Suppression of *Microdochium nivale* by Phosphite in Cool-season Amenity Turfgrasses

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Abstract

The ascomycete fungus *Microdochium nivale* (Fr.) Samuels and Hallett (teleomorph *Monographella nivalis* (Schafnitt) is one of the most ubiquitous and damaging pathogens of cool-season amenity turfgrasses. Current control measures rely on inputs of chemical fungicides, making alternative means of disease reduction desirable. Phosphite (PO_3^{3-}), which is derived from the alkali metal salts of phosphorous acid (H_3PO^{3-}), has proven efficacy in reducing susceptibility to oomycete pathogens. The aims of this research were to determine if PO_3^{3-} treatments to amenity turfgrasses can suppress the incidence of *M. nivale* infection, to determine the processes involved in such suppression and to assess the effect PO_3^{3-} treatment had on turfgrass growth and quality. The research produced significant and novel data. *In vitro* inhibition of *M. nivale* mycelial growth was determined by amending PDA with PO_3^{3-} and phosphate (PO_4^{3-}), with concentrations from 0.5 to 1000 µg/ml. It was determined that PO_3^{3-} concentrations of 100 µg/ml and above, fully inhibited mycelial growth, with EC_{50} values from 35.95 to 48.22 µg/ml. PO_4^{3-} caused no inhibition. Microscopic analysis of hyphal morphology showed distinct irregularities in *M. nivale* growing on PO_3^{3-} amended PDA, while on PO_4^{3-} amended PDA, mycelial growth was normal. Further *in vitro* studies determined PO_3^{3-} was fungistatic rather than fungicidal, and that the presence of PO_3^{3-} in growth media significantly inhibited conidial germination. Field trials determined significantly lower percentages of *M. nivale* incidence on PO_3^{3-} treated plots of turfgrass, when compared with untreated controls, with the addition of PO_3^{3-} significantly enhancing fungicide efficacy. Turfgrass quality on all PO_3^{3-} treated plots was significantly better than either control or PO_4^{3-} treated plots. Analysis of PO_3^{3-} treated turfgrass tissues using High Performance Ion Chromatography, determined rapid *in planta* accumulation, symplastic mobility and no conversion to PO_4^{3-}. The data also indicate that PO_3^{3-}, applied sequentially at four week intervals, would maintain leaf tissue amounts of approximately 2000 ppm, but would lead to cumulative accumulations in meristematic tissues. Furthermore, PO_3^{3-} applications applied sequentially in excess of a six month period, can lead to increases in soil P levels. In phosphorus (P) deficient rootzones foliar-applied PO_3^{3-} does not supply an available form of P and can repress plant P deficiency responses. In P sufficient rootzones foliar-applied PO_3^{3-} increases plant biomass, with a reduction in root to shoot ratios. Assessment of turfgrass infection incidences determined *M. nivale* hyphae are the main source of inoculum and that infection was by means of stomatal penetration. Conidia produced via sporodochia following infection, are the means of propagation and dispersal. Analyses of infected turfgrass confirmed that increased synthesis of phenolic compounds and H_2O_2 are a component of initial defence responses and that PO_3^{3-} pre-treatment, enhanced these responses. In conclusion, this work has shown that phosphite, when applied sequentially, as a component of a balanced nutrient programme, will suppress *M. nivale* incidence, increase the efficacy of turfgrass fungicides and lead to an enhancement of turfgrass quality. The results of this research will lead to changes in golf green management procedures, resulting in reduced requirements for chemical plant protectants, with added benefits of cost savings and a possible reduction in environmental impact.

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Table of Contents

List of Figures

List of Tables

List of publications

Dempsey, J. Wilson, I. Spencer-Philips, PTN and Arnold, D. (2014). Phosphite mediated inhibition of *Microdochium nivale*. Proceedings of the 4th European Turfgrass Society Conference, July 2014, Osnabrueck, Germany. European Turfgrass Society.

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1. Introduction and literature review

1.1 General Introduction

Worldwide, amenity turfgrasses provide surfaces for numerous sports and recreational facilities, parks, home lawns and general ground cover in many diverse areas. Numerous genera of grasses are used; in temperate climates, cool-season species, using C3 photosynthesis predominate. Amenity turfgrasses in temperate climates, are dominated by the festucoids, some of the more common species used being *Poa* spp*. Lolium* spp*., Festuca* spp*. Agrostis* spp. (Christians, 2005; Turgeon, 2005). Disease prevention and control is a major factor in the successful management of amenity turfgrasses, with pathogenic fungi being the major infectious agents of disease (Beard and Oshikazu, 1997; Vargas, 2005). Disease management is one of the more contentious and problematic areas of turfgrass maintenance, with managers using numerous cultural and chemical methods as part of Integrated Pest Management (IPM) programmes to reduce disease incidence and severity. *Microdochium nivale* (teleomorph *Monographella nivalis* (Schafnitt)), (Smiley *et al.*, 1992) is an ascomycete facultative parasite, which is the causal agent of the most important and common turfgrass disease of temperate climates, Microdochium patch, infecting most cool season turfgrass species (Smiley *et al.*, 1992; Beard and Oshikazu, 1997; Mann, 2002a; Vargas, 2005). While IPM is used to limit this disease, utilisation of chemical fungicides is the foremost tool used. This gives rise to a number of contentious issues:

- adverse public opinion due to perceived high frequency of use;
- associated costs;
- inhibition of non-target beneficial microorganisms;
- development of fungicide-resistant populations;
- the possibility that fungicide usage will be reduced by legislative restrictions.

This ensures that research into alternative methods of reducing susceptibility to this pathogen is desirable. One such possibility is the use of compounds such as phosphite (PO_3^{3-}, Phi), as part of IPM programmes (Cook *et al.*, 2006). Phi is an anion of phosphorus (P) and has been used extensively to control numerous phytopathogens. It has been shown to inhibit disease development via direct fungistatic means and indirectly, through stimulation of plant defence responses (Fenn and Coffey, 1984; Fenn and Coffey, 1987; Grant *et al.*, 1990b; Jackson *et al.*, 2000; Mc Carren *et al.*, 2009).

1.2 Review of Literature

This review explores the infection processes of phytopathogens, in particular *M. nivale*, and how challenged plants respond via a range of constitutive and induced defence mechanisms. Phosphorus nutrition, the differences between phosphate and phosphite and the role of phosphite as a plant nutrient are examined. Phosphite's role as part of an IPM programme to reduce *M. nivale* susceptibility in amenity turfgrasses is the primary focus of this research, therefore, the fungistatic properties of phosphite are detailed, as are its abilities to stimulate or enhance defence mechanisms in plants.

1.2.1 Cool season turfgrasses

Numerous species of Poaceae are used for amenity turfgrass purposes, with the choice of species primarily depending on factors such as intended use, playing surface properties, soil type, aesthetic value and climate (Turgeon, 2005). They provide groundcover for recreational facilities and high quality playing surfaces for numerous sports, such as football, rugby, tennis, athletics and the main focus of this research, golf. In temperate climates, cool-season species using C3 photosynthesis predominate. These species are adapted to favourable growth where temperatures are not extreme in the winter or summer, with optimum growing temperatures ranging from 15° to 25° C. These turfgrasses are generally found in temperate and subarctic climates and may become dormant or injured during high temperatures (Turgeon, 2005). Most cool season turfgrass exhibit a surge of growth in the spring, which then slows down or stops during the warmer summer months. Growth then increases again during cooler temperatures in the autumn, but during the winter months growth will significantly slow or even cease (Christians, 2005) (Fig. 1-1). It is during periods of excessive summer heat or winter dormancy that cool-season turfgrasses experience greatest disease pressures.

Figure 1-1. Seasonal growth patterns of cool- and warm-season turfgrasses, used with permission from N. Christians (Christians, 2005).

Desirable turfgrass species for amenity use vary according to climatic region and proposed usage, and four of the most widely used species are used in this research:

- *Agrostis stolonifera* L.;
- *Agrostis canina canina* L.;
- *Lolium perenne* L.;
- *Poa annua* L.

1.2.1.1 *Agrostis stolonifera*

Agrostis stolonifera L. (Creeping Bentgrass), is a fine-textured stoloniferous species, which is currently the most widely used cool-season turfgrass for putting green establishment worldwide (Turgeon, 2005). It has a wide range of cultivars, with varying degrees of leaf fineness, shoot density, growth habits and disease susceptibility (Smiley *et al.*, 1992; Beard and Oshikazu, 1997; Vargas, 2005), (Fig. 1-2).

1.2.1.2 *Agrostis canina canina*

Agrostis canina L. *ssp. canina* (Velvet Bentgrass), is an extremely fine-textured, moderately stoloniferous turfgrass, which forms a velvety sward of very high density. It is less adapted to climatic ranges and is less widely used than *A. stolonifera,* with a more limited range of available cultivars (Turgeon, 2005) (Fig. 1-2).

Figure 1-2. Examples of cool-season turfgrass swards. A: *Agrostis stolonifera* golf green. B: *Agrostis canina canina*.

1.2.1.3 *Lolium perenne*

Lolium perenne L. is a competitive, cool season grass that is well adapted to moderate temperatures and is known for its rapid germination and establishment, tolerance to traffic, resistance to insects and stress. Because of this, it is often used in mixtures with other species for use in a wide range of sports surfaces (Fig. 1-3).

Figure 1-3. *Lolium perenne* playing surface. Typical example of *L. perenne* football surface.

1.2.1.4 *Poa annua*

Poa annua L. is regarded as a successful weed species within golf greens in most parts of the world (Beard, 1982). Although rarely planted intentionally, the adaptability of *P. annua* and its tolerance of extremely low heights of cut (2-4 mm), compacted soil and shade, ensures it is in fact, the predominant species in the sward of most temperate golf greens (Hagley *et al.*, 2002). It is a species which includes numerous biotypes ranging from coarse-leaved, true annuals to fine-leaved, perennials, as found in golf green swards (Beard, 1999) (Fig. 1-4). One of its major liabilities is its susceptibility to, and slow recovery from, turfgrass diseases and its susceptibility to *M. nivale* is a major reason for its status as a weed species (Mann, 2004a; Vargas, 2005).

Figure 1-4. *Poa annua* golf green sward.

These four species are used worldwide, to provide playing surfaces for many sports,

all are susceptible to common turfgrass pathogens, and in particular *M. nivale,* thus making them ideal candidates for this research.

1.2.2 Turfgrass disease, golf course factors

Disease prevention and management on golf courses is one of the more contentious and problematic areas of turfgrass maintenance. Turfgrass managers employ numerous cultural and chemical methods as part of Integrated Pest Management (IPM) programmes to reduce disease incidence and severity.

Requirements for a successful infection by a plant pathogen is often illustrated by use of the 'disease triangle' (Fig. 1-5). In temperate climates the host (susceptible turfgrasses) and the pathogen are present throughout the golf course. The area of greatest disease pressure and symptom development is the golf green. This is due primarily to the environmental factors created by high levels of traffic and the intense maintenance regimes employed.

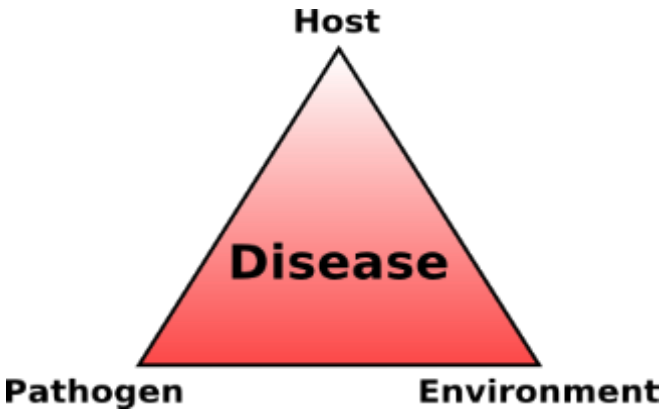

Figure 1-5. The disease triangle. The disease triangle is used to illustrate the interaction of factors required for successful infection of plants by a pathogen.

To achieve acceptable playing surfaces, modern golf greens are maintained at mowing heights typically of 2 to 3 mm and receive minimal nutrient and irrigation inputs. These maintenance regimes are specified so tightly that any imbalance can lead to an increase in environmental conditions conducive for disease development. The timing and amounts of nutritional inputs are an example of the fine balances required: too little nutrition can lead to a weakened plant, while excessive amounts, especially in autumn or winter, can lead to soft tissues, more susceptible to disease (Mann, 2004a). A further example would be an increase in the thatch layer. Beard (2002) describes thatch as "an intermingled organic layer of dead and living shoots, stems, and roots of grasses that develops between the turf canopy of green vegetation and the soil surface". Pathogens can inhabit these plant residues and an increase in thatch depth can lead to higher levels of inoculum. Thatch layers should be kept to a minimum as excessive depths can lead to environmental conditions advantageous for the pathogen (Mann, 2004a).

1.2.3 Plant pathogens

As in general plant disease occurrences, in amenity turfgrasses, phytopathogenic oomycetes and fungi are the most damaging, forming a large diverse group of organisms, which have a unique lifestyle, a worldwide distribution and many varied habitats (Isaac, 1992; Beard and Oshikazu, 1997; Knogge, 1998; Feys and Parker, 2000; Talbot, 2004; Vargas, 2005). Management of amenity turfgrasses, as used in sports and recreational complexes worldwide, has to deal with a wide array of these phytopathogens, employing numerous techniques, involving many person-hours and high financial costs, in order to maintain acceptable levels of turf quality. An understanding of these phytopathogens' biology and lifestyle is therefore a crucial factor when determining treatments or procedures to inhibit or reduce the occurrence and damage caused by them.

1.2.3.1 Oomycetes

While oomycete pathogens are not directly involved in this research, previous studies with oomycetes in areas of turfgrass disease, plant defence responses and the fungistatic properties of phosphite are relevant. Oomycetes are part of the oomycota group of microorganisms which display fungal-like growth morphology, producing mycelium similar to fungi. Previously they were considered to be true fungi and part of the *Mycota*. Research using systematics, molecular biology and ultrastructural processes, re-classified them as *Chromista* or *Straminipila*, depending on different authorities and their view on ancestry of this group of organisms, more similar to chromophyte algae and heterotrophic protoctista than fungi (Campbell and Reece, 2002; Kamoun, 2003; Van West *et al.*, 2003; Lutzoni and Kauff, 2004). A major distinction between oomycetes and fungi, is the methods by which they synthesise amino acids and also differences in the composition of the cell walls. Oomycete cell walls are composed of glucans and small amounts of hydroxyproline and cellulose rather than glucans and chitin as in fungi (Agrios, 2005). These differences are of particular relevance when determining the efficacy and mode of action of fungistatic compounds (Campbell and Reece, 2002; Lutzoni and Kauff, 2004; Agrios, 2005; Ott, 2005; Mclaughlin *et al.*, 2009).

There have been many studies involving oomycetes and the use of phosphite as both a direct and an indirect inhibitor of their growth (Fenn and Coffey, 1987; Guest and Grant, 1991; Daniel and Guest, 2005), and these studies have been used in helping to understand possible similar effects phosphite may have on fungal growth.

1.2.3.2 Fungal pathogens

Fungi make up a diverse kingdom composed of a wide range of eukaryotic, spore bearing organisms, which interact extensively with plants, animals, bacteria and other organisms. They are classified into a number of divisions, depending upon their morphological features, reproductive methods and means of nutrient acquisition (Isaac, 1992; Agrios, 2005). Fungi are classified as either *Myxomycota* (slime moulds) or *Eumycota*, which are the true fungi (Isaac, 1992). The *Eumycota* are divided into five divisions (Agrios, 2005). The *Mastigomycota* predominantly aquatic fungi, flourishing under moist conditions and characteristically produce motile cells and therefore are known as zoosporic fungi. The *Zygomycota* produce sexual spores known as zygospores, as well as asexual sporangiospores. The *Deuteromycota* lack a known sexual cycle of reproduction and are therefore said to be "imperfect". The *Basidiomycota* produce large fruiting bodies - basidiocarps, such as a typical mushroom. The final division is the *Ascomycota* (Agrios, 2005), which is of particular interest here, as it contains the pathogen of this research *M. nivale.*

1.2.3.3 Ascomycota

The *Ascomycota*, commonly referred to as ascomycetes, employ a heterotrophic, absorptive, nutrition process, enabling them to obtain nutrients from preformed organic compounds. A positive aspect of this mode of nutrition is that they are major decomposers and re-cyclers of organic materials in the soil (Mclaughlin *et al.*, 2009). A negative aspect is that they have evolved methods to obtain their nutrient requirements which include infection of living plant tissues (Dickinson and Lucas, 1982; Isaac, 1992). The ascomycetes include a wide range of important phytopathogens, including the powdery mildews and the pathogen causing Dutch elm disease (*Ophiostoma* spp.). *M. nivale* is an ascomycete and causal agent of numerous diseases and disease complexes in many species of the Poaceae.

1.2.4 Microdochium nivale

Microdochium nivale (Fries) Samuels & Hallett (teleomorph *Monographella nivalis* (Schafnitt)) is an ascomycete pathogen and causal agent for many disease complexes in numerous species of cereals, forage and turf grasses (Smiley *et al.*, 1992; Tronsmo *et al.*, 2001; Pronczuk *et al.*, 2003). The taxonomy of *M. nivale* is varied with a number of nomenclature changes since having first been described as *Lanosa nivalis* by Fries in 1825 (Jamalainen, 1943). Prior to 1980, it was known as *Fusarium nivale* Ces. Ex. Berlese and Vogl., this despite not having a pediciallate basel foot cell in the conidia (Diamond and

Cook, 1997). It is still commonly known as Fusarium patch in sportsturf management. Identification was based on conidial morphology, which was also used in the early 1930s to categorise *F. nivale* into two varieties *F. nivale* var. *nivale*, and var. *majus.* This separation was based primarily on the difference between their average conidial sizes, the conidia of *M. nivale* var. *majus* are larger in size (width from 4.2-6.0 μm, length 15-33 μm) than those of *M. nivale* var. *nivale* (width no larger than 3.8 μm, length 8-27 μm), and possess more septa (1-7 compared to 0-3) (Wollenweber, 1930; Wollenweber, 1931; Gerlach and Nirenberg, 1982). This identification based on conidial morphology was not accepted by many researchers however, as it was found that many individual isolates of these species fall within an ambiguous range, rendering morphological differentiation alone unreliable (Litschko and Burpee, 1987; Lees *et al.*, 1995). This separation of the species into two variations was confirmed later by Gams and Muller (1980), Nirenberg (1981) and Gerlach and Nirenberg (1982), however, none could determine any significant differences between the two varieties using any other morphological features. The classification was changed in 1980, from *Fusarium* to a new genus *Gerlachia* and the species named *G. nivalis* var. *nivalis* and *G. nivalis* var. *major*. This removal from the *Fusarium* genus was on the basis that it was an outsider in the genus, as the conidia lacked any sign of foot-cell differentiation; it was entirely light-dependent for sporulation and also on the reaction to certain fungicides (Gams and Muller, 1980). Following this, Samuels and Hallet (1983) compared *G. nivalis* with species of *Microdochium* and found them to be congeneric. *Gerlachia* was considered to be a taxonomic synonym of *Microdochium*, and Samuels and Hallet proposed the new designation of *M. nivalis* var. *nivale* and *M. nivalis* var. *majus. M. nivale* remained divided into two varieties, although this separation was not universally accepted as the use of the identifying morphological characteristics for taxonomic purposes was questioned (Nelson *et al.*, 1983). Litschko and Burpee (1987) for example, were unable to differentiate selected isolates on the basis of conidial morphology, conidiogenesis, response to fungicides or asexual compatibility among thalli, and suggested that distinct biotypes of *M. nivale* did not exist. Other research, however, suggested there were in fact two distinct varieties. Lees *et al*. (1995) using random amplified polymorphic DNA (RAPD) and separated isolates of *M. nivale* from wheat into two sub-groups which corresponded to the morphologically-defined varieties. This work was supported by Parry *et al*. (1995) who used polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region, followed by restriction enzyme digestion of the PCR product, to distinguish two distinct varieties within *M. nivale. M. nivale* is now acknowledged to be two distinct species,

Microdochium nivale (Fr.) Samuels and Hallett and *Microdochium majus* (Wollenw.) Glynn and Edwards, comb. nov.. This is due to the work of Glynn *et al*. (2005) who defined them as separate species based on a number of characteristic sequence polymorphisms in the translation elongation factor 1 alpha gene (TEF-1a), as well as the reported biological differences of these two fungal species. Both *M. nivale* and *M. majus* are constituents of a series of disease complexes causing pre- and post-emergence death of wheat, barley and oat seedlings, leading to reduced establishment and reductions in grain yield. In mature plants they are causal agents of wheat head blight, foot rot and ear infection and *Microdochium* Leaf Blotch of oats (Pettitt *et al.*, 1993; Humphreys *et al.*, 1995; Clement and Parry, 1998; Pronczuk *et al.*, 2003; Cockerell *et al.*, 2009). There are reports indicating host specificity (Maurin *et al*., 1995) and specialisation to tissue types (Lees *et al*., 1995). Analyses of Canadian turfgrass isolates using RAPD and Restriction Fragment Length Polymorphism (RFLP), found only *M. nivale* (Mahuku *et al.*, 1998). This was supported in a European study by Hofgaard *et al.* (2006), when they concluded that isolates of *M. nivale* were more pathogenic on *Lolium perenne* than *M. majus.* In cereals, Simpson *et al.* (2000) determined that *M. majus* selectively infected wheat and oats, while *M. nivale* preferably infected rye. However, the opposite was concluded in a more recent study by McNeil *et al.* (2012) who identified *M. nivale* as the major pathogen in oat with *M. majus* infecting barley.

1.2.4.1 *M. nivale* and turfgrasses

In amenity turfgrasses *M. nivale* infects most cool season species, each year affecting 100% of golf courses in the UK and Ireland (Smiley *et al.*, 1992; Beard and Oshikazu, 1997; Mann, 2002a; Vargas, 2005). *M. nivale* is regarded as a highly opportunistic pathogen, due to its ability to attack plants over a wide range of environmental conditions. Infection takes place primarily in moist conditions below 18° C, with optimum occurrence between 0° and 6°, and under prolonged snow cover, *M. nivale* causes pink snow mould (Smiley *et al.*, 1992; Beard and Oshikazu, 1997).

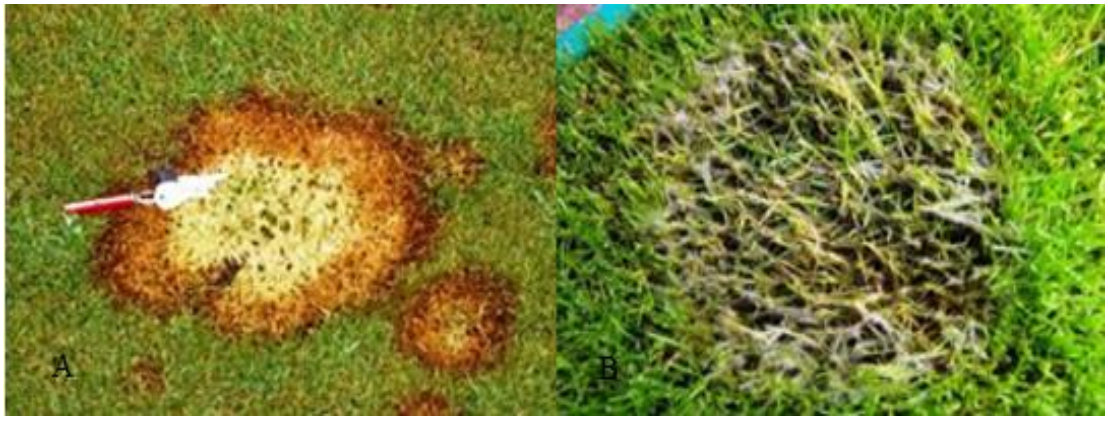

Figure 1-6. Examples of *Microdochium nivale* infection of turfgrass from Ireland. Both examples above show the typical radial growth pattern common to *M. nivale* infection. A: infection patch on golf green. B: infection patch on a greenhouse turfgrass sample showing mycelial growth.

In the northern hemisphere, greatest disease pressure is between October and March each year, although infection symptoms can be observed almost year round. Symptoms initially appear as small circular spots, orange/brown in colour, one to two cm in diameter, which can increase and coalesce to form large irregular shapes greater than 20 cm across. Significant and long lasting damage can occur to both the visual and playing qualities of fine turf surfaces (Mann, 2004a), as shown in Figs 1-6 and 1-7.

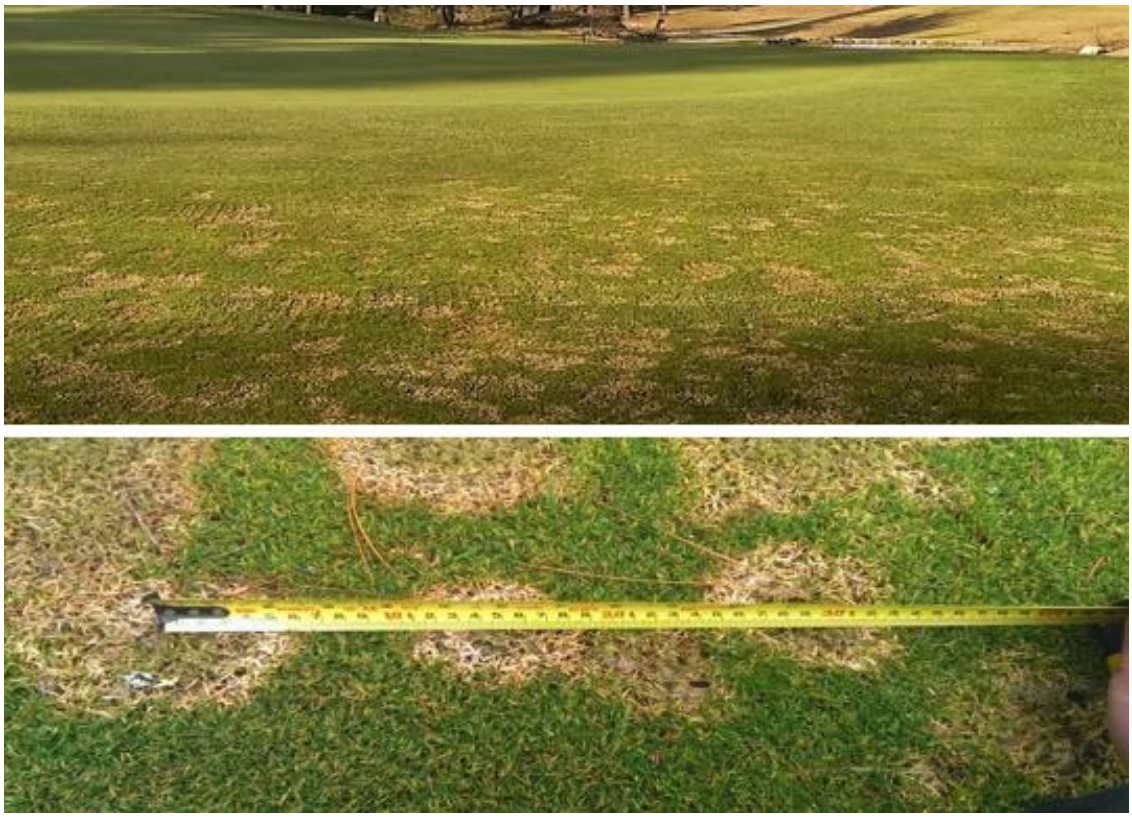

Figure 1-7. Surface damage caused by *Microdochium nivale* infection on golf greens in British Colombia, Canada, used with permission from J. Haines (Haines, 2014)

The mycelium varies from sparse to densely flocculated, and white to pinkish/white in colour. Chlamydospores have not been reported and although the teleomorph has been observed on cereals, it has not been found on turfgrasses (Tronsmo *et al.*, 2001). Macroconidia are the asexual spore, and are curved, falcate, tapering towards each end with a pointed apex and a wedge-shaped, rounded base (Fig. 1-8). Conidia are often used as a means of identification; in *M. nivale* they have zero to three septa, predominantly one, with a maximum length of 27 µm, while in *M. majus* they have predominantly 3 or more septa. Lees *et al.* (1995) concluded that conidial width could distinguish the narrower var. *nivale* from var. *majus*, a distinction not universally accepted, with some researchers questioning the validity this method of morphological characterisation for taxonomic purposes (Litschko and Burpee, 1987; Krans and Morris, 2007).

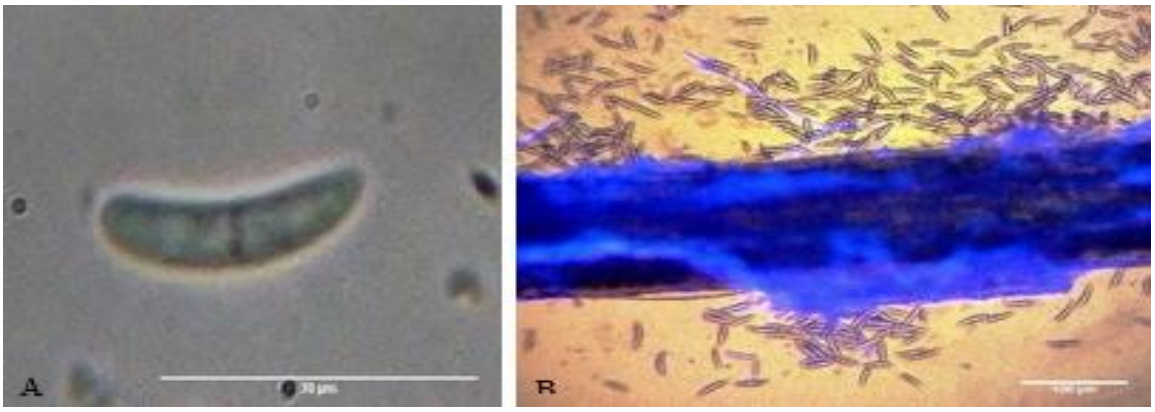

Figure 1-8. *Microdochium nivale* conidia. *M. nivale* reproductive structures. A: single conidium showing one septa, B: a mass of conidia emanating from an infected turfgrass leaf.

The conidia can be formed sparsely in aerial mycelium, but more commonly in sporodochia, which results in large numbers of airborne conidia. This is the most common and prolific means of dispersal and a major source of inoculum. The conidia can lie inactive, for example in partially decomposed organic matter such as a thatch layer in a golf green, until dormancy is broken by environmental changes, allowing for activation of the spore's metabolism and germination. The ease of dispersal of conidia and its ability to remain dormant is particularly significant from the turfgrass management perspective, because as well as being dispersed through natural means, conidia and mycelia can be transported between sites on maintenance equipment, golf shoes and golf clubs (Beard, 1982).

1.2.4.2 *M. nivale* infection process

Hemi-biotrophic fungi such as *M. nivale,* have evolved methods which allow them to gain access to plant tissues, to optimise growth, obtain their nutrient requirements from within

the plant and to reproduce, thereby continuing the cycle of re-infection (Knogge, 1996). Many fungal infections begin with spore attachment to host surfaces and continue with spore germination, host recognition, formation of infection structures, and penetration of host tissues. Hyphae then spread intra- or inter-cellularly throughout plant tissue continuing to branch within the host plant until the pathogen reproduces and infection is either halted or the plant dies (Agrios, 2005). Some fungal pathogens form haustoria which are thought to provide one avenue through which nutrients are absorbed, this is not likely to be the case for endophytic fungi however, for even if haustoria are formed, the intercellular hyphae provide an extensive interface for nutrient uptake from the apoplast (Spencer-Phillips, 1997).

In turfgrass, the cuticle is the first line of defence, providing a physical barrier, impervious to many pathogens, but against which some have evolved means to overcome. In many foliar pathogens, direct penetration of the cuticle is the common strategy, with appressoria formed which can breach this outer layer (Isaac, 1992; Talbot, 2004). Other pathogens bypass the cuticle and penetrate by way of natural openings, for example stomata or wounds such as made during maintenance operations (Knogge, 1998; Turgeon, 2005). With turfgrasses there is no conclusive determination of the *M. nivale* infection process in the literature. There are however, a number of studies detailing these processes in species of cereals (Clement and Parry, 1998; Kang *et al.*, 2004; Dubas *et al.*, 2010; Żur *et al.*, 2011).

1.2.4.3 *M. nivale* infection of cereals

With cereals, reports of infection processes vary. Kang *et al.* (2004) used electron microscopical studies of wheat infection to show that following inoculation, conidia germinated on the host surfaces, from which germ tubes and dense mycelial growth were formed. Infection hyphae penetrated the epidermal cell wall directly with a penetration peg and then spread rapidly in host tissues both inter- and intra-cellularly. There was no entering of the host tissues via stomata. Dubas *et al.* (2010) in a study of infection of triticale (a cross between wheat (*Triticum vulgare*) and rye (*Secale cereale*)), determined that infection was by hyphal growth, beginning at soil level, which proceeded to the sheaths and leaves of the plants, but that penetration occurred only through the stomata, from which haustoria were formed leading to growth into the plant cells. Zur *et al.* (2011) studying *M. nivale* infected *Secale cereale* L., confirmed that numerous hyphae penetrated the leaves via the stomata within days following inoculation with *M. nivale*. The mycelium had grown from the soil and progressively penetrated the crown cortex cells, entered the vascular tissues and spread

throughout the intercellular spaces. They also observed swollen hyphae which appeared to form vesicle-like structures resembling haustoria, however, electron microscopy would be required to confirm proper haustorium formation in living plant cells.

1.2.4.4 *M. nivale* infection of turfgrasses

Until recently there were no detailed published studies on infection process and colonisation of turfgrasses by *M. nivale*. Jewell and Hsiang (2013) studied the infection processes of both *M. nivale* and *M. majus* in *T. aestivum* and also in *Poa pratensis,* (Kentucky bluegrass, a commonly used turfgrass) and determined that both pathogens, regardless of the host species of origin, colonised and penetrated the leaf tissues via the stomata, but contrary to the studies mentioned in the previous section, haustoria-like structures were not observed. Prior to the Jewell and Hsiang study, reports indicated that the infective propagules were either mycelia or conidia or ascospores (Parry *et al.*, 1995; Mahuku *et al.*, 1998; Tronsmo *et al.*, 2001; Mann, 2004a). For example, inoculation of *L. perenne* with *M. nivale* conidia did not produce any disease symptoms, but when mycelial inoculum was used, severe disease symptoms occurred (Pronczuk and Messyasz, 1991). Mahuku *et al.* (1998) used RAPD and RFLP analyses to show that ascospores were the major source of inoculum for *Microdochium* snow mould patches on turfgrasses. Turfgrass pathologists generally consider the most common means of infection is by conidia and mycelia, disseminated from infested soil or plant debris (Mann, 2004a; Mann, 2004b; Turgeon, 2005; Vargas, 2005). This is supported by the fact that *M. nivale* has good saprotrophic abilities and can grow over and through soil, especially at low temperatures, and has also been shown to survive for periods of between 13 and 52 weeks in infected wheat straw (Domsch *et al.*, 1980). This has highly significant implications for its propagation in golf greens, where the upper soil profiles often contain a layer of semi-decomposed plant debris.

1.2.5 Chemical controls

M. nivale infection control in turfgrasses is achieved by implementing IPM programmes (Beard and Oshikazu, 1997), which reduces disease incidence to acceptable pre-determined thresholds. Despite this however, the use of chemical fungicides is still the foremost control tool deployed. The available arsenal of chemical plant protectants is wide, and varies in their uptake and biochemical mode of action. Most fungicides approved for turfgrass use are either contact, which remain on the outside of the leaf, or acropetal penetrants, which are mesosystemic and translaminar, thus having limited vascular mobility. With the exception of phosphite based products, no registered turfgrass fungicides are fully systemic. The

available chemistries of protectants vary in their biochemical mode of action. Pathogen activity is inhibited by interference with fungal mitosis, reduction of respiratory enzyme activity, inhibition of sterol, DNA and RNA synthesis, limitation of amino acid assimilation or inhibition of ATP production (Smiley *et al.*, 1992; Yang *et al.*, 2011). Chemical plant protectants are an integral part of IPM and while the efficacy and safety of these products is not disputed, their use can give rise to a number of contentious issues: adverse public opinion due to the perception of high frequency of use, associated costs of chemicals, possible inhibition of non-target beneficial microorganisms, development of fungicide-resistant populations, and possible legislative restrictions. This ensures that development of alternative means of reducing disease susceptibility is desirable. It has been suggested that reliance on these plant protectants could be reduced by stimulation or enhancement of inherent plant defences.

1.2.6 Plant defences

The interaction between plants and their pathogens is complex, involving a wide array of defence strategies. Initially, a fungal pathogen has to break several lines of defence measures before it achieves its target - a living cell. These include constitutive protections, antifungal preinfectional metabolites and a secondary arsenal of inducible defence processes to further combat the invading pathogen. Tuzun (2001) comments that the priming of plants with an inducing compound can incite an effective defence response upon subsequent encounters with pathogens. One of the objectives of this research is to determine if prior treatment with Phi can prime turfgrasses defences, thus allowing for reduced susceptibility to *M. nivale*.

1.2.6.1 Constitutive defences

A plant's first line of defence includes an outer protective cuticle formed mostly from cutin, suberin and waxes, which provides a physical barrier and antifungal compounds, preinfectional metabolites, prohibitins or phytoanticipins, which inhibit spore germination and germ tube elongation. These defences are constitutive and permanently in place, providing a generalised protection throughout the plants lifetime (Yang *et al.*, 1997; Grayer and Kokubun, 2001; Lack and Evans, 2002; Ridge, 2002; Taiz and Zeiger, 2006). Constitutive chemical defences can exist in healthy plants in their biologically active form, or as inactive precursors, which are then activated in response to tissue damage or pathogen elicitation. The activation of these chemical defences involves enzymes breaking down the preformed compounds, releasing the biologically active products. These preformed compounds have been referred to as phytoanticipins, which are defined as low molecular

weight antimicrobial compounds, which are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents (Vanetten *et al.*, 1994). A large number of phytoanticipins have been identified which exhibit antifungal activity, and these include phenols and phenolic glycosides, unsaturated lactones, sulfur compounds, saponins, cyanogenic glycosides and glucosinolates (Osbourne, 1996).

1.2.6.2 Induced defences

As well as these constitutive defences, plants also produce a broad, complex array of induced defences and interconnected signaling pathways, which combine to combat the invading microorganism (Agrawal *et al.*, 1999; Campbell and Reece, 2002; Taiz and Zeiger, 2006). Important for the success of this inducible defence system is rapid recognition of the pathogen by the plant, such as by recognition of elicitors (Ebel and Cosio, 1994; Hahn, 1996; Montesano *et al.*, 2003).

1.2.6.3 Elicitors

The term elicitor is used for compounds stimulating the induction of defence responses or enhanced resistance (Ebel and Cosio, 1994; Hahn, 1996). They include exogenous elicitors of pathogen origin and endogenous elicitors released by the plants as a result of the pathogenic actions. The range of elicitors is wide, with varied chemical structures that include oligosaccharides, peptides, proteins and lipids, and are usually found in low concentrations. Many are derived from pathogen cell wall fragments or produced from the action of cell wall degrading enzymes (Ebel and Cosio, 1994; Montesano *et al.*, 2003). Treatment with chitin and chitosan, components of fungal cell walls, for example, has stimulated defence responses in many plants, including species of Poaceae (Pearce and Ride, 1982; Vander *et al.*, 1998). Fragments derived from the pathogen's physical or enzymatic penetration of plant cell walls, have also triggered defence responses (Hématy *et al.*, 2009). Once the plant recognises the incursion of the pathogen a series of interconnected biochemical defence responses are deployed. One of the first of these is the hypersensitive response (HR).

1.2.6.4 Hypersensitive response

One of the most studied responses of induced defences is the HR (Goodman and Novacky, 1994; Huang *et al.*, 2004), in which cells surrounding the site of pathogen penetration switch on genes encoding for pathogenesis related proteins, before activating programmed cell

death (PCD) (De Gara *et al.*, 2003). Reactive Oxygen Species (ROS) are induced by various stresses (Dat *et al.*, 2000) including pathogen challenge and elicitor recognition (Desikan *et al.*, 1998). ROS are a key element in HR, as not only can they act directly to impair the pathogen, but also act as stress indicators and molecular messengers (Knight and Knight, 2001; Mittler *et al.*, 2004). ROS are formed through successive one electron reductions of molecular oxygen including, from most oxidised to most reduced: superoxide (O_{2}^{-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH); the reactive nitrogen species - nitric oxide (NO) is also a key component in this signaling cascade (Dixon *et al.*, 1994; Lamb and Dixon, 1997; Thatcher *et al.*, 2005; Egan *et al.*, 2007; Rookes *et al.*, 2008; Zhang *et al.*, 2009).

1.2.6.5 Hydrogen peroxide

H_2O_2 is a relatively stable ROS (Wojtasek, 1997) and plays a major role in HR. Upon elicitation of pathogen challenge, it has a direct antimicrobial effect, as part of a rapid, localised, transient, oxidative burst, directly impairing the pathogen. As well as this, H_2O_2 acts as an endogenous signal for defence gene activation (Neill *et al.*, 2002), has a key role in PCD (Desikan *et al.*, 1998) and stimulates numerous modifications to strengthen cell walls (Egan *et al.*, 2007). The timely production of H_2O_2 and its accumulation in cells has been used to determine the efficacy of a plants response to pathogen challenge. For example, the role of H_2O_2 in HR was validated by both Thordal-Christensen *et al.* (1997) and Huckelhoven *et al.* (1999) when, using histological stains, they showed accumulations of H_2O_2 in barley leaves at sites of infection of powdery mildew *(Blumeria graminis f.sp. hordei),* in cell wall appositions and in cells undergoing HR. More recently Dubas *et al.* (2010), again using fluorescent staining techniques, observed H_2O_2 accumulations in tritacle following penetration by *M. nivale,* not only in the epidermal and mesophyll cells, but significantly, in close proximity to the infection sites. The determination of the speed of synthesis and accumulations of H_2O_2 at infection sites is an excellent means to measure a plant's level of resistance or susceptibility to a particular pathogen.

That H_2O_2 plays an important role in responding to biotic stress is clear, but what is also clear is that high and unbalanced H_2O_2 levels can cause toxic effects on plants. Golebiowska *et al.* (2011) concluded that balanced H_2O_2 levels were positively correlated with resistance to pink snow mould infection (*M. nivale*) in triticale seedlings, but that higher H_2O_2 levels observed in susceptible genotypes were a result from the imbalance between H_2O_2 production and their elimination or control. Plant cells are protected against damage from

excessive ROS generated during the HR by a complex antioxidant system, which includes enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase and catalase (Tuzun, 2001).

1.2.6.6 Systemic Acquired Resistance and Salicylic acid

While HR is important in combating fungal infection as an immediate short term response, it can be viewed as a first, rapid reaction to pathogen challenge and an initial component of a symphony of complex combinations of interrelated signaling compounds and synthesis of anti-microbial molecules. The arsenal of plant defence compounds which are actively involved in longer term resistance is varied and includes jasmonic acid (JA), ethylene (ET), plant specific phenolic compounds and salicylic acid (SA) (Agrios, 2005).

Systemic Acquired Resistance (SAR) is a form of induced resistance whereby plants, preconditioned by prior pathogen infection or treatments, form increased resistance or tolerance to further pathogenic challenges (Tuzun, 2001; Campbell and Reece, 2002; Durrant and Dong, 2004). SA plays a significant role as the main chemical regulator, although it is not necessarily the actual signal molecule for SAR induction (Balmer *et al.*, 2013). The role of SA in SAR was first recognised in the 1990s and then confirmed in 2001, when a number of studies determined that increased levels of SA, not only at infection sites but also systemically, in tissues away from the infection area, is a requirement for SAR to be expressed (Delaney *et al.*, 1994; Mauch-Mani and Métraux, 1998; Dong, 2001). In support of this, Gaffney *et al.*(1994) were able to show that removal of SA from transgenic plants prevented the induction of SAR. As well as the pathogenically triggered induction of SAR, the exogenous application of SA or its functional analogues, such as thiadiazole-7-carbothioic acid S-methyl ester (BTH), has led to its induction in monocots (Dong, 2001; Hofgaard *et al.*, 2005). Morris *et al.* (1998) for example, determined exogenous treatment with BTH was as effective as the fungicide metalaxyl in inducing resistance to downy mildew in maize, while Bertini *et al.* (2003) concluded that in *T. aestivum*, antimicrobial pathogenesis related (PR) proteins, are strongly induced after both SA and BTH treatment. On the contrary, however, Hofgaard *et al.* (2005) concluded that treatment with BTH did not enhance resistance to *M. nivale* in *L. perenne*.

SAR induction leads to a restriction of pathogen growth and suppression of disease symptoms, when compared to non-induced plants infected by the same pathogen (Hammerschmidt, 1999). SAR leads to the coordinated activation of genes encoding for PR proteins, thus allowing for the accumulation of antimicrobial compounds, such as

phytoalexins (Daniel and Purkayastha, 1995; Hammerschmidt, 1999; Vranova *et al.*, 2002; Van Bel and Gaupels, 2004; Ponce *et al.*, 2009).

1.2.6.7 Phenolic compounds and phytoalexins for defence

Plants, like all other living organisms, possess primary metabolic pathways by which they synthesise and utilise essential compounds such as sugars, amino acids and nucleotides for their normal growth, development and reproduction. However, as well as these primary metabolites, plants also produce secondary metabolites which, while not essential for basic metabolic processes, are necessary for their survival ability (Ridge, 2002). Phytoalexin is a generic term covering a range of chemically diverse secondary metabolites which have antimicrobial properties They can be described as low molecular weight antimicrobial compounds produced by plants in response to infection or stress (Kuc, 1995), they accumulate at infection sites and are a means of resistance to pathogens (Stevenson *et al.*, 1997). They are undetected in plants before infection, but are detectible after attack, which gives evidence of their inducible nature (Taiz and Zeiger, 2006). Stimulation of phytoalexin synthesis can lead to a decrease in pathogenic damage (Hain *et al.*, 1993; Kuc, 1995; Heil and Bostock, 2002). The idea that defences can be activated after infection was first hypothesised by Muller *et al*. (1940) who determined that prior inoculation of potato tubers with *Phytophthora infestans* induced resistance to a following challenge by inoculation with *P. infestans.* They hypothesised that the tuber tissue, in response to the first infection, produced substances, phytoalexins, that inhibited further growth of the pathogen and also protected the plant against later infection by other compatible pathogens (Hammerschmidt, 1999).

There are numerous studies giving evidence of phytoalexins in the Poaceae. In barley (*Hordeum vulgare*), phenolic compounds have been identified as phytoalexins in plants challenged with the pathogen *Erysiphe graminis* (powdery mildew) (Christensen *et al.*, 1998; Ropenack *et al.*, 1998; Kruger *et al.*, 2002). Oat (*Avena sativa*), produces avenanthramides as phytoalexins (Ishihara *et al.*, 1999; Okazaki *et al.*, 2004), and wheat (*Triticum* spp.) produces hydroxycinnamic acid amides such as feruloylagmatine (Bélanger *et al.*, 2003; Jin *et al.*, 2003; Remusborel *et al.*, 2005). Studies with rice (*Oryza sativa*) also identify a number of chemically diverse phytoalexin compounds (Grayer and Kokubun, 2001; Rodrigues *et al.*, 2004; Peters, 2006). Phenolic compound accumulation was detected in a study at the site of pathogenic hyphae penetration and it was concluded that this reaction was part of the triticale defence system against *M. nivale* (Dubas *et al.*, 2010). There is little

evidence to determine the composition of phytoalexins in turfgrasses, however, Pociecha *et al.* (2009) confirmed that increased levels of phenolic compounds gave rise to higher resistance to *M. nivale* in *Festulolium* spp.

Stimulation of phytoalexins by various means has been demonstrated in many plant systems and has led to a decrease in damage by pathogens (Hain *et al.*, 1993; Kuc, 1995; Heil and Bostock, 2002). One such compound is Phi and it has been the subject of many studies over the past number of years.

1.2.7 Phosphorus in plant metabolism

Phosphorus (P) is an element required by all living organisms for growth and development. P is a major plant nutrient used in many metabolic processes: it is vital for cell division, early root formation, energy transfer, and it is a component of adenosine triphosphate (ATP), nucleic acids, nucleotides, coenzymes, phospholipids and phosphoproteins (Campbell and Reece, 2002; Ridge, 2002).

In nature, because of its reactivity, P does not occur as a free element and is only found in combinations with other elements, such as oxygen (O) or hydrogen (H). The P cycle occurs by the oxidation and reduction of P compounds by electron transfer reactions and when fully oxidised the product is orthophosphate (PO_4^{3-}; Pi). In soils at neutral pH, the Pi ion is present as a mixture of HPO_4^{2-} and H_2PO_4 and because it cannot be oxidised further, it is incorporated by means of phosphorylation directly into the plant cells (Mcdonald *et al.*, 2001).

Pi is the sole P-containing nutrient important for optimal plant growth and development and is required in amounts second only to Nitrogen (N) (Campbell and Reece, 2002). But because of its insoluble mineral form, it is largely unavailable to plants, leading to the widespread use of Pi containing fertilisers (Raghothama and Karthikeyan, 2005). Over the past number of years, however, Phi has increasingly been used as an alternative form of P nutrition in many crop systems (Raghothama and Karthikeyan, 2005).

1.2.8 Phosphite

Phi for plant use is derived from the alkali metal salts of phosphorous acid, H_3PO_3, which upon disassociation releases the phosphite ion (HPO_3^{2-}) and when fully oxidised PO_3^{3-}, Phi is formed (Guest and Grant, 1991; Rickard, 2000). Pi and Phi are chemically very similar, and both ions are formed with a central P atom. With Pi the atom sits at the centre of a tetrahedron, with oxygen atoms distributed at each point, forming a symmetrical structure, with the charge distributed evenly among these four oxygen atoms (Fig. 1-9). With Phi

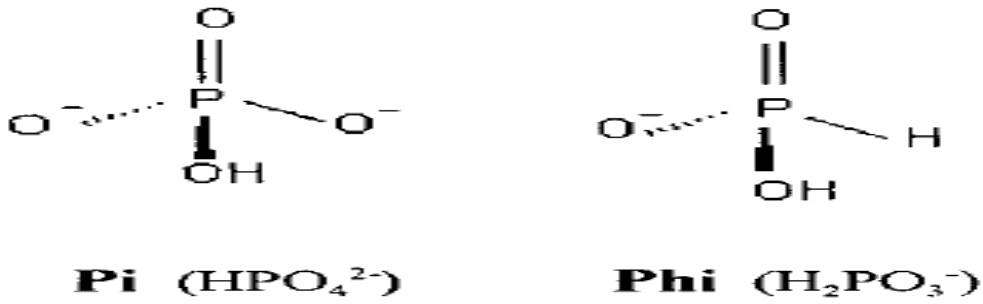

Figure 1-9. Comparison of molecular structure of phosphite and phosphate. Diagrams showing the similarity of the chemical compounds of A: phosphoric acid, HPO_4 and B: phosphorous acid, H_2PO_3, used with permission from A. McDonald (Mcdonald *et al.*, 2001)

however, one of the oxygen atoms is replaced by a hydrogen atom and although the P atom is still at the centre of the tetrahedron, the symmetry of the Pi ion is lost. For Pi to be metabolised in any living organism, enzymatic catalysation is required. The enzyme Pi binding sites recognise three of the four oxygen atoms, binding the Pi ion on the enzyme surface. Both the shape of the molecule and the charge distribution influences this process. The remaining oxygen molecule protrudes and is available to interact with other molecules in a range of metabolic processes. Because of the molecular shape of Phi however, only one face of the tetrahedron can bind to the enzyme, and this leaves the H atom and not the O atom exposed, ensuring the Phi cannot be metabolised as with Pi (Fig. 1-9).

1.2.8.1 Phosphite for plant use

Prior to use on any plant system the pH of phosphorous acid needs to be modified to prevent phytotoxicity (Ouimette and Coffey, 1988). In the 1980s the first marketable phosphite product was produced by reacting phosphonic acid with ethanol, forming ethyl-phosphonate and then combining with aluminium ions. The resulting product is referred to as Fosetyl Al or aluminium tris (O-ethyl phosphonate) (Fig. 1-10). Following uptake by plants, ethyl phosphonate is hydrolysed in the plant to phosphorous acid, and then to Phi and is successful as

a preventative treatment in controlling oomycete pathogens (Guest and Grant, 1991; Cook *et al.*, 2009).

Today however, phosphorous acid is most commonly neutralised by combining with an alkali salt, typically potassium hydroxide (KOH), although other alkali salts can be used (Ouimette and Coffey, 1990). The resulting solution contains mono- and di-potassium salts of phosphorous acid, forming potassium dihydrogen phosphite (KH_2PO_3) or dipotassium hydrogen phosphite (K_2HPO_3), commonly referred to as potassium phosphite (Fig. 1-10).

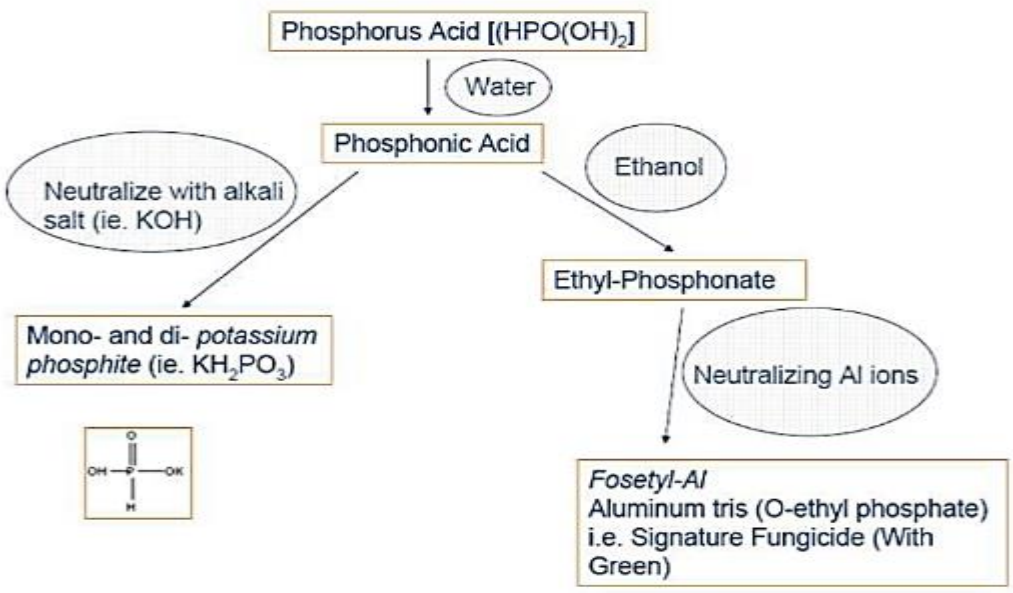

Figure 1-10. Representation of the process of producing potassium phosphite and Fosetyl-AL from phosphorous acid.

Potassium phosphite is the active substance in numerous products used in commercial horticulture and turfgrass management since the 1990s Most are described as fertilisers, and some as biostimulants (Landschoot and Cook, 2005). Phi containing products can be promoted legally as fertilisers, because after pH neutralisation they contain cations usable as plant nutrients, such as K^+, $NH4^+$, Ca^{2+}, Mg^{2+}, Cu^{2+} or Zn^{2+}, with the result that there are numerous Phi based P fertilisers currently being marketed and the list of products is increasing annually, however, the promotion of Phi as a plant nutrient is subject to controversy (Thao and Yamakawa, 2009).

1.2.8.2 Phosphite as a source of P nutrition

Results of studies researching the value of Phi as a supplier of P nutrition to many plant systems are mostly negative and at best inconclusive. The inability of Phi to provide P in a

metabolically usable form was first reported in a 1950s study, which concluded that Phi was a very poor source of P for crops (Macintire *et al.*, 1950), although they did report a favourable growth response the year following the application. This late response was explained by soil scientists from the University of California, who determined that oxidation of Phi to Pi occurred, mediated by soil microorganisms, and reported that several species of bacteria, actinomycetes, and fungi were able to assimilate Phi and release Pi in synthetic culture solutions. They also found that these organisms would not use Phi until most Pi was depleted (Adams and Conrad, 1953).

Interest in Phi as a nutrient source was re-kindled in 1990 when Lovatt (1990) reported that application of K_3PO_3 to P deficient citrus plants restored normal plant growth, and that soil or foliar applications of Phi could replace Pi as a source of P in avocado. Since then a number of studies have reported positive physiological responses from a wide range of Phi treated plants (Albrigo, 1999; Lovatt, 1999; Rickard, 2000; Vincelli and Dixon, 2005; Cook *et al.*, 2006; Dempsey and Owen, 2010).

However, despite these positive correlations of Phi application and growth, the majority of studies, as detailed in the review by Thao and Yamakawa (2009), have determined that Phi is not metabolised by plants, even though it is absorbed and translocated well. For example, Thao *et al.* (2008a) stated that Phi cannot be used as a P fertiliser and has no beneficial effect for spinach via either root or foliar application. Hydroponically grown and Phi treated tomato and pepper plants exhibited a significant reduction in growth compared with Pi treated plants (Forster *et al.*, 1998). A negative growth response from Phi fertilisation was also reported in *Brassica nigra* seedlings (Carswell *et al.*, 1996), in *Brassica napus* (Singh *et al.*, 2003) and in *Ulva lactuca* (Lee *et al.*, 2005).

1.2.8.3 Phosphite and turfgrass nutrition

The P concentration of dried turfgrass clippings is usually less than 0.5% (Turgeon, 2005), but despite this and as with many cultivated plants turfgrasses require P as a regular fertiliser input. Research into Phi specifically as a turfgrass fertiliser was published by Butler *et al.* (2009). The effects of Phi and Pi treatments on *A. stolonifera* in a greenhouse study were determined by the weekly grass dry weights, leaf tissue phosphorus content and measurement of the dry root weights. It was concluded that Phi applications have limited influence on turfgrass growth and development, when applied to a newly sown turfgrass sward. With regards to the effect of Phi on turfgrass quality, Horvath *et al.* (2007) carried out field trials at a number of locations in the United States, assessing the impact of a range

of Phi products on *A. stolonifera* (creeping bentgrass, Penn A4). Results showed that no Phi product consistently provided a significant increase in turf quality or colour. Improved turfgrass quality following sequential treatment with Phi was reported, however, by Cook *et al*. (2006) on a mixed sward of *A. stolonifera* and *P. annua* and by Dempsey and Owen (2010) on an *A. stolonifera* sward.

1.2.8.4 The effects of phosphite on the phosphate deficiency response

As seen above, there are many reports of the inability of Phi to supply nutritional P and importantly there is evidence that Phi application negates the P deficiency response, thus reducing a plant's adaptations that enable it to survive in low P situations (Forster *et al.*, 1998; Mcdonald *et al.*, 2001; Schroetter *et al.*, 2006; Thao *et al.*, 2008b; Thao and Yamakawa, 2010).

When under stress from P deficiency, plants deploy a number of physiological responses, such as increased phosphatase activity, modification of root systems and synthesis of high affinity transporters for P (Jiang *et al.*, 2007). The presence of Phi in the plant system can inhibit gene expression related to these compensatory responses. Enhanced root growth or an increased root to shoot ratio are definitive responses to P limitation, and these responses were strongly inhibited by Phi in *B. nigra* (Carswell *et al.*, 1996), tomato (Varadarajan, 2002), celery, spinach and komatsuna (Thao and Yamakawa, 2008; Thao *et al.*, 2008a; Thao *et al.*, 2008b). P starvation-induced root development in *A. thaliana* was also significantly reduced by Phi treatment (Ticconi *et al.*, 2001). Schroetter *et al.* (2006) determined a negative growth response to Phi application of maize (*Zea mays*) growing under P limited conditions, a response which was absent in P sufficient conditions. Fabricio *et al.* (2012) concluded that foliar-applied KH_2PO_3 caused harmful effects to common bean (*Phaseolus vulgaris*) growing in P-limited soil.

A number of researchers have claimed that these deleterious effects were a result of using Phi in excessive amounts or as a sole P source (Lovatt and Mikkelsen, 2006). It is also suggested that Phi, if used at appropriate rates, can provide stimulation to plants that may not occur with Pi alone and that a combination of Phi and Pi can be more effective than either ion alone (Young, 2004). Forster *et al.* (1998) found that Phi, while ineffective as a sole P source, did lead to an enhancement of tomato plants when applied in combination with Pi, compared to plants receiving Pi alone.

Most studies reporting growth enhancement to Phi were carried out under field conditions (Lovatt, 1990a; Albrigo, 1999; Rickard, 2000; Watanabe, 2005), where phytopathogens could have influenced plant growth and development. While the value of Phi as a plant nutrient may be inconclusive, it has proven efficacy as an inhibitor of phytopathogens (Fenn and Coffey, 1984; Fenn and Coffey, 1987; Grant *et al.*, 1990b; Jackson *et al.*, 2000; Mc Carren *et al.*, 2009). The possibility that any growth enhancement in the field was due to the ability of Phi to inhibit phytopathogen activity is a factor which must be considered.

1.2.8.5 Phosphite in disease control

The fungicidal properties of Phi were discovered at Rhone-Poulenc Agrochemical Laboratories in France during the 1970s (Guest and Grant, 1991). They discovered that phosphite salts were effective in controlling diseases caused by a group of oomycete fungi in the *Peronosporales* order (*Phytophthora*, *Plasmopara*, *Pythium* and others). Soon after this discovery, Fosetyl Al was formulated under the trade name Aliette and released for commercial use (Guest and Grant, 1991).

1.2.8.6 Phosphite disease control in turfgrass

Fosetyl-Al was the first Phi based product specifically for turfgrass use. Initially it was introduced to control *Pythium* spp. but was subsequently combined with the dithiocarbamate fungicide mancozeb,(Beard and Oshikazu, 1997), which improved turf quality and controlled Summer Decline of bentgrass (Cook *et al.*, 2006). A number of other trade products were then formulated based on Phi and used in combination with other fungicides to control Yellow Tuft (caused by *Schlerophthora macrospora*) and Summer Stress Complex (Cook *et al.*, 2006; Schroetter *et al.*, 2006).

In turfgrass, apart from Dempsey and Owen (2010), Phi research has been focused primarily into its value in controlling diseases such as Pythium (*Pythium* spp.) and Anthracnose (*Colletotrichum cereale*) and as a means to improve the overall quality of turfgrass swards (Sanders, 1983; Vincelli and Dixon, 2005; Cook *et al.*, 2006; Horvath *et al.*, 2007; Cook *et al.*, 2009). There is no definitive published research in the literature into the ability of Phi to either reduce susceptibility to *M. nivale* or be responsible for inducing or enhancing defences in turfgrass. There are however, many examples of successful inhibition of phytopathogens by Phi in a wide range of plant systems, although the means by which this is achieved is still debated (Abbasi and Lazarovits, 2006). Studies have been published showing Phi inhibiting phytopathogens by both direct fungistatic means and indirectly

through stimulation of host defence processes (Fenn and Coffey, 1984; Fenn and Coffey, 1987; Grant *et al.*, 1990b; Jackson *et al.*, 2000; Mc Carren *et al.*, 2009).

1.2.8.7 Direct mode of suppression

Pythium spp. are oomycetes, responsible for foliar and root diseases in numerous plant species, including turfgrasses. Suppression of *Pythium* by Phi under field conditions was reported by Sanders in 1983, but the same report determined no *in vitro* inhibition of the pathogen, and it was concluded that *in planta* control resulted from stimulation of host defence responses. However, Fenn and Coffey (1984) concluded that mycelia of four *Pythium* spp. were inhibited *in vitro* when corn meal agar (CMA) was amended with Phi, at concentrations between 276 and 552 µg ml. In 2001, isolates of *P. cinnamomi* were tested *in vitro* for sensitivity to Phi and EC_{50} values (Effective Concentration which reduces growth by 50% of control growth) were determined ranging from 4 to 148 µg ml (Wilkinson *et al.*, 2001). More recent research reports that Phi reduced mycelial growth by 50%, with EC_{50} values for *Pythium* spp isolates between 38.7 and 220.8 µg/ml (Cook *et al.*, 2009).

As well as inhibiting *in vitro* mycelial growth, it has been shown that Phi in the growth media can cause adverse morphological changes in the hyphae of oomycetes (Daniel *et al.*, 2005; Wong, 2006). Phi treatment led to convolution and collapse of the cell walls of *P. cinnamomi* (Daniel *et al.*, 2005), while Wong (2006) clearly showed Phi causing deformation and lysis of reproductive spores of five *Phytophthora* species. This direct mode of inhibition seems to involve disruption of the pathogen's metabolism. For example, a study with three *Phytophthora* species showed that Phi interfered with Pi metabolism in pathogen cells, by causing an accumulation of polyphosphate and pyrophosphate, diverting ATP from other metabolic pathways, thus resulting in a decrease in growth (Niere *et al.*, 1994). A direct mode of suppression was also indicated by a study showing that Phi inhibited enzymes of the glycolytic and phosphogluconate pathways, disrupting metabolism in *P. palmivora,* by competing with Pi as an allosteric regulator on several enzymes (Stehmann and Grant, 2000).

1.2.8.8 Direct suppression of fungal pathogens

While the majority of studies on Phi mediated inhibition of phytopathogens have been with oomycetes, there are a number of studies on its effects on fungal pathogens. Mycelial growth of *Armillaria mellea* (Vahl ex Fr) Kummer, a basidiomycete, was inhibited by KH_2PO_3 with an EC_{50} of 18.6 µg/ml (Aguín *et al.*, 2006). There are also a number of studies into Phi effect on ascomycete pathogens. Reuveni *et al.* (2003) reported mycelial growth of *Alternaria*

alternata was sensitive to Phi with an EC_{50} value of 278 µg/ml and an EC_{90} value (Effective Concentration which reduces growth by 90% of control growth) of 515 $\mu g/ml^{-1}$. Burpee (2005) reported sensitivity to Phi of *Colletotrichum graminicola* (Ces.) Wils, with an EC_{50} value of 121.9 mg/ml. *Alternaria alternata* and *Botrytis cinerea* were both assessed for sensitivity to H_3PO_3, with mycelial growth in both significantly inhibited (Mills *et al.*, 2004). Reduced growth of *Fusarium culmorum* and *F. graminearum* was reported by Hofgaard *et al*. (2010) in KH_2PO_3 amended PDA. This same study included the effects of Phi on *M. majus.* At the lowest KH_2PO_3 concentration used (10 µl/ml), mycelial growth of *M. majus* was reduced by more than 90%, with full inhibition at concentrations of 100 µl/ml.

Studies on disease incidence in Poaceae determined that Phi mediated significant reductions of *M. majus* in winter wheat (*Triticum aestivum*) (Hofgaard *et al.*, 2010) and of *M. nivale* in *A. stolonifera* (Dempsey and Owen, 2010). Neither study, however, determined whether the disease reduction was caused by either stimulation of plant defences or direct (fungistatic) means.

1.2.8.9 Effects on reproductive structures

As well as inhibiting mycelial growth it has been shown that Phi in the growth medium can cause adverse morphological changes in the reproductive spores and also directly inhibit sporangia production, zoospore release and sporulation of many species of oomycete (Coffey and Joseph, 1985; Wilkinson *et al.*, 2001; Mccarren, 2006). Coffey and Joseph (1985) reported *in vitro P. cinnamom*i chlamydospore production was reduced by 50% by Phi amendments of 15–44 µl/ml. Chlamydospores of four isolates of *P. cinnamomi* grown on media with 100 µl/ml of Phi showed significantly lower germination rates when compared with those in unamended media (Mccarren *et al.*, 2009). Phi treatment led to convolution and collapse of the cell walls of *P. cinnamomi* (Daniel *et al.*, 2005), while Wong (2006) showed Phi causing deformation and lysis of reproductive spores of five *Phytophthora* species. There are also some similar reports with regard to fungal pathogens. Mills *et al*. (2004) reported that H_3PO_3 not only reduced mycelial growth but also caused complete inhibition of sporulation of *Alternaria alternata* and *Botrytis cinerea,* and full inhibition of spore germination of *Fusarium solani.* Reuveni *et al*. (2003) reported inhibition of *A. alternata* conidial germination with Phi EC_{50} and EC_{90} values of 229 and 531 µg/ml.

1.2.8.10 Indirect mode of suppression

Studies into the indirect inhibition of phytopathogens by Phi have also been published. *Vigna unguiculata* (cowpea) seedlings inoculated with *Phytophthora cryptogea* and floated

on a Phi solution exhibited reduced lesion development (Saindrenan *et al.*, 1988). The authors reported that in Phi treated leaves, larger quantities of phytoalexins (phaseollidin and kievitone) were produced compared to un-treated leaves, and that host defence reactions are involved in the mode of action of phosphite (Saindrenan *et al.*, 1988). Jackson *et al*. (2000) inoculated *Eucalyptus marginata* with a soil borne oomycete pathogen, and concluded that when Phi accumulations in the root were low, host defences were stimulated, but when Phi levels were high, direct inhibition of the pathogen occurred. Daniel *et al*. (2005) examined the effect of Phi application on *P. cinnamomi* infection of *Xanthorrhoea australis*, showing that Phi induced intense, rapid, cellular responses to pathogen challenge and suppressed pathogen ingress in both seedlings and cell cultures. In untreated *X. australis* seedlings, hyphal growth was found to be both inter- and intra-cellular 24 h post-inoculation but, in Phi-treated plants, growth of *P. cinnamomi* remained intercellular, even 72 h post inoculation. Phenolic compounds were deposited around infection sites in adjacent, uninfected cells, and they suggested that Phi increased the efficacy of host defences. Daniel and Guest (2006) concluded that in *A. thaliana* inoculated with *P. palmivora*, Phi induced rapid defence responses, including release of superoxide, localised cell death and an increase in phenolic compounds. Lobato *et al.* (2011) showed that potato tubers, following foliar treatment with Phi, exhibited a reduced susceptibility to *P. infestans*, *F. solani* and *Pectobacterium carotovorum* (previously known as *Erwinia carotovora*) infections. They suggested that Phi induced a systemic defence response in the treated plants, based partly on their findings of increased levels of phytoalexins. Reduced disease susceptibility following Phi treatment in potato tubers was also reported by Olivieri *et al.* (2012) who suggested that Phi induced molecular modifications in potato tuber periderm and cortex that enhanced disease resistance. Eshraghi *et al*. (2011) demonstrated that *A. thaliana* treated with Phi and inoculated with *P. cinnamomi* zoospores exhibited increased levels of H_2O_2 production at the site of hyphal penetration, with significant differences evident between the amount of H_2O_2 production between the Phi-treated and non-Phi-treated plants. They also concluded that Phi primed plants for a rapid response to infection involving heightened activation of a range of defence responses.

1.3 Aims and Objectives

It is quite clear from the published literature that *M. nivale* is a major turfgrass pathogen, causing significant and costly damage to numerous sports' surfaces. To control this pathogen, turfgrass managers implement IPM programmes that include extensive reliance on chemical plant protectants. This research into an alternative means to reduce susceptibility to *M. nivale* is based on the proven efficacy of Phi to suppress phytopathogens in a wide range of plant species. As an inhibitor of phytopathogens, it is clear from the published research that Phi has a complex mode of action. Evidence shows Phi acting directly as a fungistat and indirectly via stimulation of host defences. Synthesis of defence related compounds, however, come at a cost to the plant, and therefore the nutritional status and overall health of the plant is vital in its response to pathogen challenge. Intensely managed turfgrasses have limited resources to divert to defence compounds, as in order to obtain optimum playing surfaces turfgrass nutrition is kept to minimal levels. The use of Phi as a plant nutritional input is controversial and not conclusive, but there are data supporting enhanced plant quality and extra benefits from inclusion of Phi in a nutritional programme. Additionally, published research indicates that Phi may be able to reduce the occurrence *of M. nivale* and lead to an enhancement of turfgrass quality. Thus the possibility of plant health enhancement by Phi is an important factor when considering its role as a suppressor of *M. nivale*, and merits investigation.

Aims: the primary aims of this research are to determine if Phi treatments to amenity turfgrasses can suppress the incidence and severity caused by *M. nivale*, to investigate the processes involved in such suppression, and to assess any effects foliar treatments of Phi may have on turfgrass growth and quality.

Specific objectives are to:

- determine if Phi treatment reduces *M. nivale* occurrence in turfgrass;
- determine if Phi has fungistatic or fungicidal properties against *M. nivale*;
- describe the uptake, vascular translocation, accumulation and fate of Phi in treated turfgrass tissues;
- assess the value of Phi as a source of P nutrition in turfgrass;
- demonstrate whether Phi treatment enhances turfgrass growth and quality;
- describe the infection processes of *M. nivale* in turfgrasses;

- to determine whether Phi can activate biochemical defence responses in turfgrass either prior to and/or during infection, and if this then leads to an inducement of systemic acquired resistance.

1.3.1 Null hypotheses

- Phosphite treatments to amenity turfgrass will have no effect on the incidence of *Microdochium nivale*.
- Phosphite treatment to amenity turfgrass will have no effect on its growth and development.

2 *In Vitro* study on the fungistatic properties of phosphite

2.1 Introduction

The use of *in vitro* studies is an established method for determining the efficacy of compounds to inhibit the growth of, or kill plant pathogenic organisms (Mann, 2002; Glynn *et al.*, 2008; Hofgaard *et al.*, 2010).

With oomycetes, Phi has proven efficacy in inhibiting *in vitro* mycelial growth, causing adverse hyphal morphology and reducing the percent germination of reproductive structures (Coffey and Bower, 1984; Fenn and Coffey, 1984; Coffey and Joseph, 1985; Darakis *et al.*, 1997; Wilkinson *et al.*, 2001; Daniel *et al.*, 2005; Mccarren, 2006; Wong, 2006; Garbelotto *et al.*, 2008; Mccarren *et al.*, 2009).

Less research has been published into the *in vitro* effects of Phi on fungal pathogens than with oomycetes, but some have produced interesting and relevant data (Reuveni *et al.*, 2003; Mills *et al.*, 2004; Burpee, 2005; Aguín *et al.*, 2006; Hofgaard *et al.*, 2010). However, there has been no published data on the *in vitro* effect Phi may have on *M. nivale.*

When compiling a disease protection programme, an important factor is determining whether a compound is fungicidal or fungistatic. A fungicidal compound kills the pathogen while a fungistat prevents or inhibits fungal growth without killing it (Agrios, 2005). It is possible that at sufficient concentrations, fungistatic compounds will fully prevent fungal growth and sporulation, but upon removal the effects are reversed and growth will re-commence.

2.2 Aims and Objectives

The aims of this section of the research were to determine the effects Phi has on the *in vitro* growth and development of *M. nivale,* with the objectives being:

- To assess the inhibitory effects Phi may have on the *in vitro* mycelial growth of *M. nivale.*
- To determine if such inhibition is fungistatic or fungicidal.
- To assess the effect Phi has on conidial germination and growth.

2.3 Materials and methods

Four separate experiments were used to assess the effects of Phi on: 1) the *in vitro* growth of *M. nivale;* 2) the fungistatic properties of Phi; 3) the effects of Phi on *M. nivale* hyphal morphology; 4) effects of Phi on *M. nivale* conidial germination.

2.4 *M. nivale* mycelial inoculum

Four isolates of *M. nivale*, designated MN1 to MN4, were used in the experimental procedures. Two isolates were obtained from infected golf greens on Irish golf courses, Royal Curragh Golf Club (MN1) and Slade Valley Golf Club (MN2) and two isolates from the Sports Turf Research Institute, Bingley, UK, (MN3 and MN4).

The *M. nivale* isolates from Ireland (MN1 and MN2) were obtained by collecting infected turfgrass plugs, placing in sealable plastic bags, Fig. 2-1A, into which was placed wet tissue paper, to enhance mycelial growth. The plugs were kept in darkness at 19^{o} +/- 2^{o} C for 48 hours, after which all had produced copious amounts of mycelium. Infected leaves were detached, rinsed in sterile distilled water (SDW) and surface sterilised in a 1% NaOCl solution. After rinsing in fresh SDW, they were plated onto potato dextrose agar (PDA,) 19g/l (Himedia Potato Dextrose Agar, Sparks Laboratory Supplies, Dublin), amended with 1 ppm streptomycin. The plates were incubated in darkness at 19^{o} +/- 2^{o} C to allow fungal colonies to develop, Fig. 2-1B.

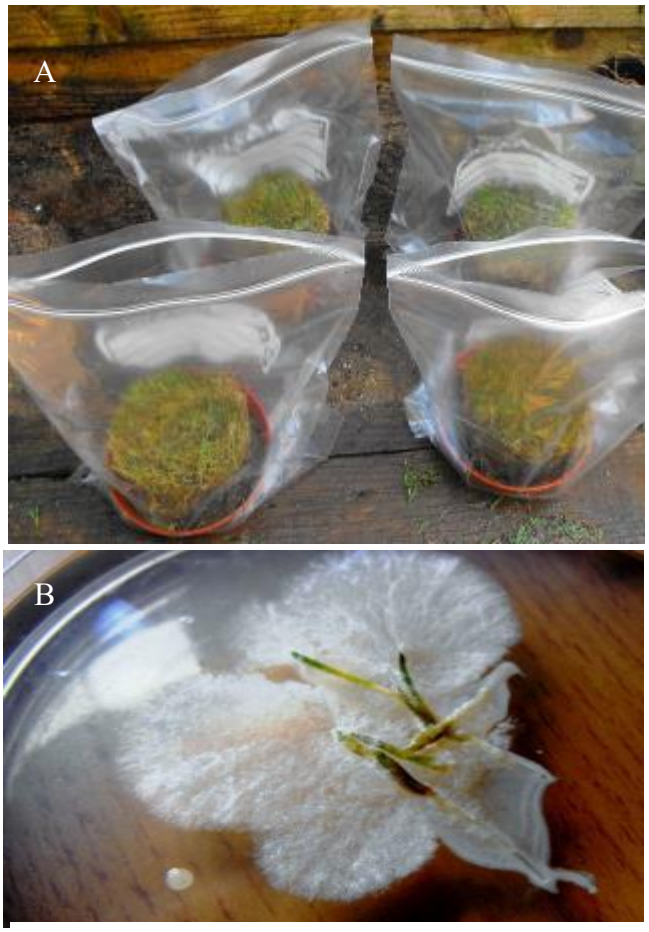

Figure 2-1 *M. nivale* infected turfgrass used as source of isolates used for experimental procedures. A: Infected turfgrass plugs in sealable plastic bags. B: *M. nivale* mycelium growing from infected turfgrass leaves.

M. nivale isolates were cultivated by cutting a 5 mm plug of agar from the actively growing edge of the colonies and re-plating them. After a number of isolation procedures, *M. nivale* mycelium was either used immediately for experimental procedures or stored on PDA slopes at -20 ° C. The MN3 and MN4 isolates were maintained on PDA slants and stored as above until used in experimental procedures. All *M. nivale* isolates were originally identified on the basis of colony characteristics, conidial morphology and on re-infection symptoms, as in Kock's postulates. These identifications were later confirmed following DNA extractions in TrisEDTA buffer and testing by polymerase chain reaction (PCR), using primers, EFniv-F/EF-Mic-R , as described by Glynn *et al.*(2005) (Crops Research, Oak Park, Teagasc, Carlow). To induce conidiation, agar plugs cut from actively-growing colony margins, were placed centrally on PDA plates. They were incubated in darkness for 48 hours, after which they were exposed to UV light at room temperature. Conidiation was induced within 48 hours of exposure. Conidia were harvested by flooding the plate with SDW and scraping with a sterile rod. Conidia were used immediately thereafter.

2.4.1 PDA amendments

2.4.1.1 H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH

H_3PO_3 and H_3PO_4 amendments were obtained from 1 M solutions of reagent grade phosphorous and phosphoric acids. KH_2PO_3 and KH_2PO_4 amendments were prepared by titrating 1 M solution phosphorous and phosphoric acids with 6 M reagent-grade potassium hydroxide (KOH) to pH 6.5. KOH amendments were prepared from 6 M potassium hydroxide, all amendments were serial diluted to required concentrations, chemicals supplied by Lennox Laboratory Supplies, Dublin. Concentrations of 0 (control), 10, 50 100, 250 and 500 $\mu g/ml^{-1}$ were used to assess in a number of experiments.

2.4.1.2 Commercial Phi

Commercially available Phi products labeled for either amenity or horticulture were used:

1. TKO Phosphite 0:29:26, (29% KH_2PO_3, Growth Products, New York)
2. Naturfos–WSP 0:59:39, (59% KH_2PO_3, Daymsa, Zaragoza, Spain)
3. PK Fight 0:0:28, (22% KH_2PO_3, Floratine, Collierville, TN)
4. Turfite 0:0:24 (24% NH_2PO_3, Headland Amenity, UK)
5. PK Plus 3:7:18 (14% KH_2PO_3, Grigg Bros, Idaho, USA)

These were used to amend PDA with 0 (control), 10, 50 100, 250 $\mu g/ml^{-1}$, PO_3^{3-}.

All reagent grade and commercial Phi compounds were added to autoclaved PDA via filter sterilisation, after cooling to 50° C, this was to ensure concentrations were accurate and that no oxidation of Phi to Pi occurred, as the addition of Phi to a medium prior to autoclaving can result in Phi being oxidised to Pi {Komorek, 1997 #2386}.

Assessments

2.4.2.1 Experiment 1: Effects on *in vitro* mycelial growth of *M. nivale*

Experiment 1 assessed the effect of a range of compounds on the *in vitro* mycelial growth of *M. nivale*. Five laboratory grade compounds and five commercial Phi products were used. Each utilised five different concentrations with six replications for each. Agar plugs, 5 mm in diameter, cut from actively-growing colony margins of *M. nivale,* were transferred to the centre of amended and control PDA. The plates were incubated in darkness at 18° +/- 2° C, six replicates were used for each amendment. Colony diameters were determined by measuring from either edge of fungal mycelial development in two directions at 90° to one another and reported in mm, minus the initial 5 mm inoculum. Mean values of each of the six replicates were used to calculate – mean daily growth (MDG) and percent relative growth (PRG) on amended PDA, compared to 0 $\mu g/ml^{-1}$ control PDA (calculated as – ((radial growth on amended PDA/radial growth on control PDA) × 100). PRG was used to calculate percent inhibition (calculated as 100-PRG = percent inhibition).

The effective concentration that reduced mycelial growth by 50% (EC_{50}) and 90% (EC_{90}) were determined by probit transforming the percent inhibition and regressing against the Log_{10} of amendment concentrations.

2.4.2.2 Experiment 2: Assessment of Phi as fungicide or fungistat

Mycelial plugs, 5 mm in diameter, from actively growing colony margins, were placed into 10 mL SDW containing 0 (control), 10, 50, 100 and 250 $\mu g/ml^{-1}$ of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH, 6 replications for each concentration. They were incubated in darkness at 18° +/- 2^0 C for 10 days. The plugs were retrieved, rinsed twice in SDW and transferred onto fresh PDA. Growth responses were measured and the presence or absence of growth determined if the concentrations were fungicidal or fungistatic. *M. nivale* colony diameters were determined by measuring the colony radius at four points on each plate, from the edge of the initial inoculum to the extreme area of fungal mycelial

2.4.2.3 Experiment 3: Effects of Phi on hyphal morphology

Experiment 3 microscopically assessed the effects of Phi on hyphal morphology from samples collected from Experiment 1.

Mycelial sections were collected from the outer edge of colonies growing on amended and unamended PDA. Treatment effect on *M. nivale* hyphal morphology was determined by light and fluorescence microscopy. The fluorescence indicator dye, Calcofluor white was used to visualise hyphae as in Dubas *et al.* (2010). Both light and fluorescence microscopy were performed by means of a Bresser epifluorescence microscope. Images were recorded using a Canon D1100 camera and processed by Adobe Photoshop version 5.0 LE (Adobe Systems, Inc., San Jose, CA).

2.4.2.4 Experiment 4: Effects of Phi on the conidial germination of *M. nivale*

Experiment 4 assessed the effect of a range of compounds on *M. nivale* conidial germination. Five laboratory grade compounds were used at five different concentrations with six replications for each.

M. nivale conidial suspensions were filtered through sterile cheesecloth, to remove mycelium, then 50 µl were transferred to 1.5 ml tubes and mixed with 1 ml solutions of 0 (control), 10, 50, 100 and 250 $\mu g/ml^{-1}$ concentrations of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4, and KOH. Aliquots (50 µl), of the mixtures were pipetted onto depressions in microscope slides then placed immediately on moist tissue paper in 9 cm. petri dishes and sealed, 6 replications for each concentration, Fig. 2-2. They were then incubated in darkness at 18° +/- 2^0 C for 48 h.

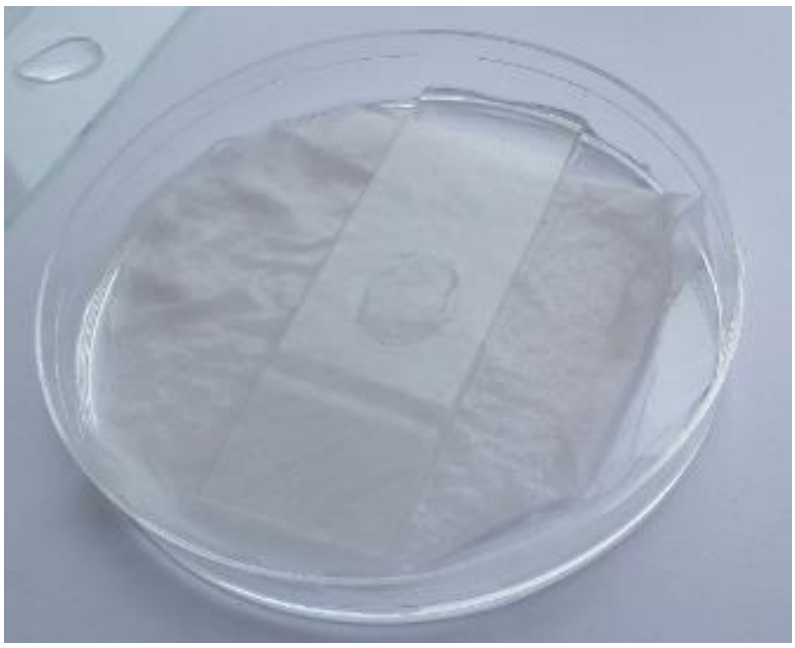

Figure 2-2 Petri dishes used for assessment of treatment effect on conidial germination.

Following this, the samples were agitated for one hour, then 20 µl were pipetted onto fresh slides.The number of germinating conidia were counted and percent germination calculated (conidia germinated/total conidia x 100). Conidia were considered to be germinated when the germ tube extended to at least twice the length of the conidia itself (Mills *et al.*, 2004).

2.5 Data analysis

All experiments were a randomised complete design with six replications. Descriptive statistics are presented with mean values ± 95% confidence intervals. Measurement of mycelial growth of *M. nivale* isolates were used to calculate MDG, PRG, percent inhibition and colony diameters. Paired samples t-tests were used to assess for any significant differences between the four isolates. Data were assessed prior to analyses to ensure they met the requirements for the relevant statistical methods used. Residual analyses were performed to test for the assumptions of the two-way Anova, outliers assessed by inspection of boxplots, normality assessed using Shapiro-Wilk's normality test (Shapiro and Wilke, 1965 and homogeneity of variances was assessed by Levene's test (Levene, 1960. Two-way Anova, assessed significant effects and interactions on MDG, percent inhibition, the fungicidal or fungistatic properties of Phi, colony diameters and on the percent germination of conidia. Where required, data were suitably transformed prior to analyses and back-transformed for presentation of charts. Where there were significant effects or interactions, one-way Anova, followed by Tukey HSD post hoc tests, at a significance level of $p < 0.05$, were used to determine and separate statistical differences. For calculation of EC_{50} and EC_{90} values, probit analysis was used to transform percent inhibition from sigmoid to linear data and then regress against the Log10 of amendment concentrations. One-way Anova then assessed for significant differences between compounds. All data analysis was performed using the statistical programme SPSS Statistics 21. Additional statistical data tables are available in the document 'Appendices to the Thesis'.

2.6 Results

Experiment 1, the effects on *in vitro* mycelial growth of *M. niva**le.***

2.6.1 Mean daily growth rates of *M. nivale* on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA

Measurement of mycelial growth of *M. nivale* isolates were carried out 4 dpi and MDG rate calculated. Paired samples t-tests determined there were no significant ($p > 0.05$) differences in responses between the four isolates, (MN1, MN2, MN3 and MN4), Table 2-1, therefore, the data were pooled to produce mean MDG values, which were used for statistical analyses. Descriptive statistics for the pooled MDG are shown in Table 2-2.

Table 2-1 paired samples t-tests comparing MDG of four isolates of *M. nivale* growing on H_3PO_3, H3PO4, KH_2PO_3, KH_2PO_4 and KOH amended PDA.

		df	t	Sig. (2-tailed)
Pair 1	Mean daily growth MN1 - Mean daily growth MN2	149	1.124	.264
Pair 2	Mean daily growth MN1 - Mean daily growth MN3	149	0.825	.411
Pair 3	Mean daily growth MN1 - Mean daily growth MN4	149	1.544	.126
Pair 4	Mean daily growth MN2 - Mean daily growth MN3	149	0.678	.499
Pair 5	Mean daily growth MN2 - Mean daily growth MN4	149	1.523	.131
Pair 6	Mean daily growth MN3 - Mean daily growth MN4	149	.987	.327

Table 2-2 Descriptive statistics for MDG rates of *M. nivale* growing on H_3PO_3, H3PO4, KH_2PO_3, KH_2PO_4 and KOH amended PDA.

Concentration	Compound	Mean mm	Std. Deviation	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0 µg/ml	H_3PO_3	10.85	0.07	10.76	10.93
	H_3PO_4	10.86	0.06	10.78	10.95
	KH_2PO_3	10.83	0.12	10.74	10.91
	KH_2PO_4	10.95	0.13	10.87	11.04
	KOH	10.88	0.12	10.80	10.97
10 µg/ml	H_3PO_3	8.79	0.06	8.66	8.92
	H_3PO_4	11.00	0.22	10.87	11.12
	KH_2PO_3	7.87	0.16	7.74	8.00
	KH_2PO_4	10.92	0.07	10.79	11.05
	KOH	10.94	0.18	10.81	11.07
50 µg/ml	H_3PO_3	4.61	0.12	4.49	4.73
	H_3PO_4	10.40	0.17	10.28	10.52
	KH_2PO_3	3.80	0.07	3.68	3.92
	KH_2PO_4	10.11	0.20	9.99	10.23
	KOH	9.98	0.12	9.86	10.10
100 µg/ml	H_3PO_3	1.14	0.06	1.04	1.25
	H_3PO_4	9.98	0.11	9.88	10.09
	KH_2PO_3	0.87	0.05	0.77	0.98
	KH_2PO_4	10.02	0.18	9.91	10.13
	KOH	9.15	0.17	9.05	9.26
250 µg/ml	H_3PO_3	0.00	0.00	-0.10	0.10
	H_3PO_4	9.55	0.17	9.46	9.65
	KH_2PO_3	0.00	0.00	-0.10	0.10
	KH_2PO_4	9.82	0.05	9.72	9.92
	KOH	8.72	0.19	8.62	8.82

Two-way Anova, using MDG as dependent variable and treatment compounds and compound concentrations as independent variables determined significant ($p < 0.05$) effects and interactions as shown in Table 2-3. The interaction represents the combined effects of compounds and concentrations on MDG and as this was significant it indicated that while the effect compounds had on MDG was significant the level of effect was influenced by the concentration of compounds used. As the effect of both compounds and concentrations were very significant one-way Anova were used to determine the effect on MDG the compounds used had at each level of concentration used, Tukey HSD at $p < 0.05$, was then used to separate statistical differences between compounds, as shown in Fig. 2-3.

Table 2-3 Two-way Anova of MDG as dependent variable with treatment compounds and compound concentrations as independent variables.

	df	f	p	η2
Compound	4,125	15488.820	< .001	0.998
Concentration	4,125	9302.005	< .001	0.997
Compound*concentration	16,125	1727.428	< .001	0.995

As would be expected, there were no significant ($p = 0.285$ by one-way Anova, df = 4, 25, Fstat 1.33) differences in MDG rates between the amendment compounds at the 0 $\mu g/ml^{-1}$ concentration. Significant ($p < 0.5$) differences in growth rates were however, determined between compounds at all other amendment concentrations assessed

At 10 $\mu g/ml^{-1}$ amendment concentrations, a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 558.349) difference in growth rates was determined. The H_3PO_4, KH_2PO_4 and KOH amended PDA, were statistically ($p > 0.05$) the same, the H_3PO_3 rates significantly ($p < 0.05$) lower, while the KH_2PO_3 were significantly ($p < 0.05$) lower that all other amendments.

At the 50 $\mu g/ml^{-1}$ amended concentrations, a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 3173.120) difference in growth rates was also determined. The H_3PO_3 and the KH_2PO_3 growth rates were significantly ($p < 0.05$) less than all others, with the KH_2PO_3 significantly ($p < 0.05$) less than the H_3PO_3. The H_3PO_4, and KH_2PO_4 amendments were statistically the same ($p = 0.144$), and the KH_2PO_4 and KOH were statistically the same ($p = 0.829$), but the KOH rates were significantly ($p = 0.021$) less than the H_3PO_4.

At the 100 $\mu g/ml^{-1}$ amended concentrations, a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 8615.760) difference in growth rates was also determined, with the H_3PO_3 and the KH_2PO_3 growth rates were significantly ($p < 0.05$) less than all others with no significant difference ($p = 0.066$) between the KH_2PO_3 and the H_3PO_3. The H_3PO_4, and KH_2PO_4

amendments were statistically the same ($p > 0.05$), with the KOH growth rates significantly ($p < 0.05$) lower than the H_3PO_4 and KH_2PO_4 amendments.

At the 250 µg/ml^{-1} amended concentration, significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 11730.173) differences were again determined. The H_3PO_3 and the KH_2PO_3 growth rates were again significantly ($p < 0.05$) less than all others. The H_3PO_4 rates were significantly ($p = 0.042$) less than the KH_2PO_4 and the KOH rates were statistically ($p < 0.05$) less than the H_3PO_4 and the KH_2PO_3.

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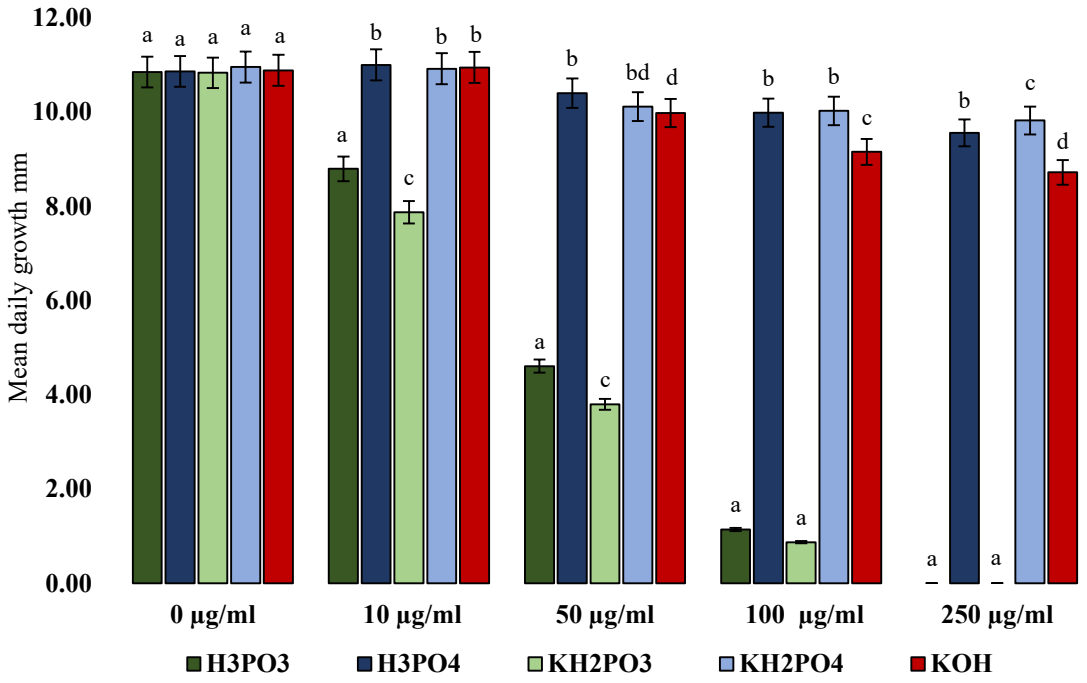

Figure 2-3 Mean daily growth rates on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA. Mean daily growth (MDG) rates in mm, of *M. nivale* growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of H_3PO_3, H_2PO_4, KH_2PO_3, KH_2PO_4 and KOH. Measurements were calculated from pooled data of each of the four *M. nivale* isolates, n=6, by measuring the colony radii at four points on each plate, 4 dpi. MDG were calculated as (mm day^{-1}). Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.2 Mean daily growth on commercial Phi amended PDA

Measurement of mycelial growth of *M. nivale* isolates established on PDA, amended with the range of commercial Phi products were carried out 4 dpi. The four *M. nivale* isolates, (MN1, MN2, MN3 and MN4), were used for the study and paired samples t-tests determined there were no significant ($p > 0.05$) differences in responses between the four isolates, Table 2-4. Therefore, as with the previous study using reagent grade compounds, the data were pooled to produce mean MDG values, which were used for statistical analyses. Descriptive statistics for the MDG rates of *M. nivale* on the amended PDA are shown in Table 2-5.

Table 2-4 paired samples t-tests comparing MDG of four isolates of *M. nivale* growing on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA.

		df	t	Sig. (2-tailed)
Pair 1	Mean daily growth MN1 - Mean daily growth MN2	149	1.235	.219
Pair 2	Mean daily growth MN1 - Mean daily growth MN3	149	1.342	.181
Pair 3	Mean daily growth MN1 - Mean daily growth MN4	149	0.982	.328
Pair 4	Mean daily growth MN2 - Mean daily growth MN3	149	0.948	.345
Pair 5	Mean daily growth MN2 - Mean daily growth MN4	149	1.036	.302
Pair 6	Mean daily growth MN3 - Mean daily growth MN4	149	0.736	.463

Table 2-5 Descriptive statistics for MDG rates of *M. nivale* growing on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA.

Concentration	Compound	Mean (mm)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0 µg/ml	TKO	10.33	0.07	10.25	10.40
	Naturfos	10.34	0.06	10.26	10.42
	PF Fight	10.31	0.11	10.23	10.39
	Turfite	10.43	0.12	10.35	10.50
	PK Plus	10.36	0.11	10.28	10.44
10 µg/ml	TKO	9.19	0.06	9.11	9.27
	Naturfos	8.93	0.18	8.85	9.01
	PF Fight	9.08	0.18	9.00	9.15
	Turfite	9.52	0.06	9.44	9.60
	PK Plus	9.26	0.15	9.18	9.33
50 µg/ml	TKO	4.76	0.12	4.68	4.83
	Naturfos	4.25	0.11	4.17	4.32
	PF Fight	4.02	0.10	3.94	4.10
	Turfite	4.78	0.12	4.70	4.86
	PK Plus	5.02	0.13	4.94	5.10
100 µg/ml	TKO	1.20	0.06	1.12	1.28
	Naturfos	1.36	0.07	1.28	1.44
	PF Fight	1.07	0.06	0.99	1.15
	Turfite	1.04	0.05	0.96	1.12
	PK Plus	1.23	0.06	1.15	1.31
250 µg/ml	TKO	0.00	0.00	-0.08	0.08
	Naturfos	0.00	0.00	-0.08	0.08
	PF Fight	0.00	0.00	-0.08	0.08
	Turfite	0.00	0.00	-0.08	0.08
	PK Plus	0.00	0.00	-0.08	0.08

Two-way Anova, using MDG as dependent variable and treatment compounds and compound concentrations as independent variables determined significant ($p < 0.05$) effects and interactions as shown in Table 2-6. As with the reagent grade compounds the interaction effect was statistically significant, indicating that effect on MDG depended on the concentration of compounds used. One-way Anova were then used to determine if the compounds used caused significant effects on MDG at each level of concentration used, Tukey HSD at $p < 0.05$, was used to separate statistical differences between compounds, as shown in Fig. 2-4.

Table 2-6 Two-way Anova of MDG as dependent variable and treatment compounds and compound concentrations as independent variables.

	df	f	p	η2
Compound	4,125	46.769	< .001	0.599
Concentration	4,125	70623.389	< .001	1.000
Compound*concentration	16,125	27.672	< .001	0.78

The commercial Phi products produced significant ($p < 0.05$) reductions in *M. nivale* mycelial growth over the range of amendment concentrations when compared with 0 $\mu g/ml^{-1}$ controls, Fig. 2-4. There were some statistical differences between products, but overall, the trend was similar to the reagent grade Phi, with the MDG rates reducing in direct correlation with increasing concentrations of PO_3^{3-}.

As with the reagent grade compounds, there were no significant ($p = 0.295$, by one-way Anova, df = 4, 25, Fstat 1.305) differences in MDG rates between amendment compounds at the 0 $\mu g/ml^{-1}$ concentration range. However, significant ($p < 0.5$) differences in growth rates were determined between compounds at all other amendment concentrations.

At 10 $\mu g/ml^{-1}$ concentrations, significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 15.082) differences in MDG rates were determined. The Turfite amendment allowed for significantly ($p < 0.05$) higher growth rates than all other products used. The lowest growth rates were in the Naturfos amendments, significantly ($p < 0.05$) lower than all other product, with the exception of the PK Fight ($p = 0.375$). There were no differences between the growth rates in the TKO and PK Fight ($p = 0.646$), between TKO and PK Plus ($p = 0.916$), and between PK Fight and PK Plus ($p = 0.206$).

At the 50 $\mu g/ml^{-1}$ concentrations, there was also a significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 79.231) difference in MDG rates. Growth rates in the PK Plus amendment was significantly ($p < 0.05$) higher than all other products used. The lowest growth rates were in the PK Fight amendments, significantly ($p < 0.05$) lower than all other product, with the Naturfos producing the second lowest rate, significantly ($p < 0.05$)

less than all others, with the exception of the PK Fight. There were no differences between the growth rates in the TKO and Turfite ($p = 0.996$).

At the 100 µg/ml^{-1} concentrations, a significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 26.999) difference in MDG rates was determined. The highest growth rates were in the Naturfos amendments, significantly ($p < 0.05$) greater than all others. The lowest growth rates were in the PK Fight and Turfite amendments, which were statistically the same ($p = 0.865$) but significantly ($p < 0.05$) lower than all other product amendments. There were no differences between the growth rates in the TKO and PK Plus ($p = 0.909$). As there was full inhibition of mycelial growth at the 250 µg/ml^{-1} amendment concentrations the data are not shown in Fig. 2-4.

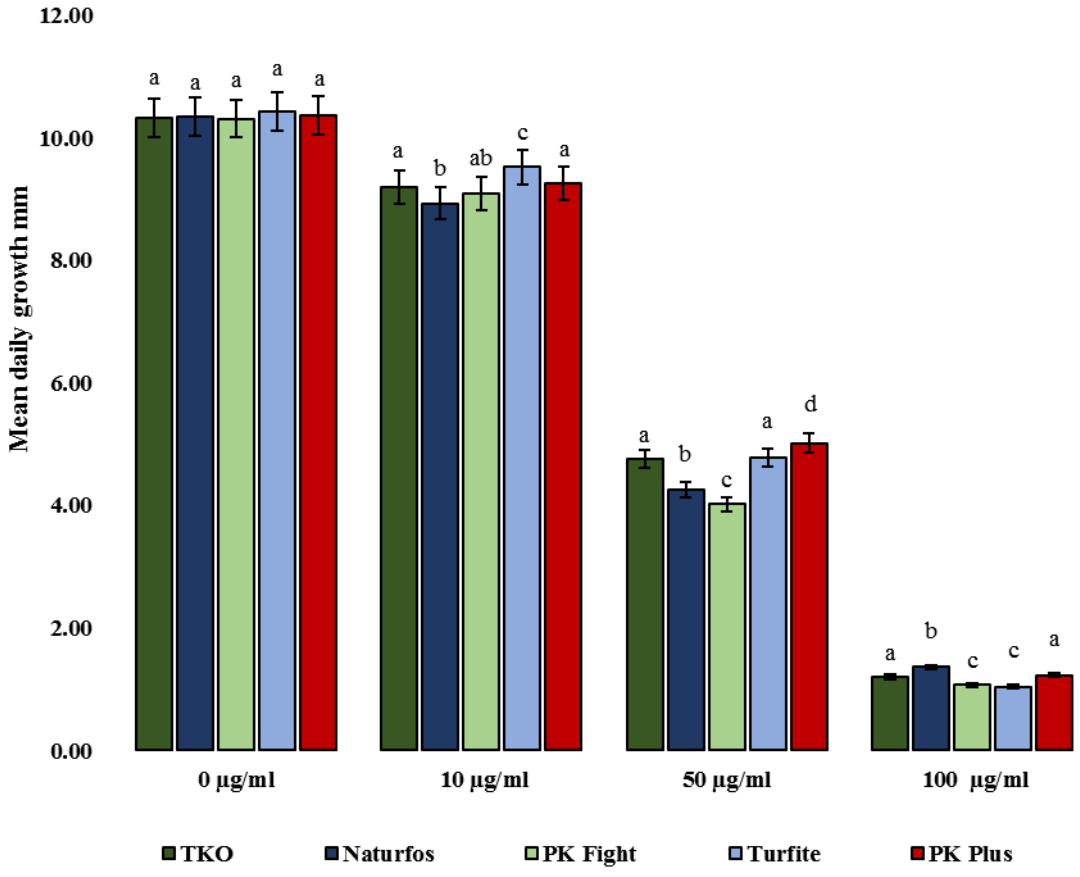

Figure 2-4 Mean daily growth rates on PDA amended with commercial Phi products. Mean daily growth (MDG) rates in mm, of *M. nivale* growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of PO_3^{3-}, derived from TKO, Naturfos, PK Fight, Turfite and PK Plus. Measurements were calculated from pooled data of each of the four *M. nivale* isolates, n=6, by measuring the colony radii at four points on each plate, 4 dpi. MDG were calculated as (mm day^{-1}). Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.3 Percent inhibition

Percent relative growth (PRG) rates, were used to determine the percent inhibition as a result of the range of compounds used. The collected data gives clear evidence that PO_3^{3-} sourced from either reagent grade compounds or commercial products, when compared to 0 $\mu g/ml^{-1}$ controls has a significant ($p < 0.05$) inhibitory effect on the *in vitro* mycelial growth of *M. nivale*, with increased percentage inhibition directly correlated to increased concentrations of PO_3^{3-}. As the effects on mycelial growth were determined as percentage inhibition, compared to mycelial growth on 0 $\mu g/ml^{-1}$ control plates, the data were arcsine transformed prior to statistical analyses and assessed to ensure they met the requirements for parametric analyses.

The analyses determined a significant ($p < 0.05$ by 2-way Anova df = 16,125, Fstat 36114.424) interaction between amendment compounds and concentrations used, with significant effect from compounds ($p < 0.05$ df = 4,125, Fstat 347542.567) and concentrations ($p < 0.05$ df = 4,125, Fstat 343425.945).

Subsequently, one-way Anova determined significant differences in growth inhibition rates at each level of amendment concentration used, with Tukey HSD at $p < 0.05$, used to separate any statistical differences between compounds.

2.6.3.1 Percent inhibition of *M. nivale in vitro* growth by H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH

At 10 $\mu g/ml^{-1}$ amendment concentrations, there was a significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 10656.897) difference between inhibition rates. The highest percent inhibition, 27.22% was from the KH_2PO_3 amendment, significantly ($p < 0.05$) greater the next highest rate of 19.23%, from the H_3PO_3 amendment, both of these percentages were significantly ($p < 0.05$) greater than the three other compounds used. Percent inhibition in the H_3PO_4 was 0.79%, with 0.35% in the KH_2PO_4 and 0.22% in the KOH amended PDA, all of which were statistically ($p > 0.05$) the same, Fig. 2-5.

At 50 $\mu g/ml^{-1}$ amendment concentrations there was also a significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 77725.958) difference between inhibition rates. The percent inhibition rates of 57.50% in the H_3PO_3 and 64.78% in the KH_2PO_3 amendments, again were significantly ($p < 0.05$) greater than all other amendments, with the KH_2PO_3 significantly ($p < 0.05$) greater than the H_3PO_3. Percent inhibition in the KH_2PO_4 of 8.07% and 8.17% in the KOH amended PDA were not significantly different ($p = 0.825$). The

percent inhibition of 4.72% in the H_3PO_4 amendments were statistically ($p > 0.05$) the lowest.

At 100 µg/ml^{-1} amendment concentrations significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 217845.431) differences were also determined. The H_3PO_3 89.45% and the KH_2PO_3 91.67%, amendments almost fully inhibited mycelial growth with significantly ($p < 0.05$) higher percentages than all other amendments. The KH_2PO_3 inhibition rate was significantly ($p < 0.05$) greater than the H_3PO_3. The 15.42% inhibition in the KOH amended PDA was significantly ($p < 0.05$) greater than the rates of 8.20% in the H_3PO_4 and 9.03% in KH_2PO_4 amendments, which were statistically the same ($p = 0.064$) and significantly ($p < 0.05$) lower than all other amendments.

Significant ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 812367.462) differences in percent inhibition rates were determined at the 250 µg/ml^{-1} amendment concentrations. The H_3PO_3 and KH_2PO_3 amendments fully inhibited mycelial growth. The KOH inhibition rate of 19.94% was next highest, significantly ($p < 0.05$) greater than the 12.30% of the H_3PO_4, which was significantly greater than the 10.70% of the KH_2PO_4 amendments.

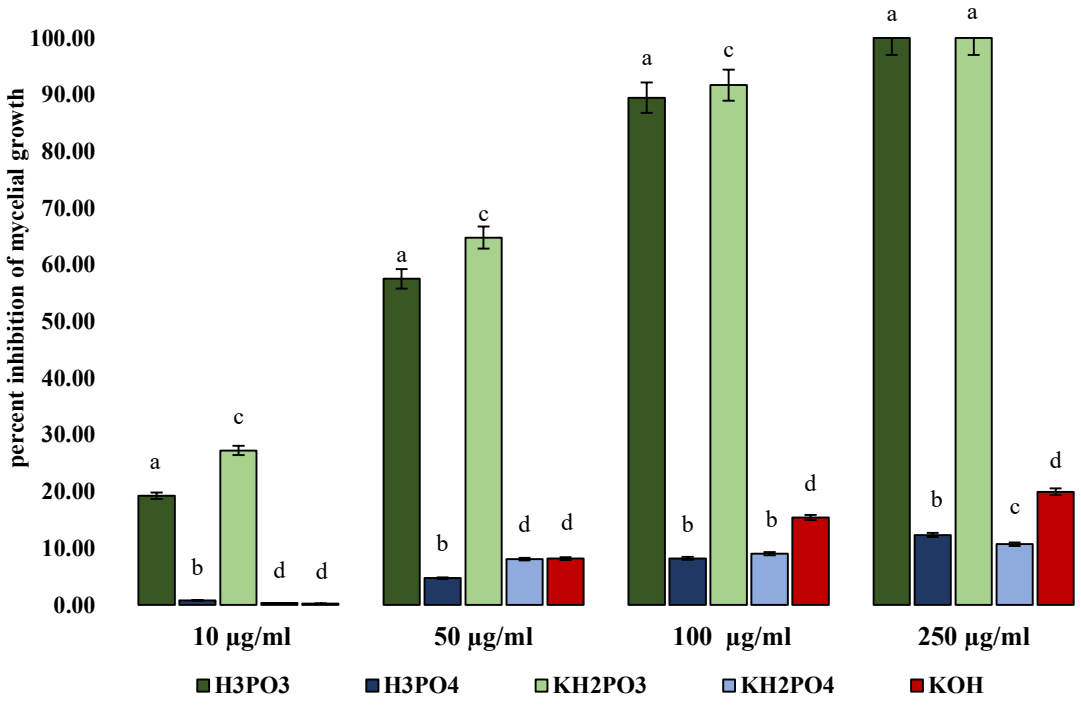

Figure 2-5 Percent inhibition of *M. nivale* mycelial growth on H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH amended PDA. Percent inhibition of *M. nivale* mycelial growth growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH. Data are mean values n=6, pooled from four *M. nivale* isolates. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.3.2 Percent inhibition of *M. nivale in vitro* growth by Phi sourced from commercial products

All the commercial Phi products used gave rise to growth inhibition in a similar manner as the reagent grade Phi. Inhibition rates varied with each product, but followed a trend of increasing inhibition with increased PO_3^{3-} concentrations. Statistical analysis determined a significant ($p < 0.05$ by 2 way Anova df = 16,125, Fstat 1341.152) interaction between amendment compounds and concentrations used, with significant effect from compounds ($p < 0.05$ df = 4,125, Fstat 1671.820) and concentrations ($p < 0.05$ df = 4,125, Fstat 51584.805). Subsequently, one-way Anova were used to determine significant effects on percent inhibition the compounds caused at each level of concentration used, Tukey HSD at $p < 0.05$, was then used to separate statistical differences between compounds.

Anova determined significant ($p < 0.05$) differences in percent inhibition rates, at each level of amendment concentration used, with the exception of the 0 $\mu g/ml^{-1}$ (control) and the 250 $\mu g/ml^{-1}$. Fig. 2-6 gives percent inhibition rates for the five products used, showing that while there were statistically different results between products at each range of amendment concentrations used, no one product gave rise to consistently less or greater inhibition rates than the others.

At the 10 $\mu g/ml^{-1}$ amendment concentration, percent inhibition rates were significantly ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 829.727) different, with significant differences determined between all five products used. Percent inhibition ranged from a low of 8.72% for the Turfite to 13.66% for the Naturfos. Inhibition rates for the other products used were 11.08% for TKO, 11.97% for the PK Fight and 10.65% for PK Plus.

At the 50 $\mu g/ml^{-1}$ amendment concentration, percent inhibition rates were also significantly ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 6360.687) different. With significant differences also determined between the five products used. Rates ranged from a low of 51.64% for the PK Plus to 61.04% for the PK Fight. Inhibition rates for the other product used were 53.91% for TKO, 58.9% for the Naturfos and 54.20% for Turfite.

At the 100 $\mu g/ml^{-1}$ amendment concentration, percent inhibition rates were significantly ($p < 0.05$, by one-way Anova, df = 4, 25, Fstat 555.784) different. Percent inhibition rates were significantly ($p < 0.05$) different between the five products used, the exception being between the TKO 88.39% and the PK Plus 88.22%, which were statistically ($p = 0.205$) the same.

The lowest inhibition rate was with the Naturfos, 86.82% with the highest rate of 90.07% being from the Turfite product, with 89.59% from PK Fight being the second highest rate. At 250 µg/ml^{-1} amendment concentrations, all five products fully inhibited mycelial growth.

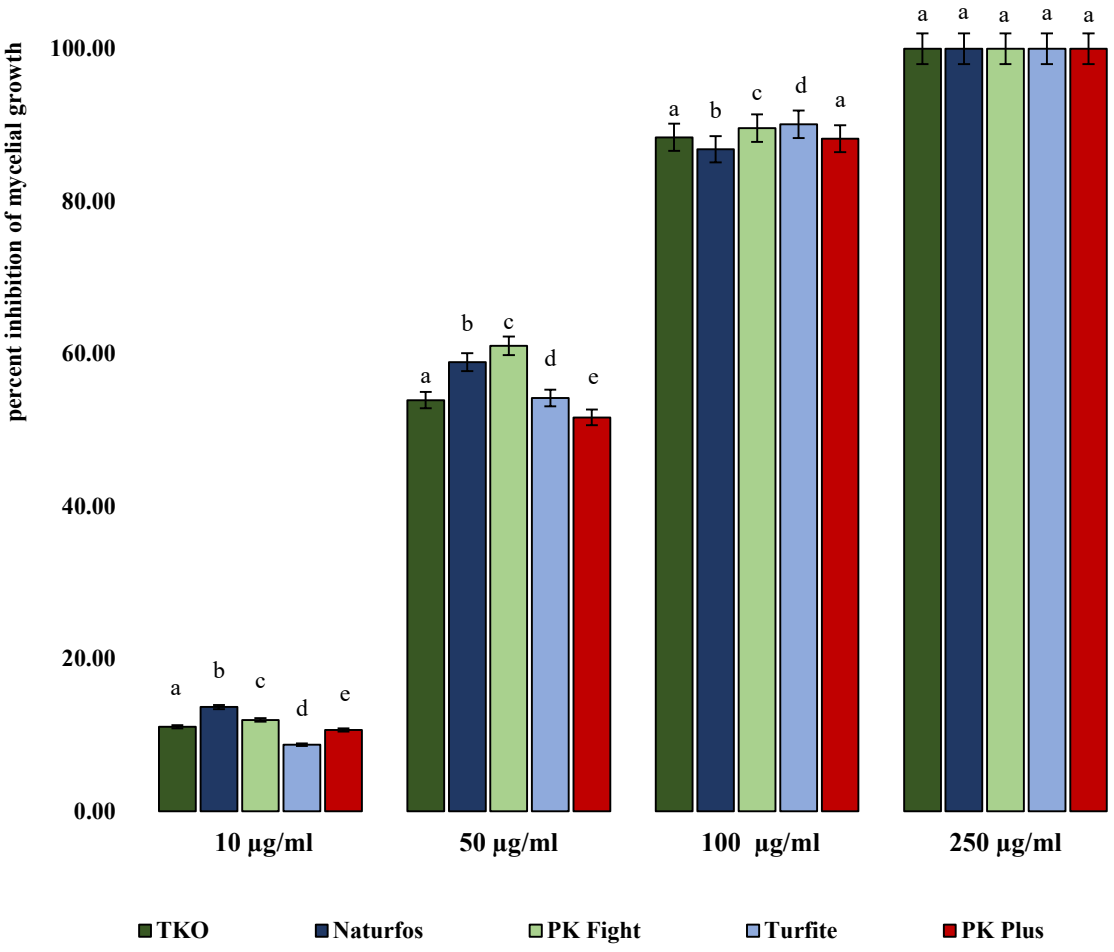

Figure 2-6 Percent inhibition of *M. nivale* mycelial growth on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA. Percent inhibition of *M. nivale* mycelial growth growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of PO_3^{3-} , derived from TKO, Naturfos, PK Fight, Turfite and PK Plus. Data are mean values, n=6, pooled from four *M. nivale* isolates. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.4 EC_{50} and EC_{90} values

EC_{50} and EC_{90} values for all amended PDA calculated at 4 dpi, are shown in Table 2-7. As there were no significant growth inhibition with the H_3PO_4, KH_2PO_3 and KOH amendments, these are not included in the table.

Table 2-7 EC_{50} and EC_{90} values, calculated by probit transforming the percent inhibition and regressing against the Log_{10} of amendment concentrations. Values are reported as $\mu g/ml^{-1}$ PO_3^{3-}, of the reagent grade and commercial Phi sources.

Compound	Log-transformed EC_{50} value	Back-transformed EC_{50} value ($\mu g/ml^{-1}$)	Log-transformed EC_{90} value	Back-transformed EC_{90} value ($\mu g/ml^{-1}$)
H_3PO_3	1.61	40.99	1.91	80.90
KH_2PO_3	1.56	35.95	1.89	77.68
TKO	1.68	47.64	1.94	87.57
Naturfos	1.65	44.58	1.93	84.36
PK Fight	1.66	45.67	1.93	85.67
Turfite	1.68	48.22	1.95	88.37
PK Plus	1.68	47.44	1.94	87.67

The EC_{50} data were analysed and the source of Phi produced significantly ($p < 0.05$, by one-way Anova, df = 6, 35, Fstat 428.703) different results. Tukey HSD post hoc analyses shows that the PK Plus (47.44 $\mu g/ml^{-1}$) TKO (47.64 $\mu g/ml^{-1}$) and Turfite (48.22 $\mu g/ml^{-1}$) were statistically ($p > 0.05$) the same and significantly ($p < 0.05$) greater than all others. The lowest EC_{50} amount was found with the KH_2PO_3 (35.95 $\mu g/ml^{-1}$), significantly $p < 0.05$) lower than all others. The H_3PO_3 at 40.99 $\mu g/ml^{-1}$, was the next lowest followed by the Naturfos (44.58 $\mu g/ml^{-1}$) and then the PK Fight (45.67 $\mu g/ml^{-1}$), Fig. 2-7 shows the back-transformed EC_{50} amounts and statistical differences between Phi sources.

The EC_{90} values were also significantly ($p < 0.05$, by one-way Anova, df = 6, 35, Fstat 729.711) different, with all compounds producing significantly ($p < 0.05$) different amounts to each other, with the exception of the TKO (87.57 $\mu g/ml^{-1}$) and PK Plus (87.67 $\mu g/ml^{-1}$) which were statistically ($p = 0.998$) the same, Fig. 2-8.

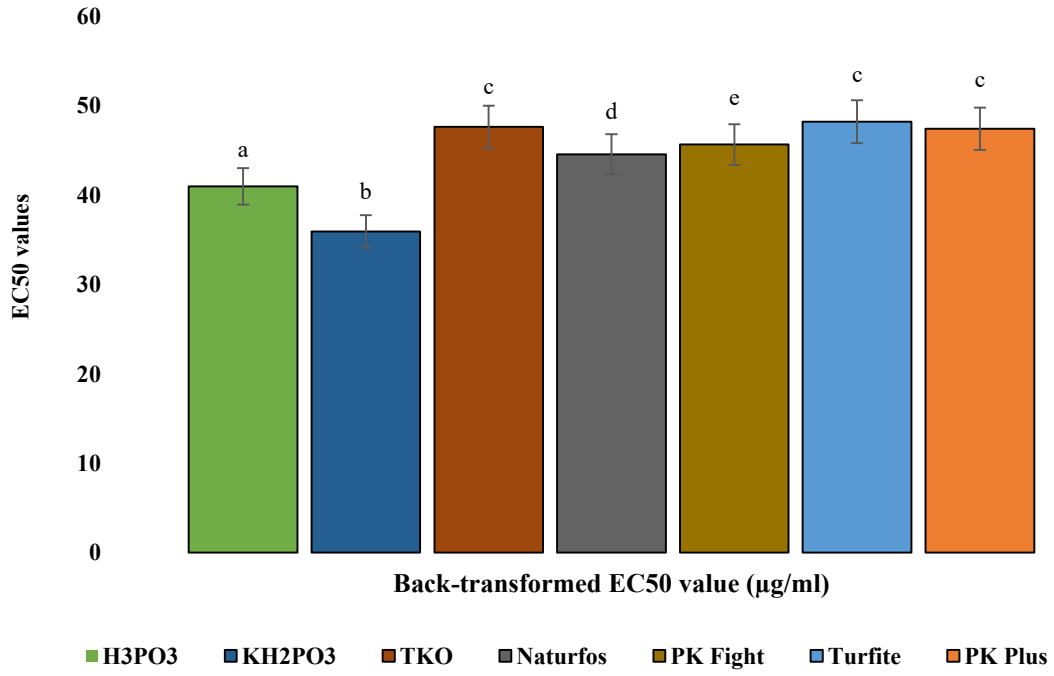

Figure 2-7 EC_{50} values of Phi sourced from reagent grade and commercial compounds. EC_{50} values calculated by probit transforming the percent inhibition and regressing against the Log_{10} of amendment concentrations, reported as $\mu g/ml^{-1}$ PO_3^{3-}, of the reagent grade and commercial Phi sources. Bars are 95% confidence intervals. Letters indicate significant differences between compounds as determined by Tukey HSD at $p < 0.05$.

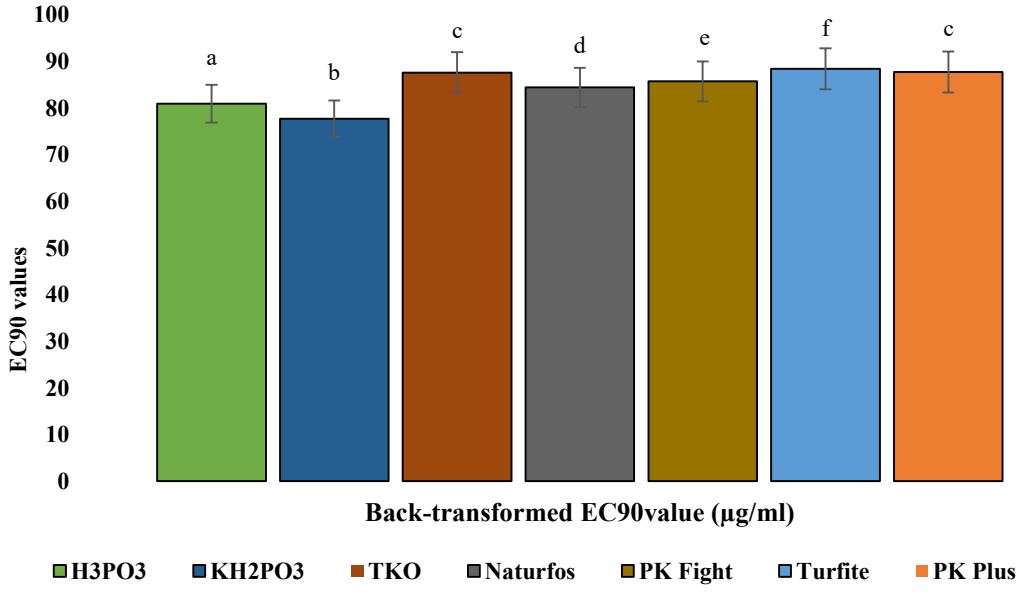

Figure 2-8 EC_{90} values of Phi sourced from reagent grade and commercial compounds. EC_{90} values calculated by probit transforming the percent inhibition and regressing against the Log_{10} of amendment concentrations, reported as $\mu g/ml^{-1}$ PO_3^{3-}, of the reagent grade and commercial Phi sources. Bars are 95% confidence intervals. Letters indicate significant differences between compounds as determined by Tukey HSD at $p < 0.05$.

2.6.5 Experiment 2, the fungicidal or fungistatic properties of Phi

Following rinsing and subsequent re-plating on PDA, *M. nivale* isolates which had been immersed in a range of concentrations of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH, were grown on and 5 days post inoculation colony diameters measured. Statistical analysis determined a significant ($p < 0.05$ by 2 way Anova df = 20,150, Fstat 118.996) interaction between compounds and concentrations used, with significant effect from compounds ($p < 0.05$ df = 4,150, Fstat 466.183) and concentrations ($p < 0.05$ df = 5,150, Fstat 4191.065) on colony diameters. Subsequently, the data file was split and one-way Anova used to determine which compounds used caused significant effects on colony diameters at each level of concentration used, Tukey HSD at $p < 0.05$, was then used to separate statistical differences between compounds.

Fig. 2-9 shows mean colony diameters in mm, 5 days post re-plating. Immersion in compounds at concentrations of 0 (control) and 10 $\mu g/ml^{-1}$, had no significant ($p > 0.05$) effect, as all colony diameters were identical at 90 mm.

There was a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 19.019) effect on colony diameters following immersion in the 50 $\mu g/ml^{-1}$ solutions. Diameters from the H_3PO_3 (86.18 mm), H_3PO_4 (86.13 mm) KH_2PO_4 (87.19 mm) and KOH (86.32 mm) were statistically ($p > 0.05$) identical, colony diameters of those which had been immersed in the KH_2PO_3 (83.8 mm) solution, were significantly ($p < 0.05$) less than all others. Immersion in the 100 $\mu g/ml^{-1}$ solutions, also had a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 83.324) effect on colony diameters. Colony diameters from the KH_2PO_3 (76.36 mm) solution was significantly ($p < 0.05$) less that all other diameters, with colonies from the H_3PO_3 (82.03 mm) solution second. The H_3PO_4 (87.69 mm) and the KH_2PO_4 (84.26 mm) produced diameters statistically ($p = 0.194$) the same, with the KOH (86.69 mm) statistically ($p = 0.526$) the same as the H_3PO_4.

Immersion in the 250 $\mu g/ml^{-1}$ solutions, had a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 303.617) effect on colony diameters. There were significant ($p < 0.05$) differences in colony diameters between all compounds used. The diameters were H_3PO_3 (71.06 mm), KH_2PO_3 (81.06 mm), H_3PO_4 (69.22 mm), KH_2PO_4 (84.48 mm) and KOH (74.27 mm).

Immersion in the 500 $\mu g/ml^{-1}$ solutions, had a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 231.344) effect on colony diameters. As with the 250 $\mu g/ml^{-1}$ solutions, there were significant ($p < 0.05$) differences in colony diameters between all compounds

used. The diameters were H_3PO_3 (54.62 mm), KH_2PO_3 (69.47 mm), H_3PO_4 (58.75 mm), KH_2PO_4 (73.84 mm) and KOH (66.47 mm).

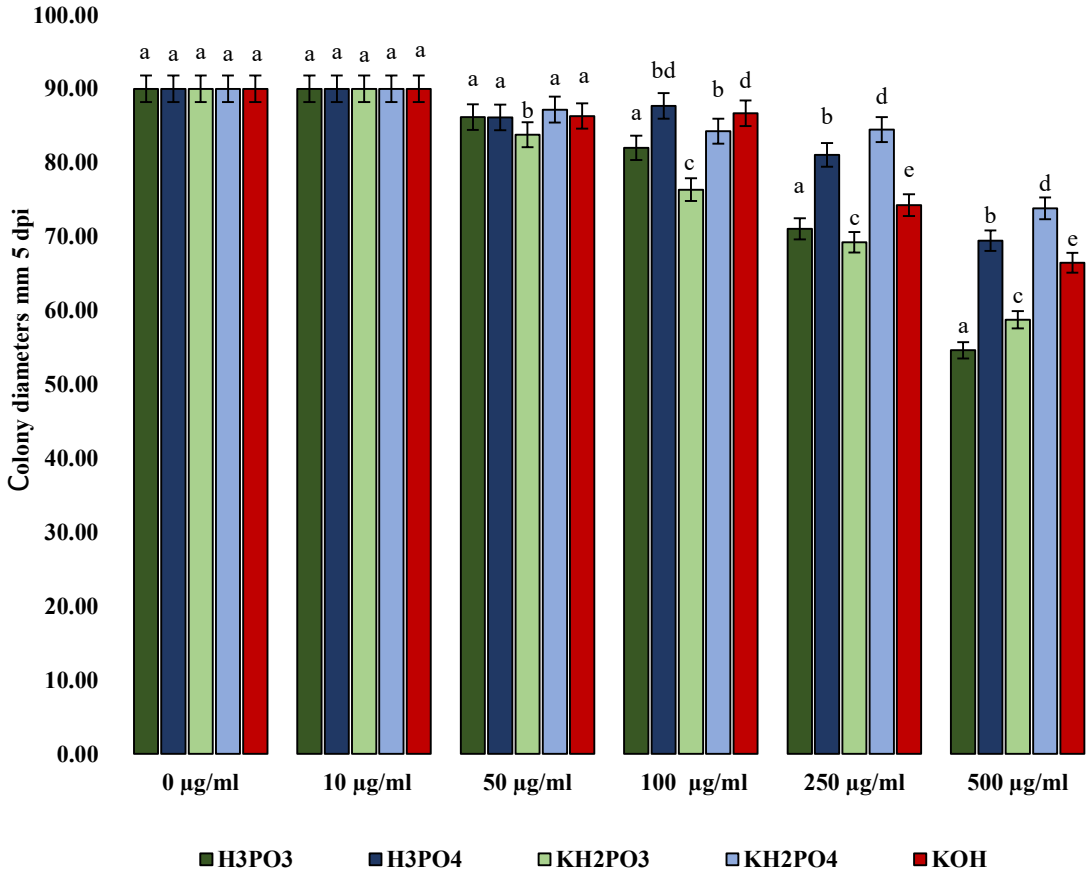

Figure 2-9 *M. nivale* colony diameters, following immersion in solutions of H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH. *M. nivale* colony diameters in mm, 5 days post inoculation, following immersion for 10 days in solutions of KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH. Data are mean values, n=6, pooled from four *M. nivale* isolates. Bars are 95% confidence intervals. Letters indicate significant differences between colony diameters at each compound concentration used, as determined by Tukey HSD at $p < 0.05$.

2.6.6 Colony diameters on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA

Further evidence of the inhibitory effect and the fungistatic rather than fungicidal properties the presence of Phi has on the *in vitro* mycelial growth of *M. nivale* is shown here. Inoculated plates were allowed to grow over 10 dpi and measurements of colony diameters recorded at 5 and 10 dpi.

At 5 dpi, *M. nivale* colonies, growing on the H_3PO_4, KH_2PO_4, KOH and the 0 $\mu g/ml^{-1}$ control plates, had grown to the maximum extent of the 9 cm petri dishes. The exception being the mycelium growing in the 250 $\mu g/ml^{-1}$ amended plates, which had attained a diameter 1 to 2 mm short of the edge of the plates, Fig. 2-10.

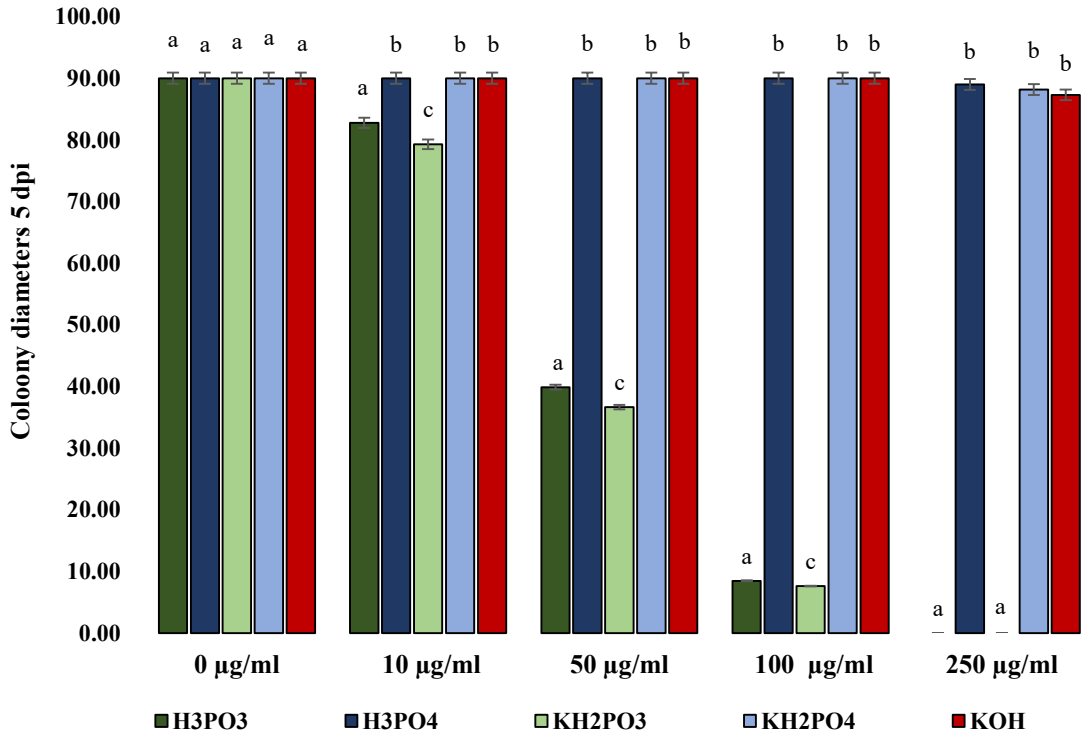

Figure 2-10 *M. nivale* colony diameters 5 dpi on H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH amended PDA. *M. nivale* colony diameters in mm, 5 days post inoculation, growing on PDA amended with 0 (control), 10, 50 100 and 250 $\mu g/ml^{-1}$ of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH. Colony diameters were determined 5 dpi by measuring the radii at four points on each plate. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.6.1 Colony diameters 5 dpi on H3PO3, and KH2PO3 amended PDA

Fig. 2-11 shows colony diameters on the H_3PO_3 and KH_2PO_3 amended plates 5 dpi. Colony diameters 5 dpi, in 10, 50, 100 and 250 µg/ml^{-1} concentrations of H_3PO_3 and KH_2PO_3 amended plates were significantly ($p < 0.05$) less than those on the H_3PO_4, KH_2PO_4, KOH and 0 µg/ml^{-1} controls.

Statistical analysis of the colony diameters on the H_3PO_3 and KH_2PO_3 amended PDA determined significant ($p < 0.05$, by two-way Anova, df = 4,50, Fstat 66.481) interaction between compounds and concentrations, a one-way Anova was then used to separate differences in colony sizes at each concentration used.

As colony sizes at the 0 (90 mm) and 250 µg/ml^{-1} (0 mm) H_3PO_3 and KH_2PO_3 concentrations were identical, Anova was not computed. At 10 µg/ml^{-1} concentration, the H_3PO_3 colony was 82.77 mm, significantly ($p < 0.05$, by one-way Anova, df = 1, 10, Fstat 115.649) greater than the colony diameter of 79.28 mm in the KH_2PO_3 amended PDA. At both the 50 and 100 µg/ml^{-1} concentrations, the colony diameters in the H_3PO_3 were significantly ($p < 0.05$, by one-way Anova, df = 1, 10, Fstat 116.513 and $p < 0.05$, by one-way Anova, df = 1, 10, Fstat 25.080) greater than the KH_2PO_3 colonies.

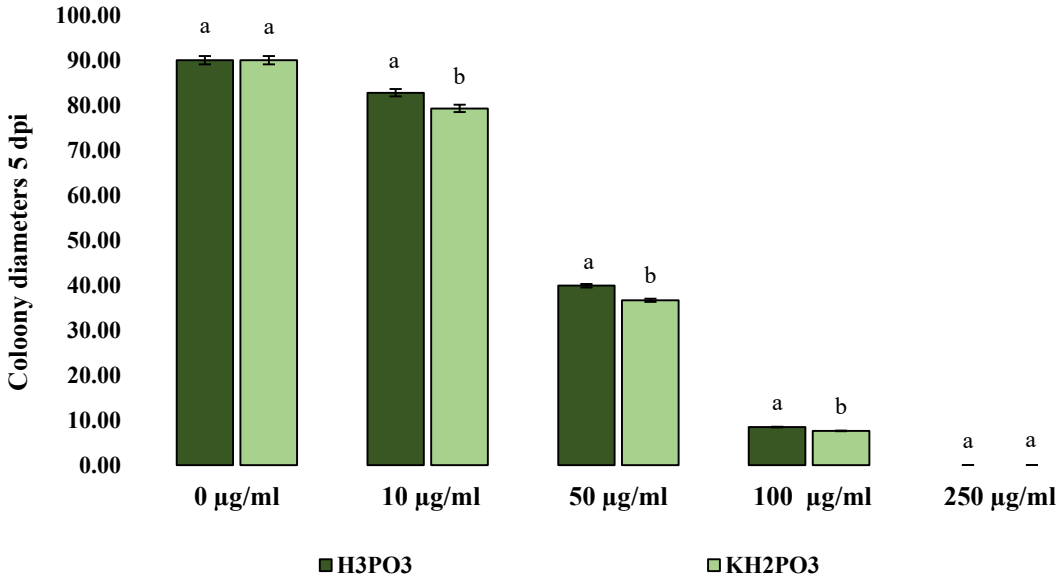

Figure 2-11 *M. nivale* colony diameters 5 dpi on H_3PO_3 and KH_2PO_3 amended PDA. *M. nivale* colony diameters in mm, 5 days post inoculation, growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of H_3PO_3 and KH_2PO_3.Colony diameters were determined by measuring the radii at four points on each plate. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.6.2 Colony diameters 10 dpi on H_3PO_3, and KH_2PO_3 amended PDA

The *M. nivale* continued to grow, but at a suppressed rate, to the end of the experimental period of 10 dpi, with growth being slower in direct correlation with increasing concentrations of PO_3^{3-} in the media. Fig. 2-12 shows colony diameters on the H_3PO_3 and KH_2PO_3 amended plates 10 dpi.

Statistical analysis of the colony diameters on the H_3PO_3 and KH_2PO_3 amended PDA determined significant ($p < 0.05$, by two-way Anova, df = 4,50, Fstat 115.396) interaction between compounds and concentrations, a one-way Anova was then used to separate differences in colony sizes at each concentration used.

As colony sizes at the 0 and 10 $\mu g/ml^{-1}$ H_3PO_3 and KH_2PO_3 concentrations were identical at 90 mm, Anova was not computed. At the 50 $\mu g/ml^{-1}$ concentration, the H_3PO_3 colony was 78.25 mm, significantly ($p < 0.05$, by one-way Anova, df = 1, 10, Fstat 156.380) greater than the colony diameter of 73.72 mm in the KH_2PO_3 amended PDA. At 100 $\mu g/ml^{-1}$ concentrations, the colony diameters in the H_3PO_3 were again significantly ($p < 0.05$, by one-way Anova, df = 1, 10, Fstat 140.858) greater than the KH_2PO_3 colonies. At 250 $\mu g/ml^{-1}$ concentrations, the colony diameters in the H_3PO_3 were again significantly ($p < 0.05$, by one-way Anova, df = 1, 10, Fstat 142.082) greater than the KH_2PO_3 colonies.

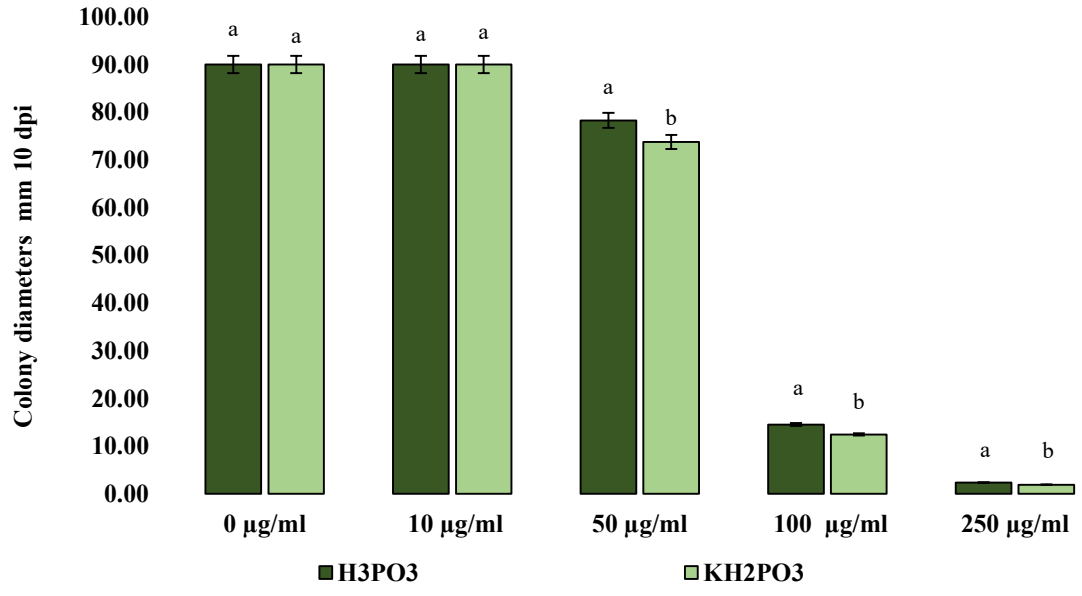

Figure 2-12 *M. nivale* colony diameters 10 dpi on H_3PO_3 and KH_2PO_3 amended PDA. *M. nivale* colony diameters in mm, 10 days post inoculation, growing on PDA amended with 0 (control), 10, 50 100 and 250 $\mu g/ml^{-1}$ of H_3PO_3 and KH_2PO_3.Colony diameters were determined by measuring the radii at four points on each plate. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.6.3 Colony diameters 5 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA

Over the 10 dpi experimental period, mycelium in amendments of 10, 50. 100 and 250 $\mu g/ml^{-1}$ grew at a suppressed rate, compared with the 0 $\mu g/ml^{-1}$ control media. At 5 dpi, *M. nivale* colonies, growing on the 0 $\mu g/ml^{-1}$ control plates, had grown to the maximum extent of the 9 cm petri dishes, with no growth determined in any of the 250 $\mu g/ml^{-1}$ amended plates.

Colony diameters 5 dpi in 10, 50 and 100 $\mu g/ml^{-1}$ concentrations are shown in Fig. 2-13. Statistical analysis determined significant ($p < 0.05$, by two-way Anova, df = 16,125, Fstat 11.955) interaction between compounds and concentrations, with significant ($p < 0.05$, by two-way Anova, df = 4, 125, Fstat 16.513) effect determined from compounds and also from concentrations used ($p < 0.05$, by two-way Anova, df = 4,125, Fstat 50050.333). One-way Anova, followed by Tukey HSD at $p < 0.05$ was then used to separate differences in colony sizes at each concentration used. Colony sizes at the 0 (90 mm) and 250 $\mu g/ml^{-1}$ (0 mm) concentrations were identical, therefore Anova was not computed.

At 10 $\mu g/ml^{-1}$ concentration, the Naturfos, Turfite and PK Plus colonies had attained the maximum diameters of 90 mm with the PK Fight colony of 87.62 mm significantly ($p = 0.008$) less than these. The TKO at 82.77 mm was significantly ($p < 0.05$) less than all others.

At 50 $\mu g/ml^{-1}$ concentrations there were no significant differences in colony diameters between any of the compounds used. The TKO was 44.77 mm, Naturfos 47.60, PK Fight 45.62, Turfite 45.94 and the PK Plus was 45.36.

As with the 50 $\mu g/ml^{-1}$ there were no significant differences in colony diameters between any of the compounds used at the 100 $\mu g/ml^{-1}$ concentrations. The TKO was 12.22 mm, Naturfos 11.70 mm, PK Fight 11.54 mm, Turfite 12.50 mm and the PK Plus was 11.37 mm.

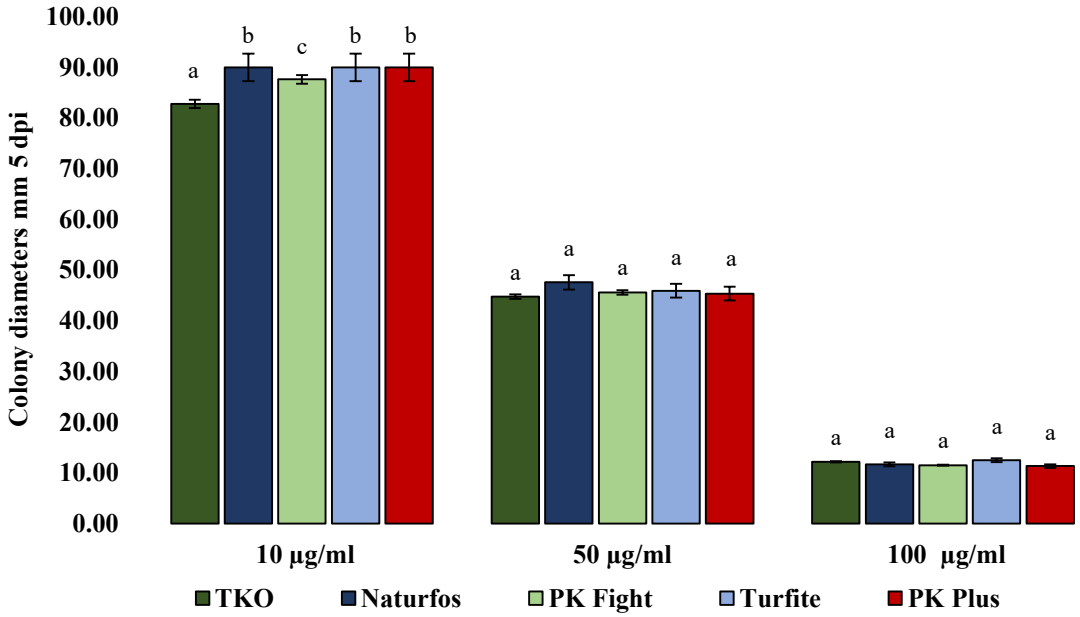

Figure 2-13 *M. nivale* colony diameters 5 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA. *M. nivale* colony diameters in mm, 5 days post inoculation, growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of PO_3^{3-}, derived from TKO, Naturfos, PK Fight, Turfite and PK Plus. Colony diameters were determined by measuring the radii at four points on each plate. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

2.6.6.4 Colony diameters 10 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA

Colony diameters 10 dpi in 50, 100 and 250 µg/ml^{-1} concentrations are shown in Fig. 2-14, colony sizes at the 0 and 10µg/ml^{-1} concentrations had attained maximum extent of 90 mm therefore they are not show in the chart.

Statistical analysis determined no significant ($p = 0.655$, by two-way Anova, df = 16,125, Fstat 0.825) interaction between compounds and concentrations, with no significant ($p = 0.720$, by two-way Anova, df = 4, 125, Fstat 0.522) effect determined from compounds. There was however, a significant ($p < 0.05$, by two-way Anova, df = 4,125, Fstat 17306.431) effect on colony sizes from the concentrations therefore, one-way Anova examined effects on colony diameters at each concentration level used.

Colony diameters at the 50 µg/ml^{-1} concentration, were not significantly ($p = 0.634$, by one-way Anova, df = 4,25, Fstat 0.647) different, with the TKO at 83.06 mm, Naturfos 82.47 mm, PK Fight 85.52mm, Turfite 84.18 mm and PK Plus 84.41 mm. At the 100

µg/ml^{-1} concentrations, the diameters were also not significantly (p = 0.346, by one-way Anova, df = 4,25, Fstat 1.174) different. The TKO were 18.55 mm, Naturfos 19.79 mm, PK Fight 18.93 mm, Turfite 18.91 mm and PK Plus 19.91 mm.

There was however, a significant (p = 0.014, by one-way Anova, df = 4, 25, Fstat 3.864) effect on colony diameters at the at the 250 µg/ml^{-1} concentration. The TKO 4.81 mm, Naturfos 4.11 mm, PK Fight 4.41 mm and the Turfite 4.98 mm were statistically (p > 0.05) the same, the PK Fight at 4.06 mm was the same as the TKO, Naturfos and PK Fight, but significantly (p = 0.035) less than the Turfite.

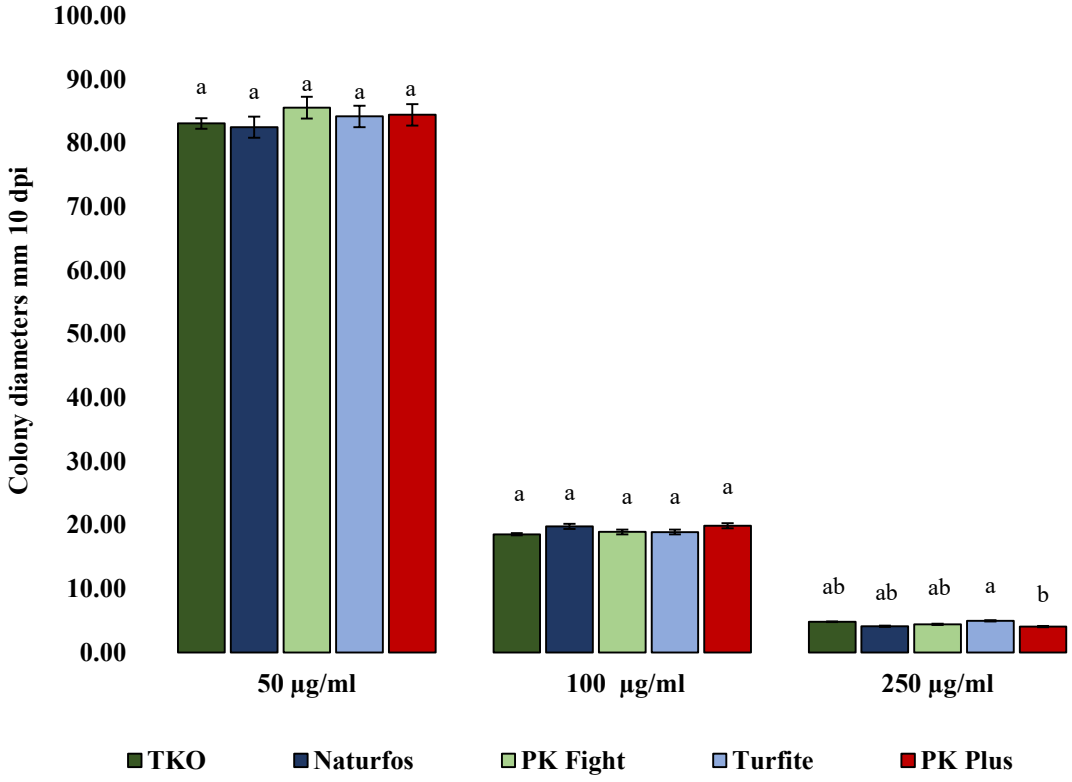

Figure 2-14 *M. nivale* colony diameters 10 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA. *M. nivale* colony diameters in mm, 10 days post inoculation, growing on PDA amended with 0 (control), 10, 50 100 and 250 µg/ml^{-1} of PO_3^{3-}, derived from TKO, Naturfos, PK Fight, Turfite and PK Plus. Colony diameters were determined by measuring the radii at four points on each plate. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at p < 0.05.

Fig. 2-15 shows comparisons between mycelial growth on 0 µg/ml^{-1} control plates and 100 µg/ml^{-1} of H_3PO_3, H_3PO_4, KH_2PO_3 KH_2PO_4 and KOH amended PDA, giving clear visual evidence of the suppressed mycelial growth in the presence of PO_3^{3-}.

Figure 2-15 Examples of colony diameters on amended PDA 5 dpi. A: Control, B: 100 µg/ml^{-1} H_3PO_3, C: 100 µg/ml^{-1} H_3PO_4, D: 100 µg/ml^{-1} KH_2PO_3 E: 100 µg/ml^{-1} KH_2PO_4, F: 100 µg/ml^{-1} KOH.

2.6.7 Experiment 3, the effects of Phi on hyphal morphology

Fig. 2-16 shows *M. nivale* hyphae, viewed using brightfield microscopy at 100X magnification. Individual hyphae as shown in Fig 2-16 A, in unamended control PDA, are visible growing with normal morphology, as evidenced by the smooth tubular formation. Hyphae grown on PO_4^{3-} (Fig. 2-16 B) and KOH (Fig. 2-16 C) amended PDA, appeared identical to those on controls. *M. nivale* grown on Phi amended PDA (Fig. 2-17) derived either from H_3PO_3 or KH_2PO_3, displayed clear disruption of hyphal morphology, when compared to hyphae on control, Pi and KOH amended PDA. In the presence of Phi *M. nivale* hyphae are swollen, stunted and short-branched with hyphal tips distorted. Further evidence of the effect Phi has on mycelial growth can be seen in Fig. 2-18, at low Phi concentrations, 10 to 50 $\mu g/ml^{-1}$ PO_3^{3-}, mycelium grew evenly, as a dense mat, while at higher PO_3^{3-} concentrations, > 75 $\mu g/ml^{-1}$, the mycelial growth was sparse and uneven. Fig. 2-19 displays mycelium growing in PO_3^{3-} and PO_4^{3-} amended PDA, the mycelium in the presence of PO_3^{3-} is dense and less flocculated than mycelium growing in the PO_4^{3-} amended PDA.

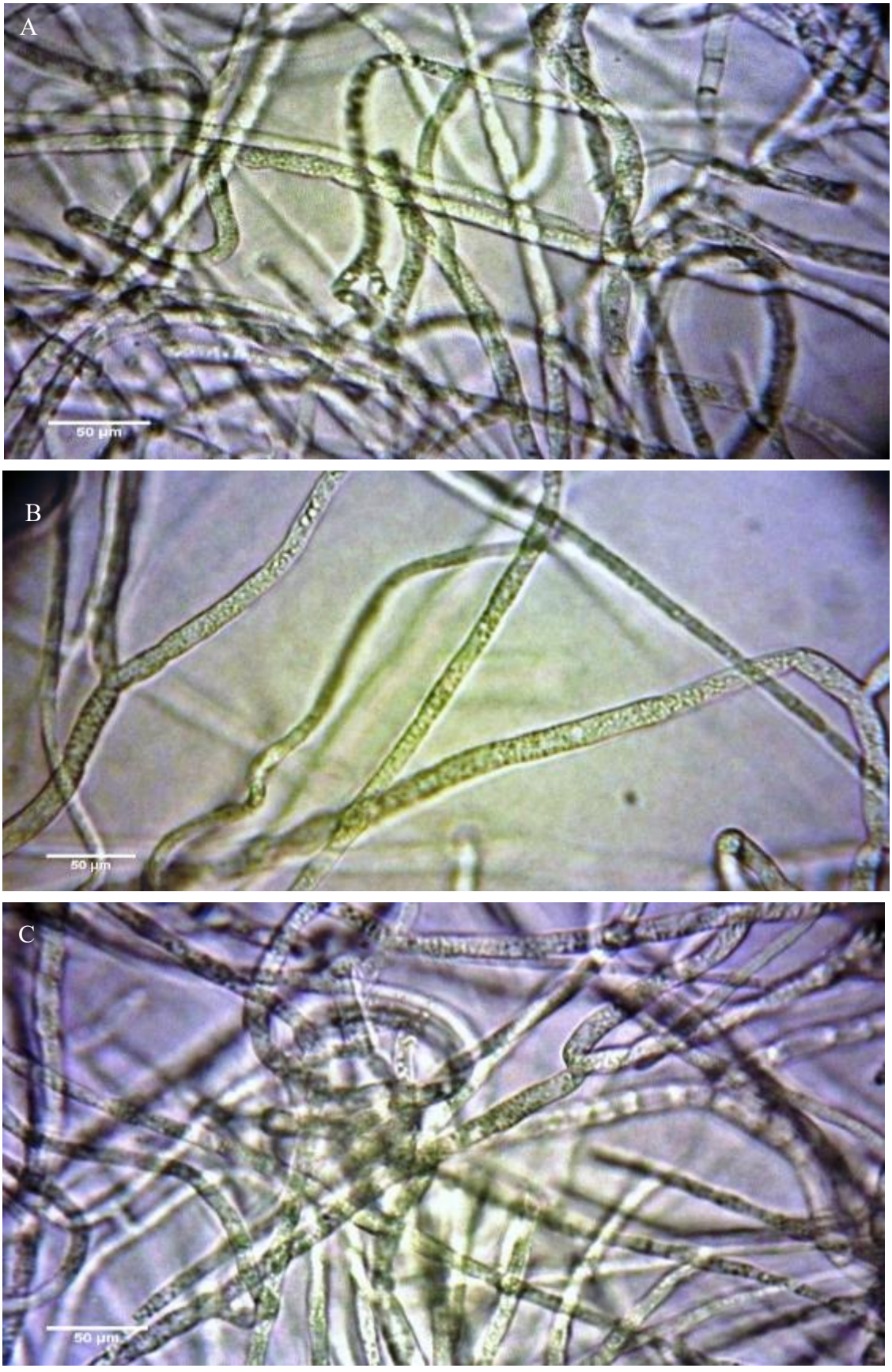

Figure 2-16 Brightfield micrographs at 100X magnification, of hyphal growth in amended PDA. A: 0 µg/ml^{-1} Control, B: 100 µg/ml^{-1} PO_4^{3-} and C: 100 µg/ml^{-1} KOH.

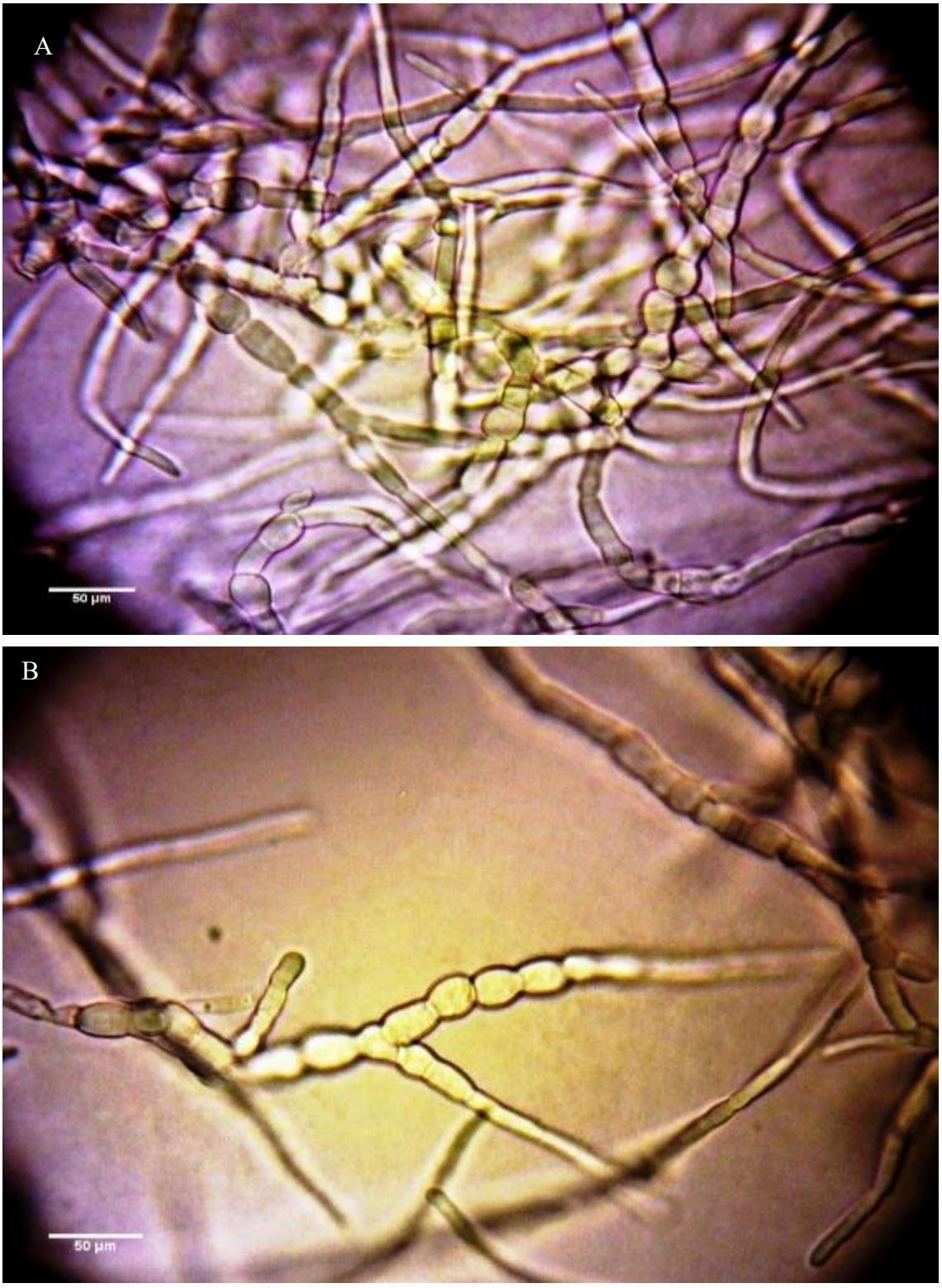

Figure 2-17 Brightfield micrographs at 100X magnification, of hyphal growth in amended PDA. A: 75 µg/ml^{-1} PO_3^{3-}. B: 100 µg/ml^{-1} PO_3^{3-}.

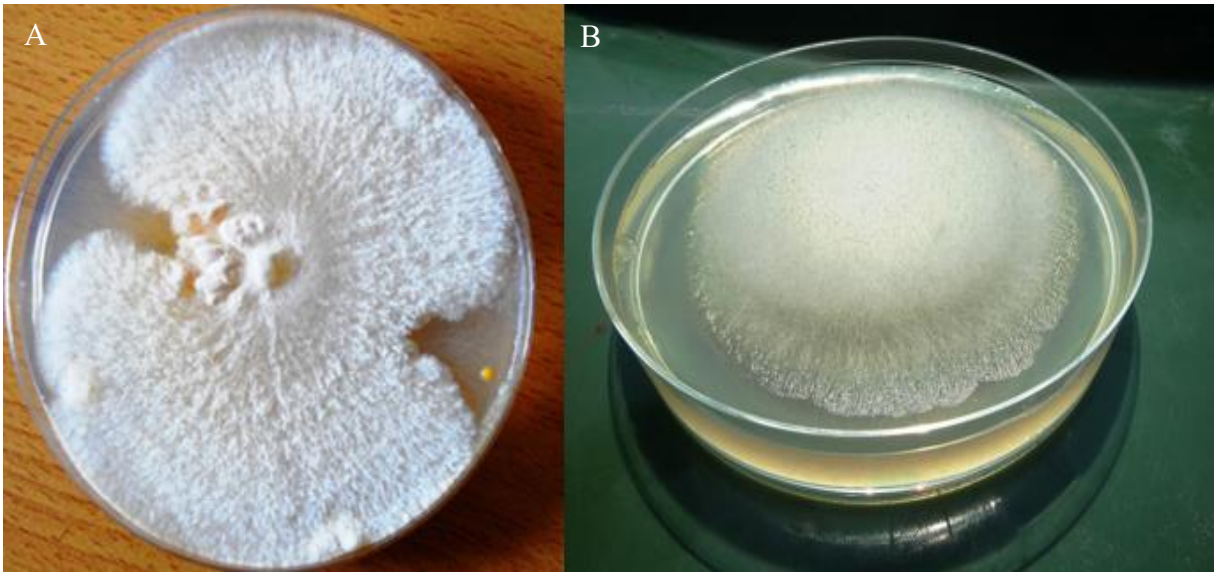

Figure 2-18 Differences in mycelial growth on amended PDA. A: PO_3^{3-} amended > 75 µg/ml^{-1} and B: PO_3^{3-} amended < 75 µg/ml^{-1}.

Figure 2-19 hyphal growth in amended PDA. A: 30 µg/ml^{-1} PO_3^{3-} , the mycelium is dense and less flocculated than in B: which is amended with 30 µg/ml^{-1} PO_4^{3-}.

2.6.8 Experiment 4, the effects of Phi on conidial germination

M. nivale conidia, in solutions of 0 (control), 10, 50, 100 and 250 μg/ml^{-1} concentrations of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4, and KOH were incubated darkness at 18° +/- 2° C for 48 h. and conidial germination was assessed. Conidia in all amendments at the 0 μg/ ml^{-1} control concentrations, did not achieve 100% germination, with the highest rate of 85.6% determined in the H_3PO_3 solution. Germination rates across the range of concentrations used are shown in Fig. 2-20. As data were determined as percentage germination, an arcsine transformation was carried out prior to statistical analyses. There was a significant ($p < 0.05$, by two-way Anova, df = 16,125, Fstat 1799.609) interaction between compounds and concentrations, with significant effect from compounds ($p < 0.05$ df = 4,125, Fstat 5845.738 and concentrations ($p < 0.05$ df = 5,125 Fstat 10422.544) on colony diameters. Subsequently, one-way Anova determined significant differences in percentage germination rates at each level of amendment concentration used, with Tukey HSD at $p < 0.05$, used to separate any statistical differences between compounds. At concentrations of 0 μg/ml^{-1} there was a significant ($p = 0.018$, df 4,25, Fstat 3.668) effect on germination rates. The only statistical ($p = 0.009$) differences were between the highest in the H_3PO_3 85.60%, the lowest in the KOH at 83.88%.

There was a significant ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 10.619) effect on germination in the 10 μg/ml^{-1} solutions. The H_3PO_3 (83.86%), H_3PO_4 (84.12%) and KOH (82.95%) were statistically ($p > 0.05$) identical, the H_3PO_3, H_3PO_4, KH_2PO_3 (85.25%) and KH_2PO_4 (85.04%) were also statistically ($p > 0.05$) the same and the H_3PO_4, KH_2PO_3 and KH_2PO_4 also statistically ($p > 0.05$) the same.

Germination rates in the 50 μg/ml^{-1} solutions were also significantly ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 483.939) different. The H_3PO_3 (73.36%) and the KH_2PO_3 (72.56%) were statistically ($p = 0.362$) the same and were significantly ($p < 0.05$) less than all others. The H_3PO_4 (83.02%) and KH_2PO_4 (82.88%) were also statistically ($p = 0.996$) identical, with the germination rate of 85.90% in the KOH significantly ($p < 0.05$) greater than all others.

Germination rates in the 100 μg/ml^{-1} solutions were significantly ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 3974.463) different. The H_3PO_3 (45.26%) and the KH_2PO_3 (45.56%) were statistically ($p = 0.986$) the same and as in the 50 μg/ml^{-1} solutions, significantly ($p < 0.05$) less than all others. The H_3PO_4 (84.90%) and KH_2PO_4 (83.69%) were again statistically ($p = 0.067$) identical, with the germination rate of 80.62% in the KOH significantly ($p < 0.05$) less than the H_3PO_4 and KH_2PO_4.

In the 250 µg/ml^{-1} solutions germination rates in the were significantly ($p < 0.05$ by one-way Anova, df = 4, 25, Fstat 16657.214) different.

The H_3PO_3 (10.45%) and the KH_2PO_3 (10.57%) were statistically ($p = 0.992$) identical and significantly ($p < 0.05$) less than all others.

The H_3PO_4 (73.97%) and KH_2PO_4 (73.75%) were again statistically ($p = 0.982$) identical, with the germination rate of 69.86% in the KOH significantly ($p < 0.05$) less than the H_3PO_4 and KH_2PO_4.

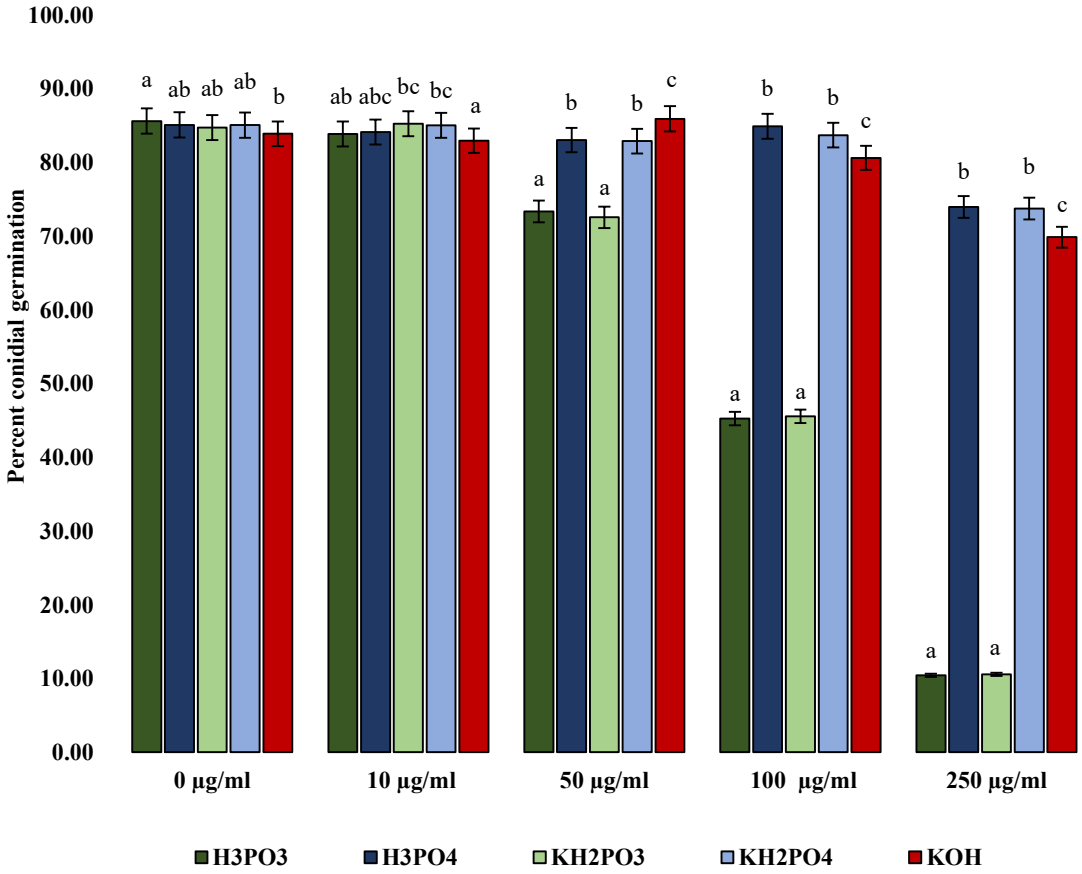

Figure 2-20 Effect of Phi on conidial germination. Percent germination of *M. nivale* conidia following immersion in solutions of 0 (control), 10, 50, 100 and 250 µg/ml^{-1} concentrations of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4, and KOH and re-plating on PDA and incubation at 18° +/- 2^0 C for 48 h. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars are 95% confidence intervals. Letters indicate significant differences between compounds at each amendment concentration, as determined by Tukey HSD at $p < 0.05$.

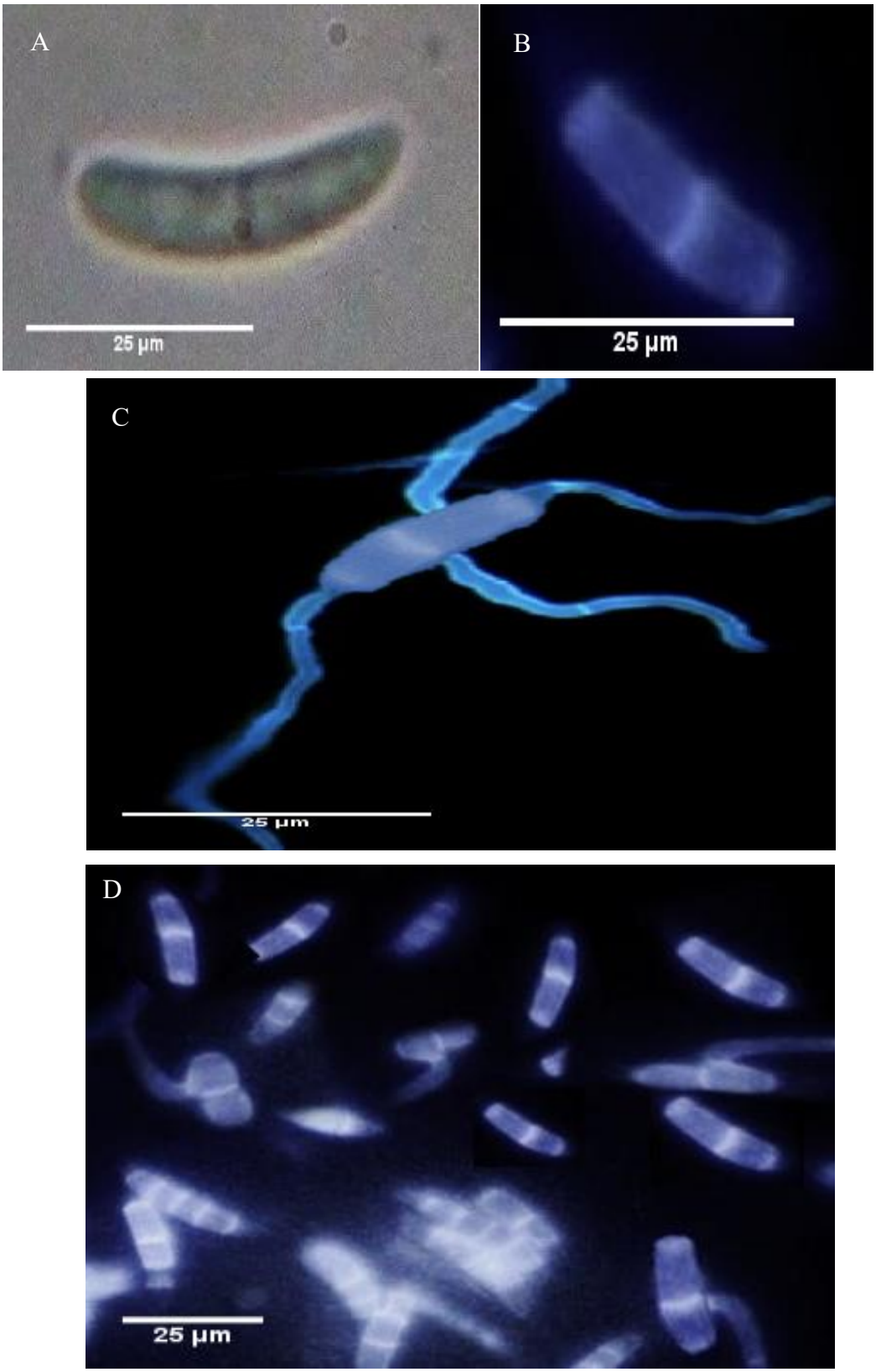

Figure 2-21 Conidia in Phi amended solutions. A and B: non-germinated conidium, C: germinating conidium, D: sample view of conidia. A viewed using brightfield microscopy, B, C and D viewed using fluorescence microscopy in UV light following staining with Calcofluor white.

2.7 Discussion

The major questions posed at the start of this study were: does Phi induce any inhibitory effects on the *in vitro* mycelial growth and on the conidial germination of *M. nivale*? These were emphatically answered by the results, which clearly show Phi has a direct mode of action, leading to significant suppression both of mycelial growth and conidial germination.

2.7.1 The effects of Phi on the *in vitro* mycelial growth of *M. nivale*

The level of *in vitro* suppression of *M. nivale* growth achieved here, across the full range of Phi amendments used was not expected. Prior to the start of this study, there was little evidence to support the premise that Phi had direct fungistatic properties against ascomycetes. It was expected that none of the Phi amendments used would significantly inhibit growth, and that there would be no significant differences between the Phi, Pi and KOH amendments. The majority of research with Phi and *in vitro* phytopathogen suppression have been with oomycetes (Coffey and Bower, 1984; Smillie *et al.*, 1989; Jee *et al.*, 2002; Landschoot and Cook, 2005; Garbelotto *et al.*, 2008; Cook *et al.*, 2009). While these studies have clearly shown that Phi inhibits mycelial growth, interferes with morphological development and reduces reproductive spore germination, there were no such data supporting the efficacy of Phi in suppressing the *in vitro* growth of ascomycetes, with only a very limited number of results published on this subject (Reuveni *et al.*, 2003; Burpee, 2005).

The four isolates of *M. nivale* used in this study, were sourced from different geographical locations, two from Ireland and two from the UK, and as shown in Tables 2-1 and 2-4 there were no statistical ($p > 0.05$) differences between isolates, with major growth suppression in the presence of Phi. Despite these data, replication of these studies using a wider population of isolates would be of great interest and should form part of further research, which should also include isolates of *M. majus*.

This present study, as evidenced in Figs. 2-3 and 2-4, has determined that Phi significantly reduces the *in vitro* mycelial growth of *M. nivale.* Furthermore, this adverse effect of Phi on *M. nivale*, was further reflected in the disruption of hyphal morphology, Fig. 2-17, and the reduction in conidial germination rates, Fig. 2-20.

Figs. 2-5 and 2-6, give clear evidence of the inhibitory effect Phi, sourced from either reagent grade or commercial products, has on the *in vitro* growth of *M. nivale*. When

compared with growth on 0 μg/ml^{-} (control), Pi or KOH amended PDA, Phi led to significant $p < 0.05$) reductions in growth.

Further evidences of the inhibitory effect Phi has on the mycelial growth of *M. nivale* is shown in Figs. 2-10 and 2-13. At concentrations of 10 μg/ml^{-1} and above, colony diameters on Phi amended plates, 5 dpi, were significantly ($p < 0.05$) less than colonies on 0 μg/ml^{-1}(control) or those on all amendment concentrations of H_3PO_4, KH_2PO_4 and KOH. Furthermore, colony diameters on Phi amended plates 5 dpi, were not only of less diameter than colonies on other amendments but visually, the mycelium was clearly impaired, as can be seen in the comparisons of hyphal morphology in Figs 2-16, 2-17 and in Figs 2-18 and 2-19.

The sensitivity of *M. nivale* to Phi was further evident when EC_{50} and EC_{90} values were calculated. The EC_{50} values for the Phi sources ranged from 35.95 to 48.22 μg/ml^{-1} of PO_3^{3-}. However, while Phi, sourced from reagent grade or commercial compounds, significantly ($p < 0.05$) suppressed mycelial growth, there were significant ($p < 0.05$) differences between sources. This variation in EC_{50} values could be attributed to the combinations of compounds used, for example, there were significant ($p < 0.05$) differences between the inhibitory effects of H_3PO_3 and KH_2PO_3, at all concentrations used, Fig. 2-5, with the exception of the 250 μg/ml^{-1}, where there was 100% suppression of growth. The EC_{50} and EC_{90} values, as shown in Table 2-7 and Figs. 2-7 and 2-9, highlight the significant ($p < 0.05$) differences between the inhibitory effect of each compound. Bucking and Heyser (1999) stated that the presence of K facilitates the uptake of Phi into fungal cells, maintaining that it helps retain the charge balance and pH of the fungal cell and is the counter ion to the transport of polyphosphates into the vacuole. Darakis *et al*. (1997) agreed with this and concluded the presence of K facilitated Phi uptake into *Phytophthora capsici* hyphae. If mycelial growth suppression is used as an indicator of increased Phi assimilation, then this enhanced assimilation of Phi in the presence of K was confirmed here, as statistically KH_2PO_3 produced significantly ($p < 0.05$) greatly inhibition than H_3PO_3. Further evidence for the effects the combination of compounds has on mycelial inhibition, can be seen from the data obtained from the commercial compounds. The EC_{50} and EC_{90} values of the Phi sourced from commercial compounds varied significantly ($p < 0.05$) between compound sources. Interestingly, the commercial compound with the highest EC_{50} was the Turfite, (NH_2PO_3), which is an ammonium phosphite and not potassium phosphite as are all other products used, again indicating that the presence of K can influence the efficacy of Phi inhibition.

Compared to Phi amendments, concentrations of H_3PO_4, KH_2PO_4 and KOH induced no similar significant inhibitory effects, although concentrations of these compounds from 50 to 250 μg/ml^{-1} did lead to some inhibition of growth, with rates ranging from 4.72% in the 50 μg/ml^{-1} of H_3PO_4 to 19.94% in the 250 μg/ml^{-1} KOH amendment concentration.

The inhibitory effects of Pi, at concentrations of 50 μg/ml^{-1} and above, Fig. 2-5, while significantly ($p < 0.05$) less than that of Phi, were not unexpected. Reuveni *et al*. (1996) studying the infection of cucumber (*Cucumis sativus* L.), by the ascomycete pathogen *Sphaerotheca fuliginea* (Schlecht.:Fr.), determined that infection was significantly controlled by a foliar spray treatment of KH_2PO_4. Howard (2001) determined Pi had *in vitro* fungicidal properties against a number of fungal species. However, in both these studies the concentrations of Pi used ranged from 20 mM to 750 mM, which were well in excess of concentrations used in this present study and in the Reuveni *et al*. (1996) study, infection suppression may well have been from an inducement of SAR, as these defence activation properties of Pi, are well documented (Deliopoulos *et al.*, 2010).

Any effect KOH had on mycelial growth inhibition is an area of particular interest. Levels of K, currently recommended for management of cool-season amenity turfgrasses, appeared to increase susceptibility to *M. nivale,* when compared to lower K inputs (Soldat, 2014). As stated, Phi is most commonly pH adjusted with KOH, the results here, as seen in Fig. 2-5, show that *in vitro,* KOH, at concentrations of 100 and 250 μg/ml^{-1} significantly inhibited mycelial growth compared to similar concentrations of H_3PO_4 and KH_2PO_4.

To date, there have been no published data specifically on the *in vitro* growth suppression of *M. nivale*, by Phi, however, the results here reflect the findings of Landschoot and Cook (2005), who carried out a series of *in vitro* studies using KH_2PO_3 and KH_2PO_4 amended growth medium, inoculated with the *Oomycete* pathogen *Pythium aphanidermafum.* The KH_2PO_3 inhibited growth of mycelia, but the KH_2PO_4 had no effect on growth.

The closest related research to this present study has been by Hofgaard *et al*. (2010), who examined the *in vitro* mycelial growth of *M. majus,* on PDA amended with a foliar fertiliser containing 731 g l^{-1} of a 50% KH_2PO_3 solution. At 10 μl/l^{-1}, mycelial growth was reduced by more than 90% and at concentrations above 50 μl /ml^{-1}, growth was fully inhibited. Their results appear to show Phi as having significantly lower EC_{50} values than

those reported here, it may be that *M. majus* is more susceptible to Phi than *M. nivale* or possibly a result due to differences in experimental methods.

2.7.2 Mode of inhibition

The mode of action by which Phi inhibits mycelial growth has been the subject of a number of studies. Most conclude that the main areas of inhibition involve disruption of phosphorous metabolism and inhibition of enzymes involved in the glycolytic and phosphogluconate pathways (Grant *et al.*, 1990; Niere *et al.*, 1994; Martin *et al.*, 1998; Stehmann and Grant, 2000; Mcdonald *et al.*, 2001).

Barchietto *et al.* (1991) determined that Phi interacts with Pi for the catalytic site of phosphorylating enzymes and concluded that in *Phytophthora* spp. the activity of Phi produced a physiological state similar to that produced as a result of P limitation. P deficiency in the presence of Phi was apparent in this study, evident by the disruption to hyphal morphology as displayed in Fig. 2-17. This malformation of hyphae induced by Phi/Pi antagonism was also evident in Wong (2006), who studied the effect Phi had on the hyphal morphology of *Phytophthora* spp. and reported that in the presence of Phi, hyphae were stunted and swollen, again in a manner similar to those of *M. nivale* in Fig. 2-17.

This P deficiency view is supported by the findings of Niere *et al*. (1994), who concluded that Phi inhibition in *Phytophthora* spp. was due to interference with Pi metabolism, as the presence of Phi led to increases in both pyrophosphate and polyphosphate. They concluded that increased accumulations interfered with Pi metabolism and diverted ATP from other pathways of metabolism, resulting in decreased mycelial growth rates.

Furthermore, they state that accumulation of pyrophosphate and polyphosphate will also alter the ion balance concentrations of potassium, magnesium, calcium and iron, influencing the activity of enzymes catalysing essential steps in metabolism.

2.7.3 Fungicide or fungistat

An important factor in this study was to determine if Phi acted as a fungicide and killed the pathogen or was a fungistat, and reduced or slowed the hyphal growth. Evidence of the fungistatic properties of Phi are clearly demonstrated in Fig. 2-9, when, after being immersed in a range of Phi concentrations for 10 days, *M. nivale* commenced regrowth without displaying any major malformation and in a manner similar to the samples immersed in Pi and KOH. Complimenting these data, and supporting the fungistatic rather than fungicidal properties of Phi, are that when plated on Phi amended PDA, *M.*

nivale growth, while significantly reduced, was not fully suppressed, but continued to grow at a reduced rate over 10 dpi, as demonstrated in Figs. 2-12 and 2-14.

The ability of oomycete and fungal organisms to tolerate the presence of Phi and maintain a suppressed growth rate can be explained by the findings of Dunstan *et al.* (1990, who found that *P. palmivora* was able to remove Phi from its mycelium, similarly, Smillie *et al.* (1989 found that Phi accumulated in *P. palmivora* during the first 5 days of growth, but subsequently showed a decrease in cellular Phi. Results of a metabolite profile study of *Phytophthora* spp. carried out by Grant *et al*. (1990 led them to conclude that Phi accumulation in mycelium was transient, as within 9 days Phi had completely disappeared from the mycelium. This was indicated in this present study, Figs. 2-12 and 2-14, as at the 250 $\mu g/ml^{-1}$ amendments of Phi, mycelial growth was observed at 10 dpi.

This determination of Phi as a fungistat rather than a fungicide has significant relevance to disease control programs and to the marketing of Phi products. Firstly, some legislations differentiate between fungicides and fungistats, thus affecting the marketing and pricing of Phi products. Secondly, a fungicide can be applied either as a preventative measure or as a curative and kill the pathogen. With a fungistatic compound the control programme usually requires treatment as a preventative measure, therefore requiring continuous sequential applications. The sequential application programme would ensure the Phi was *in planta,* in order to suppress pathogen growth.

2.7.4 Inhibition of conidial germination

Conidial production is vital in the spread of inoculum, therefore any reduction in numbers would have a significant impact on disease spread and incidence. The results here show that the inclusion of Phi in the propagating solution led to a significant reduction in percent conidial germination, Fig. 2-10. This inhibition of reproductive spores by Phi has been well documented in oomycetes, but less so in ascomycetes (Reuveni *et al.*, 2003; Mills *et al.*, 2004. Wong (2006 for example, determined that Phi retarded spore germination in *Phytophthora* spp. and also gave clear visual evidence that Phi caused distortion and lysis of the reproductive spores, however, in this study as shown in Fig. 2-21, while Phi inhibited spore germination, there was no visible conidial distortion.

While there are no published data on the effect Phi has on *M. nivale* conidial germination, Hofgaard *et al.* (2010 demonstrated that increasing Phi concentrations correlated directly with delays in sporulation of *M. majus* on detached wheat leaves. Based on their *in vitro* and detached leaf studies they concluded Phi can suppress fungal reproduction and slow

pathogenic growth, allowing a host plant's defence system time to react, reducing the severity of infection, a subject studied in a later chapter here.

2.8 Conclusions

This study has produced significant and novel data which is relevant to methods of turfgrass disease prevention and control.

The main conclusions of this study are that Phi:

- Suppresses *M. nivale* mycelial growth.
- Disrupts hyphal morphology.
- Inhibits conidial germination.

Both hyphae and conidia are infective propagules, providing inoculum for the diseases caused by *M. nivale.* It is clearly demonstrated here, that *in vitro*, the incorporation of Phi into the growth media, significantly suppresses the growth and development of these infective propagules.

Whether these conclusions lead to suppression of disease incidence in the field is the subject of the next section of this research.

3 Field trials to determine the effects of Phi on *Microdochium nivale* infection

3.1 Introduction

In amenity turfgrass management, *Microdochium nivale* is regarded as the most important pathogen of temperate climates, infecting most cool-season species (Smiley *et al.*, 1992; Mann, 2004a; Vargas, 2005). The search for new or improved means to reduce susceptibility to *M. nivale* is an ongoing target for turfgrass research. Field evaluations of the inhibitory effects of Phi against a range of phytopathogens has been documented in the review of literature (chapter 1). In turfgrass management Phi was first used for the control of oomycete pathogens such as *Pythium* spp.(Cook *et al.*, 2006) and subsequently, in combination with Mancozeb, a dithiocarbamate fungicide, controlled summer decline of bentgrass (Beard and Oshikazu, 1997). Controlled-environment evaluations, as in the *in vitro* studies carried out in the previous chapter, are most practical and beneficial when the results can be correlated directly with similar field trial evaluations. Apart from Dempsey and Owen (2010) , there are no field trial evaluations in the literature specifically on the suppression of *M. nivale* by Phi in turfgrasses.

There are however, published data on the effect Phi treatment has on turfgrass quality, with some reports of enhanced colour, density and uniformity, following sequential applications of Phi (Tredway and Butler, 2004; Vincelli and Dixon, 2005; Cook *et al.*, 2006; Tredway, 2006; Dempsey and Owen, 2010). These studies are not fully conclusive; Tredway and Butler (2004), for example, reported there was no improvement of potassium phosphite (Alude) and Fosetyl Al (Chipco Signature) treated *A. stolonifera* swards, but Tredway (2006) reported significant quality improvement of a *P. annua* green with the same compounds.

Turfgrass trials are often conducted using commercial products which are formulated with dyes, pigments and plant nutrients, these could have an impact on turfgrass quality or even disease susceptibility (Mudge, 1997; Vincelli and Dixon, 2005). It is therefore important, that with any field trials the compounds or formulations under scrutiny, are evaluated at equivalent concentrations of the active ingredient, in this case phosphorous acid and the effect of any nutrients included in the treatments be factored into the results and conclusions.

Also, research specifically with turfgrasses, has shown that different trial designs can have significant treatment effects; often the results are influenced not only by product formulation, but also by treatment rate and application timings (Cook, 2009).

Aims and objectives:

The aims of this section of the research were to determine if Phi, formulated in commercial potassium phosphite products, labelled for amenity turfgrass use, could reduce susceptibility to *M. nivale*, whether the addition of a biostimulant could increase the efficacy of Phi to reduce *M. nivale* infection and if the addition of Phi to standard turfgrass fungicides (iprodione and chlorothalonil) could enhance the suppression of *M. nivale* compared to the fungicides alone. Further to this, the effect Phi treatments had on turfgrass quality were also studied during these trials.

Objectives:

- Determine if Phi treatment of turfgrass in the field reduces *M. nivale* occurrence.
- Determine if Phi treatment enhances turfgrass growth and quality.

3.2 Materials and methods

Field trials were carried out over four years comprising of two series of assessments. Series one assessed the effects of a number of treatments applied bi-weekly on the incidence of *M. nivale* on three turfgrass species. Series two assessed the effects of a number of treatments applied at varying intervals and periods on the incidence of *M. nivale* on three turfgrass species.

3.3.1 Experiment location

Sites at the Royal Curragh Golf Club, Co Kildare, Ireland (53.150N / 6.800 W 110 m ASL), were established and prepared for trials during the period from May - Sept 2010. Climatically the region is defined as a temperate oceanic climate, being mild, moist and changeable with rainfall annual mean of 754 mm and air temperature mean

Total rainfall in millimetres

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2014	110.7	122.0	56.7	39.3	98.4	31.7	42.6	107.7					609.1
2013	69.5	45.2	63.3	47.5	52.8	43.2	42.7	62.9	35.1	100.4	21.2	104.7	688.5
2012	63.2	19.8	27.5	94.7	64.0	178.5	102.7	74.9	89.6	84.2	79.8	46.8	925.7
2011	34.1	76.2	15.0	30.0	51.5	65.1	53.3	51.6	76.3	165.9	54.8	53.0	726.8
2010	44.1	36.5	58.9	33.0	44.7	44.7	80.2	43.1	102.5	37.0	120.0	65.5	709.7
mean	63.8	48.5	50.7	51.9	59.1	62.5	54.2	72.3	60.3	81.6	73.7	75.7	754.3

Mean temperature in degrees Celsius

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2014	5.5	5.6	6.8	9.5	11.6	13.9	16.3	15.0					10.2
2013	5.1	4.3	3.1	6.9	10.0	13.5	17.8	15.9	13.2	11.8	6.2	6.8	9.6
2012	6.0	6.6	8.5	6.6	10.2	13.1	14.3	15.6	11.9	8.5	6.1	5.2	9.4
2011	3.6	7.0	6.3	10.9	11.4	12.4	14.1	13.6	14.0	11.7	9.9	5.7	10.1
2010	1.5	2.3	5.4	8.3	10.3	14.8	`16.0	15.0	13.5	10.1	5.0	0.4	8.5
mean	5.1	5.1	6.8	8.2	10.9	13.6	15.7	15.4	13.3	10.3	7.2	5.4	9.8

Mean 10cm soil temperature

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2014	4.7	4.8	6.7	10.6	13.0	17.3	18.6	16.4					11.2
2013	4.3	4.1	4.0	7.2	11.7	15.7	19.5	17.0	14.2	11.5	6.5	5.8	10.2
2012	5.4	6.1	8.4	8.2	12.3	15.0	16.4	16.8	13.2	9.1	5.6	4.1	10.1
2011	3.3	6.2	6.7	11.9	12.6	14.9	16.6	15.3	13.4	11.4	8.9	4.7	10.5
2010	1.2	2.3	5.1	8.1	11.8	16.8	16.3	15.7	13.8	10.4	6.1	1.3	9.1
mean	3.9	3.8	5.2	7.6	11.4	14.6	16.2	15.3	12.6	9.2	6.2	4.4	9.2

3.3.2 Trial plots and experimental design

3.3.2.1 Turfgrasses and plots

The trial plots were established on a sandy/loam soil, pH 7.1. The plots, 2 x 2 m and 2 x 1 m in size, were composed of *Poa annua* L., *Agrostis canina L. ssp. canina*, variety Avalon and *Agrostis stolonifera* L., variety Penn G-6 Fig. 3-1 A and B, and were maintained throughout the trial periods at 5 mm height of cut, using a John Deere 220 pedestrian mower, Fig. 3-1.

The *P.annua* sward previously formed part of a now disused fairway and for the 6 years previous to the trials, had been maintained as part of a turfgrass nursery. The *A. canina* ssp. *canina* sward was originally established from seed in 2005 and was moved to the trial location as turfgrass sod in 2009. The *A. stolonifera* sward was established from seed in April 2010, however, due to its poor establishment, year 1 of the trials comprised the *P.annua* and *A. canina canina* plots only, with trial extension to include the *A. stolonifera* plots from September 2011.

Figure 3-1 Trial plots at Royal Curragh Golf Club. Trial area established at Royal Curragh golf club to assess the effect of a range of treatments of the incidence of *M. nivale*. A: *A. canina canina* and *A.stolonifera* plots, B: *P. annua* plots.

3.3.2.2 Nutritional and irrigation inputs

Granular nutrient inputs (Andersons 21:3:21 were applied at the beginning of May and September each year of the trials, beginning in May 2010, at a rate of 30 g/m giving annual nutritional inputs (ANI of 126 kg N ha^{-1}, 18 kg P_2O_5 ha^{-1} and 126 kg K_2O ha^{-1}. Two applications per annum were considered sufficient, as the N source contained 15.75% polymer coated urea giving a sustained release. September can be considered as late in the season for an application of a 21% nitrogen product. This could be construed as excessive and not representative of best management practices as it could encourage *M. nivale* infection. However, as *M. nivale* infection was the focus of this study it was deemed appropriate. Further nutrient inputs were supplied as part of the treatment applications and are detailed below.

Due to the prevailing temperate climate only minimal irrigation inputs were required during periods of dry weather in order to replace water lost through evapotranspiration, no irrigation inputs were required during the experimental periods of September to March each year.

3.3.3 Treatments

3.3.3.1 Foliar treatments

All treatments were completely randomised with five replications, comprising of sequential applications, applied as a foliar spray. Applications were made using 20 l knapsack sprayers fitted with flat fan nozzles delivering a fine spray operating at 4 bar, calibrated to deliver 300 l/ha.

3.3.3.2 Experiment 1: first series, years 1 and 2

In series one of the trials, treatments were applied from September 2010 to March 2012 Table 3-2 shows treatments, rates of application, formulations used and application timings. Treatments were chosen to replicate a standard turfgrass nutritional and disease management programme, as used during the autumn, winter period. The addition of the Phi product to the programme would allow determination of any effects Phi has on disease incidence and turfgrass growth and quality.

Table 3-2 Treatments, formulations and application timings, years 1 and 2.

Treatment	Formulation and rate	Application timing
Phi	PK Plus 3:7:18 (Grigg Bros, 14% KH_2PO_3, specific gravity 1.37, pH 7.05). Applied at 20 l/ha^{-1}. ANI: 11.5 kg N ha^{-1}, 11.7 kg P ha^{-1}(in the form of P_2O_5), 57.3 kg K ha^{-1} 53.7 kg PO_3^{3-} ha^{-1}.	
Phi + Biostimulant	PK Plus (20 l/ha^{-1}) + Ultraplex Biostimulant (Grigg Bros, 5-0-3, specific gravity 1.26, pH 2.5). Applied at 20 l/ha^{-1}. ANI: 20.5 kg N ha^{-1}, 11.7 kg P ha^{-1} (in the form of P_2O_5), 61.7 kg K ha^{-1}), 53.7 kg PO_3^{3-} ha^{-1}.	
Iprodione (fungicide)	Chipco Green, (Bayer, 255 g/l iprodione) applied at 20 l/ha^{-1}.	Bi weekly from Sept 2010 to March 2011 and Sept 2011 to March 2012
Iprodione + Phi	Chipco Green (20 l/ha^{-1}) + PK Plus 3:7:18 (20 l/ha^{-1}). ANI: 11.5 kg N ha-1, 11.7 kg P ha^{-1}(in the form of P_2O_5), 57.3 kg K ha^{-1} 53.7 kg PO_3^{3-} ha^{-1}.	
NPK Control	NPK control (3:7:18 to match nutritional input of PK Plus) applied at 20 l/ha^{-1}. ANI: 11.5 kg N ha^{-1}, 11.7 kg P ha^{-1} (in the form of P_2O_5), 57.3 kg K ha^{-1}.	
Control	n/a	

3.3.3.3 Experiment 2: second series, years 3 and 4

In the second series, treatments were applied from September 2012 to March 2014. Treatment applications were similar to those in series one, but with treatments differing in timing and application intervals. The aim was to study and compare any difference in treatment responses Phi may elicit when applied bi weekly, monthly or for a limited period of just three months, compared to six months of the full trial period. Table 3-3 shows treatments, rates of application, formulations used and application timings.

Table 3-3 Treatments, formulations and application timings, years 3 and 4.

Treatment	Formulation and rate	Application timing
Phi-bi-weekly	PK Plus 3:7:18 (Grigg Bros, 14% KH_2PO_3, specific gravity 1.37, pH 7.05). Applied at 20 l/ha$^{-1.}$ ANI: 11.5 kg N ha^{-1}, 11.7 kg P ha^{-1}(in the form of P_2O_5), 57.3 kg K ha^{-1} 53.7 kg PO_3^{3-} ha$^{-1.}$	Bi weekly from Sept 2012 to March 2013 and Sept 2013 to March 2014
Phi-monthly	PK Plus (20 l/ha^{-1}). ANI: 5.37 kg N ha^{-1}, 5.46 kg P ha^{-1} (in the form of P_2O_5), 26.74 kg K ha^{-1} 25.06 kg PO_3^{3-} ha^{-1}.	Monthly from Sept 2012 to March 2013 and Sept 2013 to March 2014
Phi- bi-weekly 6 apps	PK Plus (20 l/ha^{-1}). ANI: 4.60 kg N ha^{-1}, 4.68 kg P ha^{-1} (in the form of P_2O_5), 22.92 kg K ha^{-1} 21.48 kg PO_3^{3-} ha^{-1}.	Bi weekly from Sept 2012 to November 2013 and Sept 2013 to November 2013
Chlorothalonil (fungicide)	Daconil (Syngenta, 720 g/l chlorothalonil) applied at 20 l/ha^{-1}.	Monthly from Sept 2012 to March 2013 and Sept 2013 to March 2014
Chlorothalonil + Phi	Daconil + PK Plus applied at 20 l/ha^{-1}. ANI: 5.37 kg N ha^{-1}, 5.46 kg P ha^{-1}(in the form of P_2O_5), 26.74 kg K ha^{-1} 25.06 kg PO_3^{3-} ha^{-1}.	Monthly from Sept 2012 to March 2013 and Sept 2013 to March 2014
Control	n/a	

All fungicides used during the experimental periods were applied at label rates to comply with current legislation and to ensure maximum efficacy of disease suppression.

3.3.4 Assessments

3.3.4.1 *M. nivale* incidence

All trial plots were assessed for *M. nivale* incidence monthly, by independent assessors (Royal Curragh Golf Club qualified and experienced greenkeepers), from September to March each year of the study and rated on a scale of 0-100. Evaluation assessed percentage of plot area affected by *M. nivale* disease: 0 being no incidence and 100 being 100% coverage (Bruneau *et al.*, 2000).

3.3.4.2 Turf quality

Assessment of turf quality (which excluded the impact of disease within each plot) were also determined monthly. Turfgrass quality is defined as the degree to which a turf conforms to an agreed standard that is a composite of uniformity, shoot density, leaf texture, growth habit, smoothness, and colour. This was assessed visually and marked on a scale of 1 to 10 (1 = poorest possible quality, 5 acceptable and 10 = best possible quality turf). (Horvath *et al.*, 2007; Krans and Morris, 2007).

3.3.5 Data analysis

3.3.5.1 Analysis of disease incidence

Data analyses were carried out for each year separately, as disease levels were determined as percentage incidence, arcsine transformations were carried out prior to analyses. The transformed data were then assessed to ensure they met the requirements for parametric analyses. Outliers were determined by inspection of boxplots, normality of distribution assessed using Shapiro-Wilk's normality test (Shapiro and Wilke, 1965) and homogeneity of variances assessed by Levene's test (Levene, 1960). For monthly levels of disease incidence, a two-way Anova was conducted to determine the influence and interactions of treatments, turfgrass species and application period (month) on disease incidence. Where there were significant effect and interactions, one-way Anova were used to assess treatment effect on disease incidence for each species and each month of the trial period, with significant differences then separated using Tukey HSD post hoc analyses at a significance level of $p < 0.05$. For analyses of mean levels of disease incidence for each trial period (September to March each year) a two-way Anova was conducted to determine significant effects and interactions, between treatments and turfgrass species. If there were significant effect or interactions, one-way Anova determined significant treatment effects on disease incidence within each turfgrass species and Tukey HSD post

hoc analyses used to separate differences at a significance level of $p < 0.05$. Data are reported as mean ± 95% confidence intervals, unless otherwise stated. For presentation of charts all arcsine transformed data were back-transformed to provide clearer visual displays.

3.3.5.2 Analysis of turfgrass quality

Turf quality over the four years was assessed and rated on a nominal scale of 1 to 10, which is inherently non-parametric, therefore, a Kruskal-Wallis test was conducted to determine differences in median ratings for each of the four years of the trials. Distributions of turf quality ratings were assessed by visual inspection of boxplots. Where there were significant differences, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

Data analysis was performed using the statistical programme SPSS Statistics 21.

Additional statistical data tables are available in the document 'Appendices to the Thesis'

3.4 Results

3.4.1 Disease incidence – years 1 and 2

In both years of the first series of trials, *M. nivale* incidence developed naturally, with high levels of infection from October 2010 to February 2011 and September 2011 to December 2011. In year 1, *M. nivale* became active mid October 2010, with disease incidence progressively increasing and peaking during January 2011, following a three week covering of snow in December 2010. Disease pressure declined from the end of January 2011 until environmental conditions allowed re-emergence in September 2011. In year 2, disease pressures became evident earlier than the previous year, with *M. nivale* incidence beginning mid-September 2011 and remaining at high levels to mid-December. From January to March 2012 disease pressure remained, but at a lower level than the previous months and disease incidence declined gradually through March. *A. stolonifera* plots became available for trials in September 2011 and are included in the data analyses.

3.4.1.1 Monthly disease incidence from September 2010 to March 2011 (year 1)

Levels of disease incidence on the trial plots (n=5) for each of the seven months of the first years trial were analysed, with significant ($p < 0.05$ by two-way Anova df = 30, 336, Fstat 1391.930) differences and interactions determined between species, treatments and months. There were significant interactions between treatments and species ($p < 0.05$ df = 5, 336, Fstat 4548.238), between treatments and months ($p < 0.05$ df = 6, 336, Fstat 16581.537) and between species and months ($p < 0.05$ df = 6, 336, Fstat 8783.365).

Subsequently, treatment effect on disease incidence for each species and each month were examined using one-way Anova, results shown in Table 3-4.

Table 3-4 One-way Anova showing significant differences between treatments on monthly levels of *M. nivale* incidence on *P. annua* and *A. canina* trial plots (n=5), from September 2010 to March 2011 (year 1).

P. annua Year 1 Sept 2010 to March 2011					*A. canina* Year 1 Sept 2010 to March 2011				
Month	df	f	p	η2	Month	df	f	p	η2
Sept	5,24	0	0	0	Sept	5,24	0	0	0
Oct	5,24	952.171	< .001	0.995	Oct	5,24	4036.355	< .001	0.999
Nov	5,24	14461.121	< .001	1.000	Nov	5,24	41624.405	< .001	1.000
Dec	5,24	97109.273	< .001	1.000	Dec	5,24	138914.86	< .001	1.000
Jan	5,24	102946.849	< .001	1.000	Jan	5,24	167485.344	< .001	1.000
Feb	5,24	43419.023	< .001	1.000	Feb	5,24	69011.331	< .001	1.000
March	5,24	53517.723	< .001	1.000	March	5,24	72371.556	< .001	1.000

Significant treatment effects resulting from the Anova were then separated by Tukey HSD at $p < 0.05$. In the *P. annua* plots, disease incidence was greatest in January 2011 but in the *A. canina* highest levels of disease were determined in December 2010. Treatment effect on the levels of incidence during these months are shown in Figs 3-2 and 3-3.

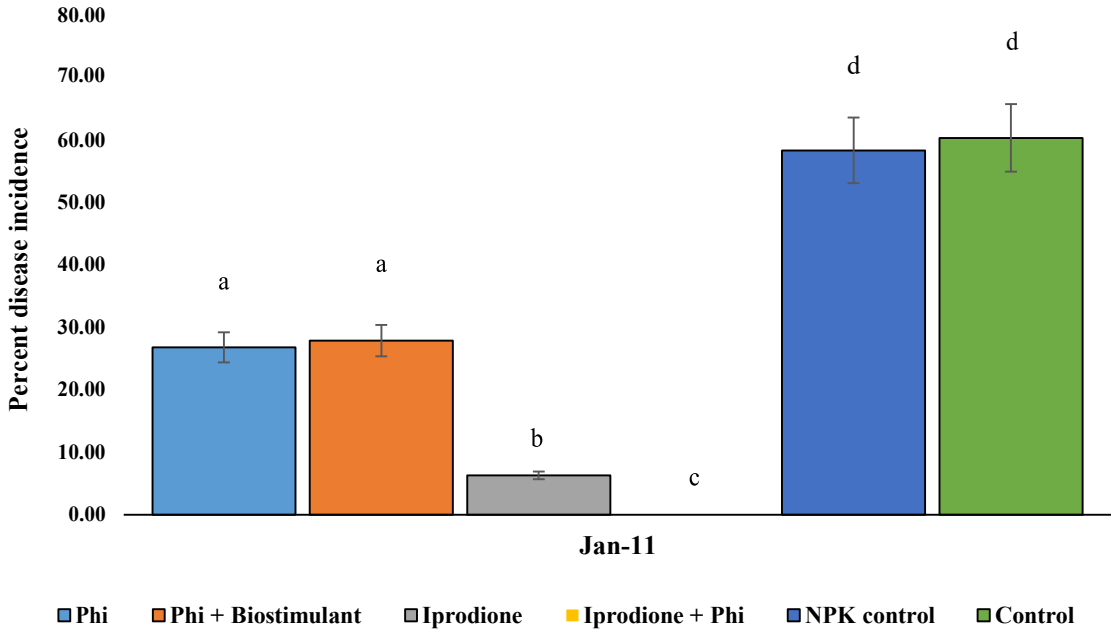

Figure 3-2 Monthly disease incidence, *P. annua*, January 2011 (year 1). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua,* during the month of greatest disease incidence in year 1 of the trial, January 2011. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

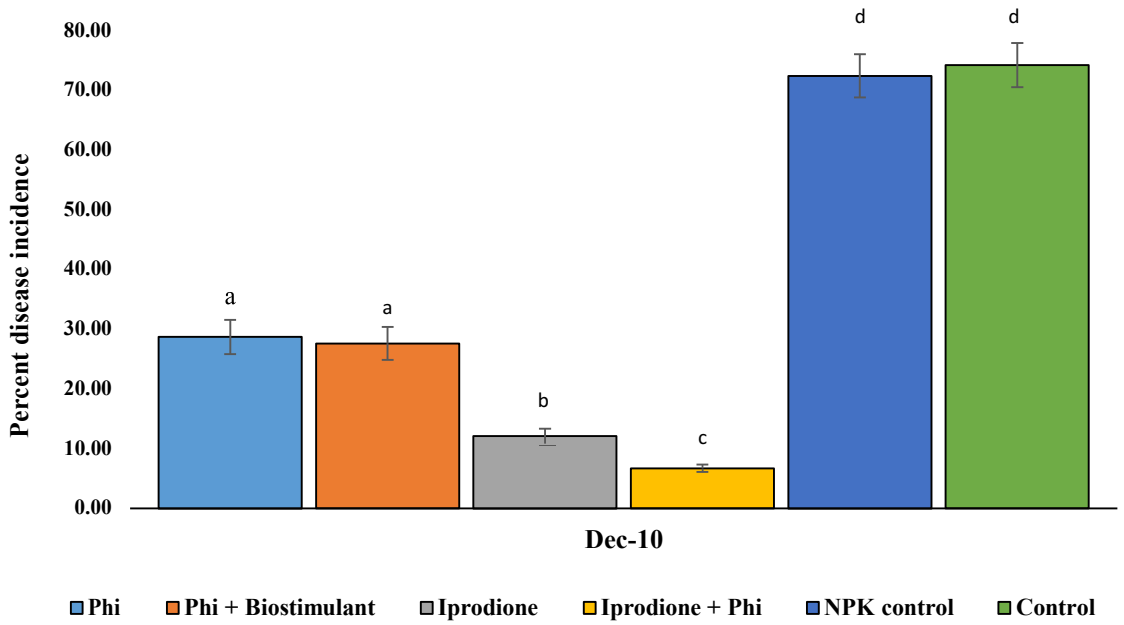

Figure 3-3 Monthly disease incidence, *A. canina*, December 2010 (year 1). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A. canina* during the month of greatest disease incidence in year 1 of the trial, December 2010. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

3.4.1.2 Mean levels of disease incidence September 2010 to March 2011 (year 1)

A two-way Anova was conducted to examine the effects of treatments and turfgrass species on mean levels of disease incidence between September 2010 and March 2011 (year 1). There was a significant ($p < 0.05$ df = 5, 48, Fstat 9.057) interaction between turf species and treatments, with significant differences on disease incidence between species ($p < 0.05$ df = 1, 48, Fstat 208.941) and from treatments ($p < 0.05$ df = 1, 48, Fstat 1996.614). Subsequent one-way Anovas determined significant treatment effects on disease incidence within *P. annua* ($p < 0.05$ df = 5, 24, Fstat 448.530) and *A. canina* ($p < 0.05$ df = 5, 24, Fstat 576.405), which were then separated by Tukey HSD at $p < 0.05$. In *P. annua,* post hoc analyses revealed significant ($p < 0.05$) differences between all treatments, with the exceptions of between Phi and Phi/Biostimulant, $p = 0.894$ and between NPK control and Control, $p = 0.986$, Fig. 3-4. In *A. canina,* significant ($p < 0.05$) differences were determined between all treatments, with the exceptions of Phi and Phi/Biostimulant, $p = 0.998$ and between NPK control and Control, $p = 0.835$, Fig. 3-4.

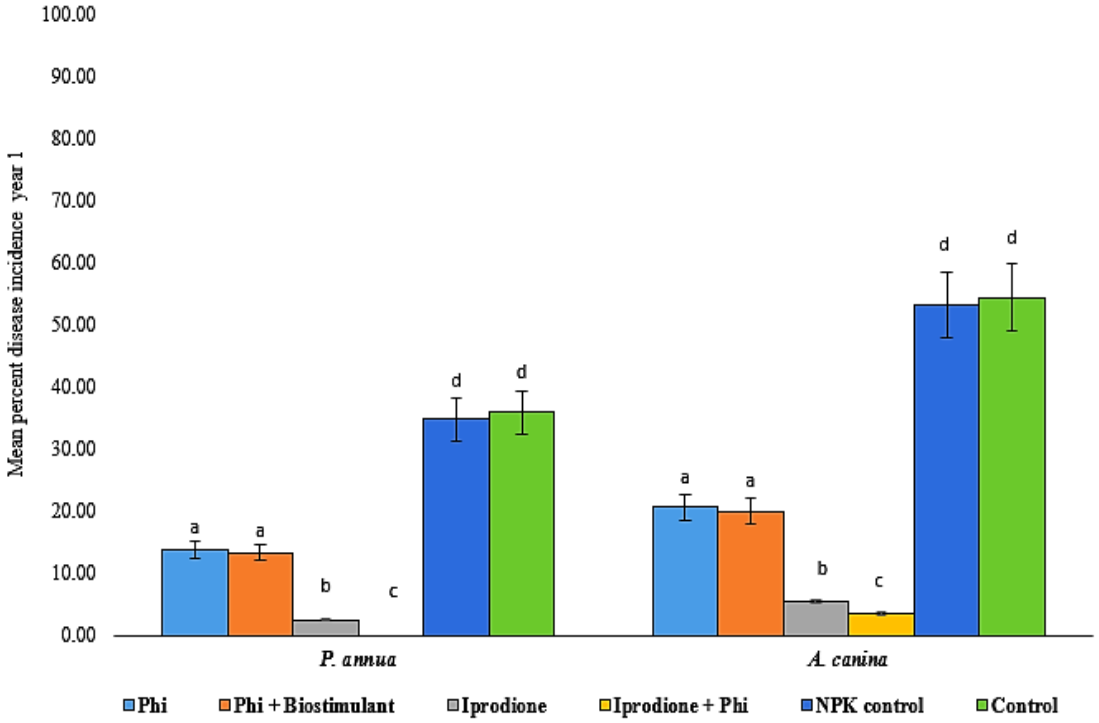

Figure 3-4 Mean disease incidence, *P. annua* and *A. canina*, from September 2010 to March 2011 (year 1). Treatment effect on mean levels of percent incidence of *M. nivale* on *P.annua* and *A. canina*, trial plots (n=5). Data are mean values from September 2010 to March 2011 (year 1). Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species, Tukey HSD $p < 0.05$.

3.4.1.3 Monthly disease incidence September 2011 to March 2012 (year 2)

The levels of *M. nivale* disease incidence and treatment effect on the trial plots (n=5) for each of the months of the second years trials were analysed with significant ($p < 0.05$ by two-way Anova df = 60, 504, Fstat 159.367) differences and interactions determined between treatments, months and turfgrass species. There were also significant interactions between treatments and species ($p < 0.05$ df = 10, 504, Fstat 1210.120), between treatments and months ($p < 0.05$ df = 30, 504, Fstat 694.392) and between species and months ($p < 0.05$ df = 12, 504, Fstat 676.645). Subsequently, treatment effect on disease incidence for each species and each month were examined using one-way Anova, Table 3-5, with significant treatment effects separated by Tukey HSD at $p < 0.05$. In the three turfgrass species trialled, disease incidence was greatest in November 2011 and treatment effect on the levels of incidence during these months are shown in Figs 3-5, 3-6 and 3-7.

Table 3-5 One-way Anova showing significant differences between treatments on monthly levels of *M. nivale* incidence on *P. annua, A. canina* and *A. stolonifera* trial plots (n=5), from September 2011 to March 2012 (year 2).

P. annua Sept 2011 to March 2012 (year 2)				
Month	df	f	p	η2
Sept	5,24	4699.628	< .001	0.999
Oct	5,24	2825.961	< .001	0.998
Nov	5,24	2147.738	< .001	0.998
Dec	5,24	2925.706	< .001	0.998
Jan	5,24	1661.402	< .001	0.997
Feb	5,24	2668.02	< .001	0.998
March	5,24	4297.851	< .001	0.999

A. canina Sept 2011 to March 2012 (year 2)				
Month	df	f	p	η2
Sept	5,24	2106.943	< .001	0.998
Oct	5,24	2988.743	< .001	0.998
Nov	5,24	2445.107	< .001	0.998
Dec	5,24	1712.823	< .001	0.997
Jan	5,24	2248.341	< .001	0.998
Feb	5,24	5302.199	< .001	0.999
March	5,24	3657.969	< .001	0.999

A. stolonifera Sept 2011 to March 2012 (year 2)				
Month	df	f	p	η2
Sept	5,24	2389.694	< .001	0.998
Oct	5,24	3254.675	< .001	0.999
Nov	5,24	1594.269	< .001	0.997
Dec	5,24	2834.858	< .001	0.998
Jan	5,24	1350.372	< .001	0.996
Feb	5,24	2025.089	< .001	0.998
March	5,24	4196.684	< .001	0.999

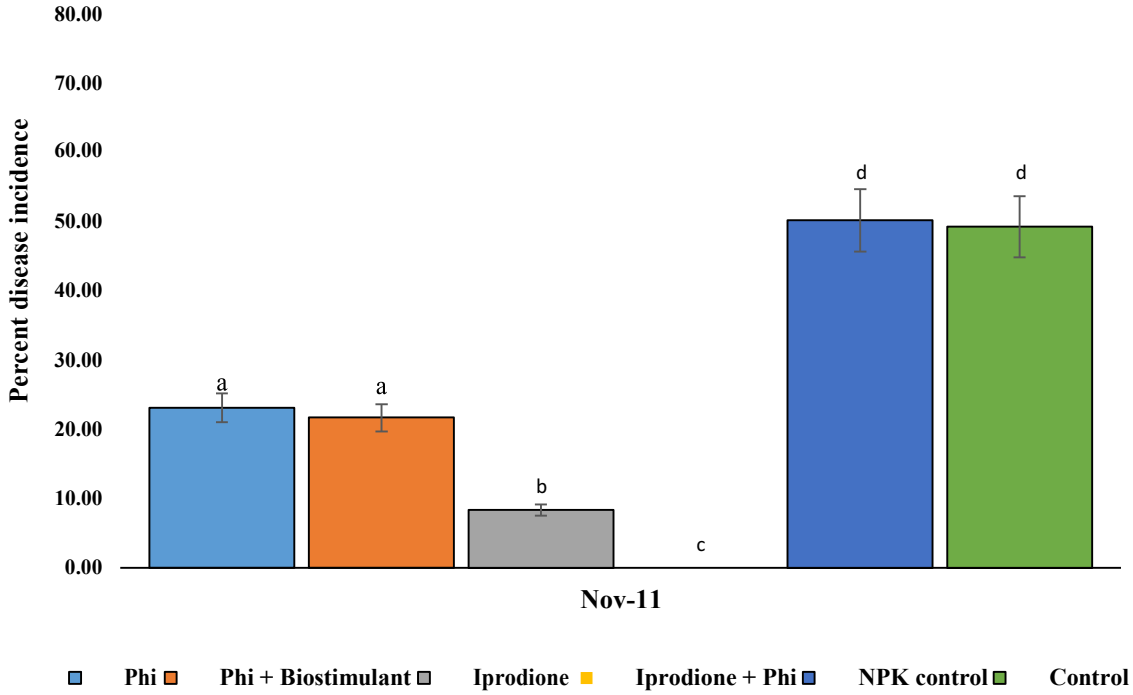

Figure 3-5. **Monthly disease incidence, *P. annua,* November 2011 (year 2).** Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua* during the month of greatest disease incidence in year 2 of the trial, November 2011. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$

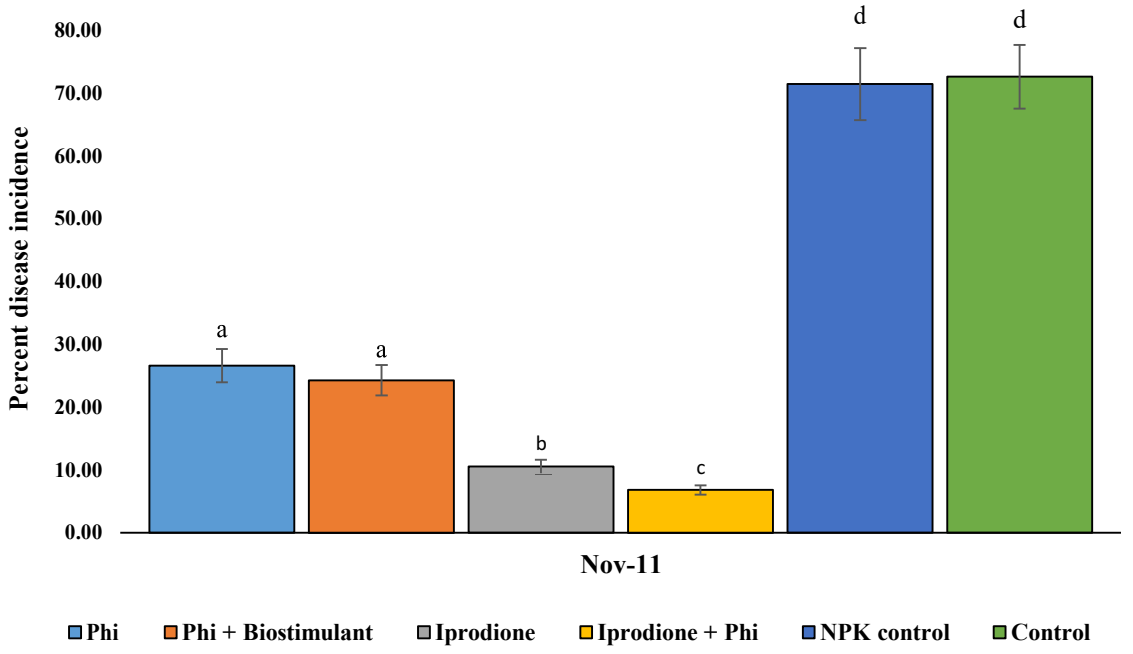

Figure 3-6 Monthly disease incidence, *A. canina,* November 2011 (year 2). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A. canina* during the month of greatest disease incidence in year 2 of the trial, November 2011. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$

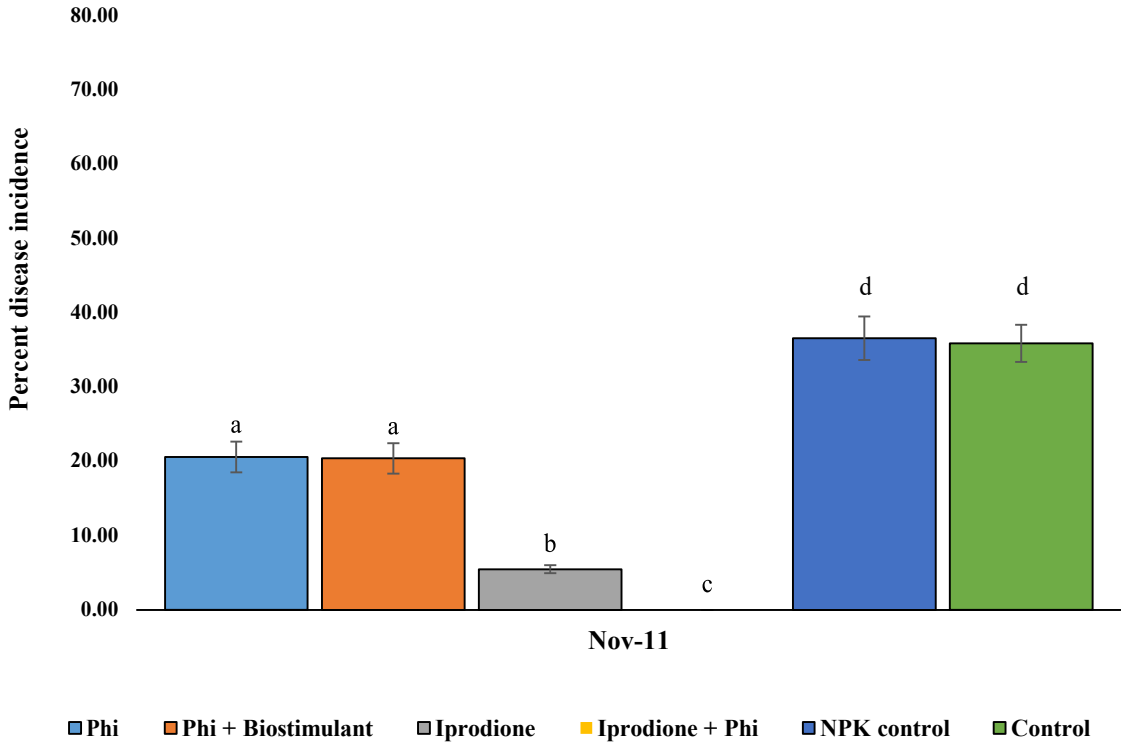

Figure 3-7 Monthly disease incidence, *A. stolonifera,* November 2011 (Year 2). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A. stolonifera* during the month of greatest disease incidence in year 2 of the trial, November 2011. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

3.4.1.4 Mean levels of disease incidence from September 2011 to March 2012

A two-way Anova was conducted to examine the effects of treatments and turfgrass species on mean levels of disease incidence between September 2011 and March 2012. There was a significant ($p < 0.05$ df = 10, 72, Fstat 20.800) interaction between turf species and treatments, with significant differences on disease incidence between species ($p < 0.05$ df = 2, 72, Fstat 149.085) and from treatments ($p < 0.05$ df = 5, 72, Fstat 1284.205). Subsequent one-way Anovas determined significant treatment effects on disease incidence within *P. annua* ($p < 0.05$ df = 5, 24, Fstat 1055.268), *A. canina* ($p < 0.05$ df = 5, 24, Fstat 637.843) and *A. stolonifera* ($p < 0.05$ df = 5, 24, Fstat 186.094), which were then separated by Tukey HSD at $p < 0.05$. In *P. annua,* post hoc analyses revealed significant ($p < 0.05$) differences between all treatments, with the exceptions of between Phi and Phi/Biostimulant, $p = 0.785$ and between NPK control and Control, $p = 0.924$. In *A. canina,* post hoc analyses revealed significant ($p < 0.05$) differences between all treatments, $p < 0.05$, with the exceptions of Phi and Phi/Biostimulant, $p = 0.367$ and

between NPK control and Control, p = 1.000. In *A. stolonifera,* post hoc analyses revealed significant ($p < 0.05$) differences between all treatments, with the exceptions of Phi and Phi/Biostimulant, p = 0.878, between NPK control and Control, p = 1.000, Fig. 3-8.

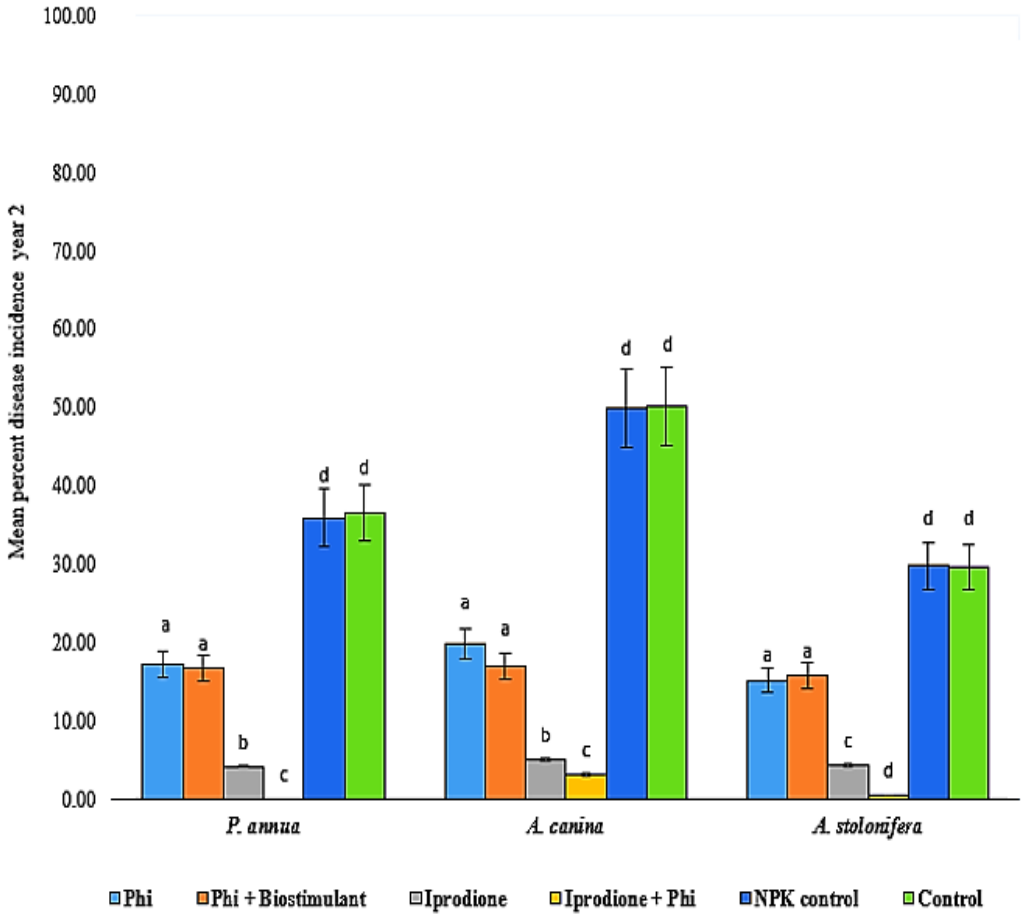

Figure 3-8 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera*, from September 2011 to March 2012. Treatment effect on mean levels of percent incidence of *M. nivale* on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are mean values from September 2011 to March 2012 (year 2). Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species, Tukey HSD $p < 0.05$

3.4.1.5 Treatment effect on mean disease incidence years 1 and 2

Percent disease incidence were arcsine transformed prior to data analysis and back-transformed to show data from the first two years trials. In year 1, Fig. 3-4, the control and NPK control plots display the highest mean values of disease incidence, ranging from 34.77, 95% CI (29.76, 39.78) percent in the NPK *P. annua* trial plots to 46.61, 95% CI (42.05, 51.16) percent in the control *A. canina* plots, levels of disease incidence which would be unacceptable on any golf green. The application of Phi reduced the incidence of the disease by more than 50%, with mean values ranging from 13.35, 95% CI (11.67, 15.03) percent in the Phi/biostimulant treated *P. annua* plots, to 17.64, 95% CI (15.44, 19.84) percent in the Phi treated *A. canina* plots, significantly ($p < 0.05$) lower than the NPK and control plots. The addition of the biostimulant to the Phi treatments, while not significantly ($p = 0.998$) reducing disease incidence, compared to the Phi treatment alone, did display a trend for slightly lower mean values, 13.35, 95% CI (11.67, 15.03) percent compared to 13.95, 95% CI (11.76, 16.14) percent in the *P. annua* treatments and 17.15, 95% CI (15.56) percent compared to 17.64, 95% CI (15.44, 19.84) percent in the *A. canina* treatments. The plots of both species which received treatment with the fungicide iprodione, displayed very low levels of disease incidence, significantly ($p < 0.05$) less than the control, NPK, Phi and Phi/Biostimulant treatments, 2.51, 95% CI (1.42, 3.60) percent in the *P. annua* plots and 4.74, 95% CI (3.94, 5.54) percent in the *A. canina* plots. The treatments which were most effective at reducing *M. nivale* incidence were the combination of iprodione and Phi, with full suppression of disease on the *P. annua* plots this combination led to significantly ($p < 0.05$) less disease incidence than all other treatments. However, despite the fact that these treatments were applied at an extreme rate on a two week cycle, *M. nivale* was not fully inhibited in the *A canina* plots, with mean incidence of disease at 2.98, 95% CI (2.22, 3.74) percent. In year 2 Fig. 3-8, treatment effect on disease incidence was similar to year 1 with the control and NPK control plots displaying the highest mean values, ranging from 29.63, 95% CI (25.83, 33.43) percent in the NPK *A. stolonifera* plots to 50.57, 95% CI (46.88, 54.25) percent in the NPK *A. canina* plots. The Phi and Phi/biostimulant treatments reduced disease incidence again by more than 50%, with mean values ranging from 15.09, 95% CI (13.51, 16.67) percent in the Phi treated *A. stolonifera* plots, to 19.76, 95% CI (17.01, 22.51) percent in the Phi treated *A. canina* plots, significantly ($p < 0.05$) lower than the NPK and control plots. The addition of the biostimulant to the Phi treatments did not significantly ($p = 0.980$) reduce disease incidence, compared to the Phi treatment

alone, but as in year 1 they did display a trend for slightly lower mean values, 16.84, 95% CI (15.26, 18.42) percent compared to 17.42, 95% CI (15.67, 19.16) percent in the *P. annua* treatments and 17.17, 95% CI (15.92, 18.42) percent compared to 19.76, 95% CI (17.01, 22.51) percent in the *A. canina* treatments, but with higher levels in *A. stolonifera* of 15.66, 95% CI (13.43, 17.98) percent compared to 15.09, 95% CI (13.51, 16.67) percent in the Phi treated plots. In the plots which received iprodione, disease incidence was significantly ($p < 0.05$) less than all treatments, with the exception of the Phi/iprodione combination, with incidence levels of: *P. annua* 4.16, 95% CI (3.27, 5.05) percent, *A. canina* 5.13, 95% CI (4.05, 6.21) percent and *A. stolonifera* 4.39, 95% CI (3.42, 5.36) percent. The treatments which were most effective at reducing disease incidence again were the combination of iprodione and Phi, significantly ($p < 0.05$) less disease incidence than all other treatments. This combination led to full suppression of disease on the *P. annua* plots, with levels of 3.16, 95% CI (2.38, 3.94) percent on the *A. canina* plots and only 0.47, 95% CI (-10, 1.05) percent on the *A. stolonifera* plots. This almost total inhibition could indicate either differing modes of suppression or a possible synergistic effect.

3.4.2 Disease incidence – years 3 and 4

Results from years 1 and 2 gave clear evidence of the inhibitory effects Phi had on the severity of infection by *M. nivale,* therefore it was decided to expand the study to include a different fungicide in years 3 and 4, and to assess the effects of varying Phi application timings and frequencies. In year 3, *M. nivale* infection again was allowed to develop naturally, with disease symptoms first appearing during October 2012. Disease incidence progressively increased and peaked at the end of November 2012. Disease pressure then declined until a re-emergence during February 2013.

3.4.2.1 Monthly disease incidence from September 2012 to March 2013 (year 3)

Monthly disease incidence levels for year 3, September 2012 to March 2013, were analysed with significant ($p < 0.05$ by two-way Anova df = 60, 504, Fstat 51.529) differences and interactions determined between species, treatments and months. There were also significant interactions between treatments and species ($p < 0.05$ df = 10, 504, Fstat 971.551), between treatments and months ($p < 0.05$ df = 30, 504, Fstat 1366.881) and between species and months ($p < 0.05$ df = 12, 504, Fstat 434.155). Subsequently, treatment effect on disease incidence for each species and each month were examined using one-way Anova, with significant treatment effects separated by Tukey HSD at $p <$

0.05 Table 3-6. In the three turfgrass species trialed, disease incidence was greatest in November 2012 and treatment effect on the levels of incidence during these months are shown in Figs 3-9, 3-10 and 3-11.

Table 3-6 One-way Anova showing significant differences between treatments on monthly levels of *M. nivale* incidence on *P. annua, A. canina* and *A. stolonifera* trial plots (n=5), from September 2010 to March 2013 (year 3).

P. annua Sept 2012 to March 2013 (year 3)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	2076.499	< .001	0.998
Nov	5,24	2308.934	< .001	0.998
Dec	5,24	3164.563	< .001	0.998
Jan	5,24	2213.136	< .001	0.998
Feb	5,24	1200.051	< .001	0.996
March	5,24	2552.607	< .001	0.998

A. canina Sept 2012 to March 2013 (year 3)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	2667.264	< .001	0.998
Nov	5,24	2994.609	< .001	0.998
Dec	5,24	2849.701	< .001	0.999
Jan	5,24	4228.057	< .001	0.999
Feb	5,24	960.275	< .001	0.995
March	5,24	3485.305	< .001	0.999

A. stolonifera Sept 2012 to March 2013 (year 3)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	2759.213	< .001	0.998
Nov	5,24	1905.981	< .001	0.997
Dec	5,24	4149.026	< .001	0.999
Jan	5,24	3066.314	< .001	0.998
Feb	5,24	2570.134	< .001	0.998
March	5,24	1127.636	< .001	0.996

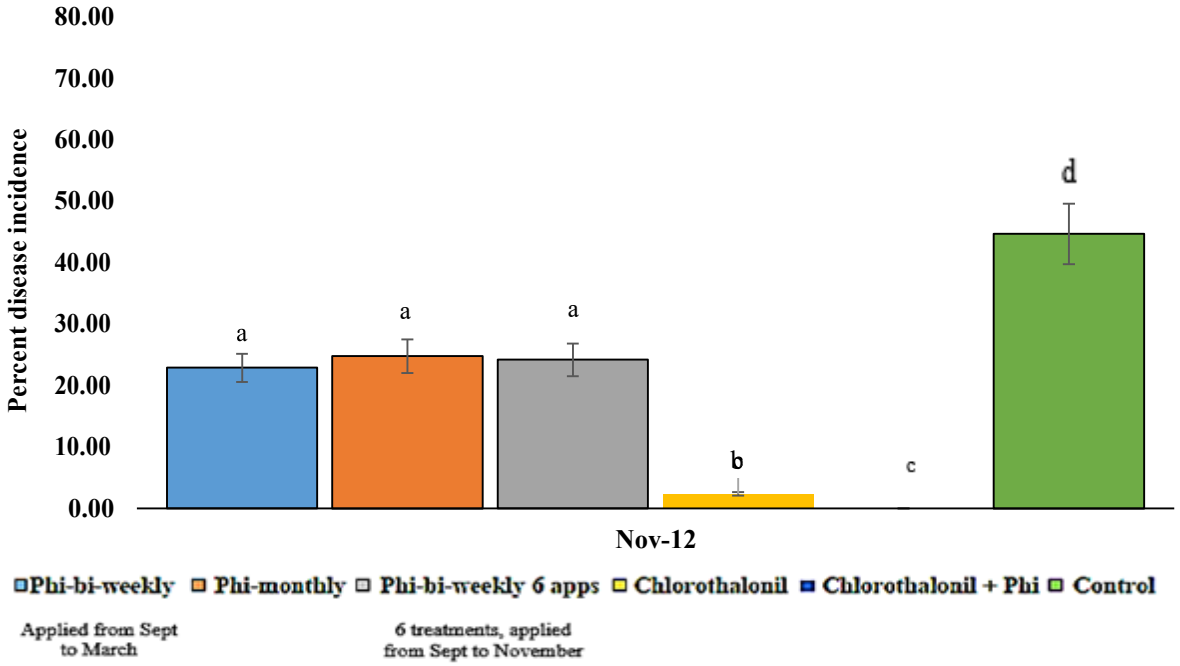

Figure 3-9 Monthly disease incidence, *P. annua,* November 2012 (year 3). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua* during the month of greatest disease incidence in year 3 of the trial, November 2012. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

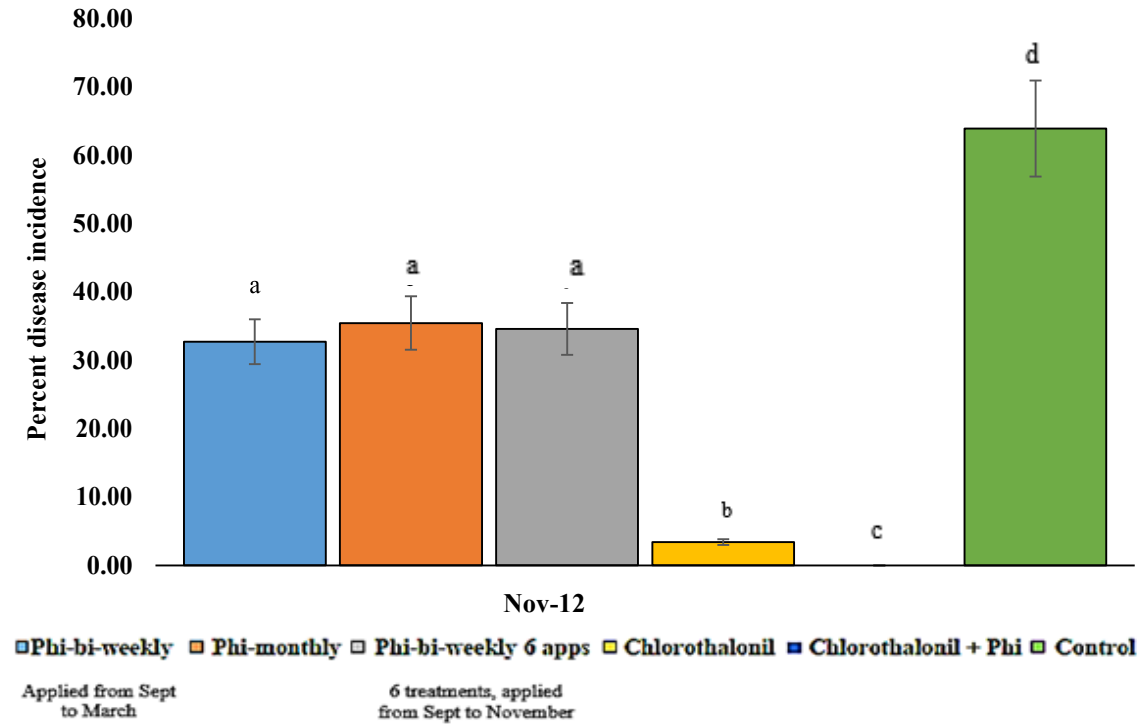

Figure 3-10 Monthly disease incidence, *A. canina*, November 2012 (year3). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A.canina* during the month of greatest disease incidence in year 3 of the trial, November 2012. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

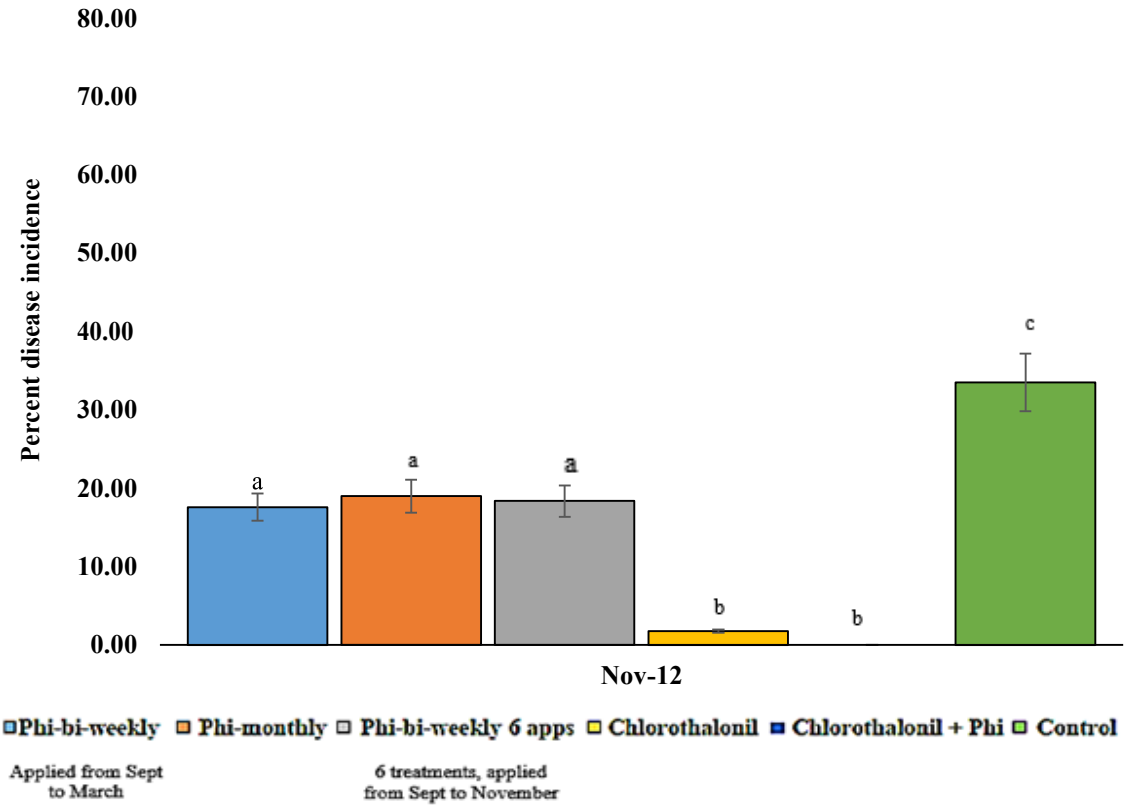

Figure 3-11 Monthly disease incidence, *A. stolonifera,* November 2012 (year 3). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A. stolonifera* during the month of greatest disease incidence in year 3 of the trial, November 2012. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

3.4.2.2 Mean levels of disease incidence from September 2012 to March 2013

A two-way Anova was conducted to examine the effects and interaction of treatments and turfgrass species on mean levels of disease incidence between September 2012 and March 2013. There was a significant ($p < 0.05$ df = 10, 72, Fstat 10.738) interaction between turf species and treatments, with significant differences on disease incidence between species ($p < 0.05$ df = 2, 72, Fstat 155.005) and from treatments ($p < 0.05$ df = 5, 72, Fstat 1166.510). Subsequent one-way Anovas determined significant treatment effects on disease incidence within *P. annua* ($p < 0.05$ df = 5, 24, Fstat 507.668), *A. canina* ($p < 0.05$ df = 5, 24, Fstat 541.319) and *A. stolonifera* ($p < 0.05$ df = 5, 24, Fstat 218.751), which were then separated by Tukey HSD at $p < 0.05$, Fig. 3-12.

In *P. annua* post hoc analyses revealed significant differences between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.136$, between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.088$ and between Phi monthly and Phi bi-weekly 6 applications $p = 1.000$. In *A. canina,* post hoc analyses revealed significant differences between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.089$, between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.062$ and between Phi monthly and Phi bi-weekly 6 applications $p = 1.000$. In *A. stolonifera,* post hoc analyses revealed significant differences between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.387$, between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.411$ and between Phi monthly and Phi bi-weekly 6 applications $p = 1.000$.

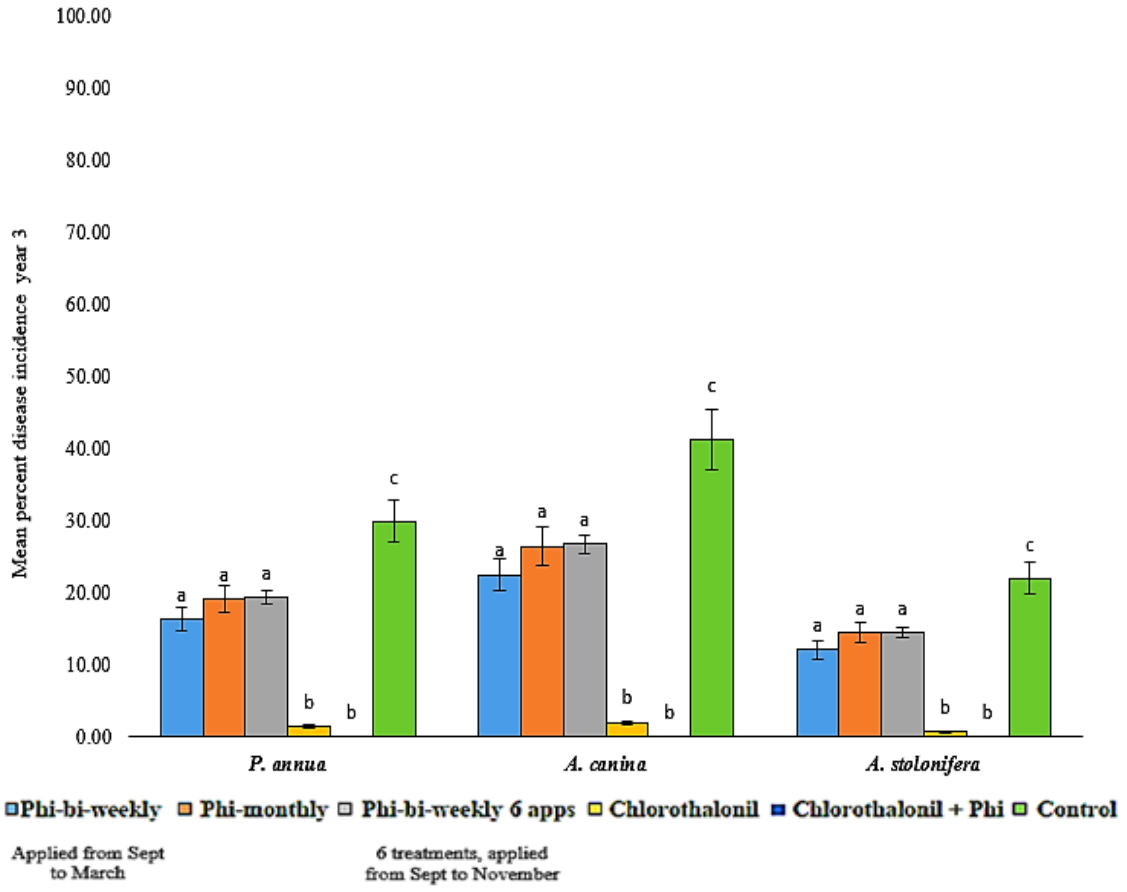

Figure 3-12 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera,* from September 2012 to March 2013 (year 3). Treatment effect on mean levels of percent incidence of *M. nivale* on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are mean values from September 2012 to March 2013 (year 3). Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species, Tukey HSD $p < 0.05$

3.4.2.3 Monthly disease incidence from September 2013 to March 2014 (year 4)

In year 4, from September 2013 to March 2014, while climatic conditions were suitable for *M. nivale* infection, disease incidence remained low, this was a general unexplained condition throughout the UK and Ireland (Golf Course managers, personal communications). Despite this, disease incidences did occur and monthly levels were analysed with significant ($p < 0.05$ by two-way Anova df = 60, 504, Fstat 212.244) differences and interactions determined between species, treatments and months. There were also significant interactions between treatments and species ($p < 0.05$ df = 10, 504, Fstat 1069.940), between treatments and months ($p < 0.05$ df = 30, 504, Fstat 2340.843) and between species and months ($p < 0.05$ df = 12, 504, Fstat 1385.277). Subsequently, treatment effect on disease incidence for each species and each month were examined using one-way Anova, with significant treatment effects separated by Tukey HSD at $p < 0.05$ Table 3-7. Figs 3-13, 3-14 and 3-15 show *M. nivale* disease incidence and significant treatment effect on *P. annua, A. canina* and *A. stolonifera* for the month with greatest disease incidence during year 4, November 2013.

Table 3-7 One-way Anova showing significant differences of treatment effect on monthly levels of *M. nivale* incidence on *P. annua, A. canina* and *A. stolonifera* trial plots (n=5), from September 2013 to March 2014 (year 4).

P. annua Sept 2013 to March 2014 (year 4)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	2281.859	< .001	0.998
Nov	5,24	2665.572	< .001	0.998
Dec	5,24	5228.386	< .001	0.998
Jan	5,24	1884.241	< .001	0.997
Feb	5,24	2076.963	< .001	0.998
March	5,24	4549.54	< .001	0.999
A. canina Sept 2013 to March 2014 (year 4)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	2648.248	< .001	0.998
Nov	5,24	1848.007	< .001	0.997
Dec	5,24	1746.317	< .001	0.997
Jan	5,24	2411.85	< .001	0.998
Feb	5,24	1804.418	< .001	0.997
March	5,24	20808	< .001	1.000
A. stolonifera Sept 2013 to March 2014 (year 4)				
Month	df	f	p	η2
Sept	5,24	0	0	0
Oct	5,24	1554.421	< .001	0.997
Nov	5,24	1048.792	< .001	0.995
Dec	5,24	2652.338	< .001	0.998
Jan	5,24	1608.181	< .001	0.997
Feb	5,24	1560.648	< .001	0.997
March	5,24	19602	< .001	1.000

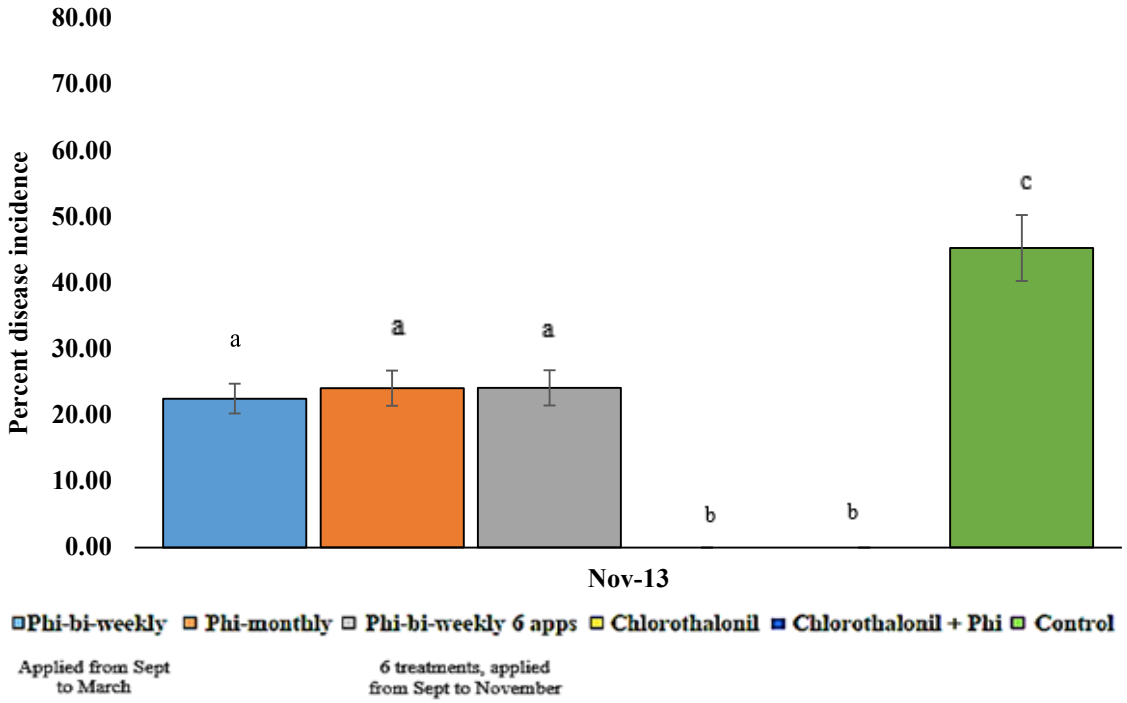

Figure 3-13 Monthly disease incidence, *P. annua,* November (year 4). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua* during the month of greatest disease incidence in year 4 of the trial, November 2013. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

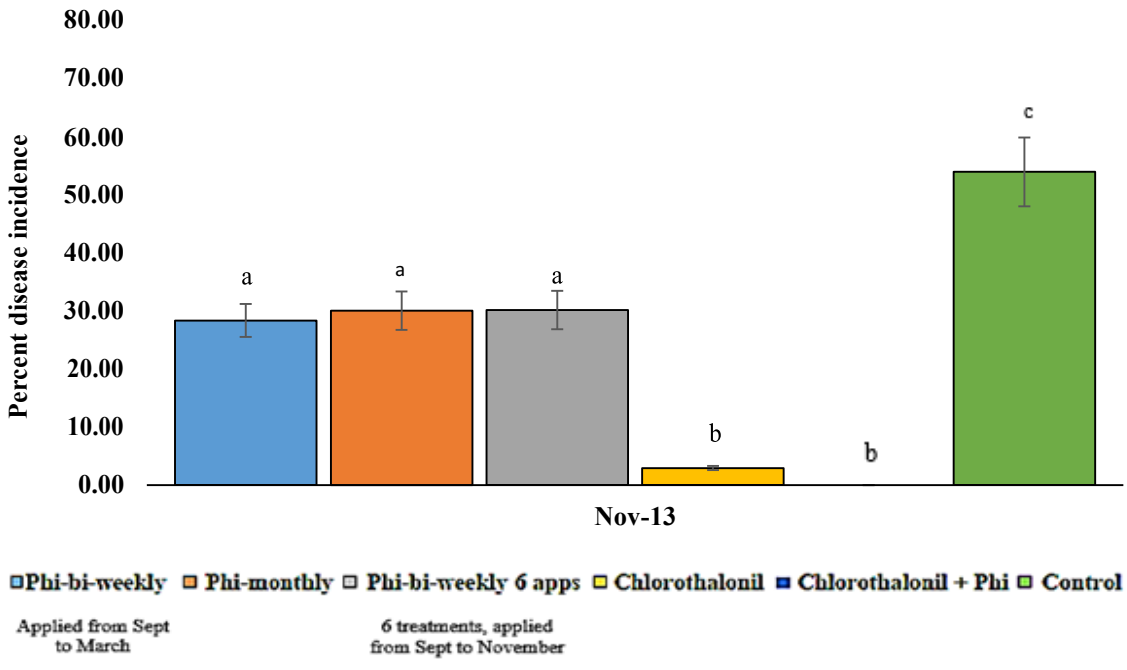

Figure 3-14 Monthly disease incidence, *A. canina*, November 2013 (year 4). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A. canina* during the month of greatest disease incidence in year 4 of the trial, November 2013. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

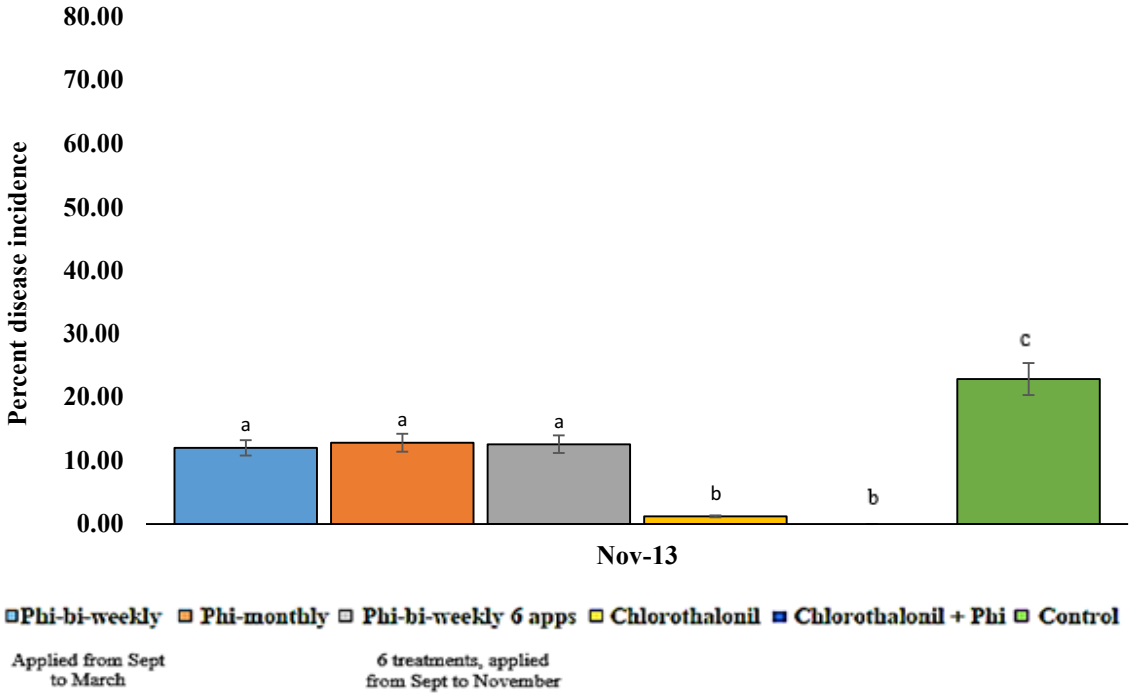

Figure 3-15 Monthly disease incidence, *A. stolonifera*, November 2013 (year 4). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *A.stolonifera* during the month of greatest disease incidence in year 4 of the trial, November 2013. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

3.4.2.4 Mean levels of disease incidence from September 2013 to March 2014

A two-way Anova was conducted to examine the effects of treatments and turfgrass species on mean levels of disease incidence between September 2013 and March 2014.

There was a significant ($p < 0.05$ df = 10, 72, Fstat 16.021) interaction between turf species and treatments, with significant differences on disease incidence between species ($p < 0.05$ df = 2, 72, Fstat 88.494) and from treatments ($p < 0.05$ df = 5, 72, Fstat 675.660).

Subsequent one-way Anovas determined significant treatment effects on disease incidence within P. annua ($p < 0.05$ df = 5, 24, Fstat 364.841), A. canina ($p < 0.05$ df = 5, 24, Fstat 330.511) and A. stolonifera ($p < 0.05$ df = 5, 24, Fstat 100.235), which were then separated by Tukey HSD at $p < 0.05$, Fig. 3-16.

In *P. annua* post hoc analyses revealed significant differences between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.059$, between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.054$ and between Phi monthly and Phi bi-weekly 6 applications $p = 1.00$. there were no differences between chlorothalonil and Phi chlorothalonil $p = 1.00$. In *A. canina,* post hoc analyses revealed significant differences

between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.148$ and between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.110$ and between Phi bi-weekly 6 applications and Phi monthly $p = 1.00$. In *A. stolonifera,* post hoc analyses revealed significant differences between all treatments, $p < 0.05$, with the exceptions of between Phi bi-weekly and Phi monthly $p = 0.272$, between Phi bi-weekly and Phi bi-weekly 6 applications $p = 0.666$, between Phi monthly and Phi bi-weekly 6 applications $p = 0.980$ and between chlorothalonil and Phi + chlorothalonil $p = 0.114$.

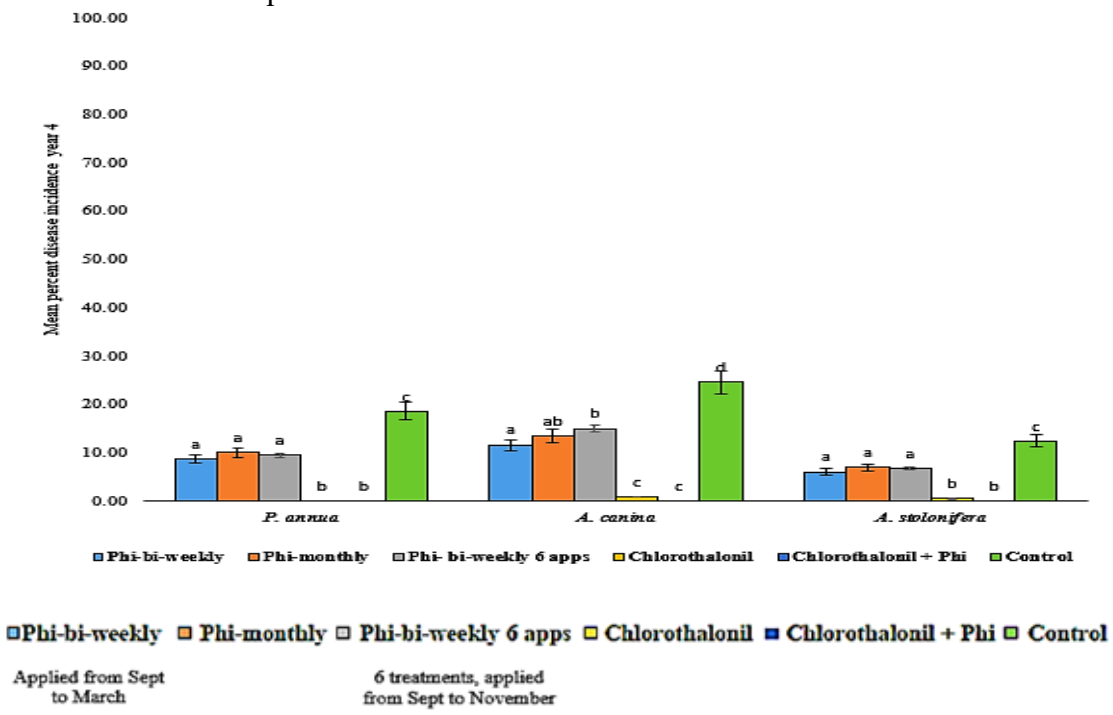

Figure 3-16 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera,* from September 2013 to March 2014 (year 4). Treatment effect on mean levels of percent incidence of *M. nivale* on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are mean values from September 2013 to March 2014 (year 4). Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species, Tukey HSD $p < 0.05$

3.4.2.5 Treatment effect on mean disease incidence years 3 and 4

Results of the mean levels of disease incidence from year 3 of the study, as shown in the back-transformed data in Fig. 3-12, show that the inclusion of Phi, as a foliar treatment, led to a significant suppression of *M. nivale* incidence. In year 3, as in years 1 and 2, the control plots displayed the highest mean levels of disease incidence with values of 29.98, 95% CI (26.37, 33.59) percent for *P. annua,* 41.27, 95% CI (38.20,44.34) percent *A. canina* and 22.04, 95% CI (19.86, 24.22) percent in the *A. stolonifera* plots. The application of Phi bi-weekly, significantly ($p < 0$ .05) reduced disease incidence, with mean values of 16.38, 95% CI (14.80, 17.96) percent for *P. annua,* 22.52, 95% CI (19.60,

25.44) percent *A. canina* and 12.13, 95% CI (8.41, 15.85) percent in the *A. stolonifera* plots. The Phi treatments applied monthly also suppressed disease incidence significantly ($p < 0.05$), compared to the control treatments with values of 19.20, 95% CI (16.80, 21.60) percent for *P. annua,* 26.49, 95% CI (22.99, 29.98) percent *A. canina* and 14.54, 95% CI (11.75, 17.33) percent in the *A. stolonifera* plots. The Phi monthly treatments, when compared to the bi-weekly treatments, gave rise to higher levels of disease incidence, but these were not statistically significant ($p > 0.05$). Disease incidence from the Phi treatments applied bi-weekly for six applications only, from September to the end of November 2013, also gave rise to a significant ($p < 0.05$), reduction in disease incidence compared to controls. The mean values for these treatments were 19.45, 95% CI (17.00, 21.90) percent for *P. annua,* 26.75, 95% CI (23.20, 30.29) percent *A. canina* and 14.49, 95% CI (11.49, 17.49) percent in the *A. stolonifera* plots.

The chlorothalonil treatments, as expected, led to the highest level of disease suppression, with 1.47, 95% CI (0.82, 2.12) percent for *P. annua,* 2.03, 95% CI (1.16, 2.90) percent *A. canina* and 0.74, 95% CI (0.39, 1.09) percent in the *A. stolonifera* plots. However, as with the iprodione treatments in years 1 and 2, *M. nivale* was not fully inhibited. The treatments which were most effective at reducing *M. nivale* incidence were the combination of chlorothalonil and Phi, with full suppression on all three species.

The mean values for disease suppression for the full trial period do not display significant differences between the three Phi treatments used. However, as can be seen in Fig. 3-17, while the Phi applied bi-weekly remained effective at reducing disease incidence during periods of high disease pressure, both the Phi monthly and the Phi bi-weekly applied for six treatments only, displayed reducing efficacy in reducing disease incidence. By February there were significant ($p < 0.05$) differences in levels of disease incidence between the three Phi treatments. In all three turfgrass species, the highest levels of disease were determined in the Phi bi-weekly six applications, with the Phi monthly treatment displaying the second highest level, Fig. 3-18.

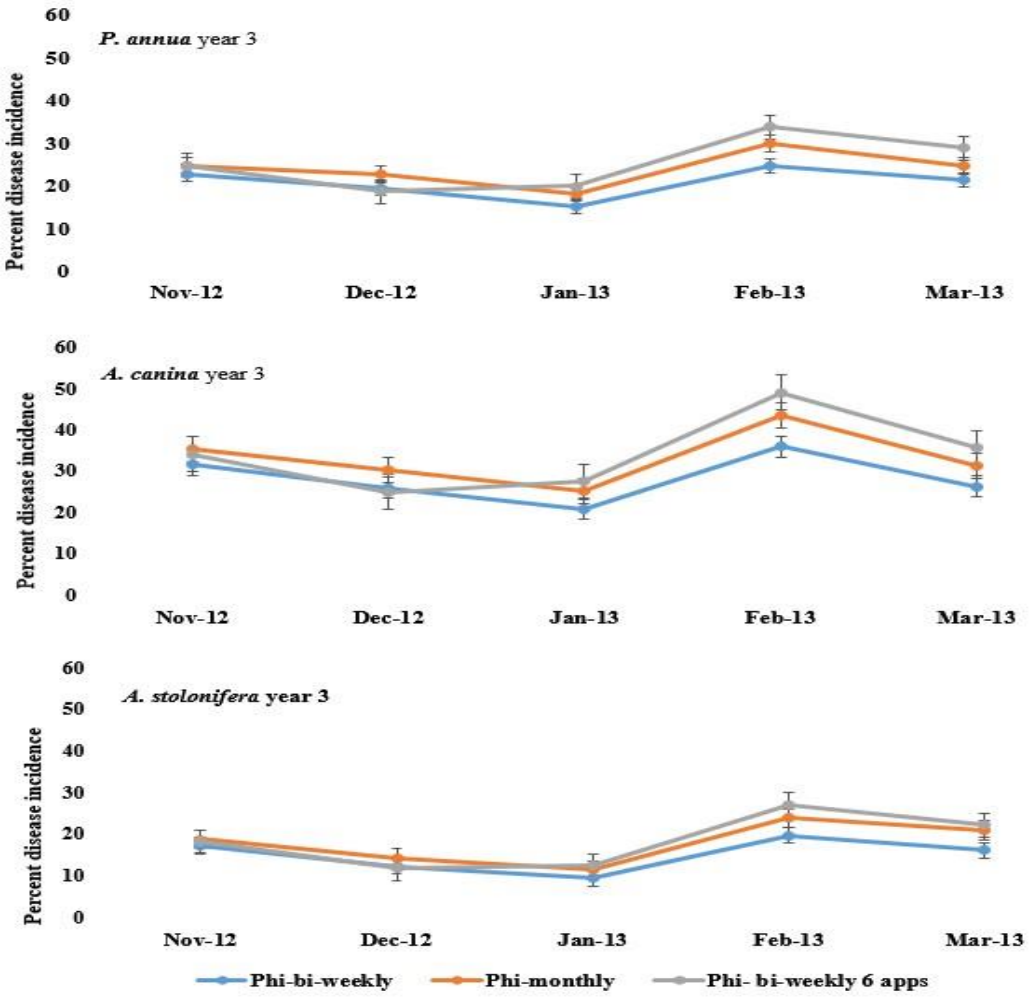

Figure 3-17 Monthly levels of disease incidence year 3. Percent incidence of *M. nivale* on trial plots of *P. annua, A. canina* and *A. stolonifera*, treated with Phi bi-weekly, Phi monthly and Phi bi-weekly 6 applications, from November 2012 to March 2013.). Data are mean values, n=5, Bars indicate 95% confidence limits.

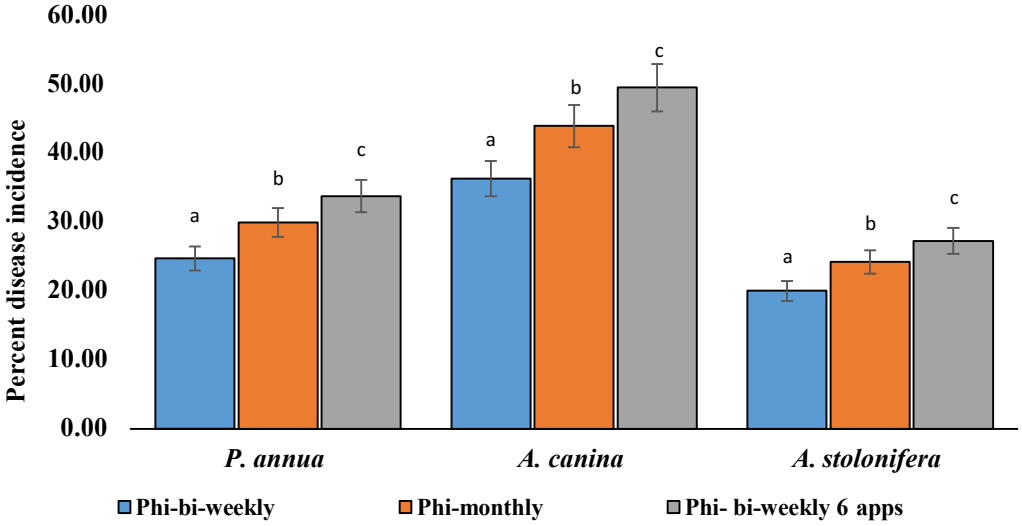

Figure 3-18 Monthly disease incidence all turfgrass species, February 2013 (year 3). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua*, *A. canina and A. stolonifera* during February 2014. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

In year 4, as shown in the back-transformed data in Fig. 3-16, control plots again displayed significantly ($p < 0.05$) higher mean levels of disease incidence than all other treatments with values of 18.55, 95% CI (15.27, 21.82) percent for *P. annua,* 24.55, 95% CI (21.40, 27.69) percent *A. canina* and 12.38, 95% CI (9.40, 15.36) percent in the *A. stolonifera* plots. The application of Phi bi-weekly, significantly ($p < 0$ .05) reduced disease incidence compared to controls, with mean values of 8.7, 95% CI (6.94, 10.46) percent for *P. annua,* 11.57, 95% CI (8.76, 14.38) percent *A. canina* and 6.04, 95% CI (4.50, 7.57) percent in the *A. stolonifera* plots. The Phi treatments applied monthly also suppressed disease incidence significantly ($p < 0.05$), compared to the control treatments with values of 10.08, 95% CI (7.99, 12.17) percent for *P. annua,* 13.46, 95% CI (11.33, 15.59) percent *A. canina* and 6.94, 95% CI (5.38, 8.41) percent in the *A. stolonifera* plots. As in year 3, the Phi monthly treatments, when compared to the bi-weekly treatments, gave rise to higher levels of disease incidence, but again there were not significant ($p >0.05$). Disease incidence from the Phi treatments applied bi-weekly for six applications only, from September to the end of November 2014, allowed for a significant ($p < 0.05$), reduction in disease incidence in the all three species compared to controls, with mean values of 9.46, 95% CI (6.40, 12.52) percent for *P. annua,* 15.00, 95% CI (12.77, 17.23) percent for *A. canina* and 6.72, 95% CI (5.15, 8.28) percent for the *A. stolonifera*. The chlorothalonil treatments again led to very high levels of disease suppression, with full suppression on the *P. annua* plots, and only 0.92, CI (0.65, 1.18) percent for *A. canina* and 0.48, CI (-0.12, 1.09) percent in the *A. stolonifera* plots. The treatments which were most effective at reducing *M. nivale* incidence were the combination of chlorothalonil and Phi, with full suppression on all three species.

As in the results from year 3, the mean values for disease suppression for the full trial period do not display significant differences between the three Phi treatments used, except within the *A. canina* results, where the Phi treatment applied bi-weekly for the full period was significantly ($p < 0.05$) less than the other two Phi treatments. Fig. 3-19 shows the levels of monthly disease incidence from November to March. There appears to be no significant differences between treatments, however, analysis of the February data show there were significant ($p < 0.05$) differences in disease incidence between the three Phi treatments. In all three turfgrass species, the highest levels of disease were again determined in the Phi bi-weekly six applications, with the Phi monthly treatment displaying the second highest level, Fig. 3-20.

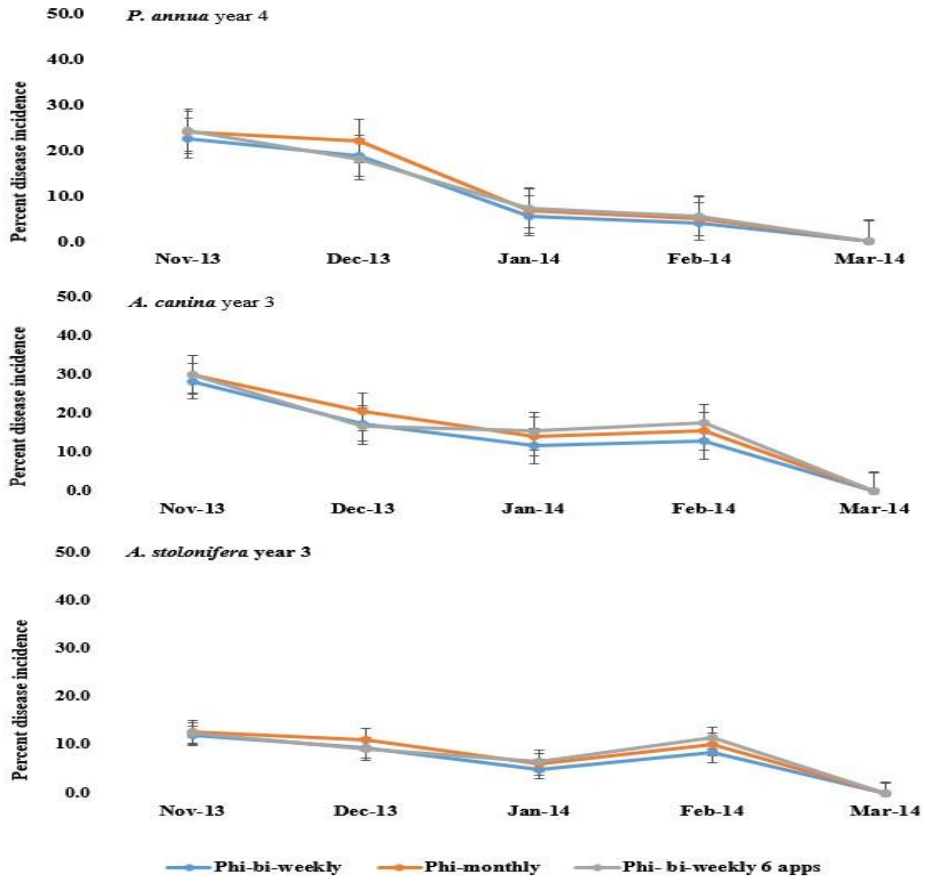

Figure 3-19 Monthly levels of disease incidence year 3. Percent incidence of *M. nivale* on trial plots of *P. annua, A. canina* and *A. stolonifera*, treated with Phi bi-weekly, Phi monthly and Phi bi-weekly 6 applications, from November 2013 to March 2014. Data are mean values, n=5, Bars indicate 95% confidence limits.

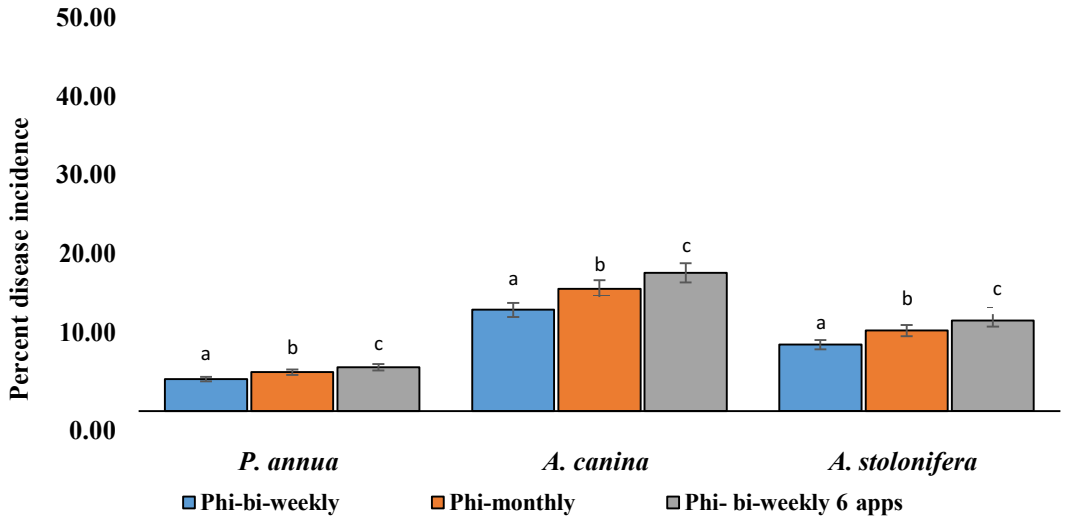

Figure 3-20 Monthly disease incidence all turfgrass species, February 2014 (year 4). Treatment effect on percent *M. nivale* incidence on trial plots (n=5), of *P. annua*, *A. canina and A. stolonifera* during February 2014. Data were arcsine transformed prior to analysis and back-transformed for this graph. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month, Tukey HSD $p < 0.05$.

3.4.3 Treatment effect on turfgrass quality

Turf quality at the beginning of each year was uniform for all treatment plots, however, as the trials progressed, turf quality in the plots receiving Phi treatments improved significantly, while the quality of the control and fungicide treated plots became progressively poorer. A Kruskal-Wallis test was conducted to determine statistical differences in turf quality ratings, firstly determining that distributions were similar for the three turfgrass species following each of the four years of treatment applications and then that ratings were not significantly different between turfgrass species:

Year 1 $\chi 2$ (1) = 0.034, p = 0.853
Year 2 $\chi 2$ (2) = 0.055, p = 0.973
Year 3 $\chi 2$ (2) = 0.116, p = 0.944
Year 4 $\chi 2$ (2) = 0.115, p = 0.944

Quality ratings however, were significantly different between treatments:

Year 1 $\chi 2$ (5) = 71.752, $p < 0.05$
Year 2 $\chi 2$ (5) = 71.986, $p < 0.05$
Year 3 $\chi 2$ (5) = 74.913, $p < 0.05$
Year 4 $\chi 2$ (5) = 76.981, $p < 0.05$

Subsequently, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. These post hoc analyses revealed the statistically significant differences in median ratings of the treatments over both years' trials.

3.4.3.1 Treatment effect on turfgrass quality years 1 and 2

In year 1, as displayed in Fig. 3-21, in the *P. annua* plots, there were significant ($p < 0.05$) differences in ratings between the Phi, Phi/Biostimulant, Phi/Iprodione and all other treatments, while in the *A. canina* plots, there were significant ($p < 0.05$) differences between the Phi, Phi/Biostimulant, Phi/Iprodione and all other treatment with significant ($p < 0.05$) differences between the iprodione and the NPK and control treatments.

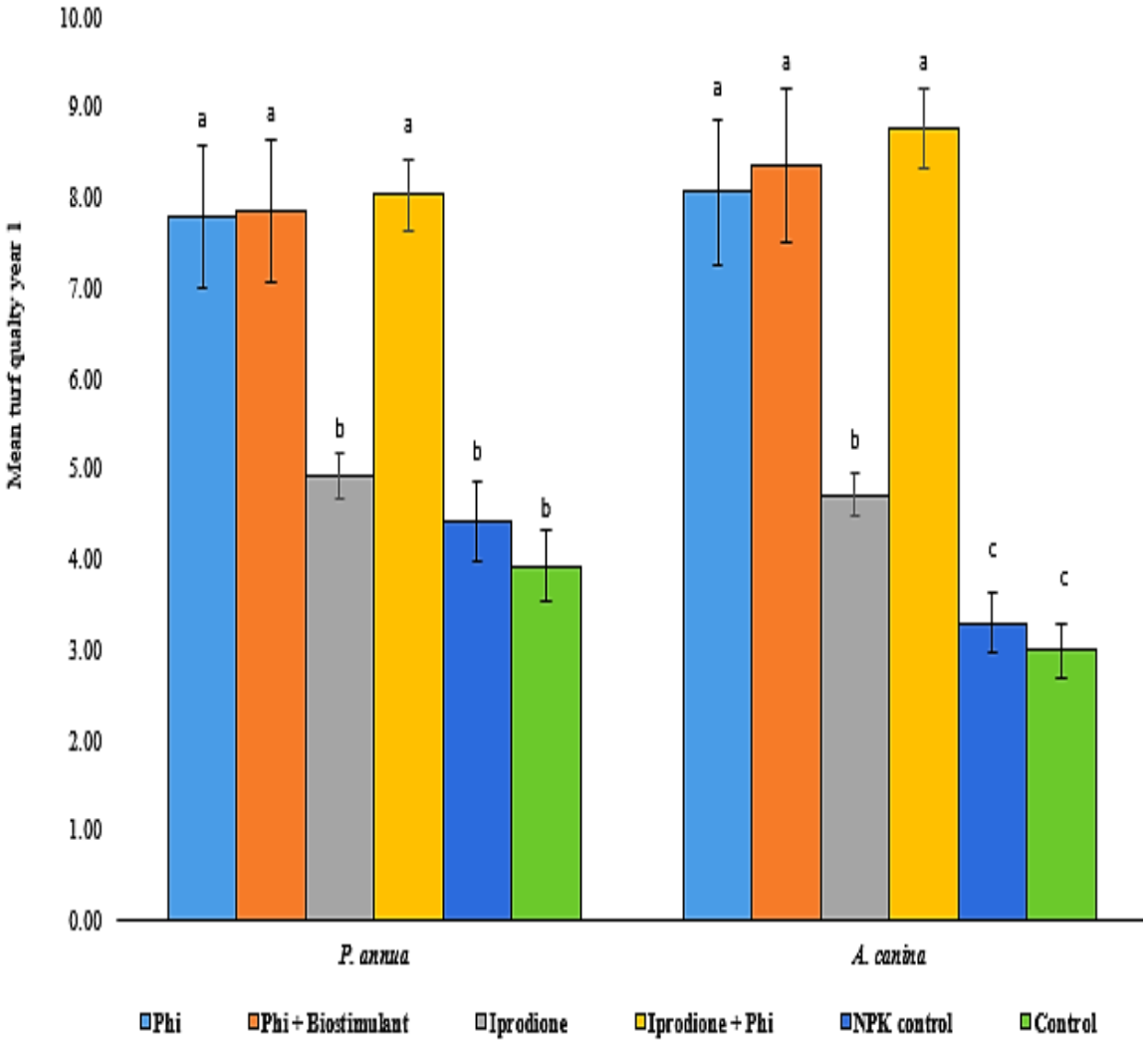

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Figure 3-21 Turfgrass quality, *P. annua* and *A. canina*, from September 2010 to March 2011 (year 1). Treatment effect on median levels of turfgrass quality on *P.annua* and *A. canina* trial plots (n=5). Data are median values from September 2010 to March 2011 (year 1). Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species following pairwise comparisons using Dunn's (1964) procedure at $p < 0.05$.

There were similar results in year 2, Fig. 3-22, with significant ($p < 0.05$) differences determined in turf quality ratings between the Phi, Phi/Biostimulant, Phi/Iprodione and the Iprodione, NPK Control and Control treatments with no other significant ($p > 0.05$) differences between any other treatment combinations.

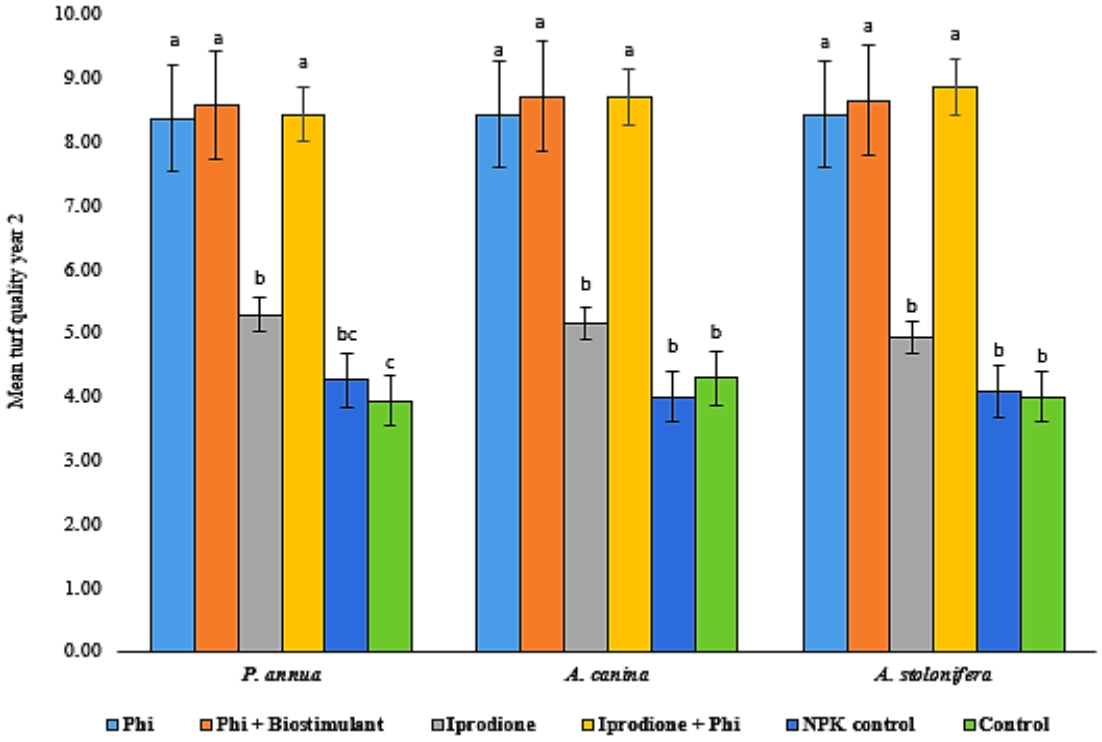


Figure 3-22 Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2011 to March 2012 (year 2). Treatment effect on median levels of turfgrass quality, assessed on a scale of 1-10, on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are median values from September 2011 to March 2012 (year 2). Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species following pairwise comparisons using Dunn's (1964) procedure at $p < 0.05$.

Figs 3-23 and 3-24 graphically illustrate the distinctions, over each year of the trial period, between plots receiving Phi and those untreated, the quality improvements clearly visible in all Phi treated plots. As the trials progressed, the quality of the control and NPK controls in the three turfgrass species, became progressively poorer, while the quality of the Phi and Phi/biostimulant treatments improved. The iprodione treated plots, even with significantly ($p < 0.05$) less disease incidence than the Phi and Phi/biostimulant treated plots, produced a poorer quality sward, with less density than the Phi treated plots. The Phi/iprodione treatment produced the highest quality ratings overall. By the end of the first two years' trial period the quality and density of all the Phi treated plots were significantly ($p < 0.05$) higher quality than all others, Figs 3-21 and 3-22.

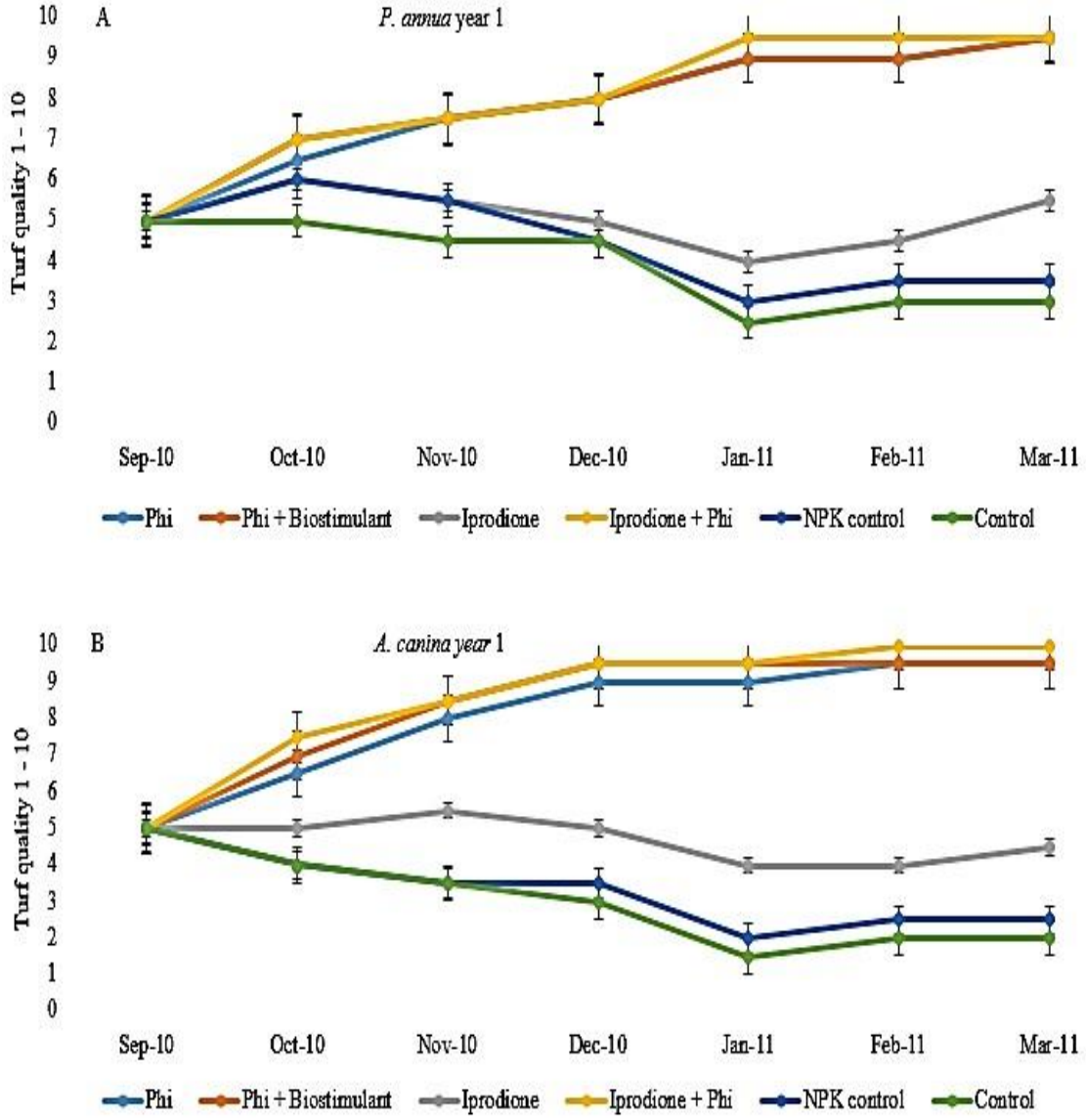


Figure 3-23 Monthly assessments of turfgrass quality, *P. annua* and *A. canina,* September 2010 to March 2011 (year 1). Treatment effect on turfgrass quality, assessed on a scale of 1-10, from September 2010 to March 2011 (year 1). A: *P. annua* and B: *A. canina*. Bars indicate 95% confidence intervals, (n=5).

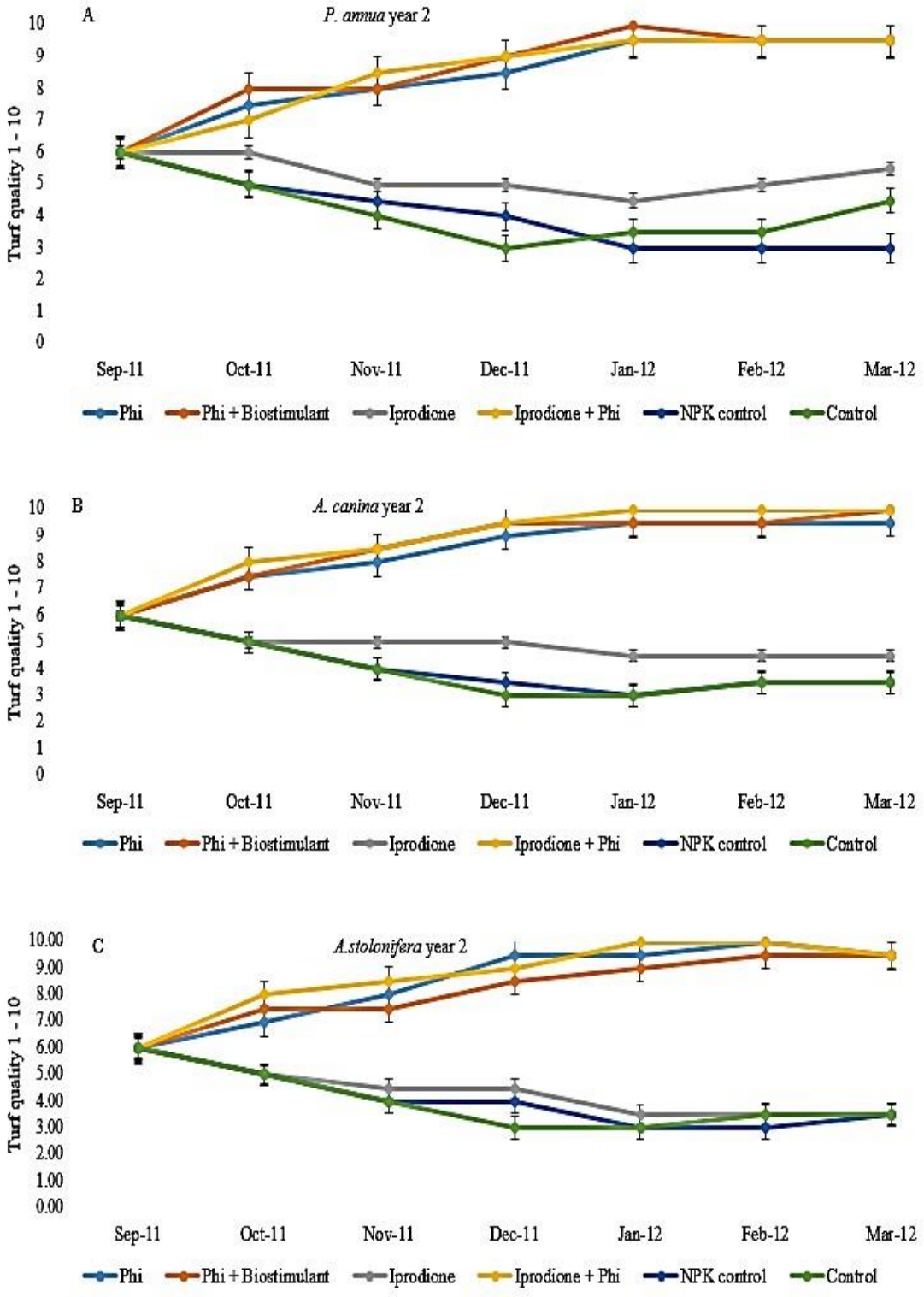


Figure 3-24 Monthly assessments of turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* September 2011 to March 2012 (year 2). Treatment effect on turfgrass quality, assessed on a scale of 1-10, from September 2011 to March 2012 (year 2). A: *P. annua*, B: *A. canina* and C: *A. stolonifera*. Bars indicate 95% confidence intervals, (n=5).

3.4.3.2 Treatment effect on turf quality years 3 and 4

In Year 3, the results in turfgrass quality ratings followed the trend of the first two years, Fig. 3-25, in that any treatments containing Phi, regardless of turfgrass species application timing or interval, led to increased turfgrass quality. Significant ($p < 0.05$) differences were determined in ratings between the control and the chlorothalonil treatments and all other treatments. There were no other significant ($p < 0.05$) differences between any other treatment combinations, with the exception of the Chlorothalonil/Phi combination which had a statistically ($p < 0.05$) higher rating than all other treatments.

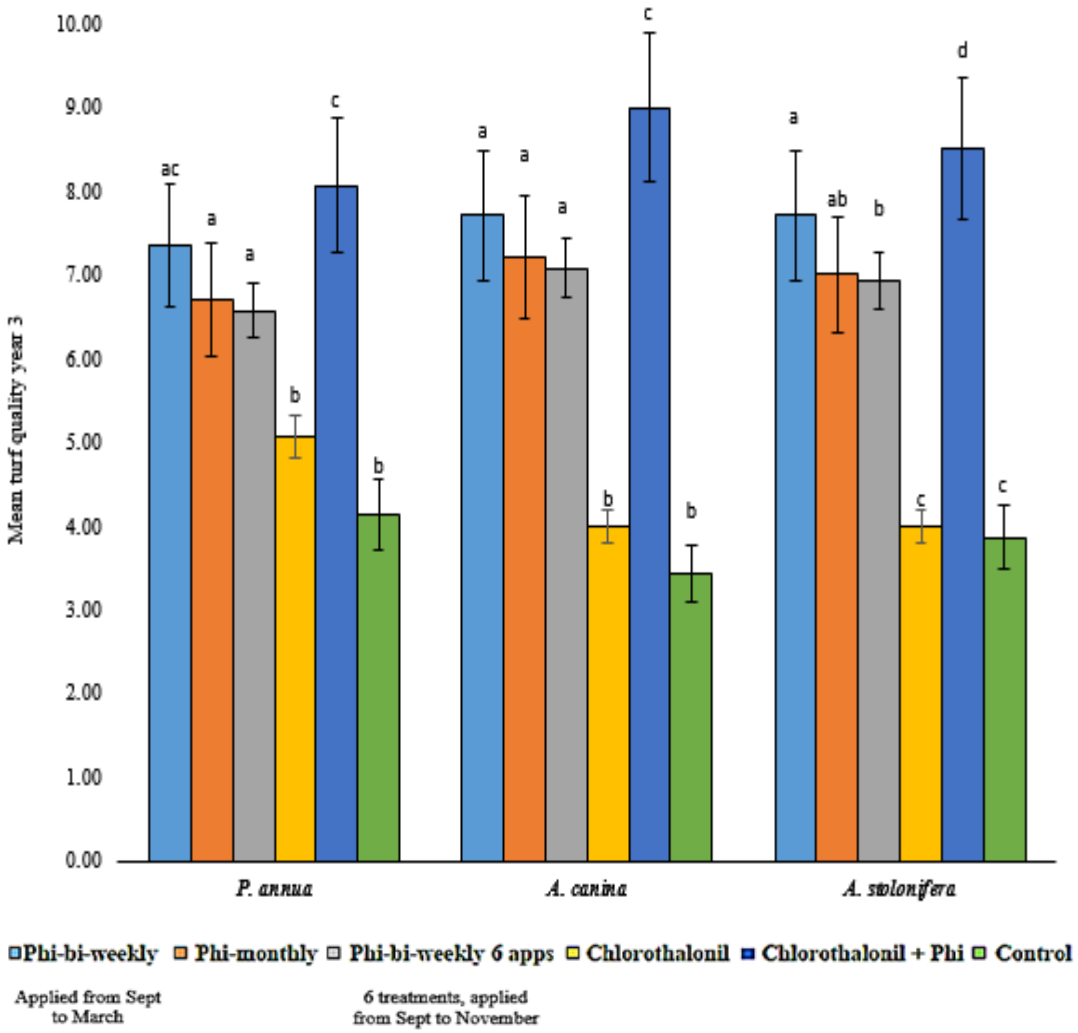


Figure 3-25 Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2012 to March 2013 (year 3). Treatment effect on mean levels of turfgrass quality, assessed on a scale of 1-10, on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are median values from September 2012 to March 2013 (year 3). Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species following pairwise comparisons using Dunn's (1964) procedure at $p < 0.05$.

In Year 4, significant ($p < 0.05$) differences were again determined in turf quality ratings between any treatments containing Phi, regardless of turfgrass species application timing or interval, Fig. 3-26. Significant ($p < 0.05$) differences were determined in ratings between the control and the chlorothalonil treatments and all other treatments. There were no other significant ($p > 0.05$) differences between any other treatment combinations.

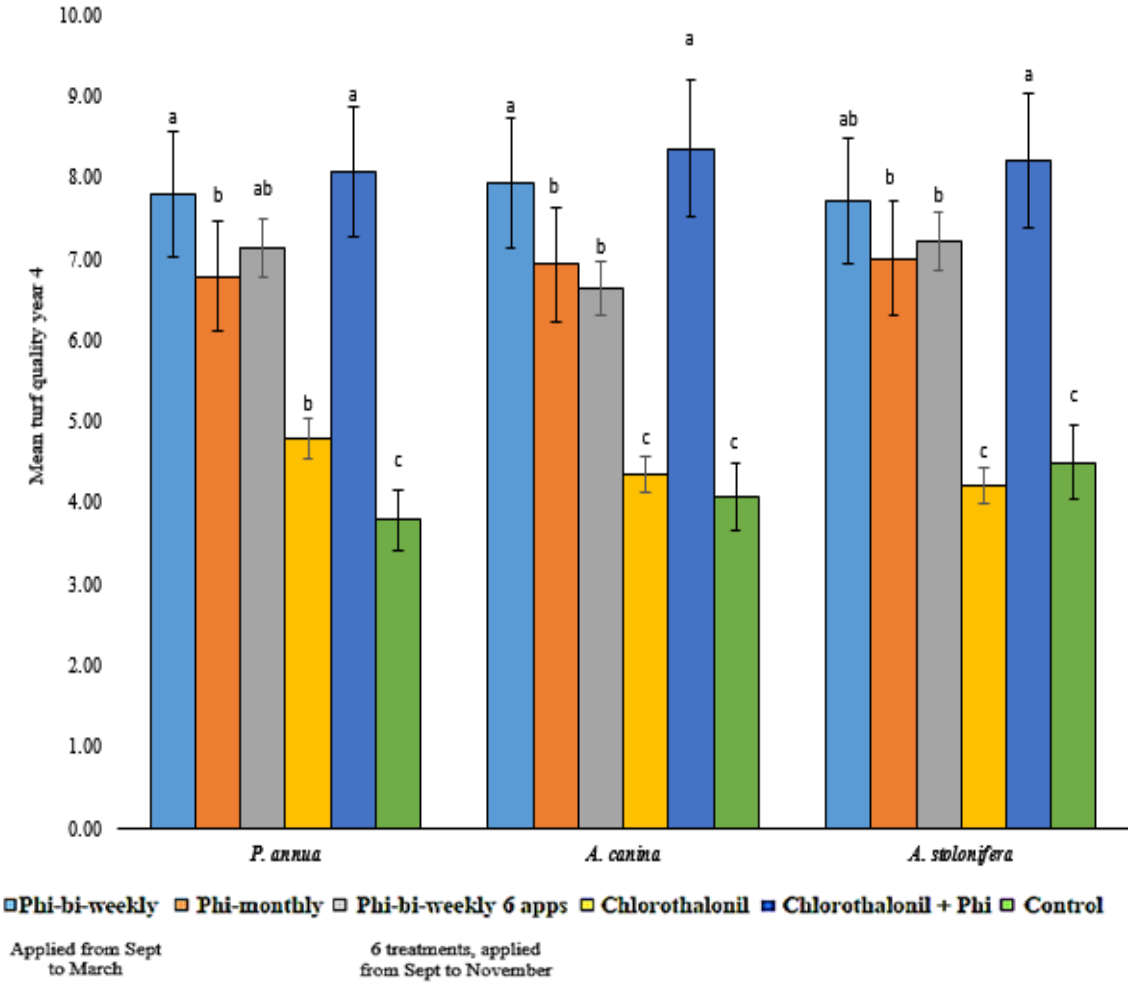


Figure 3-26 Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2013 to March 2014 (year 4). Treatment effect on mean levels of turfgrass quality, assessed on a scale of 1-10, on *P.annua*, *A. canina* and *A. stolonifera* trial plots (n=5). Data are median values from September 2013 to March 2014 (year 4). Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each species following pairwise comparisons using Dunn's (1964) procedure at $p < 0.05$.

In years 3 and 4, all Phi treatments regardless of turfgrass species, application timing or interval, led to increased turfgrass quality, while the chlorothalonil and control plots gradually became poorer, Figs 3-27 and 3-28. Statistically, all of the treatments which included Phi, were significantly ($p < 0.05$) better than the control and chlorothalonil treatments, the exception being the Phi/chlorothalonil combination, which produced the best quality swards, significantly ($p < 0.05$) better than all other treatment in year 3, Fig 3-25.

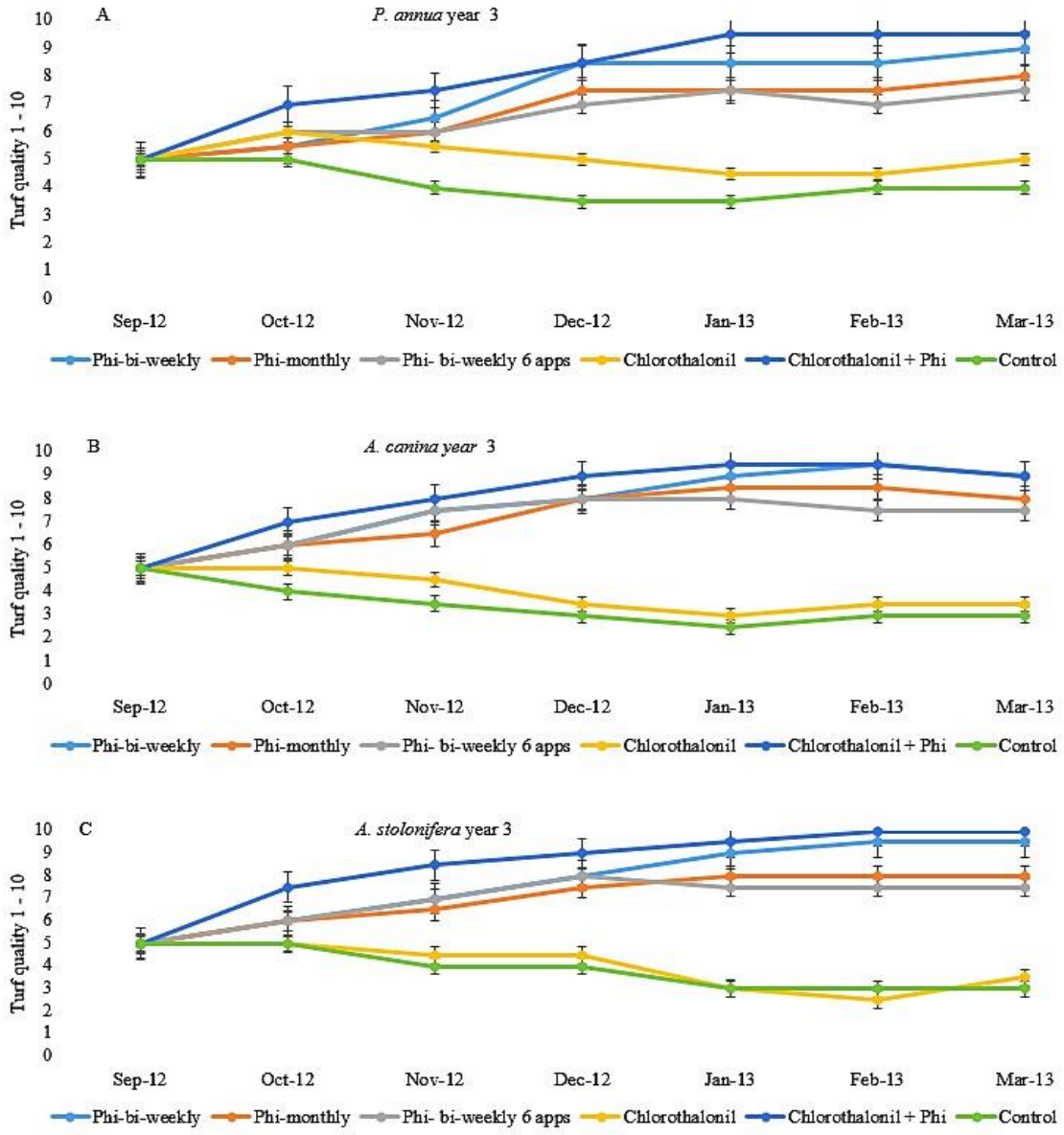


Figure 3-27 Monthly assessments of turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* September 2012 to March 2013 (year 3). Treatment effect on turfgrass quality, assessed on a scale of 1-10, from September 2012 to March 2013 (year 3). A: *P. annua*, B: *A. canina* and C: *A. stolonifera.* Bars indicate 95% confidence intervals, (n=5).

The Phi bi-weekly, continuous treatment, produced the second highest rating over both years. The Phi treatments applied bi-weekly for six applications only, from September to the end of November each year, and those receiving monthly Phi treatments, while of less quality than the Phi bi-weekly and Phi/chlorothalonil treatments were still significantly ($p < 0.05$) better than the control and chlorothalonil treatments.

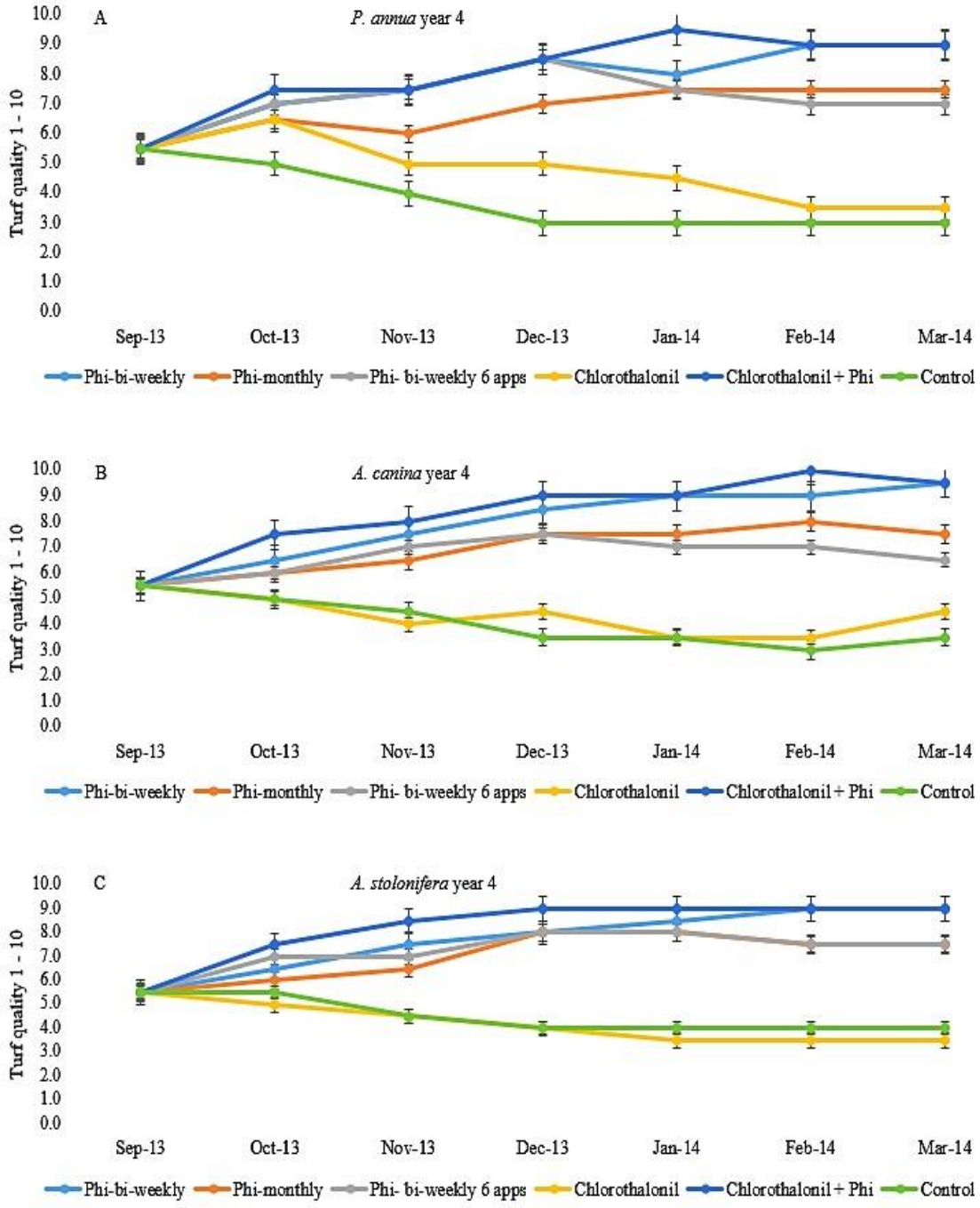


Figure 3-28 Monthly assessments of turfgrass quality September 2013 to March 2014. Treatment effect on turfgrass quality, assessed on a scale of 1-10, from September 2013 to March 2014 (year 4). A: *P. annua*, B: *A. canina* and C: *A. stolonifera.* Bars indicate 95% confidence intervals, (n=5).

Figs 3-29 to 3-35 give visual evidence of the effect treatments had on the three turfgrass species, with regard to disease incidence and severity and on turfgrass quality.

Figure 3-29 Overview of trial area at Royal Curragh golf course. Overview of trial area showing *A. canina* plots, January 2012. A: Phi, B: Phi/iprodione, C: Control.

Figure 3-30 *P. annua* trial plots, January 2012. View of the *P. annua* trial plots from January 2012. Treatments: A: Phi/iprodione, B: Control, C: Phi.

Figure 3-31 *A. canina* trial plots, January 2012. View of the *A. canina* trial plots from January 2012. Treatments: A: NPK control, B: Control C: Phi/iprodione.

Figure 3-32 *A. stolonifera* trial plots, January 2012. View of the *A. stolonifera* trial plots from January 2012. Treatments: A: Phi/iprodione, B: NPK control, C: Control, D: Phi/biostimulant.

Figure 3-33 *P. annua* trial plots February 2011. View of the *P. annua* trial plots from February 2011. Treatments: A: Control, B: Phi/iprodione.

Figure 3-34 *A. canina* trial plots, January 2012. View of the *A. canina* trial plots from January 2012. Treatments: A: Control, B: Phi/iprodione.

Figure 3-35 *A. stolonifera* trial plots, December 2011. View of the *A. stolonifera* trial plots from December 2011. Treatments: A: Control, B: Phi/iprodione.

3.5 Discussion

3.5.1 Disease suppression years 1 to 4

The data from the four years of these field trials has shown that, through periods of high disease pressure, as a result of sequential applications of Phi, there was significant and consistent reduction of *M. nivale* incidence in three commonly used cool-season turfgrasses. These trial results are supported by a previous study where significant disease reduction was reported in Phi treated *A. stolonifera* (Dempsey and Owen, 2010).

Determining the mode of action of Phi in reducing *M. nivale* occurrence in these field trials is a major objective of this research. As shown in the review of literature, there are numerous published reports showing Phi mediated reduction of plant pathogens by both direct fungistatic means and indirectly through stimulation of plant defence processes (Fenn and Coffey, 1987; Grant *et al*., 1990; Jackson *et al*., 2000; McCarren *et al*., 2009). The possibility that Phi had a direct fungistatic effect on *M. nivale* in these field trials is a distinct possibility, as the *in vitro* research in the previous chapter confirmed the fungistatic properties of Phi. Evidence of this direct inhibition is supported by the results of the second series of trials, carried out during years 3 and 4. Over both years, there were no statistical ($p > 0.05$) differences in mean levels of disease incidence between the Phi treatments applied bi-weekly and those applied monthly, as shown in Figs 3-12 and 3-16. However, the monthly Phi treatment did allow for higher levels of disease incidence and, as can be seen in Figs 3-9, 3-10, 3-11 and 3-13, 3-14 and 3-15, there were significant ($p < 0.05$) differences between both treatments in months of highest disease pressure. Likewise, the Phi treatments which were applied bi-weekly for six applications only, from September to November each trial year, were not significantly ($p > 0.05$) different, except in the *A. canina* trials in year 4, than those applied bi-weekly for the full period. Importantly however, as can be seen in Figs 3-18 and 3-20, there were significantly ($p < 0.05$) higher levels of disease incidence in the months receiving no treatments. These data would indicate that Phi on or inside the turfgrass plant inhibits the pathogens growth, as in it did in the *in vitro* studies. For Phi to suppress *M. nivale* via direct fungistatic means *in planta*, there needs to be direct contact between Phi and the pathogen. *M. nivale* infects turfgrass by entering the plant and extending hyphal growth to extract required nutrients. To suppress hyphal growth therefore, Phi needs to be taken up and translocated throughout the plants vascular system. In order to study this hypothesis, further research

into the take up, translocation and fate of Phi applied to turfgrass was required and this is detailed in the following chapter.

That disease suppression in these field trials was due to possible indirect inhibition of *M. nivale* by Phi, through stimulation or enhancement of inducible plant defence mechanisms is also a possibility. Jackson *et.al*. (2000) in a study of pathogen infected *Eucalyptus marginata* L., concluded that Phi accumulations *in planta* led to stimulation of host defences. Phi treatment resulted in induced rapid defence responses, including release of Reactive Oxygen Species, localised cell death and an increase in defence related phenolic compounds in *Arabidopsis thaliana* L. (Daniel and Guest, 2006) and Saindrenan *et al*. (1988) determined that Phi treatment enhanced the rate and magnitude of phytoalexin accumulation in *Vigna unguiculata* L.

A possible hypothesis to explain the Phi mediated disease reductions in these field trials is that *M. nivale* reduction was due to the combined effects of direct inhibition of the pathogen and enhanced host defence responses. It has been determined that Phi has fungistatic effects on *M. nivale* metabolism *in vitro*, this may also be the case *in planta*, Phi suppresses hyphal growth allowing increased time for the infected plant to respond. Phi may also induce the release of stress metabolites in *M. nivale*, enabling a more rapid recognition as a pathogen by the host plant. A more efficient recognition process allows a more rapid and hence more effective defence response, thus limiting the development of disease.

3.5.2 Turfgrass quality years 1 to 4

Turfgrass quality is a vital aspect of turfgrass management and is determined by a combination of colour, density, uniformity and texture. The search for products or procedures which improves quality is an ongoing research area. The data obtained over the four years of these trials, showed that there were significantly better visual quality and greater turf density on all Phi treated plots. These data are supported by a previous study by Dempsey and Owen (2010) who reported significant improvement of quality in Phi treated *A. stolonifera.*

In these trials all Phi treatments led to enhanced turfgrass quality, the reasons for the increase in turf quality can be attributed to a number of factors; firstly, Phi treatments could inhibit pathogenic soil microorganisms such as Oomycota and algae, and while not displaying disease symptoms could have a debilatory effect on turfgrass development. Phi has proven efficacy in reducing these organisms (Daniel and Guest, 2005; Abbasi and

Lazarovits, 2006), thus allowing for the healthier development of the turfgrass. A second possibility is that the suppression of *M. nivale* allowed unrestricted growth and development leading to increased density of the treated turfgrass. A further factor which could be considered is that Phi influenced a change in growth habit. Shcroetter *et al*. (2006) found that Phi applications to maize (*Zea mays* L.) led to an abnormality in growth habit, with the treated samples exhibiting an increase in lateral tillering, this could be a reason for the increase in turf density.

Any of the possible factors above could, separately or in tandem, have led to the improvement in the turf quality. Further study in this area was also required as, apart from the benefits to turfgrass presentation and playability brought about by this quality enhancement, the beneficial effects of Phi on turfgrass growth and development can have a significant effect on plant health and therefore its ability to synthesise defence compounds, which in turn reduces susceptibility plant pathogens.

While the ability of Phi to lessen *M. nivale* incidence and improve turf quality was clearly demonstrated during these trials, a number of anomalies do require discussion. *M. nivale* incidence was consistently and significantly ($p < 0.05$) greater on the *A. canina* plots compared to the *P. annua* and *A. stolonifera* plots. This is due to a number of factors. The siting of the *A. canina* sward, while in the same general area as the other plots, were subject to extreme shading from trees, especially during the autumn and winter months. This ensured that environmental conditions for *M. nivale* occurrence were at an optimum. The *P.annua* and *A. stolonifera* plots had optimum light conditions and excellent air movement. A second factor to be considered was that *A. canina*, when not previously hardened by low temperatures, is highly susceptible to *M. nivale* infection (Espevig, 2011). Furthermore of three varieties of *A. canina* used in the study by Espevig, the variety used in these field trials, proved more susceptible than the others.

During years 1 and 2, there were no significant ($p > 0.05$) differences in treatment effect on disease incidence between the NPK control and untreated control plots in the three turfgrass species. The nutritional inputs for each trial season supplied by the treatments were: 11.5 Kg/ha N, 26.8 Kg/ha P_2O_5 and 69 Kg/ha K_2O. It could be argued that the addition of N through periods of disease pressure would increase disease incidence, (Mann, 2004) however, while there were no significant differences, there was a trend for a lower percentage of incidence on the NPK plots. The application of relatively high amounts of K_2O could have attributed to this slight reduction. It is an area which could be studied further.

During year 4, the level of disease incidence overall was relatively low compared to the previous three years trials. This was a general phenomenon reported throughout the UK and Ireland with a number of influencing factors, such as prevailing environmental conditions and physiological status of the turfgrass swards following the summer season. A question could arise, whether or not to continue with Phi applications under these circumstances, or possibly use lower application rates or increased intervals of treatment timing. These are possibilities which require further study. However, the results of the effect Phi had on turf quality would indicate that Phi treatment should continue regardless of disease pressures as the benefits of improved sward quality would be significant. A factor which arose during these field trials was that there were significant interaction effects between turfgrass species, treatments and months for each of the four years. A significant interaction effect represents the combined effects of factors on the dependent variable, in this case disease incidence. The levels of disease incidence were significantly affected not only by treatments applied but by turfgrass species and the month of assessment. It would be expected that disease incidence would vary from month to month, so the interaction here is not unusual. The important factor however, is the significant interaction effect of turfgrass species. This in an important result as it confirms that turfgrass species will respond in different ways following Phi treatment in regard to disease levels.

3.5.3 Summary

What can be concluded from the results of these field trials is that routine and sequential applications of Phi will significantly ($p < 0.05$) reduce the incidence and severity of *M. nivale* in cool-season turfgrasses. The recommendation therefore, would be that a treatment programme of Phi, applied at 0.35g/m2 of PO_3^{3-}, should be implemented prior to occurrence of disease pressures and continued until environmental conditions preclude the chance of infection. Furthermore, the addition of Phi to standard turfgrass fungicides will significantly ($p < 0.05$) enhance the efficacy of these plant protectants in the suppression of *M. nivale.* Phi treatment will also give rise to significantly improved visual quality, uniformity and greater density when compared to untreated controls. Further work in this area would be beneficial, the persistence of Phi in treated turfgrass tissues is an important factor and is studied and assessed in a later chapter. Other areas of interest would be Phi treatment rates. These trials used a standard application rate of 0.35g/m^2 of PO_3^{3-} the question would lower levels of Phi still have significant effects on disease levels and turf quality could be studied. These results also are of particular significance to other Poaceae species, such as cereals, where *M. nivale* and *M. majus* are major pathogens. Would Phi treatment to cereal species give rise to reduced levels of disease and be beneficial in areas of increased outputs in these important commercial crops?

4 Take up of Phi in Turfgrass and its effects on growth

4.1 Introduction

Take up of Phi in a range of plant systems has been reported (Saindrenan *et al.*, 1985; Ouimette and Coffey, 1988; Roos *et al.*, 1999; Borza *et al.*, 2014), with studies concluding that not only is Phi rapidly taken up but is highly mobile within a plants vascular system. However, despite its widespread use in turfgrass management programs, there are no published data on the take up of Phi in turfgrasses. Most manufacturers of Phi products labeled for turfgrass use, state that it is rapidly absorbed and translocated through the vascular system, however there is no published research to substantiate these claims.

The primary use of foliar applied Phi, is as a means to reduce pathogen challenge, and there are much data to substantiate the efficacy of Phi in this regard (Reuveni *et al.*, 2003; Cook, 2009; Thao and Yamakawa, 2009; Silva *et al.*, 2011), there is however, an increasing use of Phi a source of P nutrition. Because of its insoluble mineral form, Pi in the soil, is largely unavailable to plants, leading to the requirement of Pi containing fertilisers (Raghothama and Karthikeyan, 2005). Phi has increasingly been used or recommended for use in many crop systems, including turfgrass. Despite some reports that Phi application led to enhanced growth responses (Lovatt, 1990a; Rickard, 2000; Vincelli and Dixon, 2005), the majority of studies have determined that Phi cannot be used directly as a nutrient source and therefore cannot complement or substitute Pi fertiliser at any rate (Thao and Yamakawa, 2009). Furthermore, the presence of Phi can inhibit Pi deficiency compensatory responses (Ticconi *et al.*, 2001). Enhanced root growth or an increase in root to shoot ratios are definitive responses to P limitation and these were strongly inhibited by Phi in *Brassica nigra* (Carswell *et al.*, 1996). Furthermore, Fabricio *et al.* (2012) concluded that foliar-applied Phi caused harmful effects to *Phaseolus vulgaris*, growing in P-limited soil.

4.2 Aims and Objectives

The aims of this section of the research were to determine the take up, translocation and fate of Phi, when applied as a foliar treatment to turfgrass, to assess the effects of long term sequential applications to turfgrass plants and the soil and to assess the role of Phi as a source of P nutrition.

The objectives were to;

- Describe the uptake, translocation, accumulation and fate of Phi in foliar treated turfgrass.
- Assess the value and effects of Phi as a source of P nutrition in turfgrass.
- Assess the effects long term sequential applications of Phi have on turfgrass plants and the soil.

4.3 Materials and methods

Three distinct experiments were carried out. Experiment 1 assessed the take up of Phi in turfgrass during two growth periods following a single treatment. Experiment 2 assessed the take up of Phi in turfgrass following sequential treatments and experiment 3 assessed the effects of Phi on turfgrass growth.

4.3.1 Establishment and maintenance of turfgrasses

Three turfgrass species, *Agrostis stolonifera* L, variety Shark, *Lolium perenne* L. variety Bargold and *Poa annua reptans* L, variety Truputt, established and maintained in greenhouses were used for this study. All samples were sown in growth vessels, filled with rootzone complying with Sports Turf Research Institute (STRI) recommendations for golf green construction in the UK (Baker, 2005). Turfgrass rootzones can be defined as the combination of substrates of gravel, sand, silt, clay, which form a matrix in which the turfgrass is established and in which it obtains its required water and nutrients for growth and development. The growth vessels were maintained in greenhouses, in Kildare, Ireland, under natural light and temperature conditions during the trial periods from January 2011 to September 2014. Two types of growth vessels were used, Fig. 4-1. The first were 110 mm diameter poly-vinyl chloride (PVC) pipes cut to 300 mm lengths, to replicate the depth of a golf green rootzone, thus allowing development of root structures similar to golf greens, and for easy manipulation of the samples during the trial period. The second were established in 400 mm by 300 mm by 120 mm growth trays. All were seeded at the optimum rate for the particular species (Butler et al., 2007; Turgeon, 2005) and allowed to establish before commencement of experimental procedures.

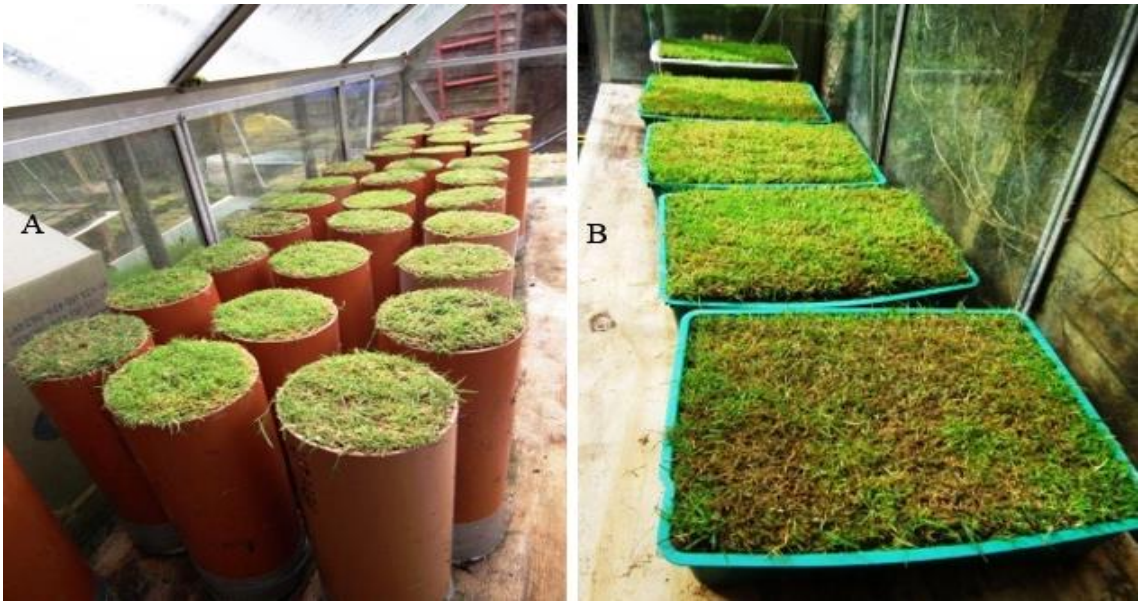


Figure 4-1 Greenhouse turfgrass samples. Growth vessels used for the range of studies, displaying the two types of vessels used. A: 110 x 300 mm tubes, B 400 x 300 x 120 mm growth trays.

4.3.2 Nutritional and irrigation inputs

Soil properties prior to seeding are shown in Table 4-1, analyses carried out by Lancrop Laboratories, York, using analytical methodology as described in brief in Table 4-2. Turfgrass growth was maintained through the trial period with regular inputs of soluble Urea, giving annual nutritional inputs (ANI) of 60 kg N ha^{-1} all other nutritional inputs were supplied as part of treatment applications. Minimal irrigation inputs were applied via a hand hose to replace water lost through evapotranspiration.

Table 4-1 Soil nutrient levels, organic matter content and Cation Exchange Capacity (C.E.C.) prior to seeding of *A. stolonifera*, *L. perenne* and *P. annua*

Organic matter %	N ppm	P ppm	K ppm	Mg ppm	Fe ppm	Ca ppm	S ppm	Zn ppm	Mn ppm	Cu ppm	B ppm	C.E.C
1.2	7.1	37	93	47	280	1548	31	1.1	6.7	1.0	0.9	6.7

Table 4-2 Description of analytical methods used to determine rootzone properties and nutrient levels prior to turfgrass establishment.

Element	Units	Digestion Extractant	Analytical Technique
Organic matter	%	Water	Weight Loss Determination
Nitrogen	ppm	Sulphuric/orthophosphoric acid digestion	Kjeldhal distillation CNS analyser
Phosphorus	ppm	Mehlick 3 solution	Solution spectrophotometry
Potassium	ppm	1M Ammonium acetate @ pH 7.0	Atomic absorption spectrometer
Magnesium	ppm	1M Ammonium acetate @ pH 7.0	Inductively coupled plasma atomic emission spectrometer (ICP-AES)
Iron	ppm	0.005 M EDTA disodium salt	ICP-AES
Calcium	ppm	1M Ammonium acetate @ pH 7.0	ICP-AES
Sulphur	ppm	Monocalcium Phosphate solution	Flow Injection analyser
Zinc	ppm	0.005 M EDTA disodium salt	ICP-AES
Manganese	ppm	1 M Ammonium acetate	ICP-AES
Copper	ppm	0.005 M EDTA disodium salt	ICP-AES
Boron	ppm	Hot water (80^{o}C)	ICP-AES
Cation Exchange	meq/100g	1 M ammonium acetate	Summation of extracted cations (K, Mg, Ca, Na, H)

4.3.3 Foliar treatments

Foliar treatments of Phi (KH_2PO_3), Pi (KH_2PO_4) and KCL (as control) were applied sequentially, at rates and timings as required by the research protocols, using 5 l pressure sprayers, operating at 3 bar, fitted with Hollow Cone (HCX) 80° nozzles delivering a fine spray calibrated to deliver 160 l/ha^{-2}. Phi and Pi treatments were prepared by titrating 1 M solution phosphorous and phosphoric acids with 6 M reagent-grade potassium hydroxide (KOH) to pH 6.5. KCl treatments were prepared from commercially available potassium chloride. All treatments were diluted to required concentrations, chemicals supplied by Lennox Laboratory Supplies, Dublin.

4.3.4 Tissue collection

Leaf tissues were collected using a scissors, crowns were harvested by removing the leaf tissues, then slicing the crowns away from the roots using a knife. Roots were collected by placing the rootzone into a 2 mm sieve and washing until all soil was removed, Fig.4-2. All tissues were washed and rinsed in SDW, then dried at 60^0 C for 48 hours prior to any analyses.

Figure 4-2 Collection of turfgrass tissues. Method used to collect turfgrass tissues for analyses. A: separation of crown and shots from roots, B: Root biomass prior to washing to remove rootzone material.

4.3.5 Take up and accumulation of Phi in turfgrass

4.3.5.1 Determination of tissue Phi and Pi content

Determination of Phi and Pi tissue content was carried out using High Performance Ion Chromatography (HPIC), using a modified version of a technique published by Roos *et al*. (1999), all analyses were carried out by OEW Laboratories, Cornwall.

4.3.5.1.1 HPIC method

The ion chromatograph consists of a Dionex ICS100 ion chromatograph equipped with an IonPac AG9-HC Guard Tube (4 x 50mm), IonPac AS9-HC Analytical Column (unheated 4 x 250mm), ASRS300 Suppressor (4mm), DS6 Heated Conductivity Cell, 25 ul injection loop. The eluent was 9 mM sodium carbonate (99.999%), degassed and pressurised to 1 bar, flowing at 1 ml/minute (approximately 2200 psi) with a single back pressure loop. Method run time was set to 18 minutes.

4.3.5.1.2 Standards

Prior to tissue analyses, tests were carried out to establish standards. The Pi standard (as PO_4 w/v) was prepared from sodium Pi monobasic anhydrous (H_2NaO_4P) and >18.2 Mohm deionised water, Phi standard (as PO_3 w/v) was prepared from sodium Phi dibasic pentahydrate (Na_2 (PHO_3).$5H_20$. Standard mixed solutions of PO_3^{3-} and PO_3^{4-} were prepared at 12.5, 25, 50, 100, 200, 500 and 1000 ppm w/v. Pi only solutions showed no evidence of Phi ions. Phi only solutions showed no evidence of Pi ions. Pi and Phi ions in 9 mM sodium carbonate eluent showed no evidence of migration over a period of two weeks.

4.3.5.1.3 Tissue analyses

The ion chromatograph was calibrated by 12.5, 25. 25, 50, 100, 200, 500 and 1000 ppm mixed Pi/Phi standards. The calibration curve was not linear over this calibration range, as a cubic curve was found to give a better fit. Samples of 0.5g of finely ground turfgrass leaf, root and crowns were weighed into 15 ml polypropylene centrifuge tubes and agitated for 2 minutes with 10.0 ml of SDW. The mixture was allowed to extract overnight at ambient temperature. The samples were agitated again for 2 minutes prior to analysis. Samples were analysed within 24 hours of extraction to avoid microbial growth. Samples were taken up in 2 ml disposable syringes from the centrifuge tubes and manually injected into the ion chromatograph, through 0.47 micron syringe

filters, into the sample loop of the Dionex HPIC system, using a 9 mM sodium carbonate eluent. The solutions did not require any additional dilution. Results were adjusted for the weights of extracted samples and reported as ppm of dried tissue weights.

4.3.5.2 Experiment 1: take up and accumulation of Phi following a single application

To assess the take up and translocation Phi in turfgrass, following a single treatment, during two contrasting growth periods, Phi was applied as a foliar treatment, as described in 4.3.3, to *A. stolonifera* and *P. annua* in February 2011 and July 2012, at a rate of 0.35 g PO_3^{3-}/m^{-2}.

4.3.5.2.1 Tissue collection and analysis

Harvesting of the leaf and root tissues was carried out at: 1, 6, 12, 24, 48 hours and 1, 2, 3, 4, and 6 weeks post application (p.a.) and Phi tissue accumulations determined.

4.3.5.3 Experiment 2: take up and accumulation Phi following long term sequential applications

To assess the effect on turfgrass tissues and rootzones of long term sequential Phi applications, foliar treatments were applied as described in 4.3.3, to *A. stolonifera* and *P. annua* sequentially, at monthly intervals, from July 2012 to July 2014. Treatments comprised of Phi and Pi applied at 0.35 g/m^2 PO_3^{3-} and PO_4^{3-} respectively. Pi was applied to assess the effect on long term soil P status compared to the Phi treatment.

4.3.5.3.1 Tissue collection and analysis

Leaf and root samples were collected at 6, 12, and 24 month intervals, from the commencement of the trial period (July 2012). Tissues were collected as in 4.3.4, immediately prior to treatment application and one week post treatment and analysed for Phi content using HPIC.

4.3.5.3.2 Soil nutrient determination

Rootzone samples were collected prior to the start of treatments and at the end of the 24 month trial period and analysed for treatment effect on nutrient content, using methodology as in Table 4-2.

4.3.6 Experiment 3, Phi as a source of P nutrition

4.3.6.1 Treatments

To assess the properties of Phi as a source of P nutrition for turfgrass growing in different soil P conditions and to determine its effect on turfgrass development, foliar treatments

were applied as described in 4.3.3, to *L. perenn*e and *P. annua*, sequentially, at two week intervals, over a six month period. These species were chosen as both have greater growth rates than *A. stolonifera,* therefore any P deficiencies would be attenuated. Two soil P levels were used, (Pi-deficient and Pi-sufficient), P deficient corresponded to 5 ppm and P sufficient 38 ppm, respectively, as determined by the Mehlick 3 method (Mehlich, 1984). Treatments comprised of Phi and Pi applied at 0.35 g/m^{-1} PO_3^{3-} and PO_4^{3-} and KCl which acted as control. Treatments were applied from March to September 2013, 13 applications in total.

4.3.6.1.1 Shoot, crown and root growth

Treatment effect on shoot growth was determined by the cumulative dry weights of clippings, collected weekly after cutting at 5 mm. Crown and roots were collected at the end of the trial, as in 4.3.4 and weighed for dry mass determination and calculation of root to shoot ratios.

4.3.6.1.2 Root to shoot ratios

Root: shoot ratios were calculated by dividing the mean dry root weights by the mean dry shoot weights.

4.3.6.1.3 Phosphorus determinations

Shoot, crown and root dry mass were analysed for P content as in Table 4-2.

4.3.7 Data analysis

Descriptive statistics were calculated and all data presented as means $\pm$ 95% confidence intervals. All treatments, unless otherwise stated, were randomised with six replications. Prior to any analyses, residuals were tested to ensure the assumptions of the two-way and one-way Anova were satisfied. Outliers were assessed by inspection of a boxplots, Shapiro-Wilk's test determined normality (Shapiro and Wilke, 1965 and homogeneity of variances assessed by Levene's test (Levene, 1960. Where significant interactions or effects were observed, Tukey HSD post hoc analyses at $p < 0.05$, separated significant differences. Phi accumulations, long term sequential Phi treatments were analysed using two-way Anova to determine statistical differences and interactions, with dependent variable of Phi accumulation in turfgrass tissues and independent variables of turfgrass species, plant tissues and month of data collection. Differences in Pi tissue accumulations and rootzones following Phi treatments were carried out using Paired-samples t-test at $p < 0.05$.

Treatment effect on leaf, crown and root development, root to shoot ratios and tissue P levels in *L. perenne* and *P. annua* growing in P sufficient and P deficient rootzones were carried out using two-way Anova to determine statistical differences and interactions, with dependent variables of tissue dry weight (growth) and independent variables of turfgrass species, plant tissues and treatments. Where significant interactions were observed, one-way Anova were used and Tukey HSD post hoc analyses at $p < 0.05$, separated significant differences.

All data analysis was performed using the statistical programme SPSS Statistics 21. Additional statistical data tables are available in the document 'Appendices to the Thesis'

4.4 Results

4.4.1 Experiment 1, take up and accumulation of Phi following a single application

Foliar Phi treatments were applied to greenhouse grown specimens of *A. stolonifera* and *P. annua*, in February 2011 and July 2012 and HPIC analyses determined Phi amounts in leaf and root tissues. Greenhouse air temperatures for both trial periods are shown in Table 4-3, mean daily levels during the February trial were 7.6 º C, while in July the mean value was 22.3 º C.

Table 4-3 Weekly temperature ranges in ^{0}C in research greenhouse during the trial periods commencing February 2011 and July 2012.

Greenhouse temperatures weekly mean values ^{0}C

Trial commencing Feb-11		
Weekending	Maximum	Minimum
08 February 2011	7.75	2.35
15 February 2011	8.75	2.75
22 February 2011	7.35	3.14
1 March 2011	11.35	5.14
08 March 2011	16.55	4.25
15 March 2011	19.28	1.26
Trial commencing Jul-12		
Weekending	Maximum	Minimum
8 July 2012	28.45	13.85
15 July 2012	34.28	11.75
22 July 2012	29.74	14.96
29 July 2012	26.25	13.45
5 August 2012	32.55	14.95
12 August 2012	33.25	14.25

4.4.1.1 Take up and accumulation of Phi in *A. stolonifera* and *P. annua,* 0 to 96 hours post treatment application in February 2011

Results from the study carried out in February 2011, show that in leaf tissues of both *A. stolonifera* and *P. annua,* Phi was rapidly accumulated, reaching a peak level at 48 h p.a. with a figure of 4886, 95% CI (4875, 4897) ppm in *A. stolonifera* and 5071, 95% CI (5060, 5082) ppm in *P. annua,* Figs 4-3 and 4-4.

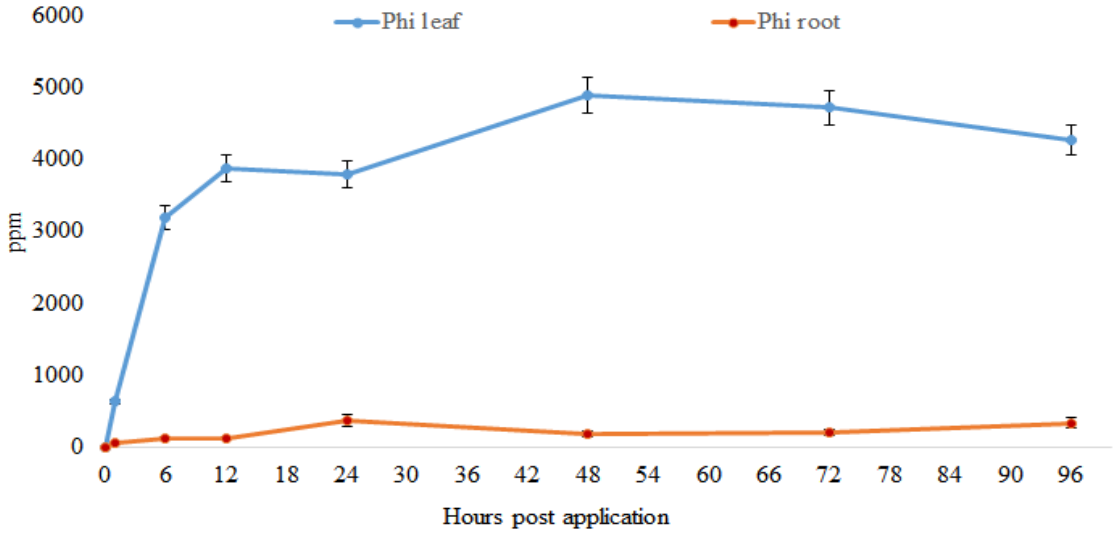


Figure 4-3 Accumulation of Phi in *A. stolonifera* leaf and root tissues, 96 hours p.a. in February 2011. Take up and accumulation of Phi in *A. stolonifera* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

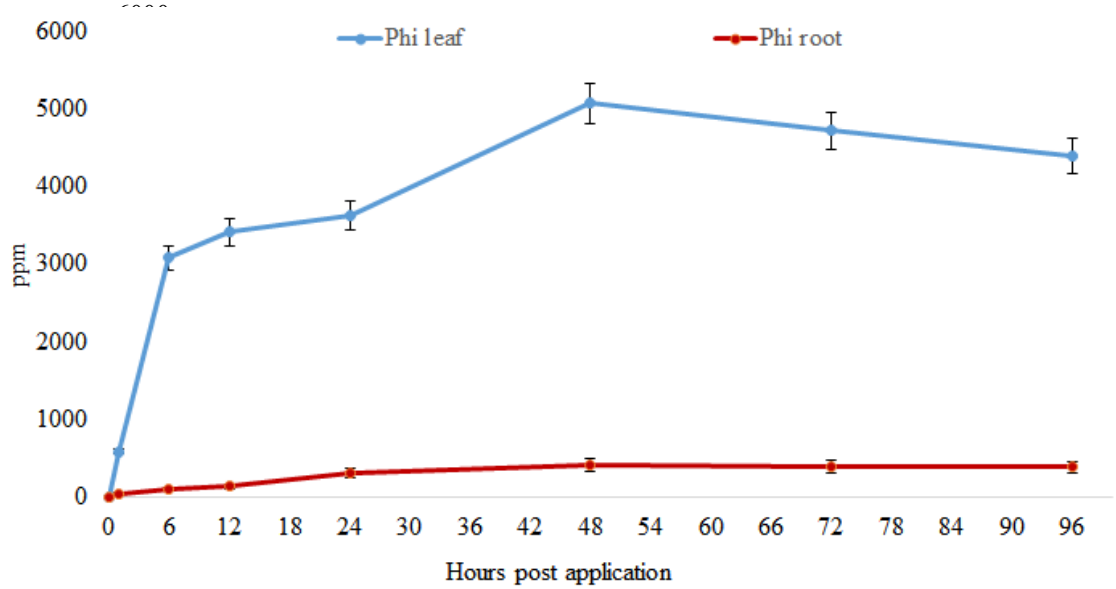


Figure 4-4 Accumulation of Phi in *P. annua* leaf and root tissues, 96 hours p.a. in February 2011. Take up and accumulation of Phi in *P. annua* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

There was rapid take up of Phi in leaf tissues, with accumulations in *A. stolonifera* of 639, 95% CI (628, 650) and 3191, 95% CI (3180, 3202) ppm, 1 and 6 h p.a. Accumulations in *P. annua* leaf tissues were similar, with figures of 585, 95% CI (574, 595) ppm and 3085, 95% CI (3074, 3095) ppm at 1 and 6 h p.a. Following the peak accumulations of 4886, 95% CI (4875, 4897 and 5071, 95% CI (5060, 5082) ppm 48 h p.a. in *A. stolonifera* and *P.annua* respectively, Phi amounts in the leaf tissues began to decrease, with levels at 96 h p.a. of 4270, 95% CI (4259, 4281) ppm and 4395, 95% CI (4385, 4406) in *A. stolonifera* and *P. annua* respectively.

In both turfgrass species, following foliar treatment with Phi, root accumulations were considerably less than in the leaf tissues. With *A. stolonifera,* accumulations were 55, 95% CI (48, 61) ppm at 1 h p.a. and 117, 95% CI (110, 123) ppm at 6 h p.a., peaking at 24 h p.a. with a level of 373, 95% CI (367, 380) ppm. Unlike Phi accumulations in leaf tissues, levels in roots remained relatively constant, with a figure of 337, 95% CI (331, 343) ppm at 96 hours p.a.

The amounts of Phi accumulations in *P. annua* roots at 1 and 6 h p.a were less than in *A. stolonifera* at the same time periods, with accumulations of 45, 95% CI (37, 53) ppm, at 1 h p.a. and 96, 95% CI (88, 104) ppm at 6 h p a. Phi amounts also peaked later than in *A. stolonifera*, 419, 95% CI (411, 427) ppm at 48 h p.a. As with the *A. stolonifera* root accumulations, Phi levels in roots in *P. annua*, remained relatively constant, with a figure of 385, 95% CI (377, 392) ppm at 96 hours p.a.

4.4.1.2 Take up and accumulation of Phi in *A. stolonifera* and *P. annua,* 0 to 6 weeks post treatment application in February 2011

Phi accumulations in February 2011, over the six week period post application, are shown in Figs. 4-5 and 4-6. Phi was rapidly accumulated, reaching a peak level in leaf tissues in both species at 1 week p.a., with a figure of 3332, 95% CI (3323, 3341) ppm in *A. stolonifera* and 4534, 95% CI (4523, 4545) ppm in *P. annua.*

At four weeks p.a. accumulations in *A. stolonifera* leaf were at 1686, 95% CI (1677, 1696) ppm, approximately 50% of the maximum, with levels decreasing steadily to 496, 95% CI (487, 505) ppm at 6 weeks p.a.

In *P. annua*, at four weeks p.a. accumulations were 1290, 95% CI (1279, 1301) ppm, 28% of the maximum levels at 1 week p.a. Phi levels, as in the *A. stolonifera* then decreased steadily to 862, 95% CI (851, 873) ppm at 6 weeks p.a.

In both turfgrass species, root accumulations were considerably less than in the leaf tissues. With *A. stolonifera* Phi accumulations were highest at two weeks p.a, with a level of 479, 95% CI (469, 489) ppm, with amounts declining over the following four weeks to 81, 95% CI (71, 90) ppm, at six weeks p.a.

In *P. annua* roots, accumulations rates were similar to the *A. stolonifera,* although Phi amounts peaked earlier at one week p.a. at 376, 95% CI (366, 386) ppm. As with *A. stolonifera*, Phi amounts declined over the following weeks, however the 163, 95% CI (153, 173) ppm, at six weeks p.a. was double the Phi levels in the *A. stolonifera* roots at six weeks p.a.

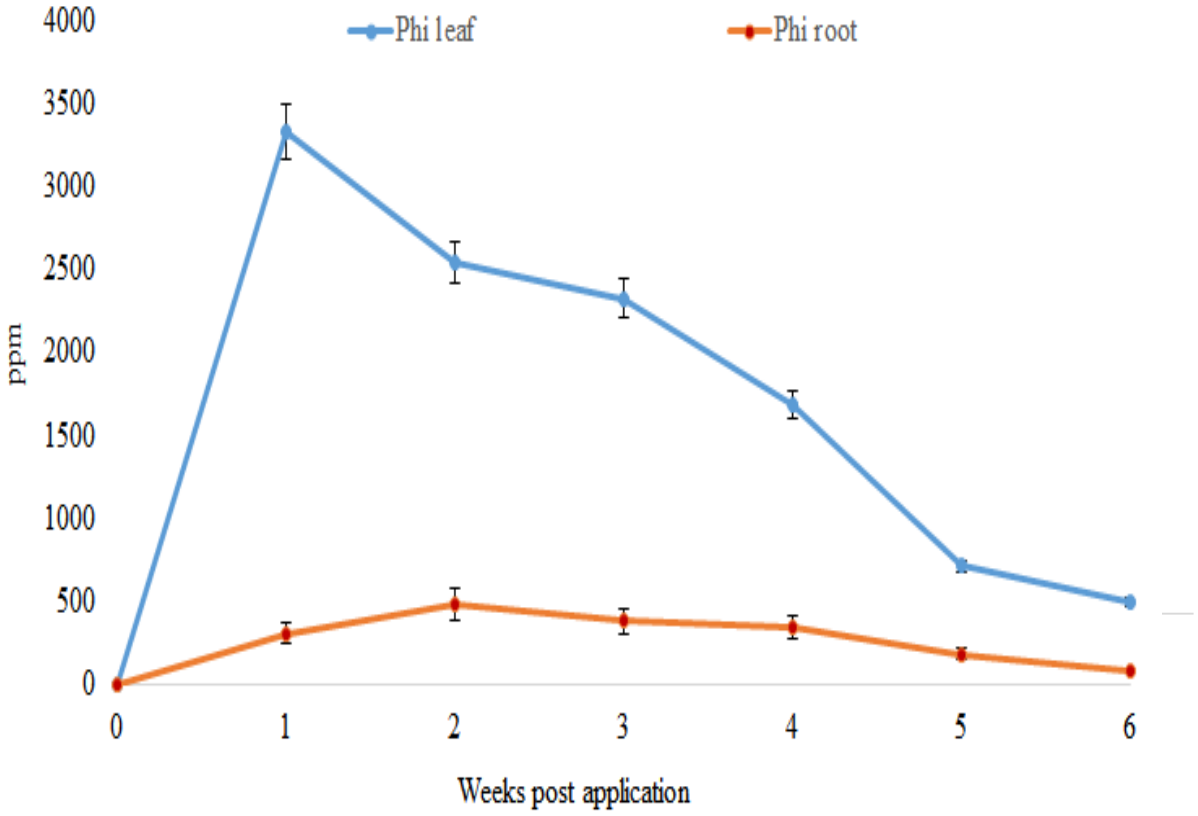


Figure 4-5 Accumulation of Phi in *A. stolonifera* leaf and root tissues, 6 weeks p.a. in February 2011. Take up and accumulation of Phi in *A.stolonifera* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

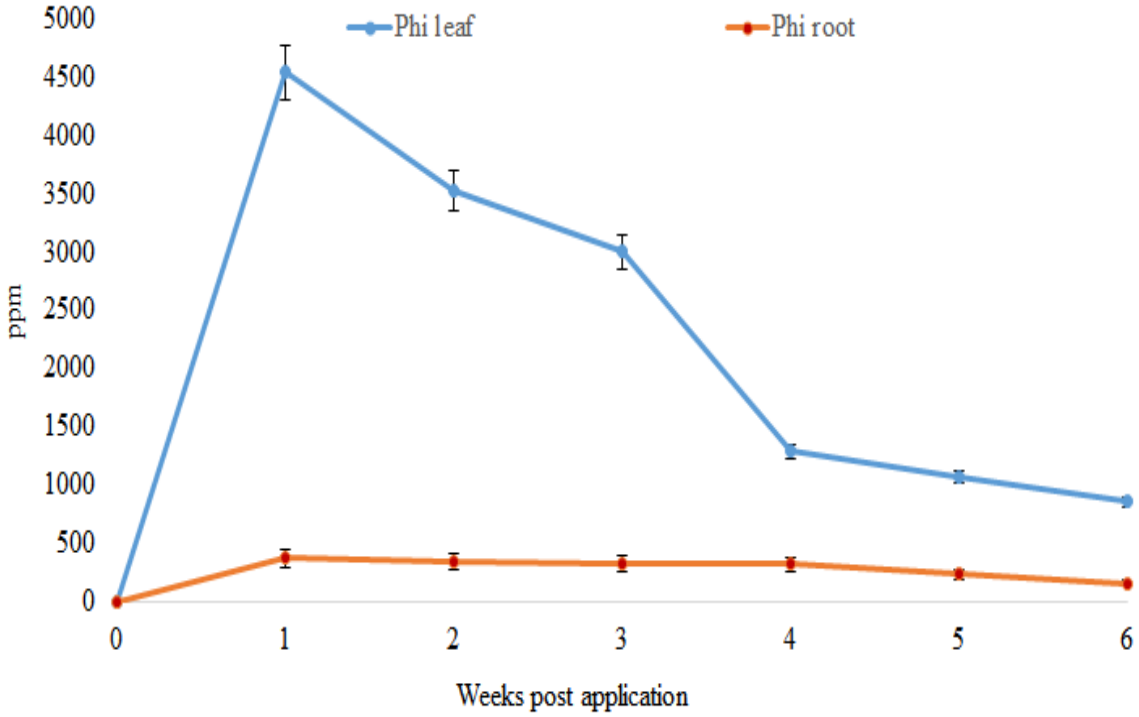


Figure 4-6 **Accumulation of Phi in *P. annua* leaf and root tissues, 6 weeks p.a. in February 2011.** Take up and accumulation of Phi in *P. annua* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

4.4.1.3 Take up and accumulation of Phi in *A. stolonifera* and *P. annua,* 0 to 96 hours post treatment application in July 2012

Results from the July 2012 study showed a similar pattern in Phi take up as that in the February 2011 study. However, as can be seen in Fig. 4-7, the higher growth rate of the turfgrasses, gave rise to an increased take up rate, compared to the February study. Phi was rapidly accumulated, reaching a peak level at 48 h p.a. with a figure of 5520, 95% CI (5509, 5531) ppm in *A. stolonifera* and 5418, 95% CI (5410, 5427) ppm in *P. annua,* Figs 4-7 and 4-8.

There was rapid take up of Phi in leaf tissues, with accumulations in *A. stolonifera* of 849, 95% CI (837, 860) and 3265, 95% CI (3254, 3276) ppm, 1 and 6 h p.a. Accumulations in *P. annua* leaf tissues were similar, with figures of 835, 95% CI (826, 843) ppm and 3194, 95% CI (3185, 3202) ppm at 1 and 6 h p.a. Following the peak accumulations at 48 h p.a. Phi amounts in the leaf tissues of both *A. stolonifera* and *P.annua*, began to decrease, with levels at 96 h p.a. of 4314, 95% CI (4302, 4325) ppm and 4452, 95% CI (4443, 4460) in *A. stolonifera* and *P. annua* respectively.

As in the February 2011 study, following foliar treatment with Phi, root accumulations were considerably less than in the leaf tissues. With *A. stolonifera,* accumulations were 53, 95% CI (45, 61) ppm at 1 h p.a. and 108, 95% CI (100, 116) ppm at 6 h p.a. Levels remained constant with the highest amount recorded at 96 h p.a. with a level of 441, 95% CI (433, 450) ppm.

The amounts of Phi accumulations in *P. annua* roots at 1 and 6 h p.a were 44, 95% CI (34, 54) ppm, at 1 h p.a. and 101, 95% CI (91, 111) ppm at 6 h p a. Phi amounts peaked earlier than in *A. stolonifera*, at 24 h p.a. with a level of 336, 95% CI (326, 346) ppm. From 48 to 96 h p.a. Phi amounts remained constant with a level of 328, 95% CI (318, 337) ppm at 96 hours p.a.

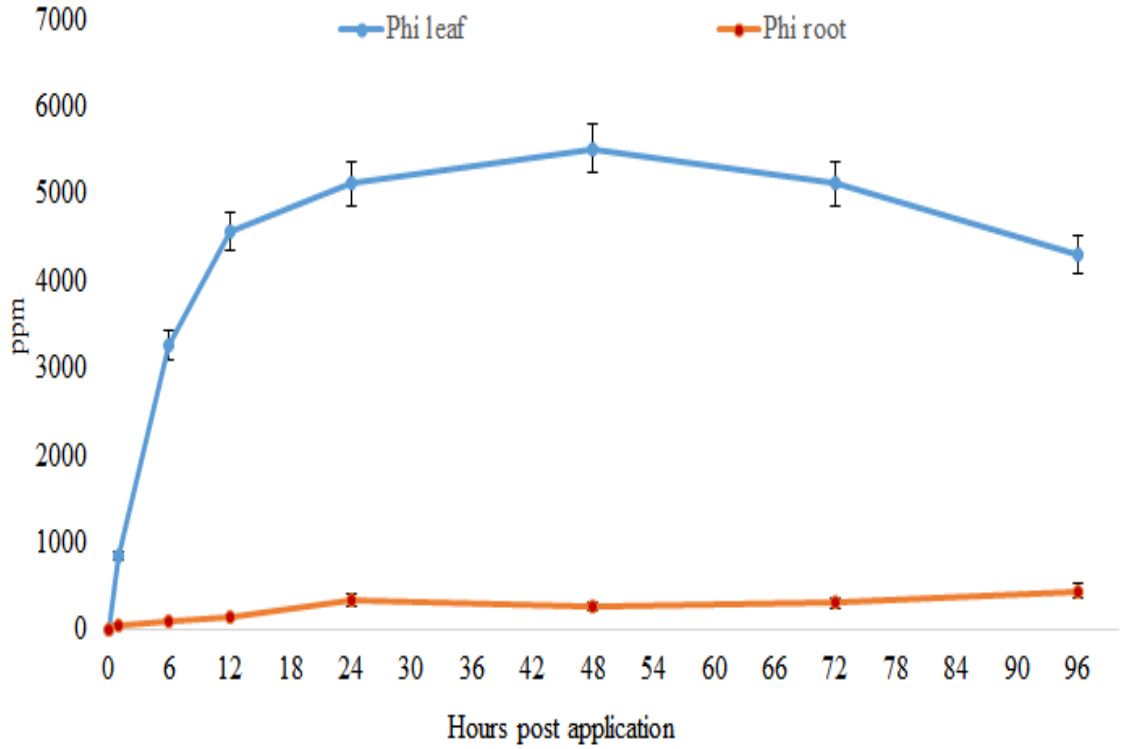


Figure 4-7 Accumulation of Phi in *A. stolonifera* leaf and root tissues, 96 hours p.a. in July 2012. Take up and accumulation of Phi in *A. stolonifera* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in July 2012. Bars indicate 95% confidence intervals, n=6.

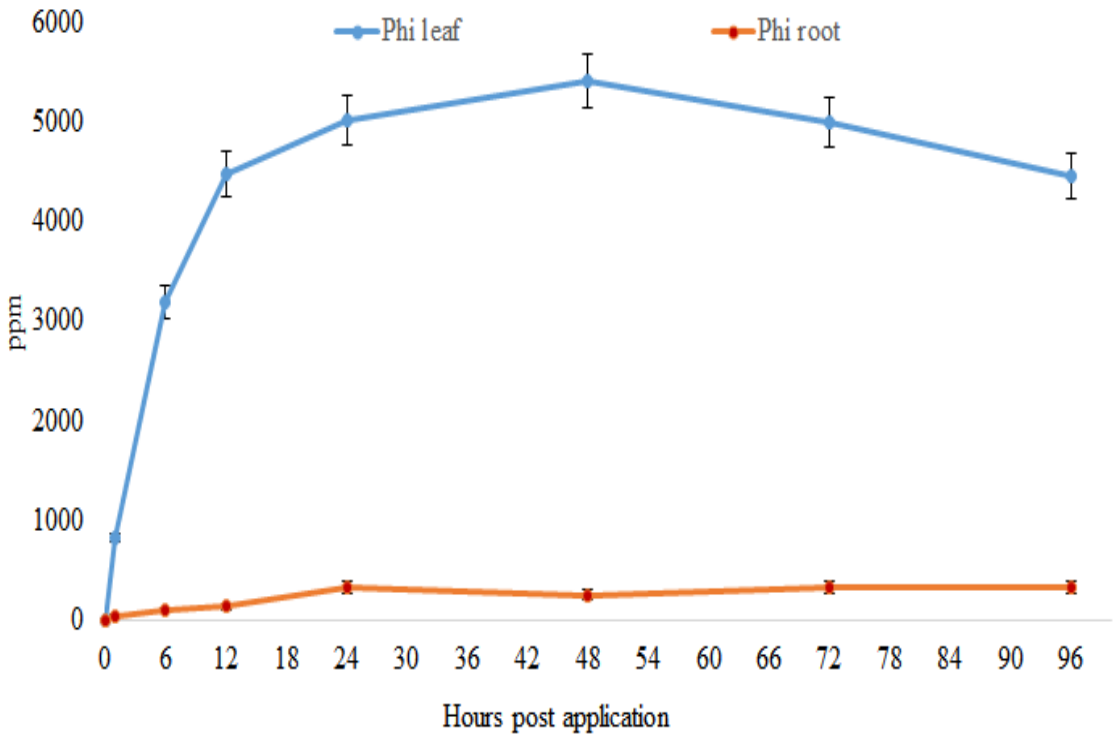


Figure 4-8 Accumulation of Phi in *P.annua* leaf and root tissues, 96 hours p.a. in July 2012. Take up and accumulation of Phi in *P. annua* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in July 2012. Bars indicate 95% confidence intervals, n=6.

4.4.1.4 Take up and accumulation of Phi in *A. stolonifera* and *P. annua,* 0 to 6 weeks post treatment application in July 2012

Phi accumulations in July 2012, over the six week period post application, are shown in Figs. 4-9 and 4-10. Phi was rapidly accumulated, reaching a peak level in the leaves of both species at 1 week p.a., with a figure of 3451, 95% CI (3442, 3460) ppm in *A. stolonifera* and 3387, 95% CI (3378, 3396) ppm in *P. annua.*

Phi accumulations in *A. stolonifera* leaf decreased to 1425, 95% CI (1416, 1434) ppm, at three weeks p.a., less than 50% of the maximum amounts recorded at one week p.a.

Phi levels decreased then steadily to 261, 95% CI (252, 270) ppm at 6 weeks p.a.

In *P. annua*, following the peak accumulation at one week p.a. amounts decreased to 1396, 95% CI (1387, 1405) ppm at three weeks p.a., less than 50% of the maximum amounts recorded at one week p.a. Phi amounts then decreased to 218, 95% CI (209, 226) ppm at 6 weeks p.a.

Root accumulations in *A. stolonifera* were highest at two weeks p.a, with a level of 463, 95% CI (456, 470) ppm, with amounts declining over the following four weeks to 256, 95% CI (249, 263) ppm, at six weeks p.a.

In *P. annua* roots, accumulations rates were similar to the *A. stolonifera,* although Phi amounts peaked later at three week p.a. at 457, 95% CI (447, 467) ppm. As with *A. stolonifera*, Phi amounts declined over the following weeks, to 313, 95% CI (303, 323) ppm, at six weeks p.a.

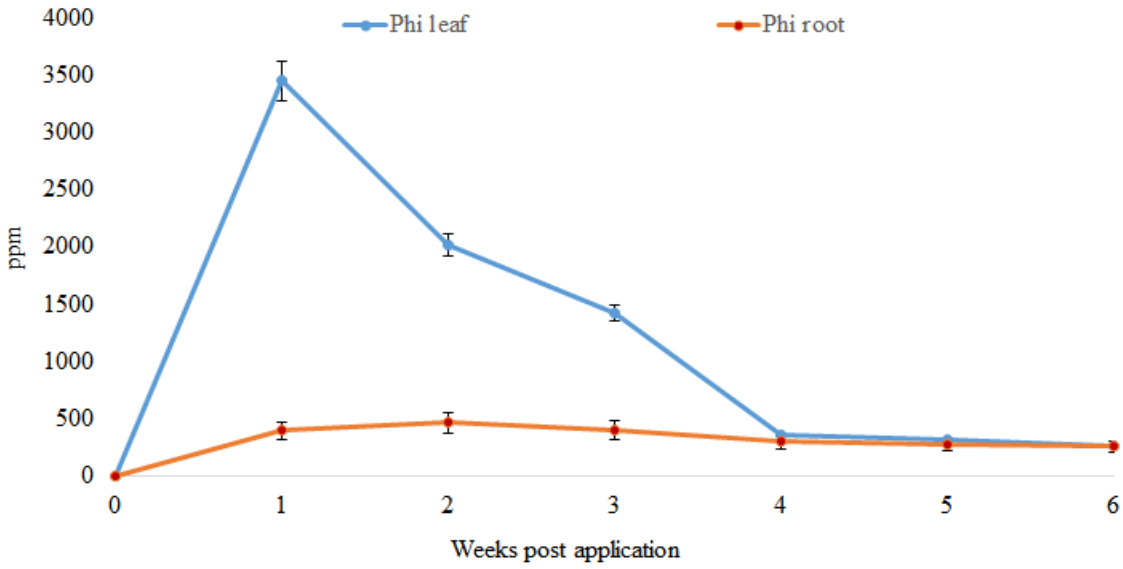


Figure 4-9 Accumulation of Phi in *A. stolonifera* leaf and root tissues, 6 weeks p.a. in July 2012. Take up and accumulation of Phi in *P. annua* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

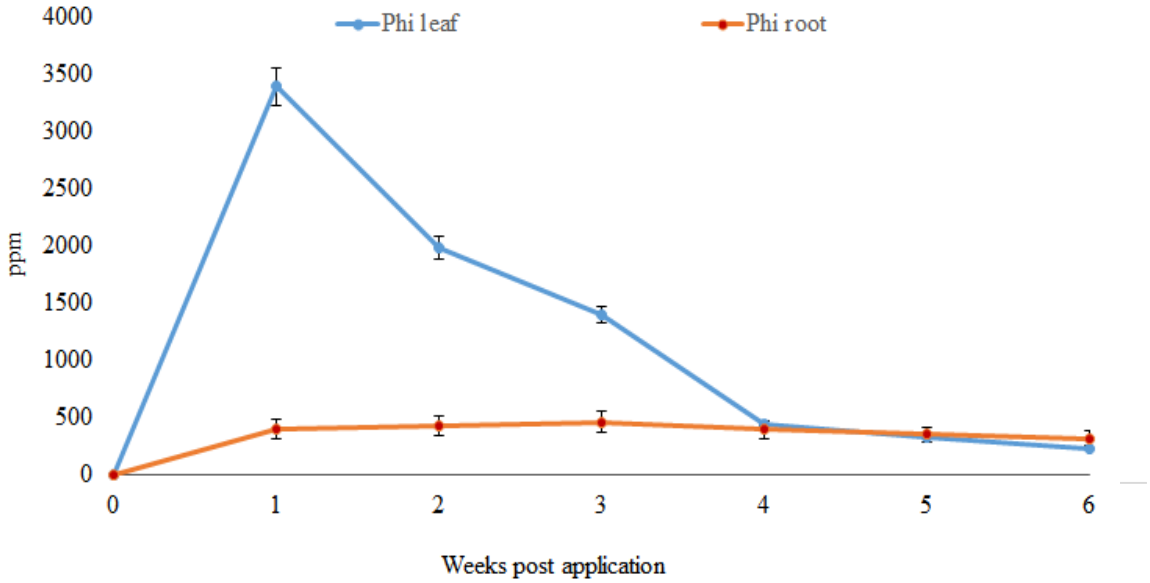


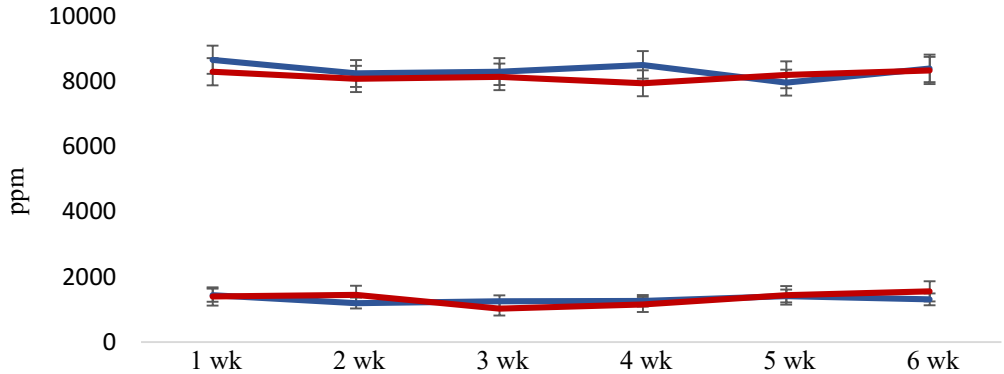
Figure 4-10 Accumulation of Phi in *P. annua* leaf and root tissues, 6 weeks p.a. in July 2012. Take up and accumulation of Phi in *P. annua* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

4.4.1.5 PO_4^{3-} determinations

Determination of PO_4^{3-} levels was an important part of this study, as the question of *in planta* conversion of PO_3^{3-} to PO_4^{3-} needed to be answered. Pi levels in leaf and root tissues were determined as part of the HPIC analyses.

In *A. stolonifera*, as can be seen in Fig. 4-11, Pi amounts in the leaf at the start of each of the studies were 8656, 95% CI (8644, 8673) ppm in February and 8287, 95% CI (8276, 8299) ppm in July, both of which are within the standard recommended levels for cool season turfgrasses. In the February 2011 study, Pi levels decreased over the six week trial period, with a reading in leaf tissues of 8390, 95% CI (8378, 8404) ppm at the conclusion, significantly ($t(5) = 39.406$, $p < 0.05$), less than the Pi amount at the beginning, indicating no *in planta* conversion of Phi to Pi. Pi levels in the root tissues followed a similar trend, with amounts at the start of the study of 1436, 95% CI (1423, 1444) ppm, decreasing significantly ($t(5) = 16.509$, $p < 0.05$), to 1314, 95% CI (1293, 1329) ppm at the conclusion.

In the July 2012 study, Pi levels in leaf tissues at the start were 8287, 95% CI (8271, 8303) ppm, levels increased to 8327, 95% CI (8310, 8351) ppm at the conclusion, but not significantly ($t(5) = 1.043$, $p = 0.345$). Pi levels in the root tissues increased significantly ($t(5) = 38.394$, $p < 0.05$) over the six week period, from 1397, 95% CI (1378, 1412) ppm at the start to 1558, 95% CI (1534, 1582) ppm at the conclusion.



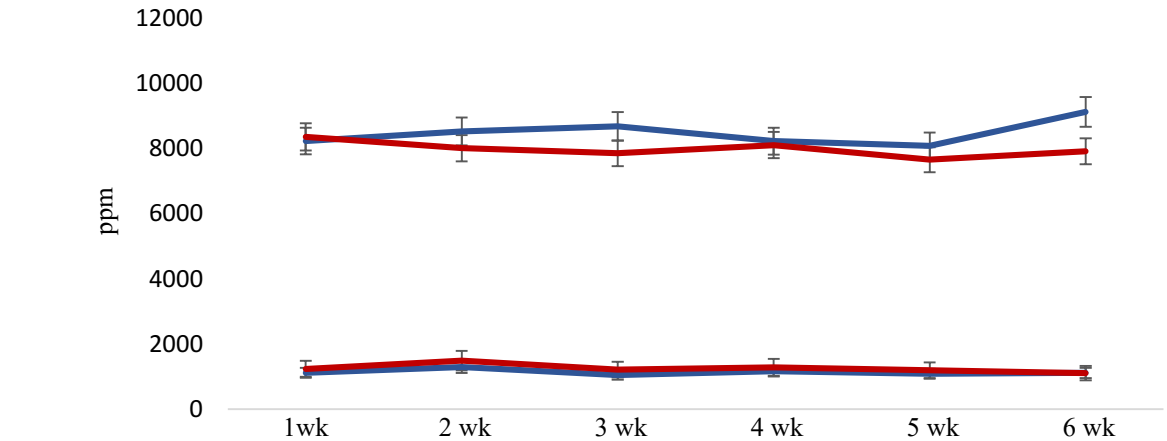
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
Leaf February	8656	8235	8293	8499	7955	8390
Root February	1436	1197	1258	1264	1412	1314
Leaf July	8287	8071	8128	7933	8193	8327
Root July	1397	1445	1025	1156	1436	1558

Figure 4-11 Pi amounts in leaf and root tissues of *A. stolonifera*. Pi amounts in leaf and root tissues of *A. stolonifera*, six weeks post treatment with Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011 and July 2012. Bars indicate 95% confidence intervals, n=6.

In *P. annua* the data were similar, Pi amounts in the leaf at the start of each of the studies were 8234, 95% CI (8222, 8245) ppm in February and 8361, 95% CI (8351, 8372) ppm in July, Fig 4-12.

In the February 2011 study, Pi levels increased over the six week trial period, with a reading in leaf tissues of 9127, 95% CI (9117, 9137) ppm at the conclusion, significantly ($t(5) = 86.254$, $p < 0.05$), greater than the Pi amount at the beginning. Pi levels in the root tissues did not vary significantly ($t(5) = 0.430$, $p = 0.685$), with amounts at the start of the study of 1113 , 95% CI (1094, 1126) ppm, and 1110, 95% CI (1090, 1122) ppm at the conclusion.

In the July 2012 study, Pi levels in the leaf, decreased significantly ($t(5) = 71.412$, $p < 0.05$), over the six week trial period, from 8361, 95% o9, 8374) ppm at the start to 7917, 95% CI (1090, 1122) ppm at the conclusion. Pi levels in the root tissues also decreased significantly ($t(5) = 18.314$, $p < 0.05$), with amounts at the start of the study of 1235, 95% CI (1224,1246) ppm, and 1104, 95% CI (1091, 1115) ppm at the conclusion.



	1wk	2 wk	3 wk	4 wk	5 wk	6 wk
Leaf February	8234	8529	8685	8230	8085	9127
Root February	1113	1289	1045	1158	1083	1110
Leaf July	8361	8014	7855	8110	7662	7917
Root July	1235	1485	1212	1280	1194	1104

Figure 4-12 Pi amounts in leaf and root tissues of *P. annua*. Pi amounts in leaf and root tissues of *P. annua*, six weeks post treatment with Phi at a rate of 0.35 g PO_3^{3-}/ m^{-2}, in February 2011 and July 2012. Bars indicate 95% confidence intervals, n=6.

4.4.2 Experiment 2, take up and accumulation of Phi in *A. stolonifera* and *P. annua* following sequential applications over two years.

Foliar Phi treatments were applied to *A. stolonifera* and *P. annua* sequentially, at monthly intervals, from July 2012 to July 2014, with Phi amounts determined in leaf and root tissues. Monthly temperature ranges are shown in Table 4-4.

Table 4-4 Mean monthly maximum and minimum temperatures in ^{0}C in research greenhouse during the trial period from July 2012 to July 2014.

Greenhouse temperatures monthly mean values ^{0}C		
Month	Maximum	Minimum
Jul-12	28.45	13.85
Aug-12	31.28	11.75
Sep-12	32.74	14.96
Oct-12	26.25	13.45
Nov-12	19.45	10.95
Dec-12	12.74	8.25
Jan-13	6.55	1.05
Feb-13	7.35	1.55
Mar-13	9.75	3.53
Apr-13	13.45	10.65
May-13	16.34	11.55
Jun-13	21.35	14.25
Jul-13	27.85	15.45
Aug-13	21.24	16.75
Sep-13	32.55	17.18
Oct-13	29.80	16.18
Nov-13	18.65	11.24
Dec-13	11.45	7.95
Jan-14	7.90	3.20
Feb-14	8.65	4.45
Mar-14	9.75	3.53
Apr-14	11.35	8.75
May-14	17.74	13.55
Jun-14	28.74	19.85
Jul-14	33.45	21.36

Descriptive statistics showing mean levels of Phi accumulations in leaf and root tissues of *A. stolonifera and P. annua,* prior to, and one week post treatment application, are shown in Tables 4-5 and 4-6.

Table 4-5 Phi accumulations (ppm) in leaf and root tissues of *A. stolonifera* and *P. annua,* prior to treatment application.

Turfgrass species	Tissue	Month	Mean	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
P. annua	leaf	Jan 13	824.67	7.37	820.87	828.47
		July 13	576.33	1.86	572.53	580.13
		July 14	657.33	5.43	653.53	661.13
	Root	Jan 13	554.17	3.19	550.37	557.97
		July 13	614.67	4.27	610.87	618.47
		July 14	599.67	3.01	595.87	603.47
A. stolonifera	leaf	Jan 13	761.17	8.28	756.04	766.30
		July 13	411.67	6.80	406.54	416.80
		July 14	492.67	3.61	487.54	497.80
	Root	Jan 13	459.17	4.88	454.04	464.30
		July 13	693.83	7.52	688.70	698.96
		July 14	787.17	4.36	782.04	792.30

Table 4-6 Phi accumulations (ppm) in leaf and root tissues of *A. stolonifera* and *P. annua,* one week post treatment application.

Turfgrass species	Tissue	Month	Mean	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
P. annua	leaf	Jan 13	4078.17	6.11	4073.48	4082.85
		July 13	3573.67	3.56	3568.98	3578.35
		July 14	3712.17	6.91	3707.48	3716.85
	Root	Jan 13	693.00	5.51	688.32	697.69
		July 13	655.67	6.86	650.98	660.35
		July 14	662.17	3.76	657.48	666.85
A. stolonifera	leaf	Jan 13	3590.33	3.56	3586.97	3593.70
		July 13	3272.17	5.71	3268.80	3275.53
		July 14	3468.83	5.38	3465.47	3472.20
	Root	Jan 13	490.00	1.90	486.64	493.36
		July 13	753.33	2.94	749.97	756.70
		July 14	835.00	3.35	831.64	838.36

Two-way Anova determined significant ($p < 0.05$) effects and interactions on Phi accumulations, Table 4-7, therefore, one-way Anova with Tukey HSD post hoc tests at $p < 0.05$ were used to determine and separate significant differences in Phi accumulations in leaf and root tissues for each species separately.

Table 4-7 Two-way Anova of Phi accumulations in turfgrass species, pre-treatment application and one week post-treatment application, showing significant interactions between species, tissues and months.

Pre-treatment				
	df	F	p	η2
Turfgrass species*Month	2,60	425.805	< .001	0.934
Turfgrass species*Tissues	1,60	5433.252	< .001	0.989
Tissues*Month	2,60	12442.196	< .001	0.998
Turfgrass species*Tissues*Month	2,60	2001.233	< .001	0.985
Post-treatment				
	df	F	p	η2
Turfgrass species*Month	2,60	6685.06	< .001	0.996
Turfgrass species*Tissues	1,60	25295.02	< .001	0.998
Tissues*Month	2,60	18844.098	< .001	0.998
Turfgrass species*Tissues*Month	2,60	319.849	< .001	0.914

4.4.2.1 Phi accumulation in *A. stolonifera* tissues, pre-treatment applications

In *A. stolonifera,* there were significant ($p < 0.05$ df = 2, 15, Fstat 4710.012) differences in mean Phi levels in leaf tissues at each of the three sampling periods. The highest leaf accumulations were recorded in January 2013, 761.17, 95% CI (756.04, 766.30) ppm, when turfgrass growth was less than the other readings, 411.67, 95% CI (406.54, 416.80) ppm in July 2013 and 492.67, 95% CI (487.54, 497.80) ppm in July 2014, indicating a more rapid loss of Phi at time of increased metabolic activity, Fig 4-13.

There were also significant ($p < 0.05$ df = 2, 15, Fstat 5177.147) differences in mean Phi levels in root tissues at each of the three sampling periods. Phi amounts were 459.27, 95% CI (454.04, 464.30) ppm in January 2013, 693.83, 95% CI (688.70, 698.96) ppm in July 2013 and 787.17, 95% CI (782.04, 792.30) ppm in July 2014, Fig. 4-13. These data determined significant ($p < 0.05$) increases in Phi amounts in root tissues following a period of sequential treatments, indicating a sink/source link with Phi accumulation and actively growing meristematic tissues.

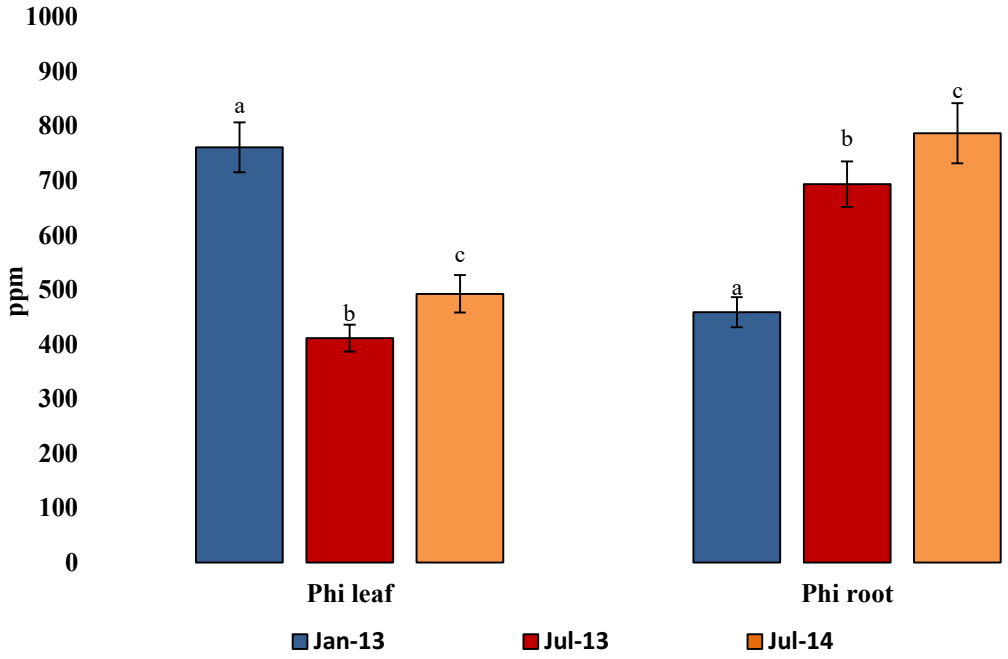


Figure 4-13 Phi accumulations in *A. stolonifera* leaf and root tissues between July 2012 and July 2014. Phi accumulations in leaf and root tissues of *A. stolonifera,* following sequential monthly applications of Phi, at a rate of 0.35 g PO_3^{3-}/ m^{-2}, between July 2012 and July 2014. Data were recorded prior to treatment application at 6, 12 and 24 months from commencement of treatments. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month as determined by Tukey HSD at $p < 0.05$.

4.4.2.2 Phi accumulation in *A. stolonifera* tissues, post-treatment applications

Phi amounts in leaf tissues one week post treatment application at each sampling period, were significantly ($p < 0.05$ df = 2, 15, Fstat 6253.506) different in leaf tissues at each of the three sampling periods. The highest leaf accumulations were recorded in January 2013, 3590, 95% CI (3586, 3594) ppm, when turfgrass growth was less than the other readings, 3272, 95% CI (3266, 3278) ppm in July 2013 and 3468, 95% CI (3463, 3474) ppm in July 2014, which, as in the pre-treatment readings, indicates a more rapid loss of Phi at time of increased growth, Fig 4-14. There were also significant ($p < 0.05$ df = 2, 15, Fstat 24933.949) differences in mean Phi levels in root tissues at each of the three sampling periods. Phi amounts were 490.00, 95% CI (488.01, 491.99) ppm in January 2013, 753.33, 95% CI (750.24, 756.42) ppm in July 2013 and 835.00, 95% CI (831.48, 838.51) ppm in July 2014, Fig. 4-14.

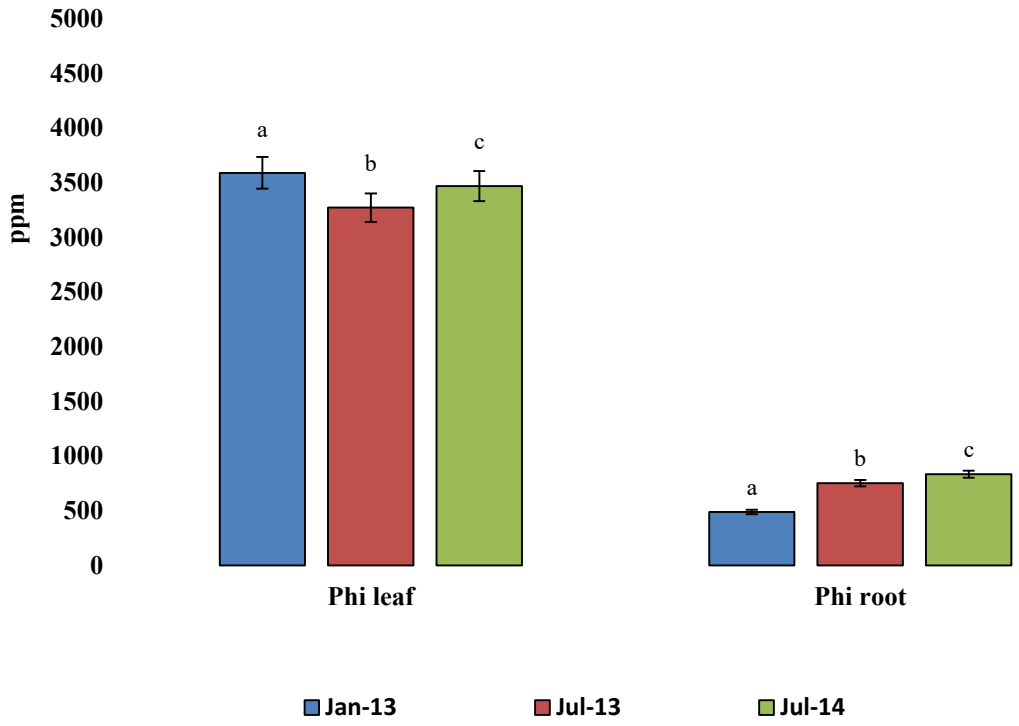


Figure 4-14 Phi accumulations in *A. stolonifera* leaf and root tissues between July 2012 and July 2014. Phi accumulations in leaf and root tissues of *A. stolonifera* following sequential monthly applications of Phi, at a rate of 0.35 g PO_3^{3-}/ m^{-2}, between July 2012 and July 2014. Data were recorded one week post treatment application at 6, 12 and 24 months from commencement of treatments. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month as determined by Tukey HSD at $p < 0.05$.

4.4.2.3 Phi accumulation in *P. annua* tissues, pre-treatment applications

Fig. 4-15 shows Phi accumulations in leaf tissues of *P. annua,* prior to treatment application, at January 2013, 824.67, 95% CI (816.93, 832.39), July 2013, 576.33, 95% CI (574.37, 578.28) and July 2014, 657.33, 95% CI (652.64, 663.03). One-way Anova determined significant ($p < 0.05$ df = 2, 15, Fstat 3310.696) differences in Phi amounts between each sampling period. Phi accumulations followed a similar trend as in *A. stolonifera* leaf tissues, in that there were significantly ($p < 0.05$) greater accumulations in January, than both of the readings from July and significantly ($p < 0.05$), greater amounts in the July 2014 samples than in those from July 2013.

Phi accumulations in root tissues displayed a similar response as in *A. stolonifera,* with significant ($p < 0.05$ df = 2, 15, Fstat 476.440) differences in amounts at each sampling period. Phi amounts were 554.17, 95% CI (550.82, 557.51) ppm in January 2013, 614.66, 95% CI (610.18, 619.15) ppm in July 2013 and599.50, 95% CI (596.50, 602.77) ppm in July 2014, Fig. 4-15.

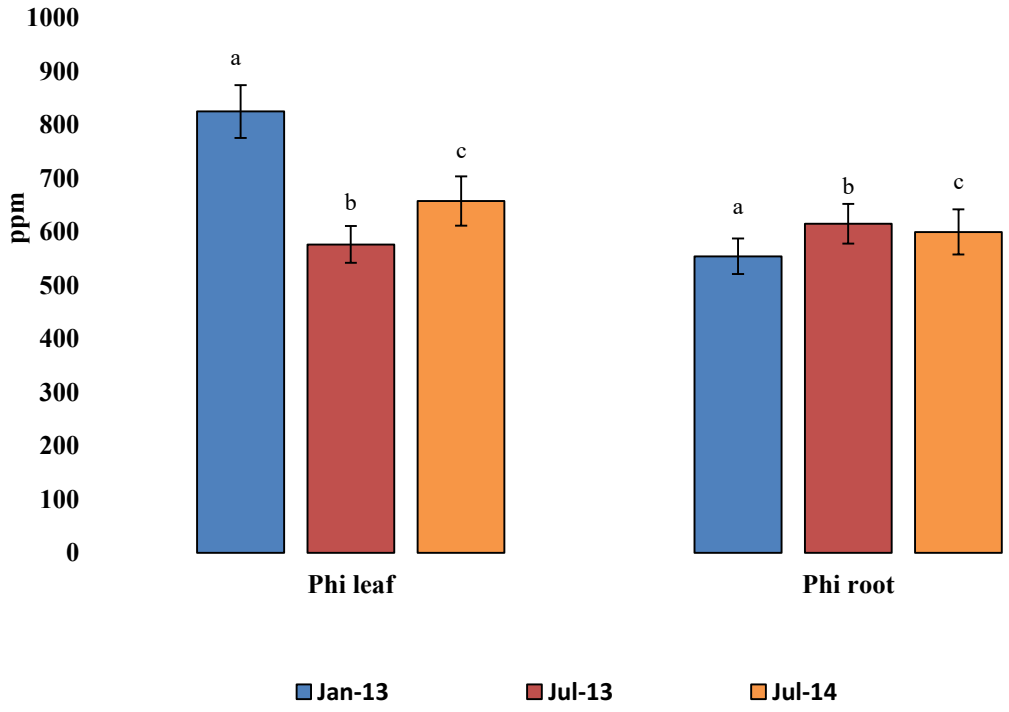


Figure 4-15 Phi accumulations in *P. annua* leaf and root tissues between July 2012 and July 2014. Phi accumulations in leaf and root tissues of *P. annua,* following sequential monthly applications of Phi, at a rate of 0.35 g PO_3^{3-}/ m^{-2}, between July 2012 and July 2014. Data were recorded prior to treatment application at 6, 12 and 24 months from commencement of treatments. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month as determined by Tukey HSD at $p < 0.05$.

4.4.2.4 Phi accumulation in *P. annua* tissues, post-treatment applications

Phi amounts in leaf tissues one week post treatment application at each sampling period, were significantly ($p < 0.05$ df = 2, 15, Fstat 12504.862) different in leaf tissues at each of the three sampling periods. Leaf accumulations, as shown in Fig 4-16, were highest in January 2013, 4078, 95% CI (4071, 4084) ppm, with amounts of 3573, 95% CI (3569, 3577) ppm in July 2013 and 3712, 95% CI (3704, 3719) ppm in July 2014, data which display a similar trend as the results determined in the *A. stolonifera* leaf samples.

There were also significant ($p < 0.05$ df = 2, 15, Fstat 2386.72) differences in mean Phi levels in root tissues one week post treatment application. Phi amounts were 693, 95% CI (687.21, 698.78) ppm in January 2013, significantly ($p < 0.05$) greater than both the July 2013 amount of 655.67, 95% CI (648.46, 662.86) ppm and the July 2014 amount of 662.17, 95% CI (658.21, 666.11) ppm, there were no significant ($p > 0.05$) differences between the July 2013 and July 2015 amounts, Fig. 4-16. This reduced cumulative accumulation of Phi in root tissues may be indicative of the shorter lifespan of roots in *P.annua* compared to the perennial growth mode of *A. stolonifera.*

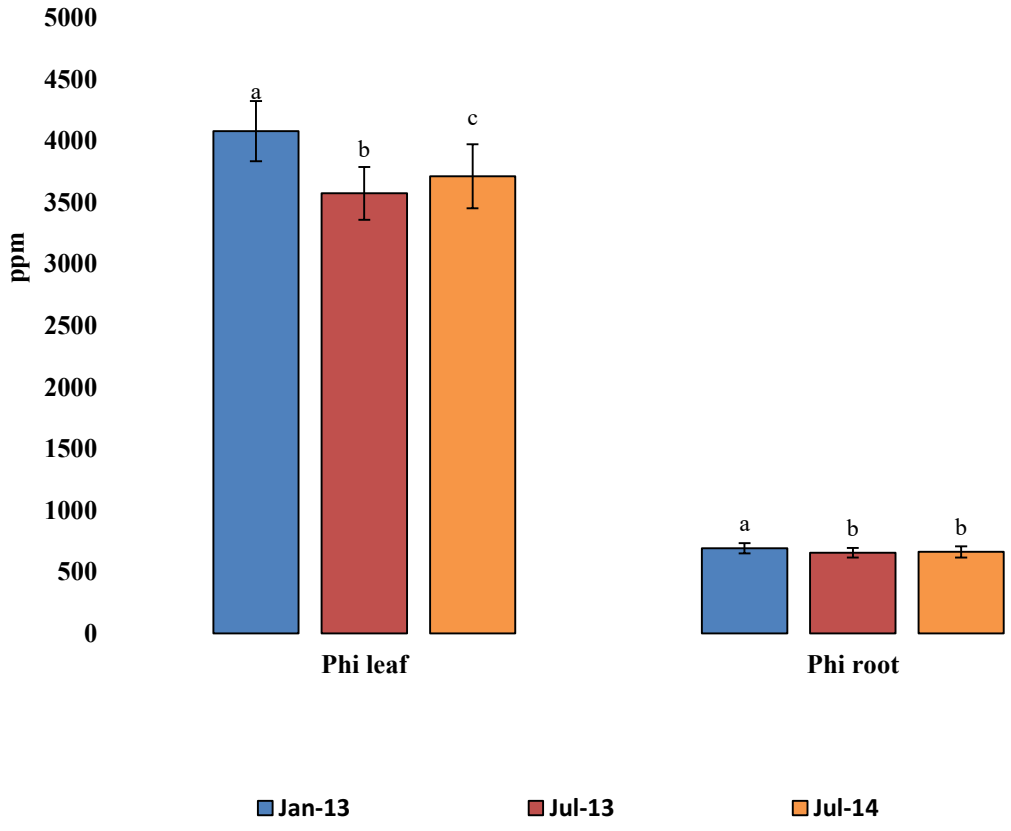


Figure 4-16 Phi accumulations in *P. annua* leaf and root tissues between July 2012 and July 2014. Phi accumulations in leaf and root tissues of *P. annua* following sequential monthly applications of Phi, at a rate of 0.35 g PO_3^{3-}/ m^{-2}, between July 2012 and July 2014. Data were recorded one week post treatment application at 6, 12 and 24 months from commencement of treatments. Bars indicate 95% confidence limits, letters indicate significant differences between treatments for each month as determined by Tukey HSD at $p < 0.05$.

4.4.2.5 Rootzone nutrient analyses

Rootzone nutrient levels prior to the start of treatment applications and at the conclusion of the study are shown in Table 4-8. The main element of interest was P and a paired-samples t-test was used to determine whether there was a statistically significant difference in soil levels following the sequential applications of P in the form of either Phi or Pi. The sequential treatments significantly ($p < 0.05$) increased soil P levels in the rootzones of both turfgrass species, over the 24 month trial. Interestingly, the rate of P increase was significantly ($p < 0.05$) greater following Phi treatment compared with the Pi treatments. In *A. stolonifera*, P levels in Phi treated rootzones increased significantly from 37 to 51 ppm, $t(5) = 20.147$, $p < 0.01$. In Pi treated rootzones, P levels also increased significantly from 37 ppm to 40 ppm, $t(5) = 3.354$, $p = 0.02$. P levels in Phi treated rootzones were significantly greater than levels in the Pi treated samples, $t(5) = 14.094$, $p < 0.01$.

The results were similar in *P. annua* rootzones, P levels in Phi treated rootzones increased significantly from 37 to 57 ppm, $t(5) = 29.277$, $p < 0.01$, while in Pi treated rootzones P levels increased from 37 ppm to 44 ppm, $t(5) = 6.575$, $p = 0.001$. P levels in Phi treated rootzones were also significantly greater than levels in the Pi treated samples, $t(5) = 14.534$, $p < 0.01$.

Table 4-8 Rootzone nutrient content (ppm) and Cation Exchange Capacity (C.E.C.), prior to the start of treatments in July 2012 and at the conclusion of treatments in July 2014.

	Treatment	N	P	K	Mg	Fe	Ca	C.E.C. (meq/100g)
				A. stolonifera rootzone				
Jul-12	Phi	6.5	37	88	46	280	1510	7.7
	Pi	6.5	37	88	46	280	1510	7.7
Jul-14	Phi	7.5	51	109	71	328	1443	7.9
	Pi	7.2	40	105	79	282	1422	8
				P. annua rootzone				
Jul-12	Phi	6.5	37	88	46	280	1510	8.2
	Pi	6.5	37	88	46	280	1510	7.6
Jul-14	Phi	7.9	57	104	73	277	1373	7.9
	Pi	6.8	44	110	77	304	1404	8.1

As Phi is generally applied in compound with K, any changes in rootzone K levels were of interest. K increased significantly ($p < 0.05$) in both rootzones over the 24 months. In *A. stolonifera* rootzones, K levels at the start of the trial were 88 ppm, increasing significantly to 109 ppm, $t(5) = 15.500$, $p < 0.01$, following Phi treatments and to 105 ppm, $t(5) = 20.821$, $p < 0.01$, after Pi treatments. K levels in Phi treated rootzones were also significantly greater than levels in the Pi treated samples, $t(5) = 3.708$, $p = 0.014$.

In *P. annua* rootzones, K levels at the start of the trial were also 88 ppm, they increased significantly to 104 ppm, $t(5) = 23.422$, $p < 0.01$, following Phi treatments and to 110 ppm, $t(5) = 21.536$, $p < 0.01$, after Pi treatments. K levels in Phi treated rootzones were also significantly greater than levels in the Pi treated samples, $t(5) = 4.772$, $p = 0.005$.

4.4.3 Experiment 3, Phi as a source of P nutrition

Phi treatments caused significant ($p < 0.05$) differences in growth responses in both *L. perenne* and *P. annua* growing in P deficient and P sufficient rootzones. In P sufficient rootzones Phi treatment increased biomass, compared with Pi and KCl treated plants. In P deficient rootzones, Phi treatment inhibited growth, producing significantly ($p < 0.05$) less biomass than the Pi and KCl treatments.

4.4.3.1 Effects of Phi treatment on leaf, crown and root development in *L. perenne* and *P. annua* growing in P sufficient rootzones

Descriptive statistics showing treatment effect on growth of leaf, crown and root tissues of *A. stolonifera* and *P. annua* are shown in Table 4-9. Over the six month trial period, in P sufficient rootzones, there were significant ($p < 0.05$) interactions between turfgrass species, treatments and tissues, Table 4-10. As a result, each turfgrass species were statistically analysed separately using one-way Anova to determine significant treatment effects on leaf, crown and root growth.

Table 4-9 Descriptive statistics of treatment effect on leaf, crown and root growth as grams of dry weight (g/dw) in *A. stolonifera* and *P. annua* growing in a P sufficient rootzone.

Species	Treatment	Tissues	Mean g/dw	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
L. perenne	KCl	leaf	3.13	0.06	3.07	3.18
		Crown	13.35	0.07	13.29	13.40
		Root	8.10	0.04	8.04	8.15
	Pi	leaf	3.15	0.06	3.10	3.21
		Crown	13.81	0.07	13.75	13.86
		Root	7.29	0.06	7.24	7.35
	Phi	leaf	3.62	0.08	3.57	3.68
		Crown	17.29	0.07	17.24	17.35
		Root	8.59	0.07	8.54	8.65
P. annua	KCl	leaf	2.83	0.07	2.77	2.88
		Crown	10.32	0.06	10.27	10.38
		Root	5.60	0.05	5.54	5.65
	Pi	leaf	3.45	0.09	3.40	3.51
		Crown	11.40	0.06	11.34	11.45
		Root	5.03	0.07	4.97	5.08
	Phi	leaf	3.79	0.06	3.74	3.85
		Crown	14.55	0.08	14.49	14.60
		Root	5.93	0.07	5.87	5.98

Table 4-10 Two-way Anova determining significant interactions between species, tissues and treatments.

P sufficient rootzones				
	df	f	p	η2
Species* treatments	2,90	118.251	< .001	0.724
Species* tissues	2,90	4683.941	< .001	0.990
Treatments* tissues	4,90	3080.634	< .001	0.993
Species* treatments*tissues	4,90	18.643	< .001	0.453

4.4.3.1.1 Treatment effect on *L. perenne* growing in a P sufficient rootzone

In *L. perenne,* there was a significant ($p < 0.05$) treatment effect on leaf, crown and root biomass, Table 4-11.

Table 4-11 One-way Anova of treatment effect on growth of leaf, crown and root tissues of *L. perenne* growth in a P sufficient rootzone.

	df	F	p	η2
Leaf	2,15	101.802	< .001	0.931
Crown	2,15	5888.251	< .001	0.999
Root	2,15	733.257	< .001	0.990

Following Tukey HSD post hoc analyses, it was determined that Phi treatment significantly ($p < 0.05$) increased dry weights in all tissue types, compared with Pi and KCl treated plants, as can be seen in Fig 4-17.

Dry weight of leaf cuttings were significantly ($p < 0.05$) greater following Phi treatment, 3.62, 95% CI (3.57, 3.86) g, compared to Pi, 3.15, 95% CI (3.10, 3.21) g and KCl (control) 3.13, 95% CI (3.07, 3.18) g. There were no significant ($p = 0.778$) differences between the Pi and KCl (control) treatments.

Crown dry weights were significantly ($p < 0.05$) greater following Phi treatment 17.29, 95% CI (17.24, 17.35) g, than both Pi 13.81, 95% CI (13.75, 13.86) g and KCl (control) 13.35, 95% CI (13.29, 13.40) g. With Pi treatments significantly ($p < 0.05$) greater then KCl (control).

Root dry weights were also significantly ($p < 0.05$) greater following Phi treatment 8.59, 95% CI (8.54, 8.65) g, than both Pi 7.29, 95% CI (7.24, 7.35) g and KCl (controls) 8.10, 95% CI (8.04, 8.15) g. However, the KCl (control) treated root dry weights were significantly ($p < 0.0\ 5$) greater than the Pi treated tissues.

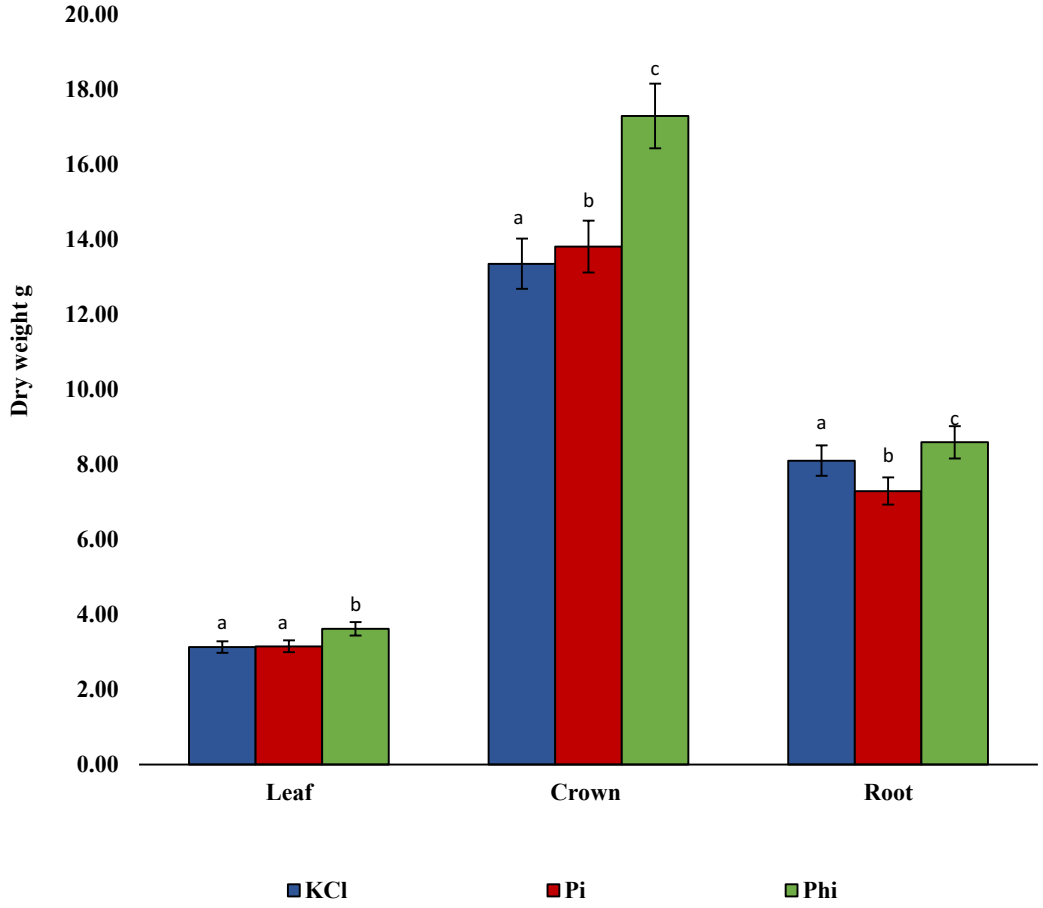


Figure 4-17 Treatment effect on the growth *L. perenne* in a P sufficient rootzone. Effect on the growth of leaf, crown and root tissues of *L. perenne,* growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p < 0.05$.

4.4.3.1.2 Treatment effect on *P. annua* growing in a P sufficient rootzone

In *P. annua,* there was also a significant ($p < 0.05$) treatment effect on leaf, crown and root biomass, Table 4-12.

Table 4-12 One-way Anova of treatment effect on leaf, crown and root tissues of *P. annua* growth in P sufficient rootzone.

	df	F	p	η2
Leaf	2,15	253.468	< .001	0.971
Crown	2,15	6470.257	< .001	0.999
Root	2,15	287.031	< .001	0.975

Following Tukey HSD post hoc analyses, as in the *L. perenne* plants, it was determined that Phi treatment significantly ($p < 0.05$) increased dry weights in all tissue types, compared with Pi and KCl treated plants, as can be seen in Fig 4-18.

Dry weight of leaf cuttings were significantly ($p < 0.05$) greater following Phi treatment, 3.79, 95% CI (3.74, 3.85) g, compared to Pi, 3.45, 95% CI (3.40, 3.51) g and KCl (control) 2.83, 95% CI (2.77, 2.88) g, with Pi treated leaf weights significantly ($p < 0.05$) greater than the KCl (controls).

Crown dry weights were also significantly ($p < 0.05$) greater following Phi treatment 14.55, 95% CI (14.49, 14.60) g, than both Pi 11.40, 95% CI (11.34, 11.45) g and KCl (controls) 10.32, 95% CI (10.27, 10.38) g. With Pi treatments significantly ($p < 0.05$) greater then KCl (controls).

Root dry weights were also significantly ($p < 0.05$) greater following Phi treatment 5.93, 95% CI (5.87, 5.93) g, compared to both Pi 5.03, 95% CI (4.97, 5.08) g and KCl (controls) 5.60, 95% CI (5.554, 5.65) g. The KCl (control) treated root dry weights were significantly ($p < 0.05$) greater than the Pi treated roots.

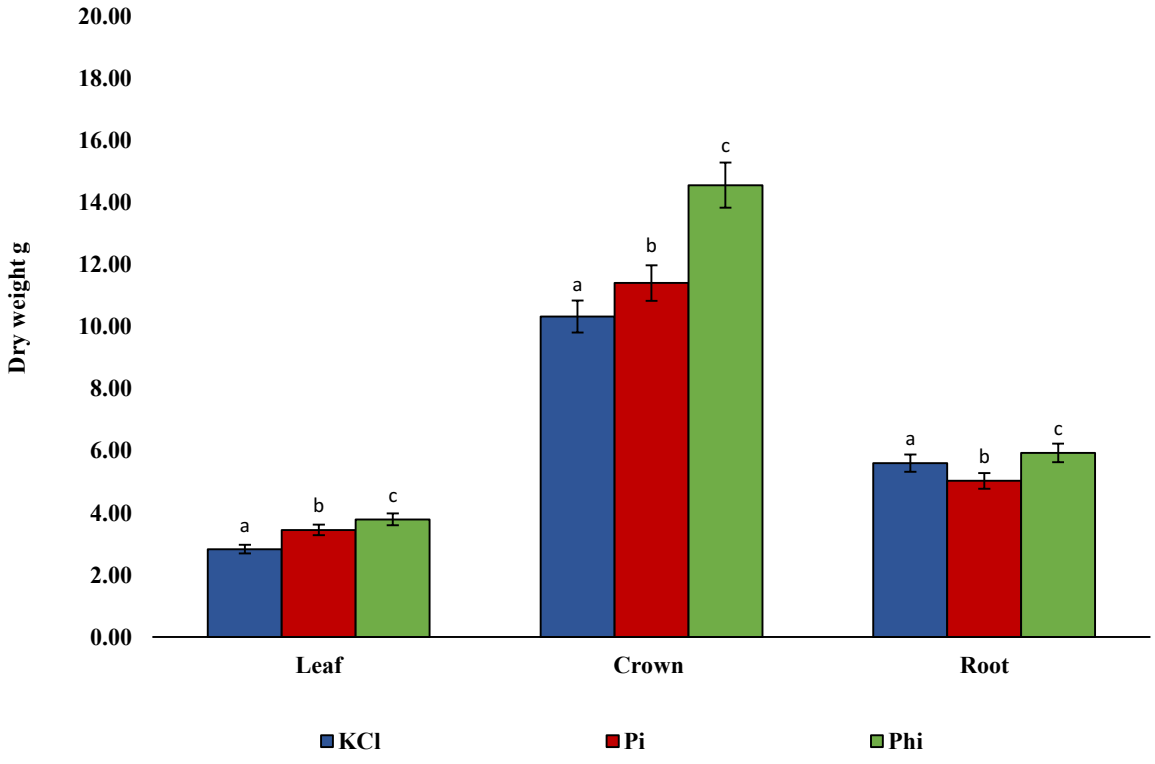


Figure 4-18 Treatment effect on the growth *P. annua* in a P sufficient rootzone. Effect on the growth of leaf, crown and root tissues of *P. annua,* growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p < 0.05$.

4.4.3.2 Effects of Phi treatment on leaf, crown and root development in *L. perenne* and *P. annua* growing in P deficient rootzones

In P deficient rootzones, over the six month trial period, Phi treatment led to reduced growth in leaf, crown and root tissues of *A. stolonifera* and *P. annua* when compared with the Pi and KCl (control) treated tissues. Descriptive statistics showing treatment effect on tissue growth are shown in Table 4-13.

Table 4-13 Descriptives statistics of treatment effect on leaf, crown and root growth as grams of dry weight (g/dw) in *L. perenne* and *P. annua* growing in a P deficient rootzone.

Species	Treatment	Tissues	Mean g/dw	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
L. perenne	KCl	leaf	2.39	0.04	2.36	2.43
		Crown	8.34	0.06	8.31	8.37
		Root	7.21	0.02	7.18	7.23
	Pi	leaf	3.04	0.04	3.01	3.07
		Crown	9.01	0.03	8.98	9.04
		Root	7.86	0.01	7.83	7.89
	Phi	leaf	1.75	0.03	1.72	1.78
		Crown	7.11	0.03	7.08	7.14
		Root	5.45	0.03	5.42	5.48
P. annua	KCl	leaf	2.15	0.01	2.12	2.18
		Crown	7.46	0.03	7.43	7.49
		Root	4.96	0.05	4.93	4.99
	Pi	leaf	2.94	0.03	2.91	2.97
		Crown	8.09	0.07	8.06	8.12
		Root	5.73	0.03	5.70	5.76
	Phi	leaf	1.46	0.04	1.43	1.49
		Crown	5.91	0.03	5.89	5.94
		Root	3.76	0.03	3.73	3.79

Two-way Anova determined significant ($p < 0.05$) interactions between turfgrass species, treatments and tissues, Table 4-14. As a result, each turfgrass species were statistically analysed separately using one-way Anova to determine significant treatment effects on leaf, crown and root growth.

Table 4-14 Two-way Anova determining significant interactions between species, tissues and treatments

P deficient rootzones				
	df	F	p	η2
Turfgrass species*treatment	2,90	10.509	< .001	0.189
Turfgrass species*tissues	2,90	5566.746	< .001	0.992
Treatments*tissues	4,90	566.778	< .001	0.962
Turfgrass species*treatments*tissues	4,90	135.852	< .001	0.858

4.4.3.2.1 Treatment effect on *L. perenne* growing in a P deficient rootzone

In *L. perenne,* there was a significant ($p < 0.05$) treatment effect on leaf, crown and root biomass, Table 4-15. Following Tukey HSD post hoc analyses, it was determined that Phi treatment significantly ($p < 0.05$) reduced dry weights in all tissue types, compared with Pi and KCl treated plants, as can be seen in Fig 4-19.

Table 4-15 One-way Anova of treatment effect of leaf, crown and root tissues of *L. perenne* growth in P deficient rootzone.

	df	F	p	η2
Leaf	2,15	1741.953	< .001	1.000
Crown	2,15	3124.365	< .001	0.998
Root	2,15	22032.343	< .001	1.000

Dry weights of *L. perenne* leaf cuttings were significantly ($p < 0.05$) less following Phi treatment, 1.75, 95% CI (1.72, 1.78) g, compared to Pi, 3.04, 95% CI (3.01, 3.07) g and KCl (control) 2.39, 95% CI (2.36, 2.42) g. The Pi treated leaf dry weights were significantly ($p < 0.05$) greater than the KCl (control) treatments.

Crown dry weights were also significantly ($p < 0.05$) less following Phi treatment 7.11 95% CI (7.08, 7.14) g, than both the Pi 9.01, 95% CI (8.98, 9.04) g and KCl (controls) 8.34, 95% CI (8.31, 8.37) g, with the Pi treatments significantly ($p < 0.05$) greater than KCl (controls).

Root dry weights were also significantly ($p <0.05$) reduced following Phi treatment 5.45, 95% CI (5.42, 5.48) g, than both Pi 7.86, 95% CI (7.83, 7.89) g and KCl (controls) 7.21, 95% CI (7.18, 7.23) g. The root dry weight following Pi treatments were significantly ($p < 0.05$) greater than KCl (controls).

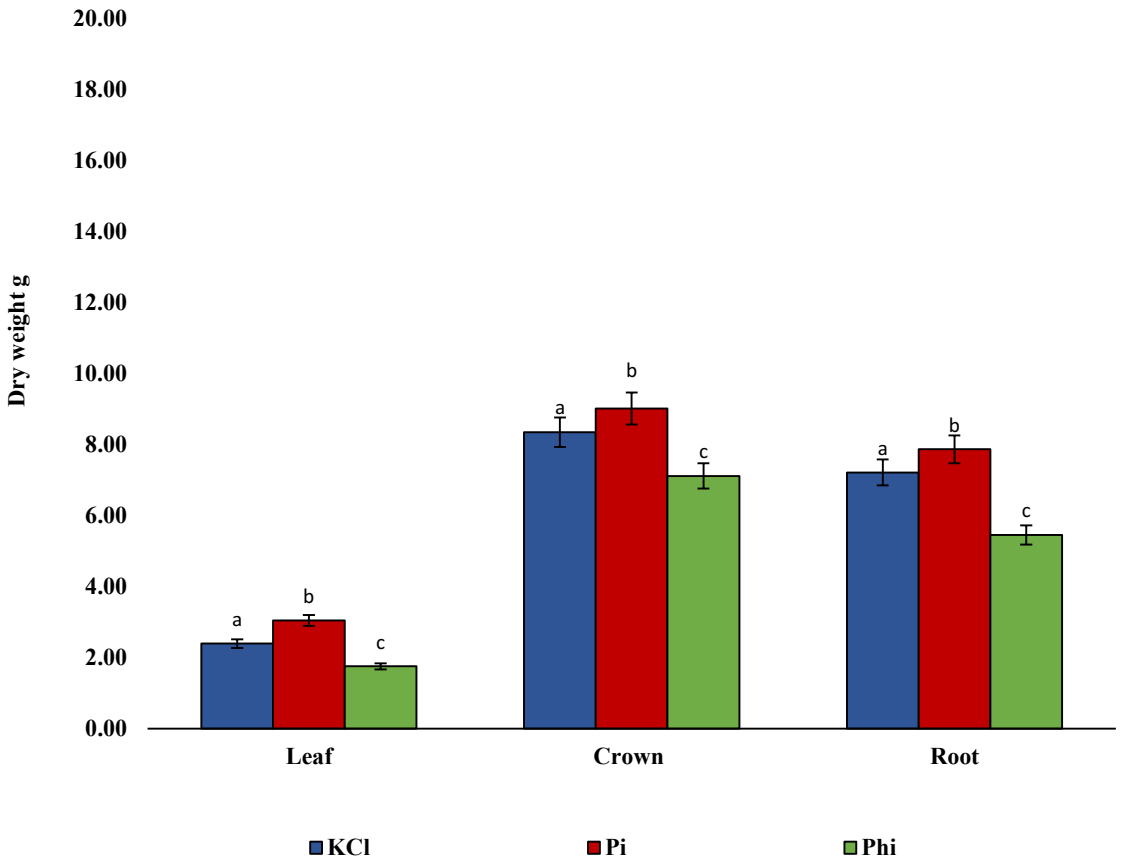


Figure 4-19 Treatment effect on the growth *L. perenne* in a P deficient rootzone. Effect on the growth of leaf, crown and root tissues of *L. perenne,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p < 0.05$.

4.4.3.2.2 Treatment effect on *P. annua* growing in a P deficient rootzone

In *P. annua* growing in the P deficient rootzones, there was also a significant ($p < 0.05$) treatment effect on leaf, crown and root biomass, Table 4-16. Following Tukey HSD post hoc analyses, it was determined that Phi treatment significantly ($p < 0.05$) reduced dry weights in all tissue types, compared with Pi and KCl treated plants, as can be seen in Fig 4-20.

Table 4-16 One-way Anova of treatment effect of leaf, crown and root tissues of *P. annua* growth in P deficient rootzone.

	df	F	p	η2
Leaf	2,15	3891.255	< .001	0.998
Crown	2,15	3208.375	< .001	0.998
Root	2,15	4839.895	< .001	0.998

Dry weights of *P. annua* leaf cuttings were significantly ($p < 0.05$) less following Phi treatment, 1.46, 95% CI (1.43, 1.49) g, compared to Pi, 2.94, 95% CI (2.91, 2.97) g and KCl (control) 2.15, 95% CI (2.12, 2.18) g. The leaf dry weights from the Pi treated plants were significantly ($p < 0.05$) greater than the KCl (control) treatments.

Crown dry weights were significantly ($p < 0.05$) less following Phi treatment 5.91 95% CI (5.89, 5.94) g, than both the Pi 8.09, 95% CI (8.06, 8.12) g and KCl (controls) 7.46, 95% CI (7.43, 7.49) g, with the Pi treatments significantly ($p < 0.05$) greater than KCl (controls).

Root dry weights were significantly ($p <0.05$) reduced following Phi treatment 1.46, 95% CI (1.43, 1.49) g, when compared with both Pi 5.73, 95% CI (5.70, 5.76) g and KCl (controls) 4.96, 95% CI (4.93, 4.99) g. The root dry weight following Pi treatments were significantly ($p < 0.05$) greater than KCl (controls).

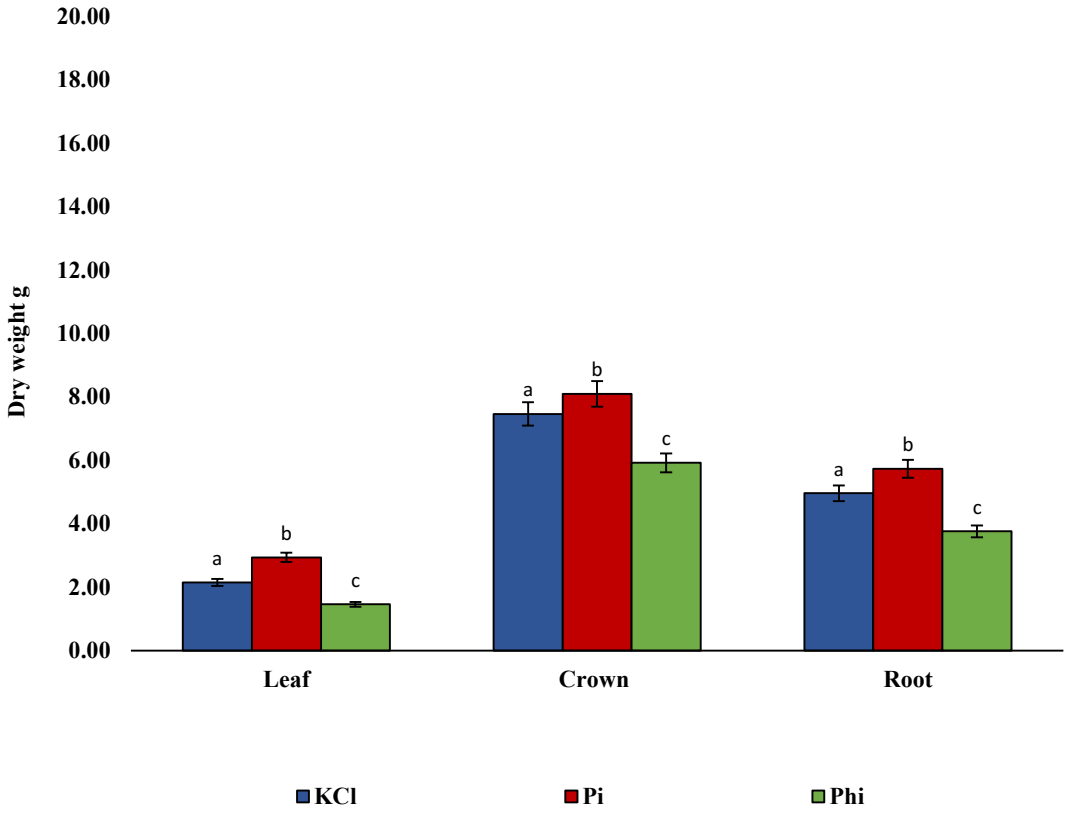


Figure 4-20 Treatment effect on the growth *P. annua* in a P deficient rootzone. Effect on the growth of leaf, crown and root tissues of *P. annua,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p <0.05$.

4.4.3.3 Treatment effect on root to shoot ratios of *L. perenne* and *P. annua* growing in a P sufficient and P deficient rootzones

There was a significant ($p < 0.05$) interaction between turfgrass species, rootzone P levels and treatments as shown in Table 4-17, this was followed up by a series of one–way Anovas to determine treatment effects on root to shoot ratios in *L. perenne* and *P. annua* growing in P sufficient and P deficient rootzones.

Table 4-17 Two-way Anova of treatment effect on root to shoot ratios

	df	F	p	η2
Turfgrass species*treatment	2,60	19.866	< .001	0.398
Turfgrass species*rootzone	1,60	621.075	< .001	0.912
Treatments*rootzone	2,60	389.704	< .001	0.929
Turfgrass species*treatments*rootzone	2,60	59.22	< .001	0.664

4.4.3.3.1 Treatment effect on root to shoot ratios of *L. perenne* growing in P sufficient and P deficient rootzones

Root to shoot ratios in *L. perenne*, growing in both rootzone types, displayed significantly ($p < 0.05$) lower ratios in the Phi treated plants than plants receiving the Pi and KCl (control) treatments, Fig 4-21. In the P sufficient rootzone there was a significant ($p < 0.05$ df = 2, 15, Fstat 521.912) treatment effect, with KCl (control) treatments producing the highest ratios, 0.607, 95% CI (0.601, 0.612), significantly ($p < 0.05$) greater than the Pi treatments of 0.528, 95% CI (0.523, 0.534), both were significantly ($p < 0.05$) greater than the Phi treated ratio of 0.495, 95% CI (0.490, 0.500).

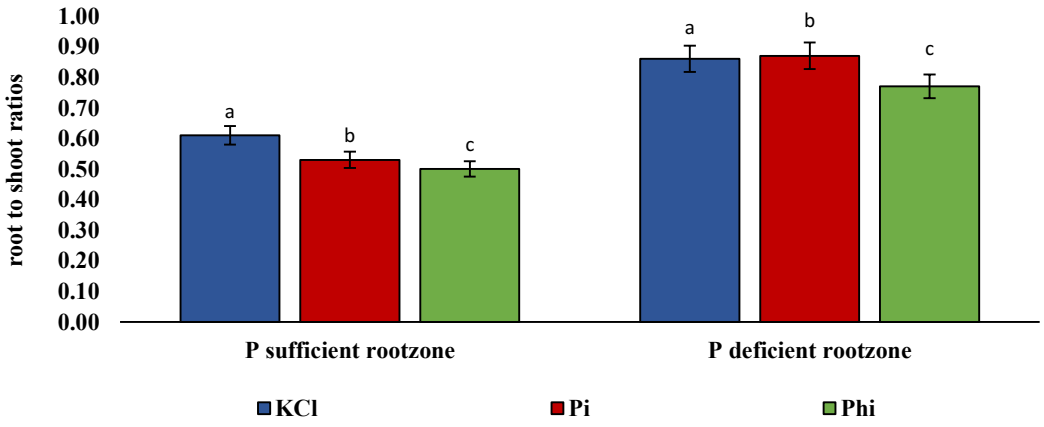


Figure 4-21 Treatment effect on root to shoot ratios of *L. perenne* growing in P sufficient and P deficient rootzones. Effect on root to shoot ratios of *L. perenne* growing in a P sufficient and deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p < 0.05$.

It was a similar result in the *L. perenne* growing in the P deficient rootzones, where a significant ($p < 0.05$ df = 2, 15, Fstat 561.618) treatment effect was also observed. Pi treatments produced the highest ratios, 0.873, 95% CI (0.868, 0.867), significantly ($p = 0.013$) greater than the KCl (control) treatments of 0.862, 95% CI (0.856, 0.867), both were significantly ($p < 0.05$) greater than the Phi treated ratios of 0.765, 95% CI (0.760, 0.770).

4.4.3.3.2 Treatment effect on root to shoot ratios of *P. annua* growing in P sufficient and P deficient rootzones

Root to shoot ratios in *P. annua* growing in in both rootzone types, as in the *L. perenne*, displayed significantly ($p < 0.05$) lower ratios in the Phi treated plants compared to the Pi and KCl (control) treatments, Fig 4-22. In the P sufficient rootzones there was a significant ($p < 0.05$ df = 2, 15, Fstat 436.293) treatment effect, with KCl (control) treatments producing the highest ratios, 0.540, 95% CI (0.533, 0.547), significantly ($p < 0.05$) greater than the Pi treatments of 0.442, 95% CI (0.435, 0.449), both were significantly ($p < 0.05$) greater than the Phi treated ratios of 0.408, 95% CI (0.401, 415). It was a similar result in the *P. annua* growing in the P deficient rootzones, where a significant ($p < 0.05$ df = 2, 15, Fstat112.333) treatment effect was also observed. Pi treatments produced the highest ratios, 0.707, 95% CI (0.700, 0.714), significantly ($p < 0.05$) greater than the KCl (control) treatments of 0.663, 95% CI (0.656, 0.670), both were significantly ($p < 0.05$) greater than the Phi treated ratios of 0.637, 95% CI (0.630, 644).

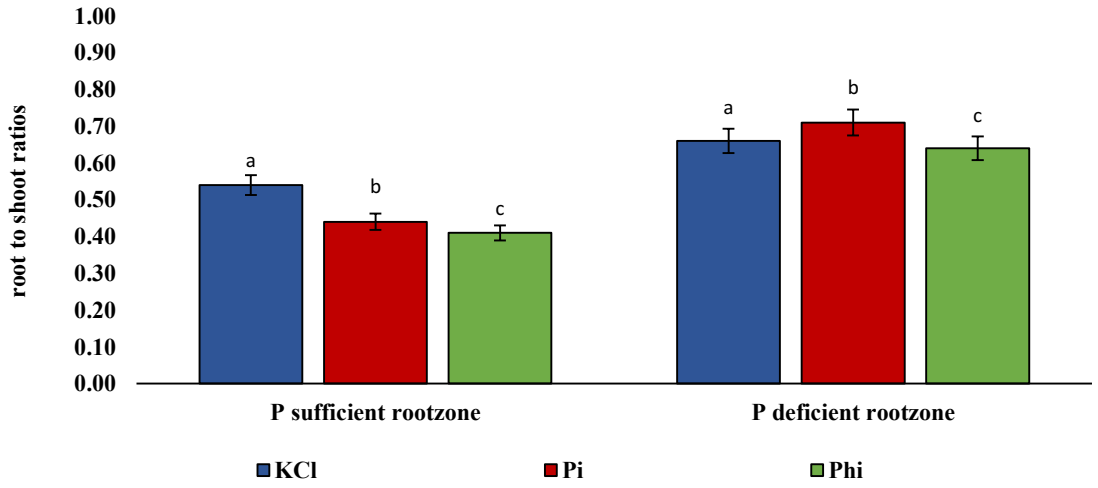


Figure 4-22 Treatment effect on root to shoot ratios of *P. annua* growing in P sufficient and P deficient rootzones. Effect on root to shoot ratios of *P. annua* growing in a P sufficient and deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at $p < 0.05$.

4.4.3.4 Treatment effect on tissue P levels of *L. perenne* and *P. annua* growing in a P sufficient rootzone

Descriptive statistics showing treatment effect on the P levels of leaf, crown and root tissues of *A. stolonifera* and *P. annua,* growing in a P sufficient rootzone, following six months sequential treatment applications, are shown in Table 4-18. Two-way Anova of tissue P levels determined significant ($p < 0.05$) interactions at the end of the two year trial period, Table 4-19, As a result, each turfgrass species were statistically analysed separately, using one-way Anova and Tukey HSD post hoc analyses, to determine significant treatment effects on leaf, crown and root P levels.

Table 4-18 Descriptive statistics of treatment effect on P levels in leaf, crown and root tissues of *L. perenne* and *P. annua* growing in a P sufficient rootzone following six months of sequential applications.

Species	Treatment	Tissues	Mean	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
L. perenne	KCl	leaf	5317.17	10.89	5308.02	5326.32
		Crown	3431.67	11.76	3422.52	3440.82
		Root	2135.33	11.11	2126.19	2144.48
	Pi	leaf	6502.67	9.03	6493.52	6511.82
		Crown	4347.00	13.22	4337.85	4356.15
		Root	2291.50	11.22	2282.35	2300.65
	Phi	leaf	6227.67	13.41	6218.52	6236.82
		Crown	4936.83	9.39	4927.69	4945.98
		Root	2788.67	9.14	2779.52	2797.82
P. annua	KCl	leaf	5253.33	11.98	5243.19	5263.47
		Crown	4818.33	13.74	4808.19	4828.47
		Root	2353.33	14.05	2343.19	2363.47
	Pi	leaf	6097.50	15.76	6087.36	6107.64
		Crown	5229.50	6.98	5219.36	5239.64
		Root	2582.83	11.00	2572.69	2592.97
	Phi	leaf	5161.33	10.19	5151.19	5171.47
		Crown	5708.00	11.87	5697.86	5718.14
		Root	3143.33	13.29	3133.19	3153.47

Table 4-19 Two-way Anova determining significant interactions between species, tissues and treatments.

	df	F	p	η2
Turfgrass species*treatment	2,90	3979.481	< .001	0.989
Turfgrass species*tissues	2,90	4683.941	< .001	0.991
Treatments*tissues	4,90	10999.45	< .001	0.998
Turfgrass species*treatments*tissues	4,90	2055.841	< .001	0.989

In *L. perenne,* there was a significant ($p < 0.05$) treatment effect on P levels in leaf, crown and root tissues, Table 4-20. As would be expected, P levels were significantly ($p < 0.05$) lower in all KCl (control) treated tissues, compared with the Pi and Phi treated tissues, Fig 4-23. In leaf tissues the highest P levels were determined following Pi treatments, with levels of 6503, 95% CI (6493, 6511) ppm, significantly ($p < 0.05$) greater than the Phi treatments of 6228, 95% CI (6217, 6237) ppm, both were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 5317, 95% CI (5307, 5326) ppm.

Table 4-20 One-way Anova of treatment effect on P levels in leaf, crown and root tissues of *L. perenne* growing in P sufficient rootzones following six months of sequential treatment applications.

	df	F	p	η2
Leaf	2,15	18242.002	< .001	1.000
Crown	2,15	15804.924	< .001	1.000
Root	2,15	6295.107	< .001	.999

In crown tissues the highest P levels were determined in the Phi treated plants, with levels of 4937, 95% CI (4926, 4946) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 4347, 95% CI (4336, 4357) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 3432, 95% CI (3422, 3442) ppm. In roots, the highest P levels were also determined following Phi treatments, with levels of 2789, 95% CI (2779, 2798) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 2291, 95% CI (2282, 2300) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 2135, 95% CI (2126, 2144) ppm.

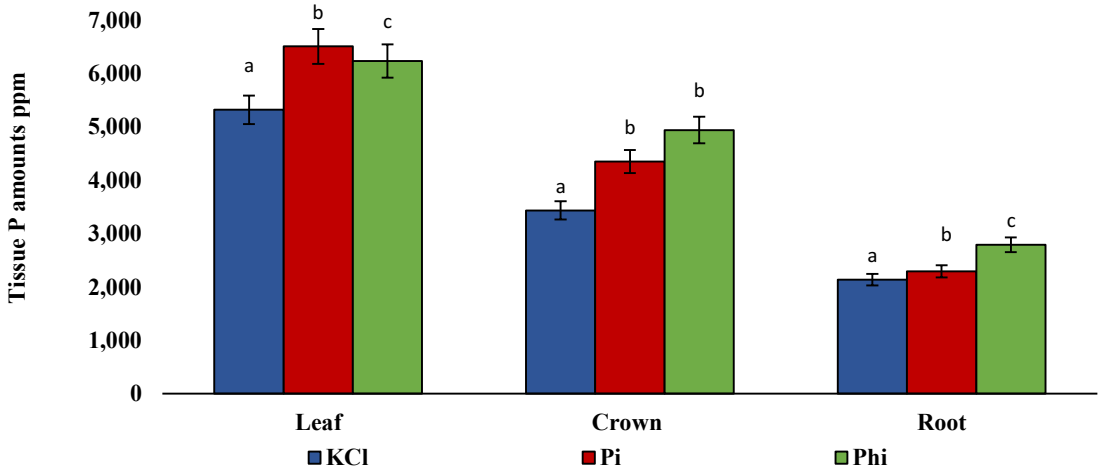


Figure 4-23 Treatment effect on P levels of *L. perenne* growing in a P sufficient rootzone. Effect on leaf, crown and root P levels of *L. perenne,* growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at $p < 0.05$

In *P. annua,* there was also a significant ($p < 0.05$) treatment effect on P levels in leaf, crown and root tissues, Table 4-21. As in the *L. perenne* plants, P levels were significantly ($p < 0.05$) lower in all KCl (control) treated tissues, compared with the Pi and Phi treated tissues, Fig. 4-24.

Table 4-21 One-way Anova of treatment effect on P levels in leaf, crown and root tissues of *P. annua* growing in P sufficient rootzones following six months of sequential treatment applications.

	df	F	p	η2
Leaf	2,15	9669.382	< .001	0.999
Crown	2,15	9436.53	< .001	1.000
Root	2,15	6004.426	< .001	0.999

In leaf tissues the highest P levels were determined following Pi treatments, with levels of 6097, 95% CI (6086, 6108) ppm, significantly ($p < 0.05$) greater than the KCl (control) treatments of 5253, 95% CI (5242, 5264) ppm, both significantly ($p < 0.05$) greater than the Phi treatment level of 5162, 95% CI (5150, 5172) ppm. In crown tissues, the highest P levels were determined in the Phi treated plants, with levels of 5708, 95% CI (5698, 5717) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 5229, 95% CI (5219, 5239) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 4818, 95% CI (4808, 4828). In roots, the highest P levels were in the Phi treated plants, with levels of 3143, 95% CI (3132, 3154) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 2582, 95% CI (2571, 2594) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 2353, 95% CI (2342, 2364) ppm.

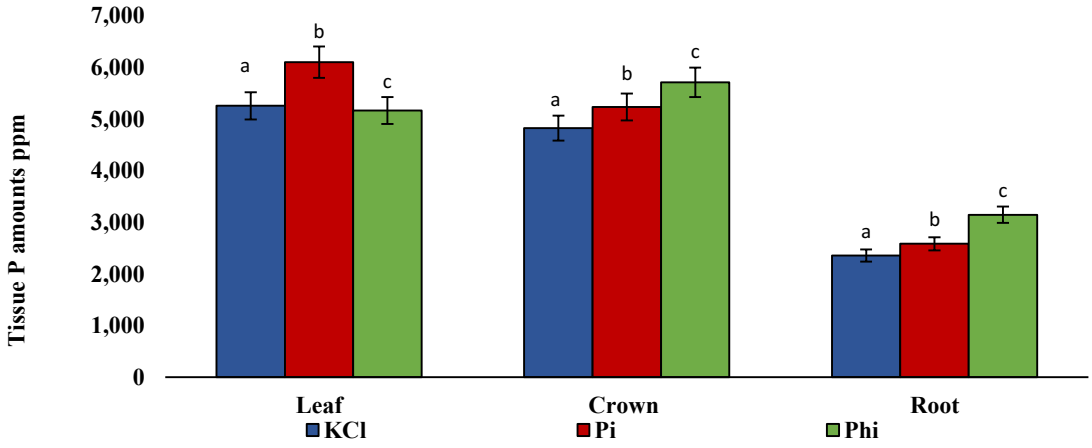


Figure 4-24 **Treatment effect on P levels of *P. annua* growing in a P sufficient rootzone.** Effect on leaf, crown and root P levels of *P. annua* growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at $p < 0.05$

4.4.3.5 Treatment effect on tissue P levels of *L. perenne* and *P. annua* growing in a P deficient rootzone

Descriptive statistics showing treatment effect on the P levels of leaf, crown and root tissues of *A. stolonifera* and *P. annua,* growing in a P deficient rootzone, following six months of sequential treatment applications are shown in Table 4-22. Two-way Anova of tissue P levels determined significant ($p < 0.05$) interactions at the end of the six month trial period, Table 4-23, As a result, each turfgrass species were statistically analysed separately, using one-way Anova and Tukey HSD post hoc analyses, to determine significant treatment effects on leaf, crown and root P levels.

Table 4-22 Descriptive statistics of treatment effect on P levels in leaf, crown and root tissues of *L. perenne* and *P. annua* growing in a P deficient rootzone following six months of sequential applications.

Species	Treatment	Tissues	Mean	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
L. perenne	KCl	leaf	4086.17	15.18	4075.74	4096.60
		Crown	2831.33	13.78	2820.90	2841.76
		Root	1585.67	7.53	1575.24	1596.10
	Pi	leaf	5214.67	9.22	5204.24	5225.10
		Crown	3556.83	17.74	3546.40	3567.26
		Root	1721.67	11.48	1711.24	1732.10
	Phi	leaf	4499.50	13.25	4489.07	4509.93
		Crown	5373.67	9.71	5363.24	5384.10
		Root	2612.17	13.03	2601.74	2622.60
P. annua	KCl	leaf	4441.33	12.77	4432.02	4450.65
		Crown	3844.33	11.99	3835.02	3853.65
		Root	1493.33	10.42	1484.02	1502.65
	Pi	leaf	5440.33	8.19	5431.02	5449.65
		Crown	4748.00	13.07	4738.69	4757.32
		Root	1910.17	7.44	1900.85	1919.48
	Phi	leaf	4653.00	11.10	4643.69	4662.32
		Crown	5569.33	14.38	5560.02	5578.65
		Root	2524.33	10.78	2515.02	2533.65

Table 4-23 Two-way Anova determining significant interactions between species, tissues and treatments

	df	F	p	η2
Turfgrass species*treatment	2,90	3393.012	< .001	0.987
Turfgrass species*tissues	2,90	10395.573	< .001	0.996
Treatments*tissues	4,90	29711.542	< .001	0.999
Turfgrass species*treatments*tissues	4,90	1603.067	< .001	0.986

In *L. perenne,* there was a significant ($p < 0.05$) treatment effect on P levels in leaf, crown and root tissues, Table 4-24. P levels were significantly ($p < 0.05$) lower in all KCl (control) treated tissues, compared with the Pi and Phi treated tissues, Fig. 4-25. In leaf tissues, the highest P levels were determined following Pi treatments, with levels of 5215, 95% CI (5204, 5225) ppm, significantly ($p < 0.05$) greater than the Phi treatments of 4499, 95% CI (4489, 4509) ppm, both were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 4086, 95% CI (4076, 4096) ppm.

Table 4-24 One-way Anova of treatment effect on P levels in leaf, crown and root tissues of *L. perenne* growing in P deficient rootzones following six months of sequential treatment applications.

	df	F	p	η2
Leaf	2,15	11946.78	< .001	0.999
Crown	2,15	51565.19	< .001	1.000
Root	2,15	15616.98	< .001	1.000

In crown tissues, the highest P levels were determined in the Phi treated plants, with levels of 5373, 95% CI (5363, 5384) ppm, significantly ($p < 0.05$) greater than the Pi treatments of3557, 95% CI (3546, 3567) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 2831, 95% CI (2821, 2842) ppm. In roots, the highest P levels were also determined following Phi treatments, with levels of 2612, 95% CI (2602, 2623) ppm significantly ($p < 0.05$) greater than the Pi treatments of 1722, 95% CI (1711, 1732) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 1586, 95% CI (1575, 1596) ppm.

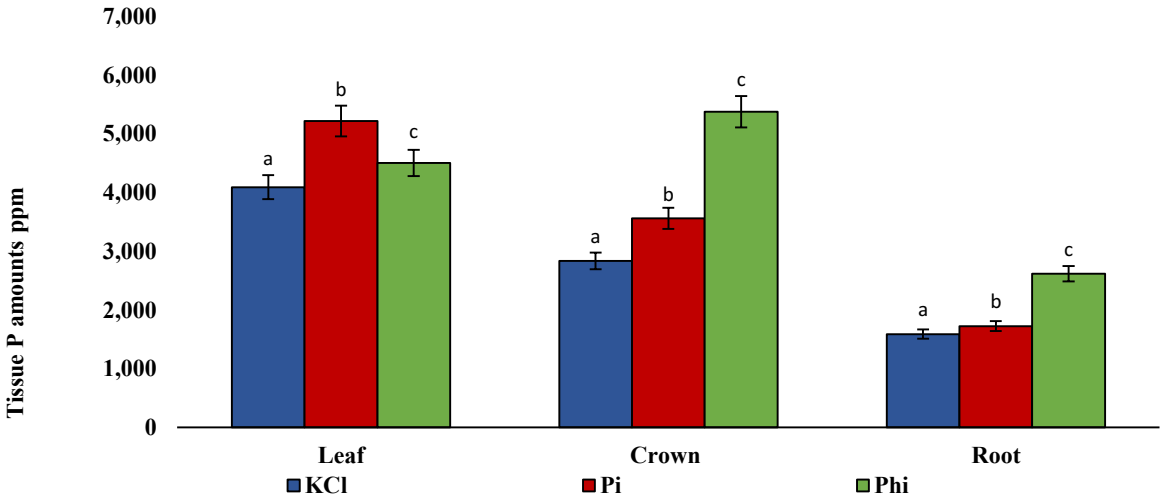


Figure 4-25 Treatment effect on P levels of *L. perenne* growing in a P deficient rootzone. Effect on leaf, crown and root P levels of *L. perenne,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at $p < 0.05$

In *P. annua,* there was also a significant ($p < 0.05$) treatment effect on P levels in leaf, crown and root tissues, Table 4-21. As in the *L. perenne* plants, P levels were significantly ($p < 0.05$) lower in all KCl (control) treated tissues, compared with the Pi and Phi treated tissues, Fig. 4-26.

Table 4-25 One-way Anova of treatment effect on P levels in leaf, crown and root tissues of *P. annua* growing in P sufficient rootzones following six months of sequential treatment applications.

	df	F	p	η2
Leaf	2,15	14117.243	< .001	0.999
Crown	2,15	25704.247	< .001	1.000
Root	2,15	17273.247	< .001	1.000

In leaf tissues, the highest P levels were determined following Pi treatments, with levels of 5440, 95% CI (5431, 5450) ppm, significantly ($p < 0.05$) greater than the Phi treatments of 4653, 95% CI (4644, 4662) ppm, both significantly ($p < 0.05$) greater than the KCl (control) treatment level of 4441, 95% CI (4432, 4451) ppm. In crown tissues, the highest P levels were determined in the Phi treated plants, with levels of 5569, 95% CI (5560, 5579) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 4748, 95% CI (4739, 4757) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 3844, 95% CI (3835, 3854) ppm. In roots, the highest P levels were in the Phi treated plants, with levels of 2524, 95% CI (2515, 2534) ppm, significantly ($p < 0.05$) greater than the Pi treatments of 1910, 95% CI (1901, 1919) ppm, both of which were significantly ($p < 0.05$) greater than the KCl (control) treatment level of 1493, 95% CI (1484, 1503) ppm.

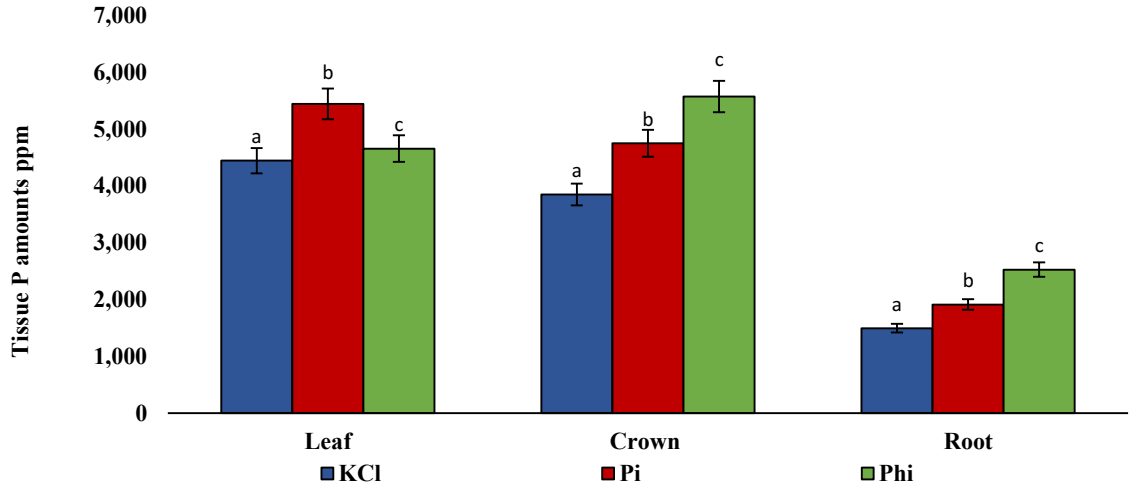


Figure 4-26 Treatment effect on P levels of *P. annua* growing in a P deficient rootzone. Effect on leaf, crown and root P levels of *P. annua,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at $p < 0.05$

4.5 Discussion

As shown in the previous chapters Phi can inhibit the *in vitro* mycelial growth of *M. nivale* and suppress disease symptoms in the field. If Phi's mode of inhibition involves the suppression of *M. nivale* hyphal growth *in planta*, it was therefore of prime importance to assess not only the take up of Phi in turfgrasses, but also the long term fate of foliar applied Phi. Prior to these analyses, there were no published data on the take up and accumulation of Phi in turfgrasses. The most relevant data on the foliar application of nutrients in turfgrasses reported on the take up and accumulation of major and minor nutrients, in particular nitrogen. These studies have shown that in turfgrasses, most nutrients are rapidly assimilated, but the speed of take up varies in correlation with nutrient compound size (Bowman and Paul, 1989; Gaussoin *et al.*, 2009; Stiegler *et al.*, 2009). Studies had been published however, showing the take up of Phi in other plant systems (Thao and Yamakawa, 2010; Borza *et al.*, 2014) and protocols had been presented for the determination of Phi accumulations in plant tissues by Saindrenan (1985) and Berkowitz *et al.* (2011) and the method adapted for these studies by Roos *et al*. (1999).

4.5.1 Phi take up in turfgrass

The HPIC analyses carried out here, produced significant and novel data. The data show that Phi, following foliar application to *A. stolonifera* and *P. annua*, is rapidly accumulated into the leaf tissues and is translocated both in the xylem and phloem, demonstrating symplastic ambimobility. The first set of treatments and analyses were carried out during February 2011, during a period of low turfgrass growth and metabolism. As this study's main focus is on the suppression of *M. nivale*, which is most active during periods of low turfgrass growth, the accumulation and persistence of Phi in turfgrass tissues during these periods, is of vital importance. Take up into the leaf tissues during February was rapid, with 60% to 79% of the maximum accumulation achieved within 6 hours of application. The level of Phi within the leaf tissues peaked at 48 hours p.a. and by 96 hours p.a. in both turfgrass species, levels had begun to decline. Over the longer study period of 0 to six week p.a., it was shown that Phi take up was indeed rapid with peak accumulations at one week p.a. and at 6 weeks p.a. had dropped to between 14% of the maximum accumulation in *A. stolonifera* and to 18% of the maximum in *P. annua* leaf tissues.

Following the first series of studies, it was theorised that take up would be more rapid during higher growth conditions, therefore a second series were carried during a period of increased growth, during July 2012. The results of this second study were similar to the first with regard to take up and translocation rates, but confirmed that Phi take up correlated positively with increased metabolic rate and that Phi tissue amounts decreased more rapidly during these periods of higher turfgrass growth rates These data are of particular significance to turfgrass managers, who implement maintenance programs, applying Phi on a 2 to 3 week cycle. The results here would indicate that during periods of high *M. nivale* disease pressure, this cycle of sequential applications would maintain Phi levels in the leaf within the range of 3000 to 3500 ppm throughout the term of the programme.

4.5.2 Phi accumulation following sequential treatments

Phi treatment as shown, gave rise to rapid take up and accumulations in all turfgrass tissues, but the fate and persistence of foliar applied Phi, following long term applications needed to be addressed. Tissue Phi levels for *A. stolonifera* and *P. annua* at 6, 12, and 24 months following the start of treatment applications were determined using HPIC analyses, with the accumulation of Phi in the different tissue types producing novel and significant results. There were significant interaction effects on Phi accumulations between turfgrass species, tissues, and months during this study. This shows there were differences in Phi take up not only between tissue types but also between turfgrass species and that take up was also significantly affected by potential growth as there were significant differences throughout the year. Over two years of sequential Phi applications, turfgrass tissues displayed a steady accumulation of Phi in meristematic regions. Phi in leaf tissue remained at constant levels, varying only with time p.a. and the metabolic rate as governed by seasonal growth rates. Sequential applications over 2 years, gave no indication of a systemic buildup of Phi in leaf tissues. This does not infer that Phi is metabolised or de-graded biochemically, but rather is physically removed, as part of the on-going mowing regime, typical of amenity turf maintenance.

Analyses of root tissues, however, produced significant results, Phi, following take up via leaf tissues, showed almost immediate translocation to the root systems of both turfgrass species. The data here show that following treatment application Phi was detected in root tissues at one hour p.a. in both turfgrass species, Figs. 4-4 and 4-5, and remained detectable throughout the six week trial period, Figs 4-6 and 4-7. This is an important point, as no other compound used for pathogen suppression in turfgrasses demonstrates symplastic ambimobility. Furthermore, sequential applications of Phi to *A. stolonifera*, gave rise to significantly ($p < 0.05$) increasing levels of accumulation, as shown in Fig. 4-13. This was also the case, but to a lesser extent, with *P. annua,* Fig. 4-14. This lower

accumulation of Phi following long term applications could be due to the shorter lifespan of *P.annua,* compared to the perennial *A. stolonifera,* with the root systems senescing more rapidly in *P.annua.* This is evidenced further by the increased levels of P found in the rootzones of *P. annua* compared to *A. stolonifera,* Table 4-8. The senescence of any turfgrass tissues which contained Phi accumulations, would give rise, over time, to increased levels of soil P. This would also be the case, although to a lesser extent, with leaf tissue, which although in the case of golf greens are collected during mowing, would eventually contribute to increased soil P content.

The increasing cumulative Phi accumulations in roots indicate a source–sink relationship, with Phi translocated to meristematic tissues undergoing rapid growth, which in turfgrasses are roots and crowns. Further evidence for this is shown in Figs. 4-23, 4-24, 4-25 and 4-26, where, following 6 months of sequential Phi applications, P levels in *L. perenne* and *P.annua*, growing in either P sufficient or P deficient rootzones, displayed greater P accumulations in crown and root tissues, than those which received Pi and KCl treatments. These data are in agreement with previously published research by Saindrenan *et al*. (1988) and who determined that Phi is translocated through the plant in association with photoassimilates and Whiley *et al*. (1995), who concluded Phi concentrations are thought to be higher in tissues of the plant undergoing rapid growth.

4.5.3 Soil P accumulations

The effect of long term sequential application of Phi, on rootzone soil was an important factor in these studies. The use of P containing fertilisers is a contentious issue worldwide, with some regions allowing P applications, subject only to confirmation of P deficiency via soil test analyses. Over two years of sequential treatments, the Phi and Pi applications supplied equivalent amounts of P, however, soil P levels in rootzones of both turfgrass species receiving Phi increased by an average 50%, from a base level of 37 ppm to 51 ppm for *A. stolonifera* and 57 ppm for *P.annua,* Table 4-8. Over the same period, soil P levels following sequential Pi applications increased by 10% from 37 ppm to 40 ppm for *A.stolonifera* and to 44 ppm for *P.annua*. This significantly ($p < 0.05$) higher level of rootzone P following Phi treatments is important. It could be due to Phi being locked into the rhizosphere by soil micro-organisms. Oxidation of Phi to Pi in soil relies on microbial activity, requiring the absorption and take up of Phi by soil bacteria and subsequent oxidisation to Pi, this however, is a slow process with a half-life of several months (Mcdonald *et al.*, 2001). P in the rootzone following Pi treatment would be less

persistent and more easily leached, bearing in mind the C.E.C. status of these rootzones are extremely low with values of on average of 8.0 meq/100g, Table 4-5.

4.5.4 Phi to Pi conversion

Determination of PO_4^{3-} levels following Phi treatment was an interesting part of this study, as the question of *in planta* conversion of PO_3^{3-} to PO_4^{3-} is often raised, with numerous suppliers claiming Phi as a source of P nutrition following *in planta* conversion of Phi plant usable Pi. The results here were conclusive, the level of Pi in leaf and root tissues were determined as part of the HPIC analyses with some significant ($p < 0.05$) differences in Pi tissue levels between the start and conclusions of the studies.

In *A. stolonifera*, leaf tissues, Fig. 4-11, Pi levels decreased over the six week trial periods of February 2011 and July 2012, significantly ($p < 0.05$) so in February, a clear indication that the application of Phi did not affect the mean level of Pi in any of the turfgrass tissues. In the *P. annua* trial, Fig. 4-12, the data were similar, with no significant ($p < 0.05$) increase in Pi levels in all tissues, with the exception of the leaf tissues during the February 2011 study. What can be concluded from both studies is that the application of Phi does not lead to *in planta* conversion to Pi.

4.5.5 Phi as nutrient source and effects on growth

As previously stated the main focus of this research is the suppression of *M. nivale* infection in turfgrasses and this research into Phi as a turf nutrient may seem out of place. However, as well as inhibiting pathogen challenge by direct fungistatic means, it is suggested that Phi suppresses pathogens via enhancement of innate plant defence mechanisms. Therefore the health status is vital in its ability to synthesis plant defence compounds. This study determined significant differences in growth response following Phi treatment, in both the P deficient and P sufficient rootzones. Studies researching the value of Phi as a supplier of P are reported in the review of literature in Chapter 0ne. What is clear, is that there is much debate regarding the value of Phi as a source of P nutrition. There are reports of both beneficial and detrimental effects on plant growth following Phi treatment, however, data on the means as to how growth enhancement came about are limited.

4.5.5.1 P deficient rootzones

It was expected prior to the start of these studies, that in P deficient rootzones, Phi treatment would inhibit growth. One reason being, that as Phi competes with Pi for uptake via the same plant transport system, (Carswell *et al.*, 1996; Danova-Alt *et al.*, 2008), this would lead to a reduction of usable P, leading to further Pi depletion. The results confirmed that Phi did inhibit growth, as shown in Figs. 4-19 and 4-20. The data show that Phi treatment led to less plant biomass in both turfgrass species. That the KCl treatment gave rise to increased growth compared to the Phi treatment is evidence that Phi not only did not provide a useable form of P, but also suppressed the P deficiency response in both species. These results agree with the findings of Ticconi *et al.* (2001), who concluded that Phi inhibited P deficiency compensatory responses in *Arabidopsis thaliana* and Fabricio *et al.* (2012), who determined foliar-applied Phi caused harmful effects to plants, growing in P-limited soils. The conclusion that Phi suppressed deficiency responses is further supported by the results of the root dry weights Figs. 4-19 and 4-20 and the root to shoot ratios Figs. 4-21 and 4-22. Varadarajan (2002) determined that Phi suppressed many of the definitive responses to P limitation, such as enhanced root growth and increased root to shoot ratios. The results here show that while there were significant ($p < 0.05$) differences in the root mass and root to shoot ratios between the KCl and Pi treatments, there was significantly ($p < 0.05$) less root growth and reduced ratios in the Phi treated plants, compared to both other treatments.

4.5.5.2 P sufficient rootzones

The results of the effects on growth from Phi treatments in P sufficient rootzones were surprising. Phi treatment significantly ($p < 0.05$) increased leaf, crown and root biomass, compared with Pi and KCl treated plants, Figs. 4-17 and 4-18. There is no evidence in the literature to support the metabolisation of Phi or it's *in planta* conversion to a plant useable form of P, this is evident also from the results of Phi treatment in the P deficient rootzones. Improved turfgrass quality, however, following sequential applications of Phi were determined in the previous chapter and also in published research (Vincelli and Dixon, 2005; Horvath *et al.*, 2007; Dempsey and Owen, 2010). Research with plant systems other than turfgrass also reported enhanced growth responses following Phi treatment (Lovatt, 1990b; Albrigo, 1999; Rickard, 2000), the reasons for the enhanced growth responses however, are not explained. Lovatt and Mikkelsen (2006) suggest Phi enhanced growth may be a growth-regulatory or phytohormonal factor, effecting sugar

metabolism, stimulation of the shikimic acid pathway, or internal hormonal and chemical changes. More recently, Zhang *et al*. (2011) concluded that while *Microcystis aeruginosa* could not utilise Phi as a sole P nutrient at any concentration, Phi, when supplied simultaneously with Pi increased cell numbers and chlorophyll content.

Root growth and development is a crucial component of all plants, but can be especially so for turfgrass, which in golf greens is maintained under highly stressed situations. Root development can determine how the turfgrass plant reacts in situations which can seriously impact on the viability and even survival of the plant. Abiotic and biotic challenges, such as drought, traffic related wear and disease pressure are constantly stressing the plants and a well-developed root system can often be the major influencing factor in the turfgrass plants success. When the root to shoot ratios were calculated, Figs. 4-21 and 4-22, it was shown that in a P sufficient rootzone, Phi treatments produced the lowest mean ratio of roots to shoots, 0.50 for *L. perenne* and 0.41 for *P.annua*, the Pi ratios were 0.53 and 0.44 with the KCl ratios 0.61 and 0.54 for *L. perenne* and *P.annua* respectively. These ratios are a direct indication of the number of roots per shoot, with the higher ratios determine the greater volume of root growth per plant. What this indicates is that while Phi treatment gave rise to increased amount of above ground biomass, it was at the expense of the development of the root systems. These results are consistent with the research by Carswell, *et al.* (1996) which included evaluations of the effect of Phi and Pi on plant nutrition and concluded that root to shoot ratios of P limited plants were typically high and that Phi treatments to these plants decreased the root to shoot ratios significantly.

Despite producing some novel and significant data, this section of the research also gave rise to a number of issues which require further study. In particular, the effects on tissue and soil accumulations following continuous sequential applications of Phi. It was shown here that meristematic tissues displayed increased accumulations over time, how this could affect plant growth and development, such as in the indicated reduction in root to shoot ratios is one area of interest. A second important factor which arose from this study, was the effect on the increasing rootzone P levels, following sequential Phi applications. Research over a longer time frame than that in this study could assess these issues, using a more disparate range of turfgrasses, growing in rootzones with varying physical and chemical properties.

4.6 Conclusions

This study determined that:

- Phi is rapidly taken up and translocated by turfgrass; and that sequential applications applied on a 3 week cycle would maintain leaf tissue accumulations of approximately 3000 ppm.
- Long-term sequential Phi treatments maintain leaf tissue accumulations, but can lead to cumulative increases in meristematic tissues and can cause increases in soil P levels.
- In P sufficient rootzones foliar-applied Phi increased biomass in all plants, but also led to a reduction in root to shoot ratios.
- In P deficient rootzones foliar-applied Phi does not supply a usable form of P and furthermore deficiency responses were repressed.

5. *M. nivale* infection and defence responses in turfgrass

5.1 Introduction

In cool season amenity turfgrasses, *M. nivale* is regarded as the most important pathogen of temperate climates, (Smiley *et al.*, 1992; Beard and Oshikazu, 1997; Mann, 2002a; Vargas, 2005). Disease symptoms have been well described, as causing small circular spots one to two cm in diameter, orange / brown in colour, which can increase to form large irregular shapes greater than 20 cm across. There are however, few detailed data on the infection process in turfgrasses, with no definitive opinion on the source of inoculum or infection process in the literature. Suggested sources of inoculum include mycelia, conidia or ascospores, the most commonly suggested means of infection being via conidia and mycelia, disseminated from infested soil or plant debris (Mann, 2004a; Turgeon, 2005; Vargas, 2005). Studies reporting *M. nivale* infection of cereals have been published (Clement and Parry, 1998; Kang *et al.*, 2004; Dubas *et al.*, 2010; Żur *et al.*, 2011), but as with turfgrass, opinions vary as to the source of inoculum and infection process. Both Dubas *et al.* (2010), and Zur *et al.* (2011) in research with triticale and Secale cereale (rye), observed hyphal growth beginning at soil level, before proceeding to infect plants via stomatal penetration. Jewell and Hsiang (2013) studying *M. nivale* infection in *Poa pratensis,* (Kentucky bluegrass), determined the pathogen colonised and penetrated the leaf tissues via the stomata.

Plants respond to pathogen challenge with a complex array of induced defences and interconnected signaling pathways, which combine to combat the invading micro-organisms, these are described in detail in Chapter one. Two important responses are studied here, synthesis and accumulation of total phenolic compounds (TPC) and Hydrogen peroxide (H_2O_2) synthesis. TPC accumulation upon pathogen challenge has been shown to be an important defence response in gramineae, Pociecha *et al.* (2009) concluded that increased levels of phenolic compounds gave rise to higher resistance to *M. nivale* in *Festulolium* spp. Dubas *et al.* (2010), observed not only phenolic compound accumulation, but also H_2O_2 accumulations in tritacle, in close proximity to the infection sites following *M. nivale* infection. H_2O_2 plays a major role in plant defence, having direct

antimicrobial properties, and, as a component of the Hypersensitive Response, is part of a rapid, localised, transient, oxidative burst, directly impairing the pathogen. The timely production of H_2O_2 and its accumulation in cells, is useful in determining the efficacy of a plants response to pathogen challenge. Huckelhoven *et al.* (1999) for example, determined that H_2O_2 accumulated in barley leaves at powdery mildew infection sites. It has been shown that Phi treatment can influence defence activation. Following Phi treatment and inoculation with *P. cinnamomi*, Eshraghi *et al*. (2011) concluded that *A. thaliana,* exhibited increased levels of H_2O_2 production, with significant differences evident between the amount of H_2O_2 production between the Phi-treated and non-Phi-treated plants.

5.2 Aims and objectives

The aims of this section of the research, were to determine the process of infection by *M. nivale* in cool-season turfgrasses and to assess initial defence reactions, specifically total phenolic compounds and hydrogen peroxide, as induced defence responses with the aim to determine the effect Phi treatment has on these responses.

Objectives were to:

- Describe the infection processes of *M. nivale* in turfgrasses, identify the source of inoculum, describe the course of mycelial growth and host penetration.
- Assessment of initial defence responses, specifically total phenolic compounds and H_2O_2, as induced defence responses.
- Determine whether Phi can enhance these defence responses either prior to and/or during infection.

5.3 Materials and methods

Three distinct experiments were carried out. Experiment 1 studied the infection process of *M. nivale* in two turfgrass species under field and greenhouse conditions. Experiment 2 assessed the accumulation of phenolic compounds in *M. nivale* infected and un-infected turfgrasses and determined the effect of Phi treatment on these accumulations. Experiment 3 assessed the effect Phi treatment had on hydrogen peroxide accumulation in two species of *M. nivale i*nfected and un-infected turfgrass

5.3.1 Plant material and growth environments

Turfgrasses from trial plots and from the golf greens of the Royal Curragh Golf Course, Co Kildare, Ireland as described in 3.3.1 and 3.3.2, provided tissue samples grown under natural environmental conditions, while controlled environment turfgrass tissues were obtained from greenhouse pot samples.

5.3.1.1 Turfgrass field samples

M. nivale infected leaf, sheath and crown tissues of *Agrostis stolonifera* and *P. annua* were collected over a four year period from infected and non-infected areas on the golf greens and the field trial plots, Figs. 5-1 and 5-2. Tissues were collected using a forceps and placed in sealable plastic bags, if not analysed within a short time period they were maintained at -20^0 C for later use. Infected turf plugs, 100 mm in diameter and 75 mm deep, Fig. 5-3, were collected using a standard hole-cutting tool, these provided not only plant tissue, but thatch layer and rootzone samples for analysis. The non-infected turfgrass samples were used to provide control samples for comparisons.

Figure 5-1 *M. nivale* infected golf green. *M. nivale* infected golf green showing typical radial infection centres.

Figure 5-2 Sources of *M. nivale* infected turfgrass. *M. nivale* infected green and trial plots which provided a source of inoculum. A: infection centre on golf green. B: infected trial plots.

Figure 5-3 *M. nivale* infected turfgrass plugs. *M. nivale* infected turfgrass plugs used to provide tissue, thatch layer and rootzone samples for analyses.

5.3.1.2 Turfgrass greenhouse samples

Two turfgrass species were used during this study, *Agrostis stolonifera* L, variety Shark, and *Poa annua reptans* L, variety Truputt, established and maintained in greenhouses as in 4.3.1, Fig. 5-4. These provided an ideal method to study not only *M. nivale* tissue infection, but also a means to track the infection process over a specific time period. As the pathogens growth radiated outward from a central infection point it was possible to study the infection timing process by examining the tissues from the outer area and working inwards to the earlier infection sites. As above, un-infected turfgrass samples were used to provide control samples for comparison.

Figure 5-4 Examples of turfgrasses used for the research. The range of turfgrasses and growth vessels used during the study.

5.3.2 *M. nivale* infection and sources of inoculum

The field samples used were allowed to become infected under natural conditions with tissues collected directly from either the trial plots or golf greens. Greenhouse samples were inoculated using either hyphal or wheat bran inoculum.

5.3.2.1 Hyphal inoculum

Hyphal inoculum was prepared by sub culturing the *M. nivale* isolates, obtained and stored as in 2.4, on PDA (19g/l^{-1}) at 20° C for five days prior to inoculation. PDA/fungal combinations were removed from the plates, placed in a glass vessel and blended with 150 ml SDW. Inoculation of turfgrass pot samples was carried out by applying 5 ml of the hyphal suspension to previously wetted leaf surfaces. Inoculated samples were maintained at high levels of relative humidity. Non-inoculated controls were prepared by spraying the leaves with SDW. A second hyphal inoculation method used a 2 mm plug of PDA, cut from the actively-growing edge of a colony and placing mycelial side down onto individual blades of turfgrass, removing after 24 hours, Fig. 5-5.

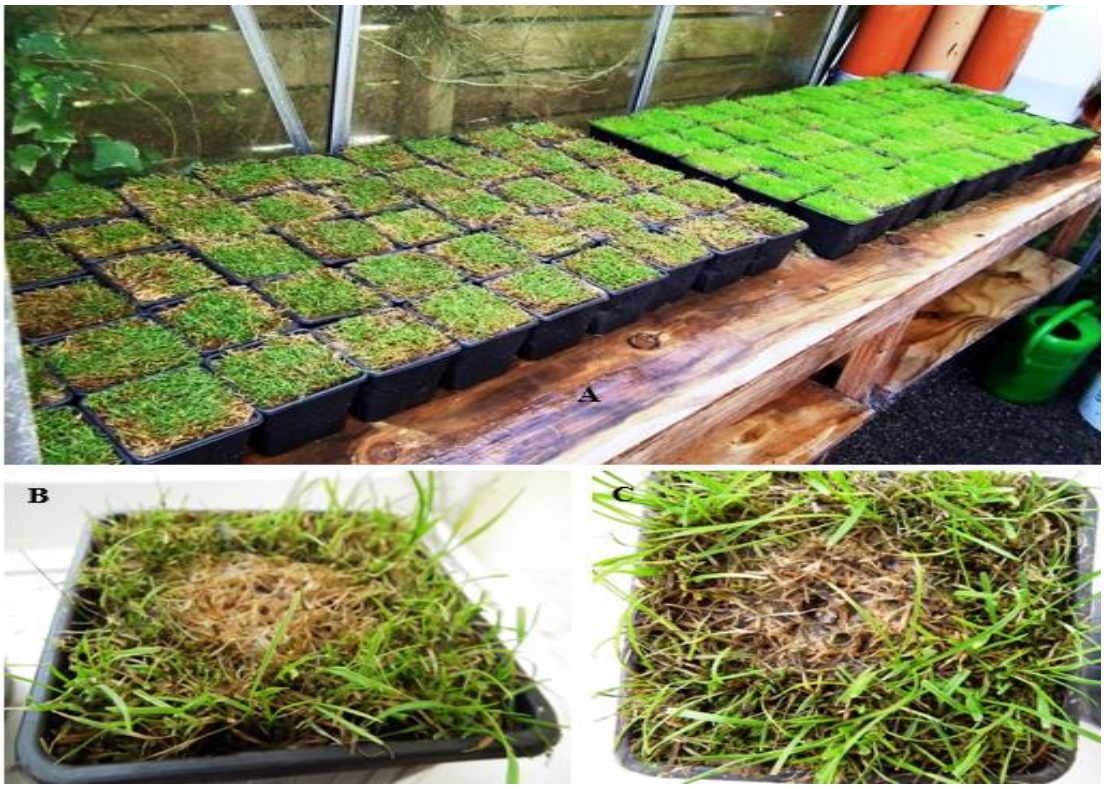


Figure 5-5 Greens house turfgrass samples. Examples of turfgrasses maintained in greenhouses which were used to provide tissue samples for analysis. A *L. perenne* and *P. annua* pots, B and C: *A. stolonifera* pots showing infection centres.

5.3.2.2 Wheat bran inoculum

Wheat bran inoculum was prepared by placing 5 mm plugs of PDA, cut from the actively-growing edge of a colony, and placing into autoclaved wheat bran in 9 cm petri dishes maintained at 20° C, Fig. 5-6. The bran was stirred daily until mycelium had grown through the bran flakes. After colonisation the bran was macerated and inoculation carried out by adding 1 g of the inoculum to the centre of a turfgrass pot. Control pots were prepared by inoculating with 1 g sterilised, macerated wheat bran. The inoculated and non-inoculated control pots were maintained at high levels of relative humidity.

Figure 5-6 Wheat bran inoculum. Autoclaved wheat bran in 9 cm petri dish, infected with *M. nivale,* which was macerated and used as a source of inoculum.

5.3.3 Evaluations and assessments

5.3.3.1 Experiment 1: Evaluation of infection process in turfgrass

Visual evaluation of the infection process in both field and greenhouse turfgrass samples was carried out using a series of microscopic analyses techniques. Hyphal fragment and was assessed on its ability to cause infection. Key defence responses in infected plants were also evaluated.

5.3.3.2 Light and fluorescence microscopy

All light and fluorescence microscopy observations were performed using a Bresser L3001 epifluorescent microscope. Light microscopy examined tissues at 100x and 400x magnification. For removal of chlorophyll prior to some microscopy studies, tissues were placed in 95% ethanol for 24 hours, the ethanol was removed and replaced with fresh ethanol for a further 24 hours. The turfgrass tissues were then removed and rinsed with SDW, tissues were mounted on glass slides with cover slips for examination.

Fluorescence microscopy was used for the visualisation of *M. nivale* infection structures, two fluorescent indicator dyes were used. Calcofluor (stock solution at 1% w/v in H_2O)was used at a concentration of 0.01% for 5 min.(Żur *et al.*, 2011)and Aniline Blue (stock solution at 0.5% w/v in H_2O was used at a concentration of 0.05%, pH 8.2 for 5 minutes (Żur *et al.*, 2011).Stained fragments of leaves, leaf sheaths, crowns, and roots were analysed on glass slides, under UV fluorescence (excitation 365 nm, dichroic mirror 395 nm. Thatch and rootzone samples were washed in SDW and agitated prior to examination.

5.3.3.3 Determination of total phenolic compounds

Total phenolic compounds (TPC were assessed by modified extraction methods as described by (Singleton *et al.*, 1965; Pociecha *et al.*, 2009; Żur *et al.*, 2011).Turfgrass tissues were collected and dried for 48 hours at 50° C. Samples were ground and 0.5g boiled in 1 ml 80% ethanol, 4 ml 80% ethanol was added and left to extract for 24 hours. The extract solution was filtered and centrifuged for 20 min at 1500 g. 20 µl was pipetted into separate 20 ml containers, 1.58 ml SDW and 100 µl of Folin-Ciocalteu reagent was added, then vortexed. The solution was left for 8 min, and 20% sodium carbonate (Na_2CO_3 solution was added and vortexed. The solutions were incubated at 20° C for 2 hours. Absorbances were read using a Cecil CE 373 spectrophometer at 765nm, and

compared with a Gallic acid standard curve. Total phenolic concentration was calculated and reported as mg/g dry weight of Gallic acid equivalents (GAE.

5.3.3.4 Determination of H_2O_2

Determination of H_2O_2 levels in turfgrass tissues were carried out by forming a titanium hydroperoxide complex, as described by Dagmar *et al*. (2001. Tissue samples (0.2 g were homogenised in liquid nitrogen, ground with 5 ml cooled acetone and the homogenate centrifuged at 6,000 g for 10 minutes. The supernatant (1 ml was put on ice and combined with 0.1 ml 5% titanium oxysulfate and 0.2 ml ammonia. The reaction mixture was centrifuged at 10,000 g for 10 min. The supernatant was discarded, and the precipitate dissolved in 5 ml 2 mM H_2SO_4. The absorbance of the resulting solution was read at 415 nm and H_2O_2 content determined using a standard curve plotted with known quantities of H_2O_2.(Wang *et al.*, 2010.

5.3.3.5 Visualisation of H_2O_2

A fluorescent stain, 3,5,3′,5′-tetramethylbenzidine-HCl (TMB, was used to visualise H_2O_2 accumulations in plant tissues, as described by Barcelo (1998. Tissues were examined by immersing in solutions containing TMB solution (0.1 mg ml^{-1} in Tris-acetate, pH 5.0 until a blue colour was observed. Assessments were performed using the Bresser L3001 fluorescence microscope.

5.3.3.6 Experiment 2: Effect of Phi on TPC in infected and un-infected turfgrass

Determination of TPC levels in *M. nivale* infected and un-infected turfgrasses was carried out by collecting tissue samples of *P. annua* and *A. stolonifera,* from field trial plots and greenhouse pot samples, over a three year period and analysing for TPC concentrations. TPC levels were determined and accumulations in infected and non-infected tissues compared.

To assess if Phi treatment stimulated increases in TPC levels in un-infected turfgrasses, tissues were analysed and compared to Pi treated and un-treated controls. Phi and Pi were applied at the standard labeled rate of 0.35g/m^{2-} of PO_3^{3-} and PO_4^{3}, Applications were made using 20 l knapsack sprayers fitted with flat fan nozzles delivering a fine spray operating at 4 bar, calibrated to deliver 300 l/ha. leaf tissues were collected from field trial plots and greenhouse pot samples of *P.annua* and *A. stolonifera*, at 0, 1, 6, 24, 48 and 72 hours post application (hpa), SDW was used as controls. The effects of Phi treatment on TPC following a single application and following sequential treatments, applied at four week intervals, over a six month period were determined.

To assess if Phi treatment stimulated increases in TPC levels in infected turfgrasses, greenhouse pot samples of *P. annua* and *A. stolonifera*, were treated with a single application and sequential applications (applied at four week intervals, over a six month period) using 20 l knapsack sprayers fitted with flat fan nozzles delivering a fine spray operating at 4 bar, calibrated to deliver 300 l/ha. Phi and Pi at 0.35g/m^{2-} of PO_3^{3-} and PO_4^3 and SDW (control) were applied. Following infection with *M. nivale* hyphal inoculum, infection diameters were measured and TPC accumulations were determined over 10 days post inoculation (dpi).

5.3.3.7 Experiment 3: Effect of Phi on H_2O_2 generation infected and un-infected turfgrass

To assess if Phi treatment stimulated increases in H_2O_2 in un-infected turfgrasses, tissues were analysed and compared to Pi treated and un-treated controls. Following Phi and Pi treatment as in 5.3.3.6, H_2O_2 concentrations were determined in tissues collected from greenhouse pot samples of *P.annua* and *A. stolonifera*, at 0, 1, 6, 24, 48 and 72 hpa. Greenhouse pot samples of *P. annua* and *A. stolonifera*, were treated with a single application and sequential applications (applied at four week intervals, over a six month period) of Phi and Pi at 0.35g/m^{2-} of PO_3^{3-} and PO_4^3 and SDW (control). Following infection with *M. nivale* hyphal inoculum, H_2O_2 concentrations in sampled leaves were determined over 10 days post inoculation (dpi).All chemicals purchased from Lennox laboratory Supplies, Dublin. Images were acquired with a Canon D1100 and processed using software programs including Photoshop and Corel Paintshop Pro X3.

5.4 Data analysis

All analyses carried out using SPSS 22.0. Descriptive statistics were calculated and presented for all data. Two-way Anova were used to determined statistical differences and interactions, using dependent variables of TPC and H_2O_2 levels in infected and un-infected tissues, and turfgrass species and treatments as independent variables. Prior to analyses, residuals were tested to ensure the assumptions of the two-way Anova were met. Outliers were assessed by inspection of a boxplots, Shapiro-Wilk's test determined normality, and homogeneity of variances assessed by Levene's test. Where statistically significant interactions were observed an analysis of simple main effects were performed reporting 95% confidence intervals and p-values Bonferroni-adjusted within each simple main effect. If interactions were not significant, main effects were analysed and pairwise comparisons run reporting 95% confidence intervals and Bonferroni-adjusted p-values. Additional statistical data tables are available in the document ‘Appendices to the Thesis’

5.5 Results

5.5.1 Experiment 1, *M. nivale* infection process

M. nivale infection followed a similar pattern, when observed in either the field or greenhouse environments, differing only in the source of inoculum. Microscopic analysis of tissues collected from infection patches allowed for determination of the progress of the infection process.

5.5.1.1 Field infections

M. nivale infection on the golf greens and trial plots, Fig. 5-7, developed naturally during the four year period of this study. Disease incidences began during September and continued until March, although there were also some incidences during other times of the year. Symptoms initially appeared as small circular spots, one to two cm in diameter and orange/brown in colour. If allowed to develop, they increased in size, reaching on occasion greater than 15 cm in diameter.

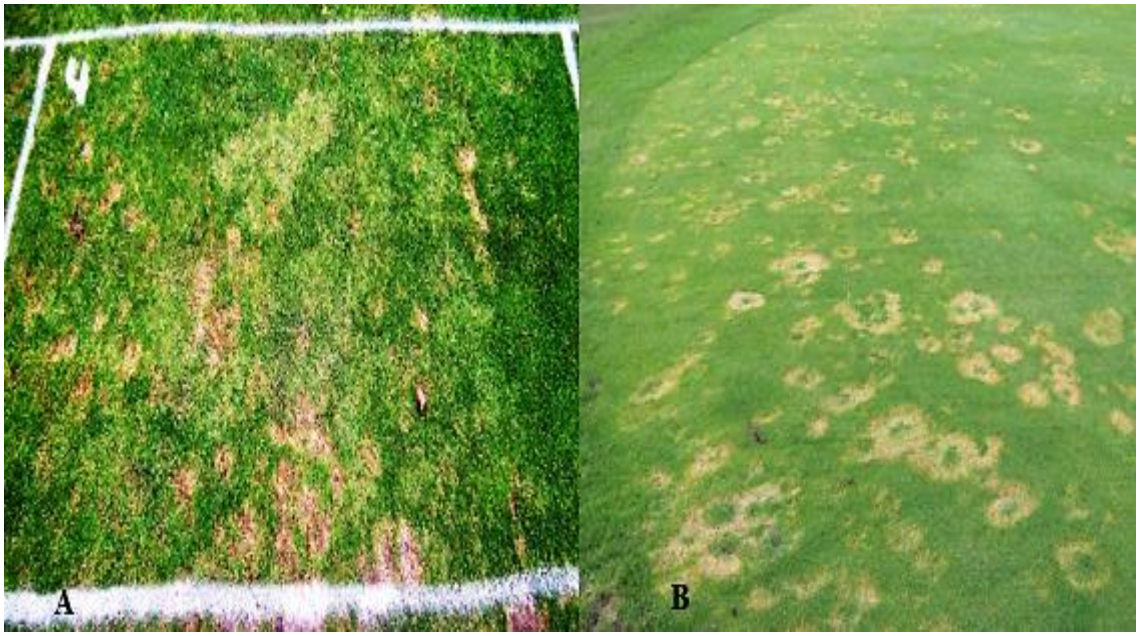


Figure 5-7 *M. nivale* infected trial plots and greens. *M. nivale* incidence as natural occurring infections observed in trial plots and golf greens. A: Trial plots. B: golf green.

Analysis of the thatch layers and the upper 5 cm from rootzones of the golf greens and trial plots showed constant levels of hyphal inoculum, Figs. 5-8 and 5-9. This was observed at all times through the year, although to a lesser extent during the period from April to September. *M. nivale* conidia were also observed, but to a much lesser extent than hyphae.

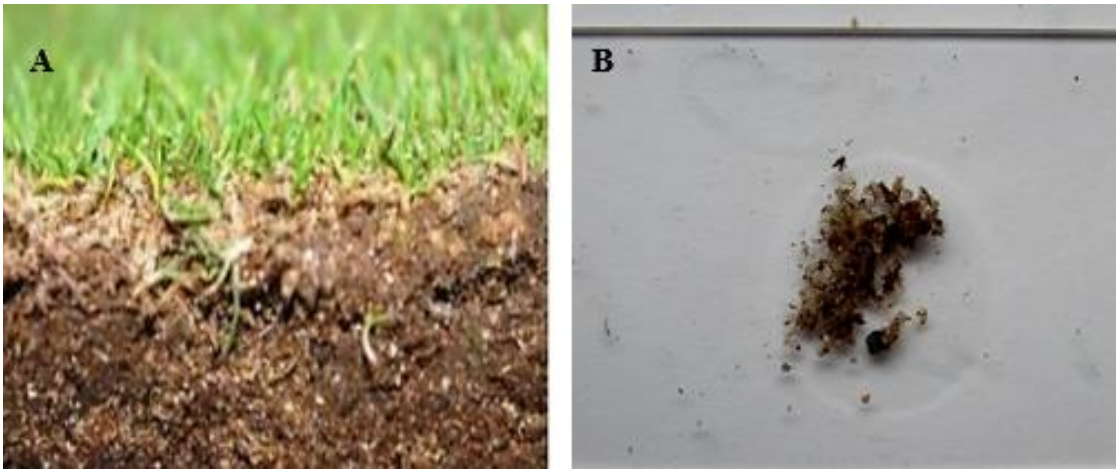


Figure 5-8 Thatch layer and golf green rootzone. Examples of A: interface of plant/thatch layer and rootzone from golf green. B: rootzone sample prior to viewing with fluorescence microscopy.

The observed hyphal levels also varied between the older golf greens (over ten years old), and more recently built sand based greens. Both hyphae and conidia remained inactive during unfavourable environmental periods, but when conditions were favourable, conidia germinated and developed hyphae. Existing hyphal mycelium within these soil/thatch layers, also began growing, Fig. 5-9, and from these, the mycelial growth emerged, growing into the crowns and then infecting the lower sheaths of the turfgrasses.

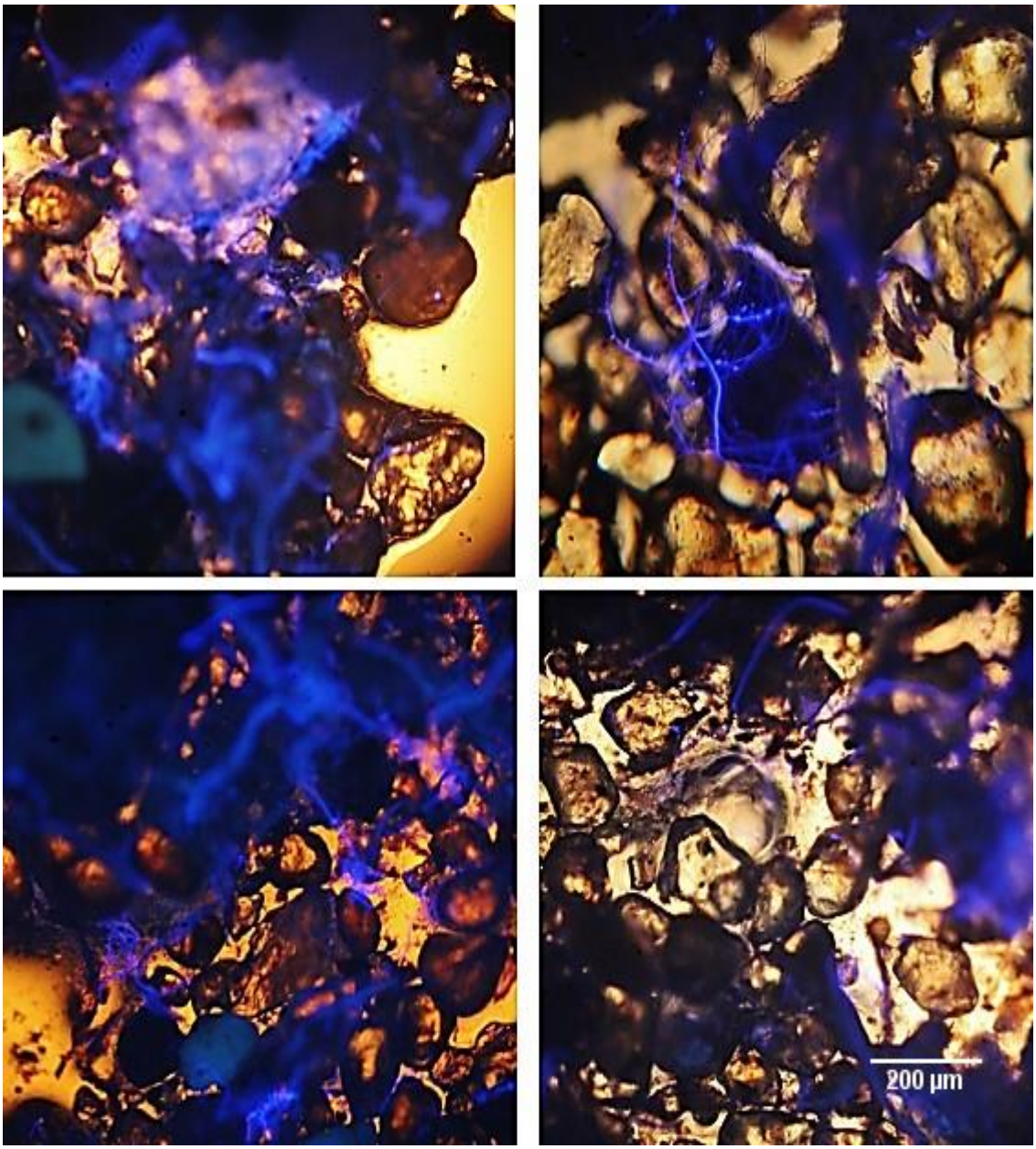


Figure 5-9 Soil samples of the upper 5 cm of a golf green. Soil samples taken from the upper 5 cm of a golf green viewed using a Bresser L3001 epifluorescent microscope, following fluorescent staining, using the indicator dyes Calcofluor and Aniline Blue. A combination of UV and bright microscopy was used to observe *M. nivale* hyphae which can be seen fluorescing, and growing through the soil particles.

5.5.1.2 Initial penetration

In the natural environment of the golf greens and trial plots, following emergence from the soil and infection of the sheaths, hyphae grew up onto the leaf surfaces, rapidly covering them in a dense mass of mycelium Fig. 5-10.

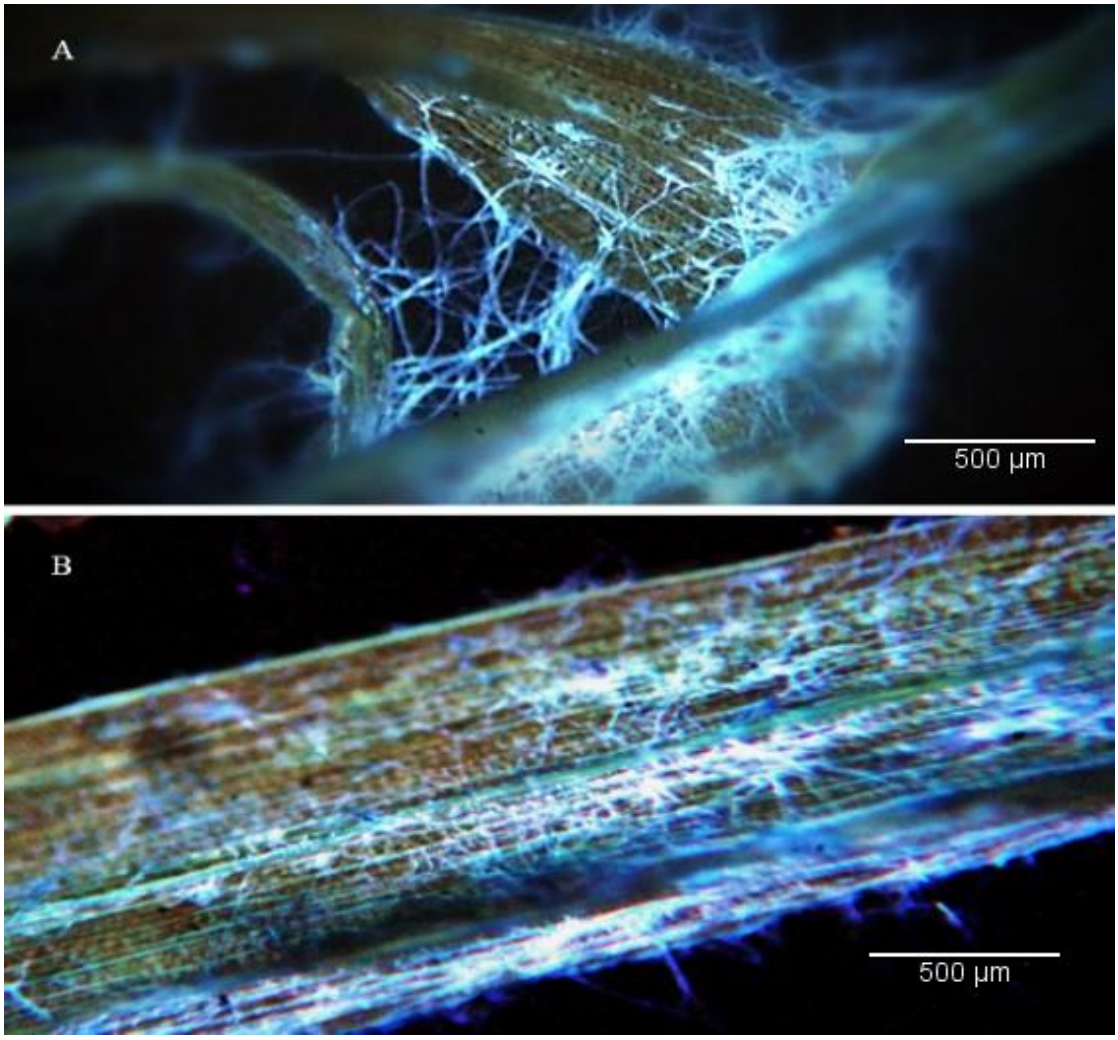


Figure 5-10 *M. nivale* hyphal growth on infected turfgrass leaves. *M. nivale* hyphae, observed using a Bresser L3001 epifluorescent microscope, following fluorescent staining, using the indicator dyes Calcofluor and Aniline Blue. Hyphae can be seen growing over the turfgrass leaves following emergence from the soil/thatch interface. A: *A. stolonifera,* B: *P.annua.*

Infection of the leaf tissues in all observed instances, occurred by the hyphae penetrating the stomata, Fig. 5-11, there was no observed incidences of formation of penetration structures.

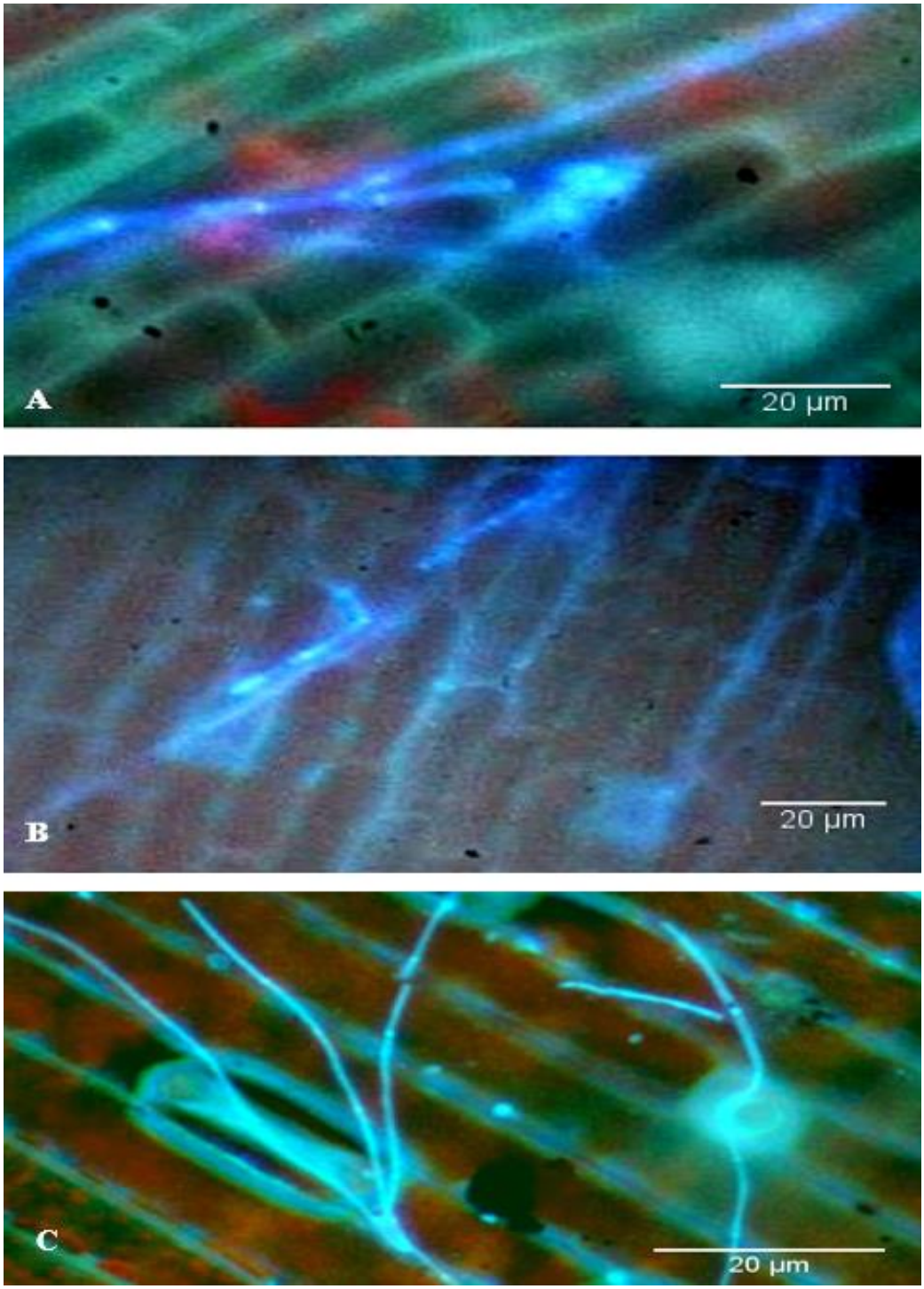


Figure 5-11 *M. nivale* hyphae entering turfgrass stomata. *M. nivale* hyphae observed under UV fluorescence, using a Bresser L3001 epifluorescent microscope, following fluorescent staining, using the indicator dyes Calcofluor and Aniline Blue. A, B and C: hyphal growth on leaf entering stomata.

5.5.1.3 Greenhouse infections

5.5.1.4 Hyphal inoculum

In the greenhouse studies, on plants inoculated with hyphal inoculum, by 4 dpi, mycelium could clearly be seen growing on the leaves Fig. 5-12. The hyphae grew from the point of inoculation, and could be observed spreading across the leaves Fig. 5-13. In pots inoculated with infested wheat bran, hyphae extended outwards from the inoculation point, by 8 dpi, a dense hyphal network was observable, displaying the radial growth pattern typical of fungal infections, Fig. 5-14. Microscopic examination of the leaf sheaths and laminae also showed that numerous hyphae had penetrated through the stomata and into the intracellular spaces.

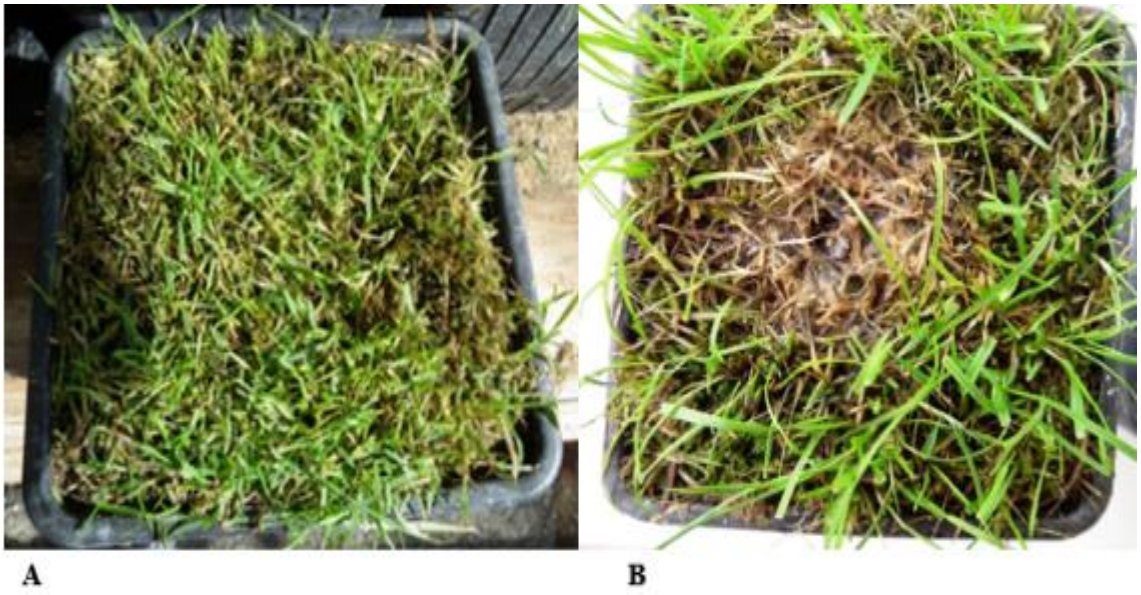


Figure 5-12 Greenhouse test pots viewed 4 dpi. Greenhouse test pots viewed 4 dpi with *M. nivale* hyphal inoculum. A: un-inoculated control. B: hyphal inoculated pot showing mycelial growth.

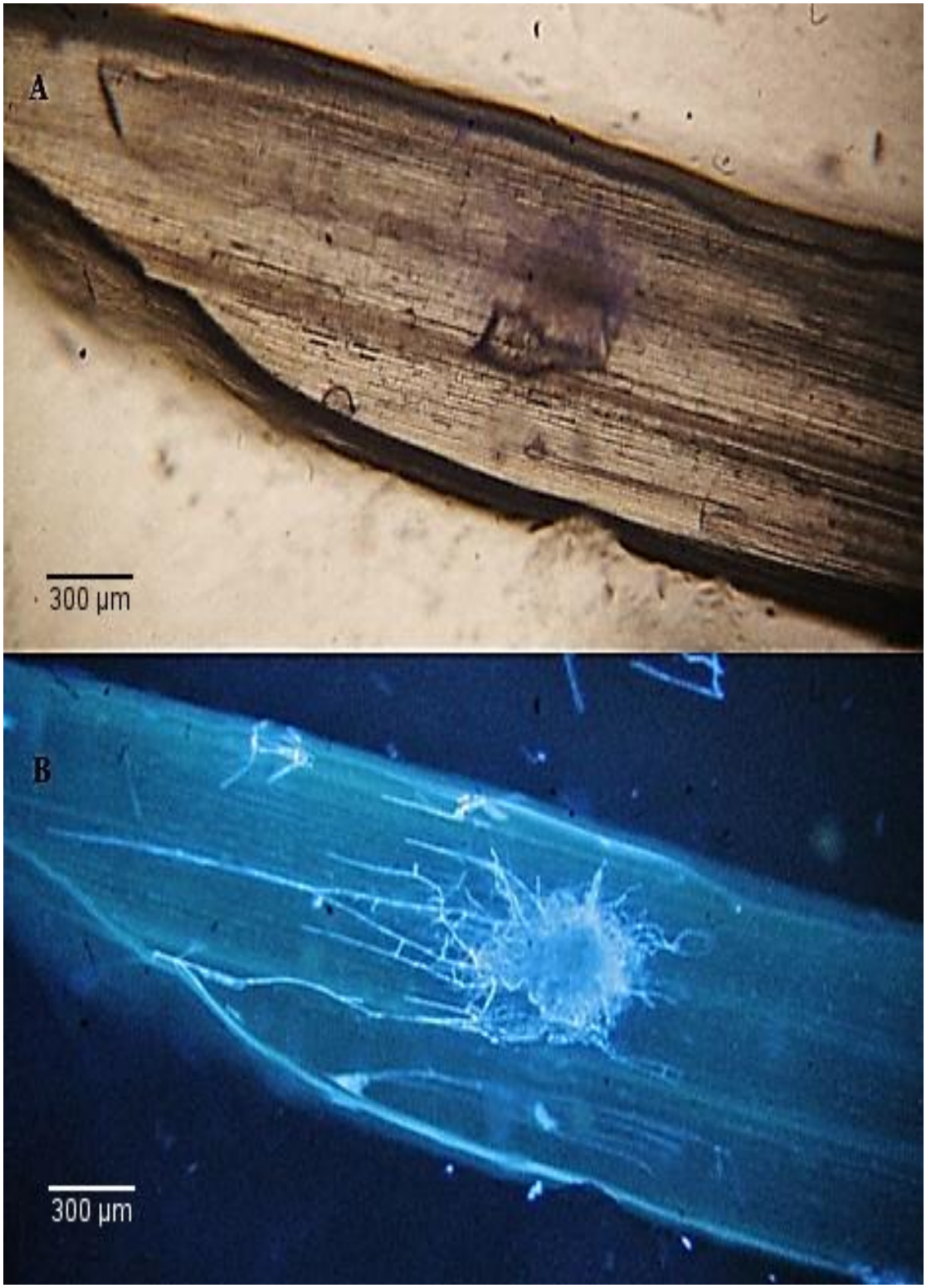


Figure 5-13 *M. nivale* hyphal growth on *P. annua*. *P. annua* leaf from greenhouse sample 4 dpi with *M. nivale* hyphal inoculum, showing inoculation centre and hyphal growth on leaf. Viewed using a Bresser L3001 epifluorescent microscope. A: viewed using light microscopy. B: viewed under UV fluorescence, following fluorescent staining, using the indicator dyes Calcofluor and Aniline Blue.

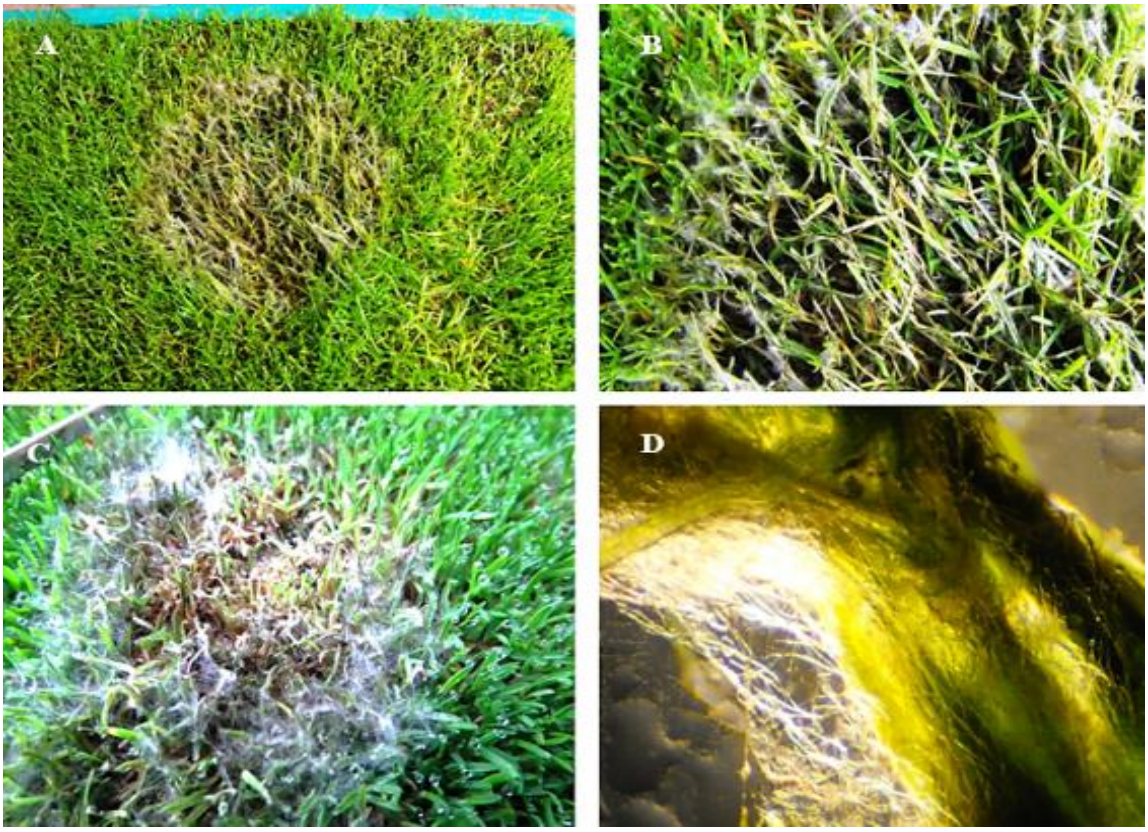


Figure 5-14 Greenhouse turfgrass samples following inoculation with *M. nivale* infested wheat bran. Greenhouse turfgrass samples 8 dpi following inoculation with *M. nivale* infested wheat bran. A: radial growth infection pattern. B: radial infection with mycelium visible. C: dense mass of mycelium on infected sample. D: hyphal growth on infected leaf.

5.5.1.5 Intracellular infection

In all cases observed, either from field or greenhouse infections, following penetration, hyphae could be observed growing through the vascular tissue and the minor veins in the leaf, Fig 5-15.

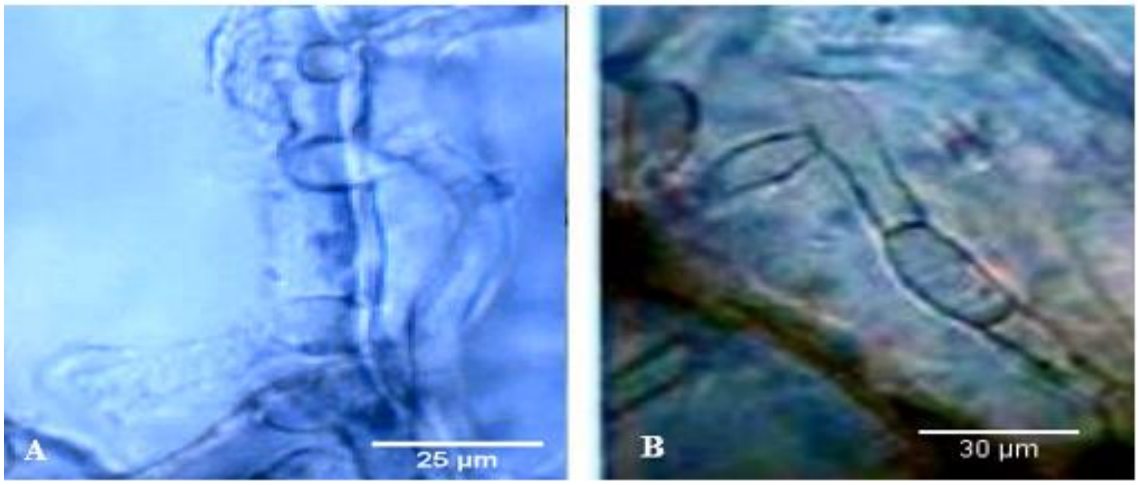


Figure 5-15 *M. nivale* hyphal growth intracellularly in turfgrass leaves. *M. nivale* hyphae observed growing through the vascular tissue and minor veins in turfgrass leaves. A: hyphae entering cell. B: hypha growing in cell.

5.5.1.6 Conidiation

Inside the turfgrass leaves, hyphae continued to extend, entering cells, causing collapse. The hyphae continued to grow, lengthening and branching, some then exited via the stomata. The cycle continued with conidiophores being formed outside the leaf, from which numerous conidia were observed being released Figs. 5-16 and 5-17.

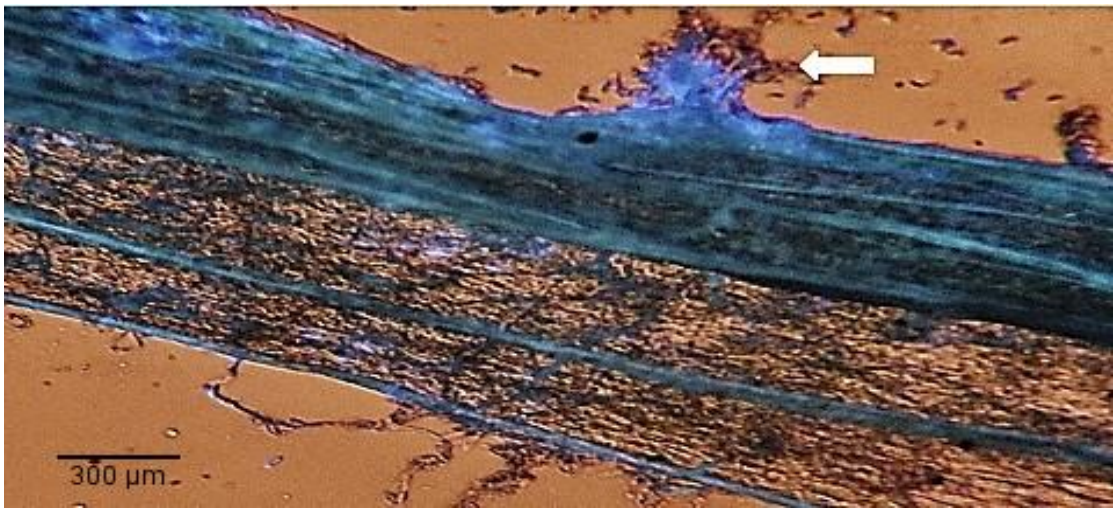


Figure 5-16 *M. nivale* infected leaf showing formation of conidiophore. *M. nivale* infected *A. stolonifera* leaf showing formation of conidiophore and the reproductive spores, conidia, indicated with arrow.

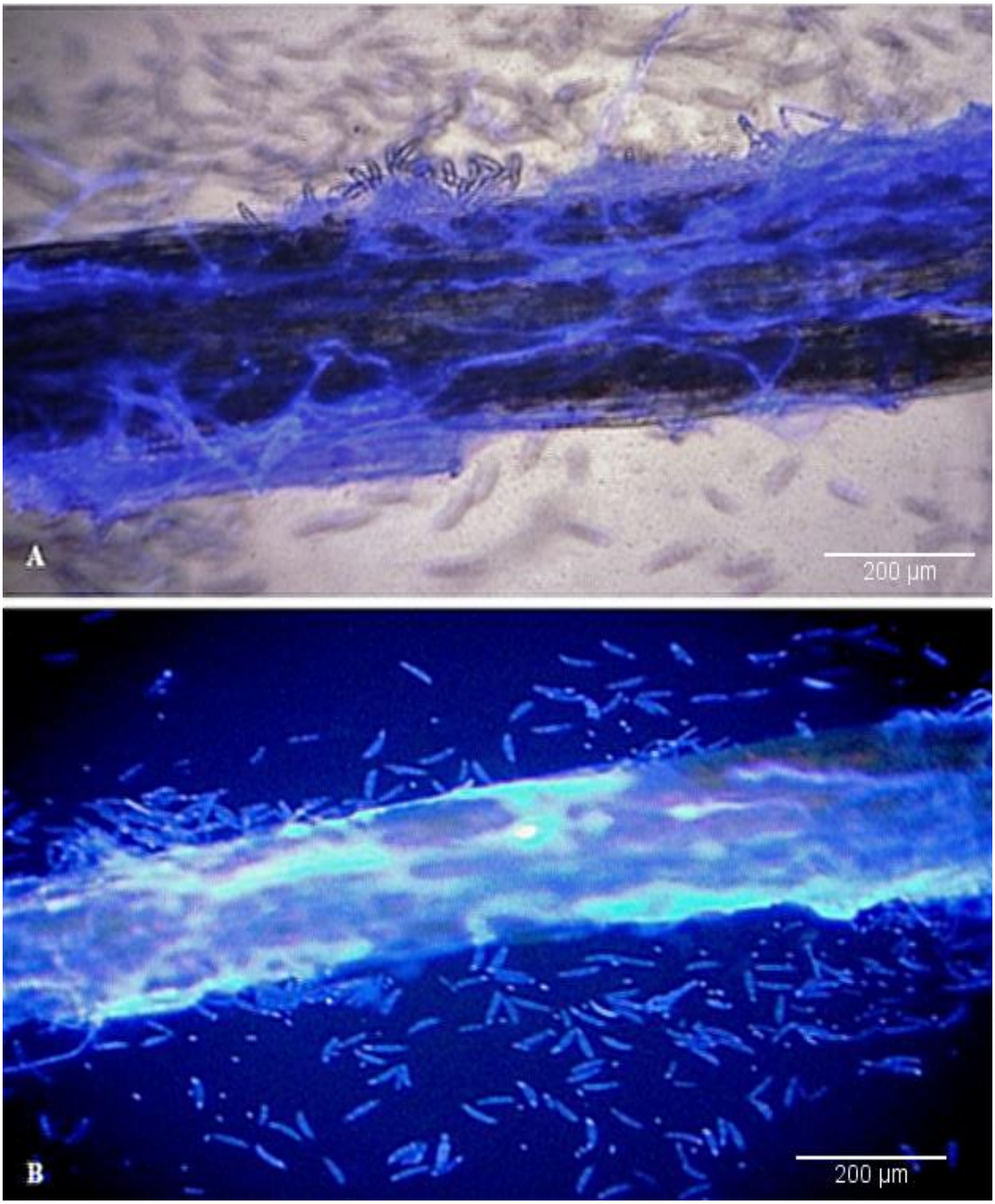


Figure 5-17 Release of conidia from *M. nivale* infected *A. stolonifera* leaf. *M. nivale* infected *A. stolonifera* leaf showing the release of numerous conidia. A and B: *A. stolonifera* leaf showing network of hyphal growth and conidia being produced.

5.5.2 Defence responses

Initiation of biochemical defences responses varied in speed of activation and concentration with turfgrass species and environmental conditions. There was a direct correlation between speed of response and susceptibility.

5.5.2.1 Experiment 2, Effect of Phi on TPC in infected and non-infected turfgrass

Mean levels of TPC were determined in infected and non-infected turfgrasses over three years, Table 5-1 shows the descriptive statistics for the field and greenhouse samples of *P. annua* and *A. stolonifera*.

Table 5-1 Descriptive statistics for TPC levels, as GAE in mg/g dw, in infected and un-infected tissues of field and greenhouse samples of *P. annua* and *A. stolonifera* turfgrasses for 2012 (year 1), 2013 (year 2) and 2014 (year 3).

Control or infected			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
Infected	TPC greens 2012	*P.annua*	10	2.45	0.03	0.01	2.43	2.48	2.41	2.52
		A.stolonifera	10	2.43	0.03	0.01	2.41	2.45	2.39	2.47
		Total	20	2.44	0.03	0.01	2.42	2.46	2.39	2.52
	TPC greens 2013	*P.annua*	10	2.25	0.04	0.01	2.23	2.28	2.20	2.31
		A.stolonifera	10	2.35	0.04	0.01	2.32	2.37	2.30	2.40
		Total	20	2.30	0.06	0.01	2.27	2.33	2.20	2.40
	TPC greens 2014	*P.annua*	10	2.39	0.02	0.01	2.37	2.41	2.35	2.42
		A.stolonifera	10	2.29	0.04	0.01	2.26	2.32	2.24	2.36
		Total	20	2.34	0.06	0.01	2.31	2.37	2.24	2.42
	TPC greenhouse 2012	*P.annua*	10	2.23	0.04	0.01	2.21	2.26	2.19	2.28
		A.stolonifera	10	2.35	0.03	0.01	2.33	2.37	2.30	2.40
		Total	20	2.29	0.07	0.01	2.26	2.32	2.19	2.40
	TPC greenhouse 2013	*P.annua*	10	2.42	0.03	0.01	2.40	2.44	2.37	2.47
		A.stolonifera	10	2.46	0.03	0.01	2.44	2.49	2.43	2.51
		Total	20	2.44	0.04	0.01	2.42	2.46	2.37	2.51
	TPC greenhouse 2014	*P.annua*	10	2.27	0.04	0.01	2.25	2.30	2.21	2.33
		A.stolonifera	10	2.56	0.04	0.01	2.53	2.59	2.50	2.62
		Total	20	2.42	0.15	0.03	2.34	2.49	2.21	2.62
control	TPC greens 2012	*P.annua*	10	2.12	0.04	0.01	2.09	2.14	2.04	2.16
		A.stolonifera	10	1.99	0.04	0.01	1.96	2.02	1.92	2.04
		Total	20	2.05	0.08	0.02	2.02	2.09	1.92	2.16
	TPC greens 2013	*P.annua*	10	1.97	0.04	0.01	1.94	2.00	1.92	2.03
		A.stolonifera	10	2.01	0.02	0.01	2.00	2.02	1.98	2.03
		Total	20	1.99	0.04	0.01	1.97	2.01	1.92	2.03
	TPC greens 2014	*P.annua*	10	2.04	0.05	0.01	2.01	2.07	1.97	2.10
		A.stolonifera	10	2.08	0.04	0.01	2.04	2.11	1.99	2.12
		Total	20	2.06	0.05	0.01	2.04	2.08	1.97	2.12
	TPC greenhouse 2012	*P.annua*	10	1.82	0.03	0.01	1.79	1.84	1.77	1.86
		A.stolonifera	10	1.94	0.04	0.01	1.92	1.97	1.90	2.01
		Total	20	1.88	0.07	0.02	1.84	1.91	1.77	2.01
	TPC greenhouse 2013	*P.annua*	10	1.89	0.02	0.01	1.87	1.90	1.85	1.92
		A.stolonifera	10	2.07	0.04	0.01	2.04	2.10	2.01	2.11
		Total	20	1.98	0.10	0.02	1.93	2.02	1.85	2.11
	TPC greenhouse 2014	*P.annua*	10	1.75	0.04	0.01	1.72	1.78	1.71	1.82
		A.stolonifera	10	1.95	0.04	0.01	1.92	1.98	1.89	2.02
		Total	20	1.85	0.11	0.02	1.80	1.91	1.71	2.02

Over the three year period, there were significant differences in TPC amounts in turfgrasses sampled from trial plots, between the infected and un-infected plants and between turfgrass species, along with a significant interaction effect, Table 5-2.

Table 5-2 Two-way Anova of TPC levels in infected and non-infected turfgrass leaf tissues, sampled from field trial plots over three years.

	Infected v non-infected controls			
	df	F	p	η2
year 1	1,36	1137.966	< .001	0.969
year 2	1,36	807.788	< .001	0.957
year 3	1,36	511.372	< .001	0.934
	Turfgrass species			
year 1	1,36	43.887	< .001	0.549
year 2	1,36	36.179	< .001	0.501
year 3	1,36	6.865	< .05	0.160
	Interaction			
year 1	1,36	18.243	< .001	0.336
year 2	1,36	7.845	< .01	0.179
year 3	1,36	28.742	< .001	0.444

Fig. 5-18 shows mean levels of TPC in field samples, as GAE in mg/g dw, in *P. annua* and *A. stolonifera* for each of the three years of assessments. In the infected samples there were significantly greater TPC amounts than those in un-infected tissues, indicating that these accumulations are part of the defence response to *M. nivale* infection.

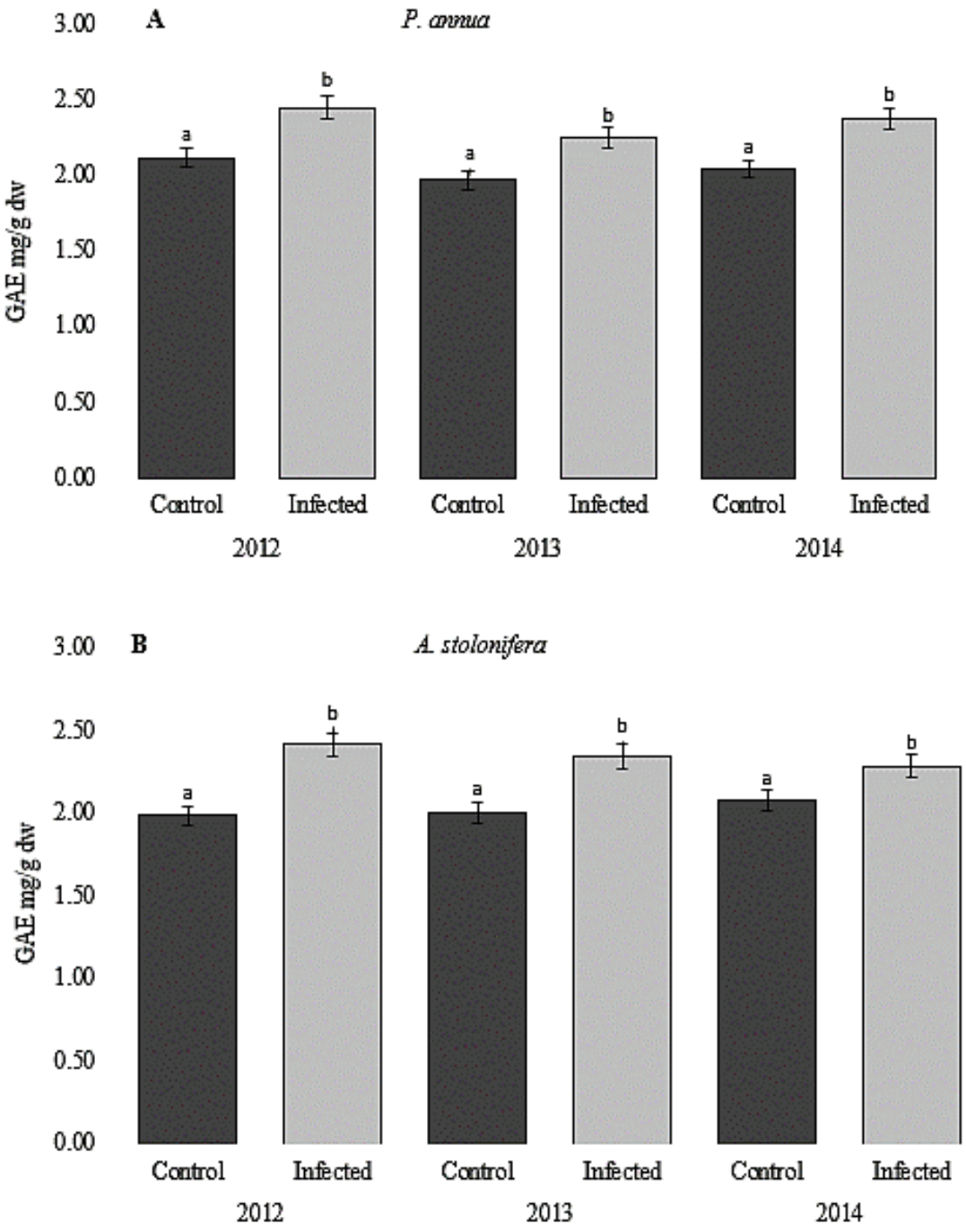


Figure 5-18 TPC as GAE mg/g dw, in infected and non-infected field trial plots. TPC as GAE mg/g dw, in infected and non-infected turfgrass leaf tissues, sampled from field trial plots over three years. A: *P.annua*. B: *A. stolonifera*. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by pairwise comparisons using Bonferroni correction at $p < 0.05$, n=10.

In greenhouse turfgrasses, two-way Anova determined there were significant ($p < 0.05$) differences in TPC concentrations between the infected and un-infected plants, turfgrass species, with a significant ($p < 0.05$) interaction effect, Table 5-3. Similarly to the field samples TPC levels, were statistically ($p < 0.05$) greater in infected tissues than non-infected plants in both turfgrass species. Significant ($p < 0.05$) differences in TPC amounts between species were also determined, with TPC levels in infected and non-infected tissues of *A. stolonifera* greater than those in *P. annua* each year, Fig. 5-19.

Table 5-3 Two-way Anova of TPC levels in infected and non-infected turfgrass leaf tissues, sampled from control and *M. nivale* inoculated greenhouse plants over three years

Infected v non-infected controls				
	df	F	p	η2
year 1	1,36	1422.825	< .001	0.975
year 2	1,36	2245.466	< .001	0.984
year 3	1,36	1934.577	< .001	0.982
Turfgrass species				
year 1	1,36	123.321	< .001	0.774
year 2	1,36	134.332	< .001	0.789
year 3	1,36	360.836	< .001	0.909
Interaction				
year 1	1,36	0.539	< .001	0.015
year 2	1,36	48.645	< .001	0.575
year 3	1,36	10.568	< .01	0.227

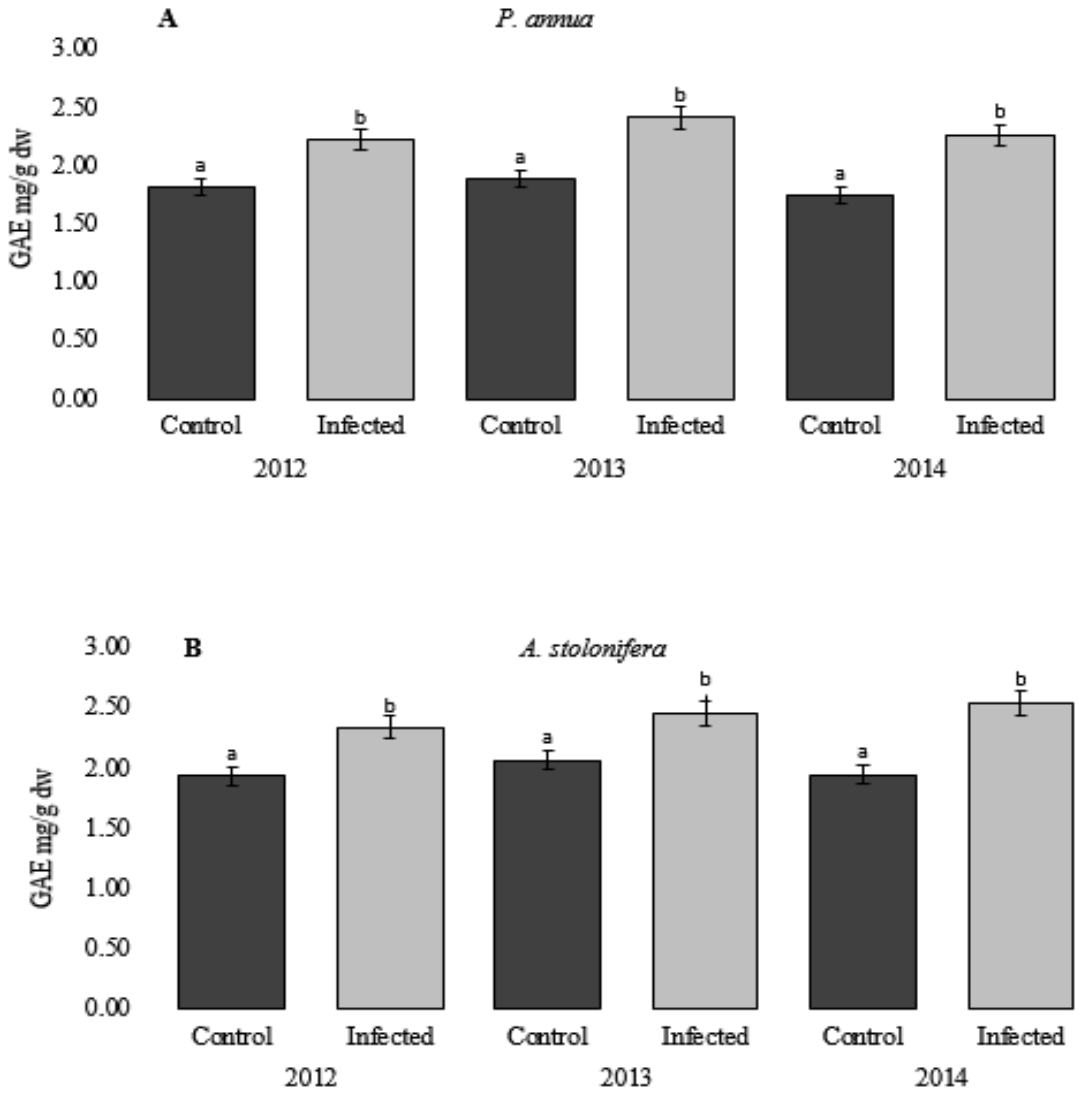


Figure 5-19 TPC as GAE mg/g dw, in infected and non-infected greenhouse turfgrasses. TPC as GAE mg/g dw, in infected and non-infected turfgrass leaf tissues, sampled from control and *M. nivale* inoculated greenhouse plants over three years. A: *P. annua*. B: *A. stolonifera.* Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by pairwise comparisons using Bonferroni correction at $p < 0.05$, n=10.

5.5.2.2 Effect of Phi treatment on TPC in non-infected turfgrass

The effects on TPC levels following a single treatment of SDW (control), Pi and Phi on *P. annua* and *A. stolonifera* tissues, sampled from field trial plots are shown in the descriptive statistics in Table 5-4. There was significant ($p < 0.05$) effect on TPC levels, from treatments and between turfgrass species at all times sampled from 0 to 72 hpa, as determined by two-way Anova, with a significant interaction at all times, with the exception of at 12 and 48 hpa, Table 5-5.

Table 5-4 Descriptive statistics showing TPC levels in SDW (control), Pi and Phi treatment treated tissues of *P.annua* and *A. stolonifera,* sampled from field trial plots from 0 to 72 hours post treatment application.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua	0 hpa	Control	10	1.93	0.04	0.01	1.90	1.96	1.88	2.02
		Pi	10	1.94	0.04	0.01	1.90	1.97	1.88	2.02
		Phi	10	1.89	0.03	0.01	1.87	1.91	1.85	1.93
		Total	30	1.92	0.04	0.01	1.90	1.93	1.85	2.02
	1 hpa	Control	10	1.88	0.03	0.01	1.86	1.90	1.85	1.93
		Pi	10	1.91	0.03	0.01	1.89	1.94	1.85	1.96
		Phi	10	1.91	0.03	0.01	1.89	1.94	1.85	1.96
		Total	30	1.90	0.03	0.01	1.89	1.92	1.85	1.96
	6 hpa	Control	10	2.02	0.04	0.01	1.99	2.04	1.96	2.08
		Pi	10	1.98	0.04	0.01	1.95	2.00	1.93	2.04
		Phi	10	2.00	0.03	0.01	1.98	2.02	1.96	2.04
		Total	30	2.00	0.04	0.01	1.98	2.01	1.93	2.08
	12 hpa	Control	10	1.89	0.03	0.01	1.87	1.91	1.85	1.93
		Pi	10	2.08	0.03	0.01	2.05	2.10	2.02	2.12
		Phi	10	2.18	0.04	0.01	2.15	2.21	2.11	2.24
		Total	30	2.05	0.13	0.02	2.00	2.09	1.85	2.24
	24 hpa	Control	10	1.97	0.03	0.01	1.95	2.00	1.93	2.03
		Pi	10	2.20	0.03	0.01	2.17	2.22	2.14	2.23
		Phi	10	2.12	0.03	0.01	2.11	2.14	2.08	2.17
		Total	30	2.10	0.10	0.02	2.06	2.14	1.93	2.23
	48 hpa	Control	10	2.02	0.04	0.01	1.99	2.04	1.96	2.08
		Pi	10	2.21	0.04	0.01	2.18	2.23	2.16	2.26
		Phi	10	2.21	0.04	0.01	2.18	2.23	2.16	2.26
		Total	30	2.14	0.10	0.02	2.11	2.18	1.96	2.26
	72 hpa	Control	10	1.95	0.04	0.01	1.92	1.98	1.88	2.01
		Pi	10	2.12	0.02	0.01	2.11	2.14	2.08	2.15
		Phi	10	2.17	0.04	0.01	2.15	2.20	2.13	2.22
		Total	30	2.08	0.10	0.02	2.04	2.12	1.88	2.22
A. stolonifera	0 hpa	Control	10	2.00	0.03	0.01	1.98	2.02	1.96	2.04
		Pi	10	2.01	0.03	0.01	1.99	2.03	1.97	2.05
		Phi	10	1.95	0.04	0.01	1.93	1.98	1.91	2.02
		Total	30	1.99	0.04	0.01	1.97	2.00	1.91	2.05
	1 hpa	Control	10	1.94	0.04	0.01	1.90	1.97	1.88	2.02
		Pi	10	1.97	0.04	0.01	1.94	1.99	1.91	2.03
		Phi	10	2.07	0.05	0.01	2.04	2.10	1.99	2.12
		Total	30	1.99	0.07	0.01	1.96	2.02	1.88	2.12
	6 hpa	Control	10	2.09	0.04	0.01	2.06	2.11	2.03	2.13
		Pi	10	2.14	0.03	0.01	2.12	2.16	2.11	2.19
		Phi	10	2.27	0.03	0.01	2.25	2.29	2.23	2.32
		Total	30	2.17	0.08	0.02	2.14	2.20	2.03	2.32
	12 hpa	Control	10	1.95	0.03	0.01	1.93	1.98	1.91	2.02
		Pi	10	2.15	0.03	0.01	2.13	2.17	2.11	2.21
		Phi	10	2.22	0.04	0.01	2.19	2.25	2.16	2.27
		Total	30	2.11	0.12	0.02	2.06	2.15	1.91	2.27
	24 hpa	Control	10	2.07	0.03	0.01	2.04	2.09	2.02	2.12
		Pi	10	2.26	0.03	0.01	2.24	2.28	2.21	2.32
		Phi	10	2.24	0.04	0.01	2.21	2.27	2.18	2.30
		Total	30	2.19	0.09	0.02	2.15	2.23	2.02	2.32
	48 hpa	Control	10	2.07	0.04	0.01	2.04	2.09	2.01	2.12
		Pi	10	2.25	0.04	0.01	2.23	2.28	2.19	2.33
		Phi	10	2.26	0.04	0.01	2.23	2.29	2.19	2.33
		Total	30	2.19	0.10	0.02	2.16	2.23	2.01	2.33
	72 hpa	Control	10	2.02	0.04	0.01	1.99	2.05	1.96	2.08
		Pi	10	2.16	0.03	0.01	2.13	2.18	2.12	2.21
		Phi	10	2.19	0.03	0.01	2.16	2.21	2.13	2.23
		Total	30	2.12	0.08	0.01	2.09	2.15	1.96	2.23

Table 5-5 Two-way Anova of TPC levels sampled from SDW (control), Pi and Phi treated tissues of *P.annua* and *A. stolonifera,* collected from field trial plots 0 to 72 hours post treatment application.

Greens				
Turfgrass species				
	df	F	p	η2
0 hpa	1,54	57.174	< .001	0.514
1 hpa	1,54	77.32	< .001	0.589
6 hpa	1,54	391.031	< .001	0.879
12 hpa	1,54	44.365	< .001	0.451
24 hpa	1,54	118.424	< .001	0.687
48 hpa	1,54	27.82	< .001	0.34
72 hpa	1,54	18.417	< .001	0.254
Treatment				
	df	F	p	η2
0 hpa	2,54	12.103	< .001	0.31
1 hpa	2,54	24.255	< .001	0.473
6 hpa	2,54	40.059	< .001	0.597
12 hpa	2,54	332.718	< .001	0.925
24 hpa	2,54	224.873	< .001	0.893
48 hpa	2,54	163.829	< .001	0.859
72 hpa	2,54	183.672	< .001	0.872
Interaction				
	df	F	p	η2
0 hpa	2,54	0.349	< .001	0.013
1 hpa	2,54	12.444	< .001	0.315
6 hpa	2,54	44.816	< .001	0.624
12 hpa	2,54	1.089	> .05	0.039
24 hpa	2,54	3.18	< .05	0.105
48 hpa	2,54	0.112	> .05	0.004
72 hpa	2,54	3.654	< .05	0.119

As shown in Fig. 5-20, following treatment application, TPC levels increased significantly ($p < 0.05$), in Pi and Phi treated tissues when compared to controls, at 12, 24, 48 and 72 hpa in *P.annua* and at 6, 12, 24, 48 and 72 hpa in *A. stolonifera*. There were also significantly ($p < 0.05$) greater amounts in Phi treated tissues compared to Pi treated tissues at 12 and 72 hpa in *P.annua* and at 1, 6 and 12 hpa in *A. stolonifera* tissues. Pi treated *P. annua* tissues, at 24 hpa were significantly ($p < 0.05$) greater that either Phi treated or controls, with no other significant ($p > 0.05$) differences between the TPC levels at other time periods.

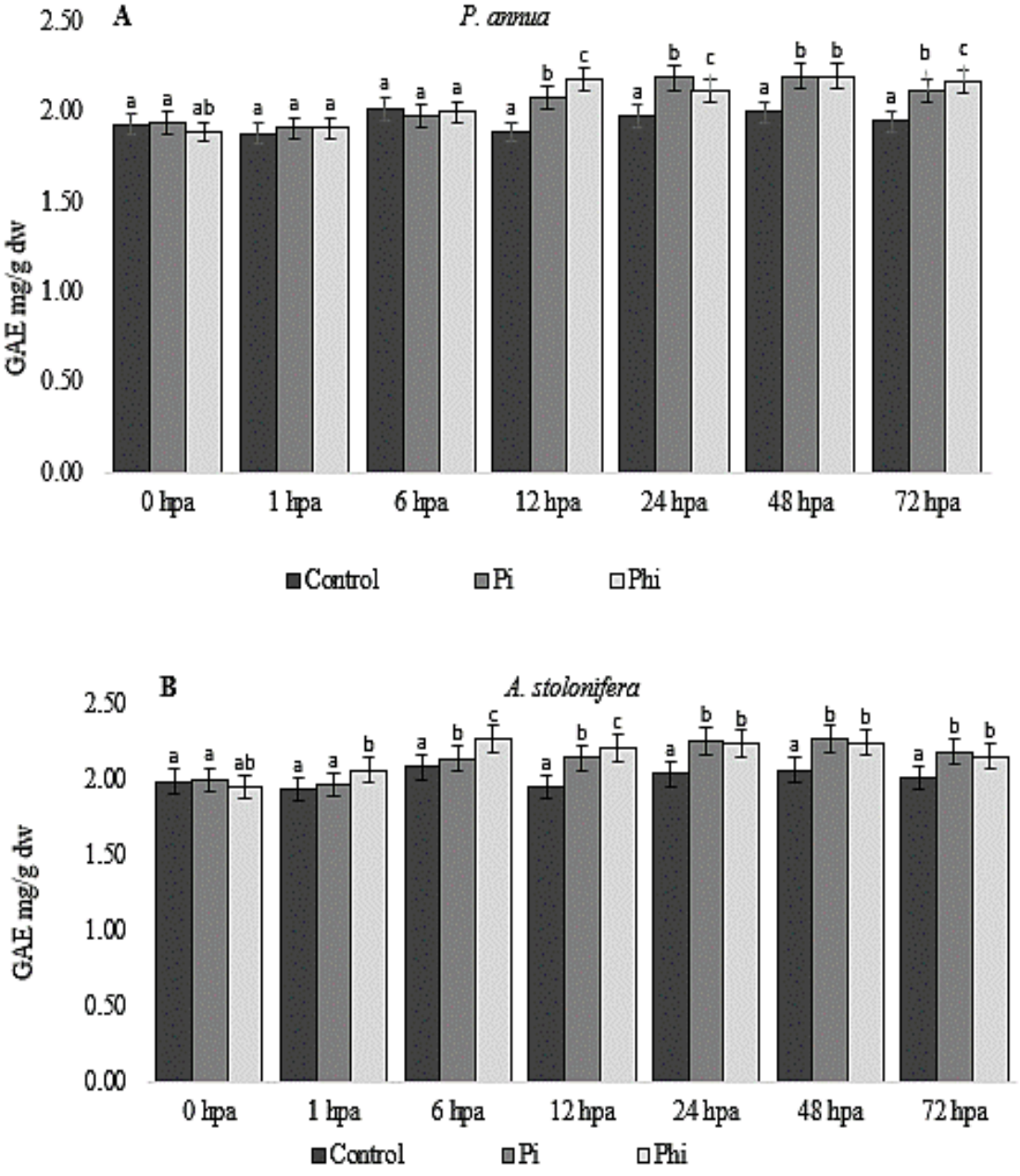


Figure 5-20 TPC as GAE mg/g dw in turfgrass tissues sampled from trial plots over 72 hours post treatment. TPC as GAE mg/g dw, of turfgrass leaf tissues, sampled from trial plots over 72 hours following SDW (control), Pi and Phi treatment. A: *P. annua*, B: *A. stolonifera*. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

In greenhouse plants, following treatment with a single application of SDW (control), Pi and Phi, TPC levels also increased, Table 5-6 show descriptive statistics.

Table 5-6 Descriptive statistics of TPC levels in SDW (control), Pi and Phi treated tissues of *P.annua* and *A. stolonifera,* sampled from greenhouse plants, 0 to 72 hours post application.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua	0 hpa	Control	10	2.11	0.03	0.01	2.09	2.12	2.06	2.14
		Pi	10	1.96	0.03	0.01	1.94	1.98	1.91	2.01
		Phi	10	2.07	0.05	0.01	2.04	2.10	1.99	2.12
		Total	30	2.04	0.07	0.01	2.02	2.07	1.91	2.14
	1 hpa	Control	10	2.01	0.03	0.01	1.99	2.04	1.96	2.06
		Pi	10	1.99	0.04	0.01	1.96	2.02	1.93	2.05
		Phi	10	2.11	0.03	0.01	2.09	2.13	2.07	2.15
		Total	30	2.04	0.06	0.01	2.01	2.06	1.93	2.15
	6 hpa	Control	10	2.16	0.03	0.01	2.13	2.18	2.12	2.21
		Pi	10	2.16	0.03	0.01	2.14	2.18	2.12	2.21
		Phi	10	2.14	0.03	0.01	2.12	2.16	2.11	2.21
		Total	30	2.15	0.03	0.01	2.14	2.16	2.11	2.21
	12 hpa	Control	10	2.10	0.04	0.01	2.08	2.13	2.06	2.17
		Pi	10	2.18	0.03	0.01	2.16	2.20	2.13	2.22
		Phi	10	2.23	0.04	0.01	2.20	2.26	2.16	2.27
		Total	30	2.17	0.06	0.01	2.15	2.19	2.06	2.27
	24 hpa	Control	10	2.01	0.03	0.01	1.99	2.04	1.96	2.06
		Pi	10	2.10	0.02	0.01	2.09	2.12	2.06	2.13
		Phi	10	2.19	0.04	0.01	2.16	2.21	2.13	2.24
		Total	30	2.10	0.08	0.01	2.07	2.13	1.96	2.24
	48 hpa	Control	10	1.96	0.04	0.01	1.94	1.99	1.91	2.02
		Pi	10	2.22	0.04	0.01	2.19	2.25	2.16	2.26
		Phi	10	2.27	0.03	0.01	2.25	2.29	2.23	2.32
		Total	30	2.15	0.14	0.03	2.10	2.20	1.91	2.32
	72 hpa	Control	10	1.99	0.02	0.01	1.97	2.00	1.96	2.03
		Pi	10	2.19	0.03	0.01	2.17	2.21	2.14	2.23
		Phi	10	2.16	0.04	0.01	2.13	2.19	2.11	2.22
		Total	30	2.11	0.10	0.02	2.08	2.15	1.96	2.23
A. stolonifera	0 hpa	Control	10	2.12	0.03	0.01	2.10	2.14	2.08	2.17
		Pi	10	2.09	0.04	0.01	2.06	2.11	2.03	2.13
		Phi	10	2.17	0.04	0.01	2.14	2.19	2.11	2.22
		Total	30	2.13	0.05	0.01	2.11	2.14	2.03	2.22
	1 hpa	Control	10	2.21	0.04	0.01	2.19	2.24	2.16	2.26
		Pi	10	2.22	0.03	0.01	2.19	2.24	2.16	2.26
		Phi	10	2.25	0.03	0.01	2.23	2.27	2.20	2.30
		Total	30	2.23	0.04	0.01	2.21	2.24	2.16	2.30
	6 hpa	Control	10	2.24	0.04	0.01	2.21	2.26	2.18	2.29
		Pi	10	2.29	0.04	0.01	2.26	2.32	2.23	2.34
		Phi	10	2.27	0.03	0.01	2.25	2.29	2.23	2.32
		Total	30	2.27	0.04	0.01	2.25	2.28	2.18	2.34
	12 hpa	Control	10	2.19	0.03	0.01	2.17	2.21	2.14	2.23
		Pi	10	2.32	0.04	0.01	2.28	2.35	2.25	2.37
		Phi	10	2.36	0.04	0.01	2.33	2.38	2.29	2.40
		Total	30	2.29	0.08	0.01	2.26	2.32	2.14	2.40
	24 hpa	Control	10	2.25	0.03	0.01	2.23	2.27	2.19	2.30
		Pi	10	2.40	0.03	0.01	2.38	2.42	2.36	2.45
		Phi	10	2.34	0.05	0.01	2.31	2.37	2.25	2.38
		Total	30	2.33	0.07	0.01	2.30	2.35	2.19	2.45
	48 hpa	Control	10	2.28	0.04	0.01	2.25	2.31	2.23	2.35
		Pi	10	2.34	0.05	0.01	2.31	2.37	2.25	2.38
		Phi	10	2.40	0.03	0.01	2.38	2.42	2.36	2.45
		Total	30	2.34	0.06	0.01	2.32	2.36	2.23	2.45
	72 hpa	Control	10	2.20	0.03	0.01	2.17	2.22	2.14	2.24
		Pi	10	2.31	0.04	0.01	2.28	2.34	2.25	2.36
		Phi	10	2.29	0.03	0.01	2.27	2.31	2.24	2.33
		Total	30	2.26	0.06	0.01	2.24	2.29	2.14	2.36

Treatments had a significant ($p < 0.05$) impact on TPC levels, Table 5-7. Unlike field tissue samples, prior to treatment application, there were significant ($p < 0.05$) differences in TPC levels in both *P.annua* and *A. stolonifera,* Fig 5-21. Despite these differences, following Phi and Pi application, TPC levels increased significantly ($p < 0.05$), when compared to controls, at 12, 24, 48 and 72 hpa in both *P.annua* and *A. stolonifera*. There were significantly ($p < 0.05$) greater amounts in Phi treated tissues compared to Pi treated and control tissues at 1, 12, 48 and 72 hpa in *P.annua* and at 48 hpa in *A. stolonifera* tissues. TPC levels in Pi treated *A. stolonifera* tissues, at 24 hpa, were significantly ($p > 0.05$) greater that either Phi treated or control tissues, with no other significant ($p < 0.05$) differences between the levels at other time periods other than at 0 hpa.

Table 5-7 Two-way Anova of TPC levels of turfgrass leaf tissues from greenhouse samples over 72 hours following SDW (control, Pi and Phi treatment.

	Turfgrass species			
	df	F	p	η2
0 hpa	1,54	85.834	< .001	0.614
1 hpa	1,54	456.306	< .001	0.894
6 hpa	1,54	168.369	< .001	0.757
12 hpa	1,54	143.583	< .001	0.727
24 hpa	1,54	673.616	< .001	0.926
48 hpa	1,54	399.696	< .001	0.881
72 hpa	1,54	332.724	< .001	0.86
	Treatment			
	df	F	p	η2
0 hpa	2,54	49.547	< .001	0.647
1 hpa	2,54	32.467	< .001	0.546
6 hpa	2,54	3.71	< .05	0.121
12 hpa	2,54	81.469	< .001	0.751
24 hpa	2,54	93.361	< .001	0.776
48 hpa	2,54	181.9	< .001	0.871
72 hpa	2,54	139.05	< .001	0.837
	Interaction			
	df	F	p	η2
0 hpa	2,54	13.525	< .001	0.334
1 hpa	2,54	9.696	< .001	0.264
6 hpa	2,54	3.376	< .05	0.111
12 hpa	2,54	2.556	> .05	0.086
24 hpa	2,54	22.474	< .05	0.454
48 hpa	2,54	47.368	< .001	0.637
72 hpa	2,54	11.327	< .001	0.296

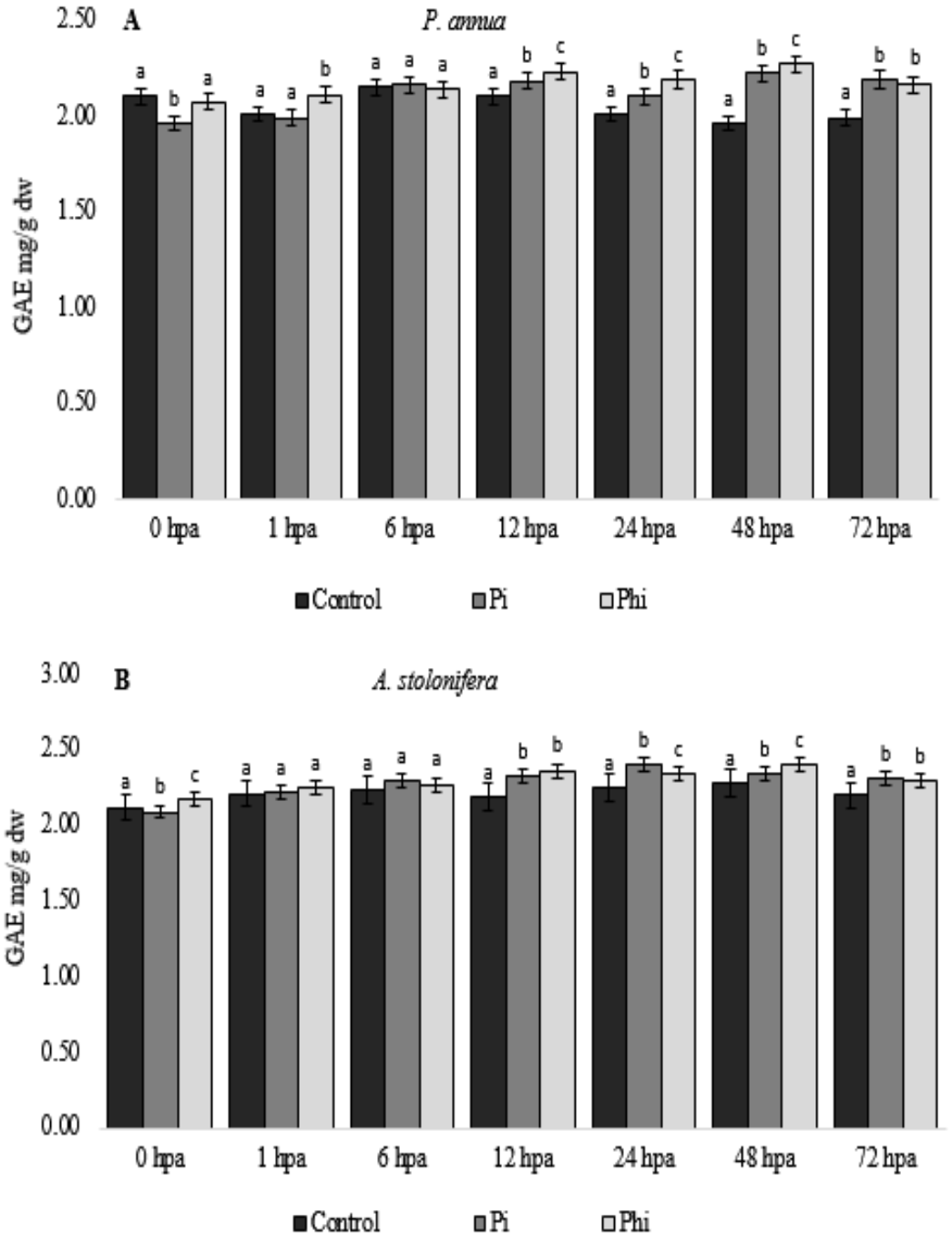


Figure 5-21 TPC as GAE mg/g dw in turfgrass tissues sampled from greenhouse turfgrasses over 72 hours post treatment. TPC as GAE mg/g dw, of turfgrass leaf tissues from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment. A: *P. annua*, B: *A. stolonifera*. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

TPC levels in *P. annua* and *A. stolonifera* tissues, sampled from trial plots and greenhouse plants, following sequential treatments, applied over a six month period, are shown in the descriptive statistics in Table 5-8.

Table 5-8 Descriptive statistics of TPC levels in SDW (control), Pi and Phi treated tissues of *P.annua* and *A. stolonifera,* sampled from field trial plots and greenhouse plants, following sequential treatments over a six month period.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua	Trial plot samples	Control	10	2.19	0.03	0.01	2.17	2.21	2.14	2.23
		Pi	10	2.26	0.04	0.01	2.23	2.29	2.19	2.33
		Phi	10	2.89	0.05	0.01	2.85	2.92	2.82	2.94
		Total	30	2.45	0.32	0.06	2.32	2.57	2.14	2.94
	Greenhouse samples	Control	10	2.08	0.03	0.01	2.06	2.10	2.04	2.12
		Pi	10	2.32	0.04	0.01	2.29	2.35	2.25	2.37
		Phi	10	3.18	0.04	0.01	3.16	3.21	3.11	3.25
		Total	30	2.53	0.48	0.09	2.35	2.71	2.04	3.25
A.stolonifera	Trial plot samples	Control	10	2.13	0.03	0.01	2.11	2.15	2.08	2.17
		Pi	10	2.41	0.04	0.01	2.38	2.43	2.36	2.47
		Phi	10	3.06	0.04	0.01	3.03	3.08	3.00	3.11
		Total	30	2.53	0.40	0.07	2.38	2.68	2.08	3.11
	Greenhouse samples	Control	10	2.00	0.03	0.01	1.98	2.03	1.96	2.06
		Pi	10	2.29	0.03	0.01	2.27	2.32	2.23	2.34
		Phi	10	2.85	0.04	0.01	2.83	2.88	2.80	2.90
		Total	30	2.38	0.36	0.07	2.25	2.52	1.96	2.90

There were significant ($p < 0.05$) increases in TPC levels, when compared to untreated controls, in Phi and Pi treated plants, in both field and greenhouse samples of both turfgrass species, Table 5-9. Treatment effects on TPC levels are shown in Fig. 5-22, with significantly ($p < 0.05$) higher levels in Phi treated tissues, when compared to Pi and controls. Pi treated tissues having significantly ($p < 0.05$) greater levels than controls.

Table 5-9 Two-way Anova of TPC levels in turfgrass tissues sampled from trial plots and greenhouse plants following six, monthly applications of SDW (control), Pi and Phi, showing significant differences and interactions between factors.

Trial plots			
	df	f	p	η2
Turfgrass species	1,54	77.565	< .001	0.59
Treatment	2,54	2640.707	< .001	0.99
Interaction	2,54	58.47	< .001	0.684
Greenhouse				
	df	f	p	η2
Turfgrass species	1,54	248.146	< .001	0.821
Treatment	2,54	4010.51	< .001	0.993
Interaction	2,54	106.367	< .001	0.798

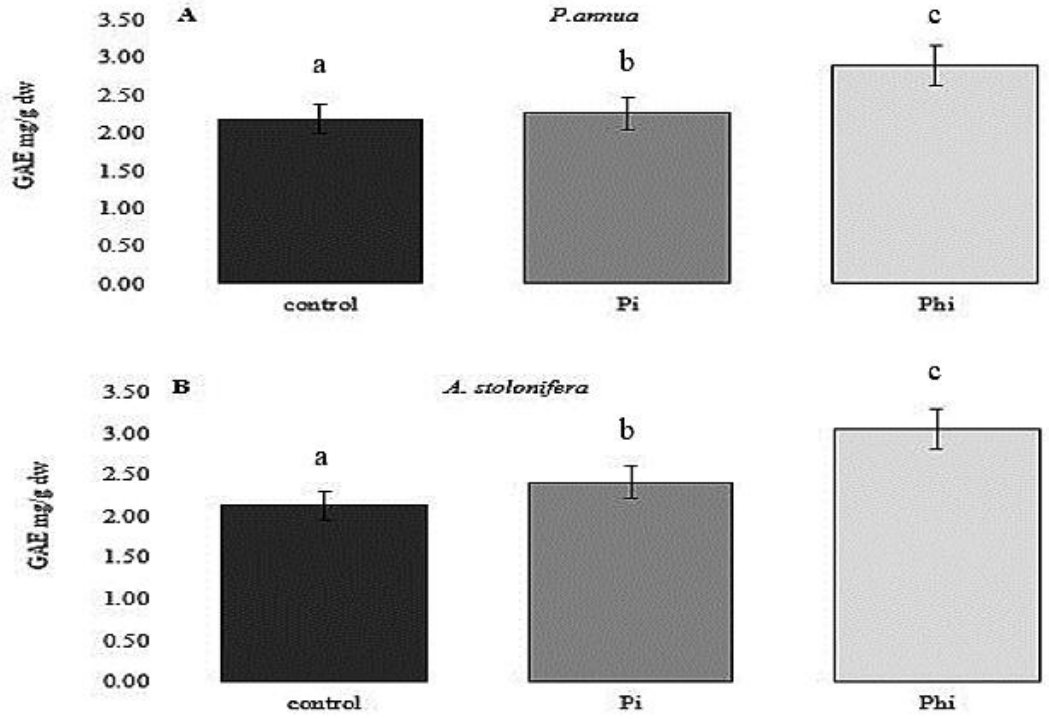


Figure 5-22 TPC as GAE mg/g dw, in turfgrass tissues sampled from field trial plots. TPC as GAE mg/g dw, in turfgrass tissues sampled from field trial plots following six, monthly applications of SDW (control), Pi and Phi. Analysis carried out 48 hpa. Bars indicate 95% confidence limits, letters indicate significant differences determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

Analyses of *P. annua* and *A. stolonifera* tissues collected from greenhouse samples, produced statistically similar results as tissues collected from the field, Table 5-9. Fig. 5-23 shows treatment effect on TPC levels, clearly showing significantly ($p < 0.05$) higher levels in Phi treated tissues, when compared to Pi and controls, with Pi treated tissues having significantly ($p < 0.05$) greater levels than controls.

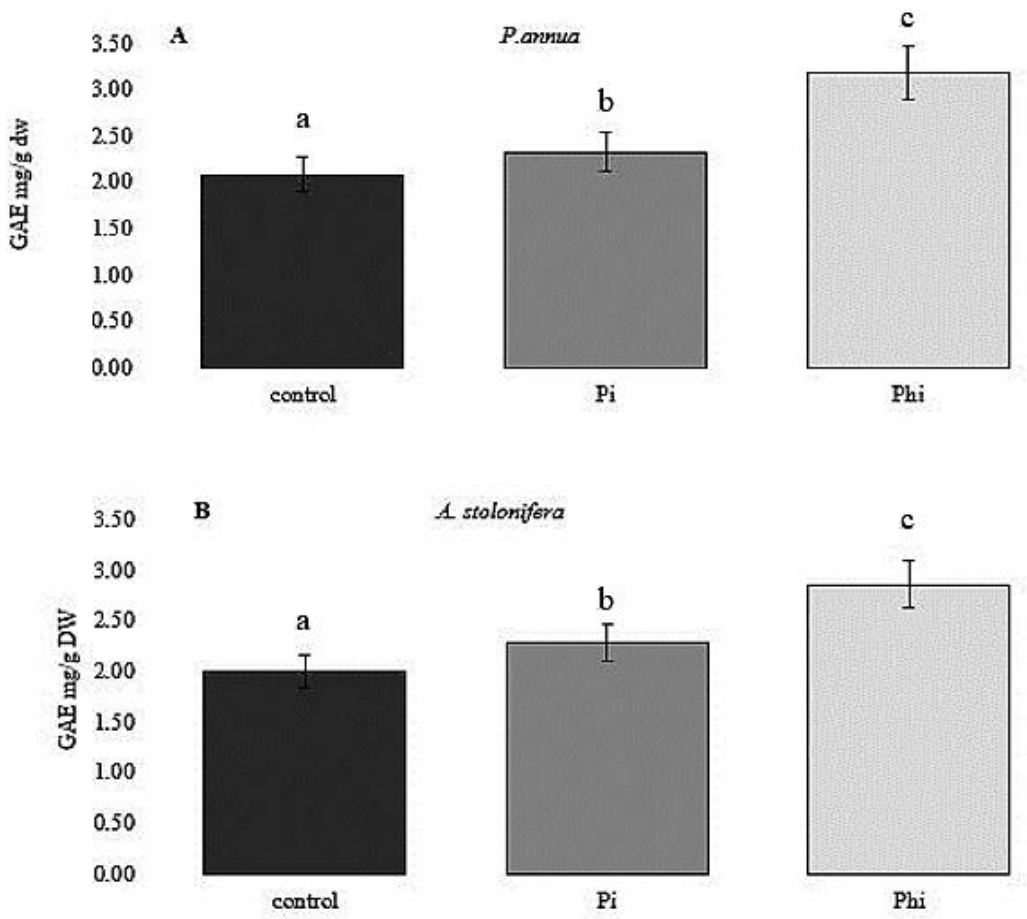


Figure 5-23 TPC as GAE mg/g dw, in turfgrass tissues sampled from greenhouse plants. TPC as GAE mg/g dw, in turfgrass tissues sampled from greenhouse plants following six, monthly applications of SDW (control), Pi and Phi. Analysis carried out 48 hpa. Bars indicate 95% confidence limits, letters indicate significant differences determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

5.5.2.3 Effect of Phi treatment on TPC in infected turfgrass

Determination of the effect Phi had on TPC in *M. nivale* infected turfgrasses was carried out following hyphal inoculation of greenhouse samples. Conditions in the greenhouse environment were ideal for *M. nivale* infection and following inoculation, there were rapid displays of disease symptoms. Infection diameters increased over 10 dpi as shown in Fig. 5-24.

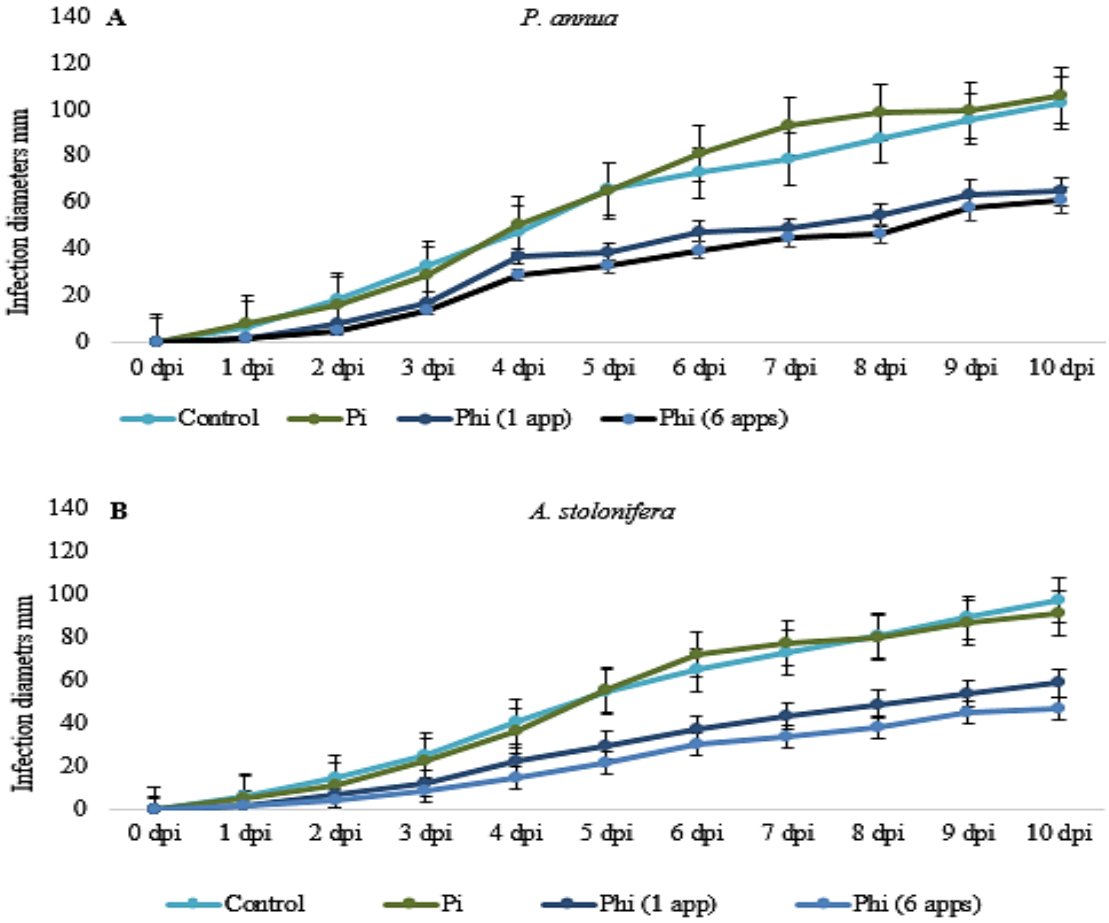


Figure 5-24 *M. nivale* infection diameters 10 dpi. *M. nivale* infection diameters in mm, 10 dpi observed in greenhouse turfgrasses treated with SDW (control), Pi, Phi (1 app) and Phi (6 apps). A: *P. annua,* B: *A. stolonifera*. Bars indicate 95% confidence limits, n=10.

Following analysis to determine TPC levels, infected tissues accumulated increasing amounts of TPC over the course of 10 dpi, descriptive statistics are shown in Table 5-10.

Table 5-10 Descriptive statistics of TPC levels in SDW (control), Pi, Phi (1 app) and Phi (6 apps) treated tissues of *P.annua* and *A. stolonifera,* collected from *M. nivale* infected greenhouse plants, over 10 days post treatment application.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua		Control	10	1.92	0.01	0.00	1.91	1.93	1.90	1.95
		Pi	10	1.99	0.06	0.02	1.94	2.03	1.90	2.10
	dpi 0	Phi 1 app	10	1.95	0.06	0.02	1.91	2.00	1.90	2.10
		Phi 6 apps	10	2.12	0.04	0.01	2.09	2.14	2.04	2.16
		Total	40	1.99	0.09	0.01	1.97	2.02	1.90	2.16
		Control	10	2.13	0.03	0.01	2.11	2.14	2.09	2.16
		Pi	10	2.22	0.03	0.01	2.20	2.25	2.19	2.28
	dpi 2	Phi 1 app	10	2.45	0.03	0.01	2.43	2.48	2.41	2.52
		Phi 6 apps	10	2.44	0.03	0.01	2.42	2.46	2.40	2.50
		Total	40	2.31	0.15	0.02	2.26	2.36	2.09	2.52
		Control	10	2.34	0.03	0.01	2.32	2.36	2.30	2.40
		Pi	10	2.34	0.03	0.01	2.32	2.36	2.30	2.40
	dpi 4	Phi 1 app	10	2.50	0.03	0.01	2.47	2.52	2.45	2.55
		Phi 6 apps	10	2.51	0.04	0.01	2.48	2.54	2.45	2.55
		Total	40	2.42	0.09	0.01	2.40	2.45	2.30	2.55
		Control	10	2.43	0.03	0.01	2.41	2.45	2.39	2.47
		Pi	10	2.53	0.03	0.01	2.51	2.55	2.50	2.58
	dpi 6	Phi 1 app	10	2.75	0.02	0.01	2.73	2.77	2.71	2.79
		Phi 6 apps	10	2.77	0.02	0.01	2.75	2.78	2.73	2.79
		Total	40	2.62	0.15	0.02	2.57	2.67	2.39	2.79
		Control	10	2.22	0.03	0.01	2.20	2.24	2.19	2.27
		Pi	10	2.32	0.03	0.01	2.30	2.34	2.29	2.37
	dpi 8	Phi 1 app	10	2.70	0.02	0.01	2.69	2.72	2.67	2.74
		Phi 6 apps	10	2.79	0.02	0.01	2.77	2.80	2.75	2.82
		Total	40	2.51	0.24	0.04	2.43	2.59	2.19	2.82
		Control	10	2.18	0.03	0.01	2.15	2.20	2.10	2.22
		Pi	10	2.24	0.02	0.01	2.22	2.25	2.20	2.27
	dpi 10	Phi 1 app	10	2.51	0.02	0.01	2.50	2.53	2.49	2.55
		Phi 6 apps	10	2.55	0.02	0.00	2.53	2.56	2.53	2.57
		Total	40	2.37	0.17	0.03	2.31	2.42	2.10	2.57
A.stolonifera		Control	10	1.79	0.03	0.01	1.77	1.82	1.75	1.86
		Pi	10	1.74	0.03	0.01	1.71	1.76	1.70	1.77
	dpi 0	Phi 1 app	10	1.78	0.02	0.01	1.76	1.79	1.74	1.80
		Phi 6 apps	10	2.03	0.04	0.01	2.00	2.06	1.97	2.10
		Total	40	1.83	0.12	0.02	1.80	1.87	1.70	2.10
		Control	10	2.08	0.04	0.01	2.04	2.11	1.99	2.12
		Pi	10	2.40	0.03	0.01	2.38	2.43	2.36	2.45
	dpi 2	Phi 1 app	10	2.53	0.02	0.01	2.51	2.54	2.50	2.55
		Phi 6 apps	10	2.59	0.05	0.01	2.55	2.62	2.50	2.65
		Total	40	2.40	0.20	0.03	2.33	2.46	1.99	2.65
		Control	10	2.43	0.03	0.01	2.41	2.45	2.39	2.47
		Pi	10	2.56	0.03	0.01	2.54	2.58	2.50	2.61
	dpi 4	Phi 1 app	10	2.58	0.04	0.01	2.55	2.61	2.50	2.64
		Phi 6 apps	10	2.72	0.03	0.01	2.70	2.74	2.67	2.76
		Total	40	2.57	0.11	0.02	2.54	2.61	2.39	2.76
		Control	10	2.44	0.02	0.01	2.42	2.45	2.40	2.46
		Pi	10	2.63	0.03	0.01	2.61	2.66	2.58	2.66
	dpi 6	Phi 1 app	10	2.84	0.02	0.01	2.82	2.85	2.79	2.86
		Phi 6 apps	10	2.94	0.03	0.01	2.92	2.96	2.90	2.99
		Total	40	2.71	0.20	0.03	2.65	2.78	2.40	2.99
		Control	10	2.60	0.02	0.01	2.58	2.61	2.57	2.64
		Pi	10	2.51	0.02	0.01	2.50	2.53	2.49	2.55
	dpi 8	Phi 1 app	10	2.79	0.02	0.01	2.77	2.80	2.75	2.82
		Phi 6 apps	10	2.81	0.02	0.01	2.79	2.82	2.77	2.84
		Total	40	2.68	0.13	0.02	2.64	2.72	2.49	2.84
		Control	10	2.47	0.03	0.01	2.45	2.49	2.44	2.51
		Pi	10	2.43	0.02	0.01	2.41	2.45	2.39	2.47
	dpi 10	Phi 1 app	10	2.74	0.02	0.01	2.72	2.75	2.70	2.77
		Phi 6 apps	10	2.80	0.02	0.01	2.79	2.82	2.77	2.84
		Total	40	2.61	0.17	0.03	2.56	2.66	2.39	2.84

There was a significant ($p < 0.05$) effect on TPC levels, from treatments and between turfgrass species at all times sampled from 0 to 10 dpi, as determined by two-way Anova, with a significant ($p < 0.05$) interaction at all times, with the exception of at 6 and 8 dpi, Table 5-11. TPC peaked in both turfgrass species between 4 and 8 dpi, levels then decreased. Tissues treated with either a single application or 6 sequential treatments of Phi accumulated significantly ($p < 0.05$) higher levels from 2 dpi, than either the Pi treated or control tissues, Fig. 5-25. In tissues receiving 6 sequential treatments of Phi, TPC levels were significantly ($p < 0.05$) greater that those receiving a single Phi treatment at 0, 8 and 10 dpi in *P. annua* and 0, 2, 4, 6, and 10 dpi in *A. stolonifera*. In Pi treated tissues TPC levels were significantly ($p < 0.05$) greater than controls at 2, 6, 8 and 10 dpi in *P. annua*, while in *A. stolonifera*, levels were greater than controls at 2, 4, and 6 dpi, but significantly ($p < 0.05$) less at 8 and 10 dpi.

Table 5-11 Two-way Anova of TPC levels in infected tissues over 10 dpi in greenhouse turfgrasses treated with SDW (control), Pi, Phi (1 app) and Phi (6 apps).

Turfgrass species				
	df	f	p	η2
0 dpi	1,72	289.129	< .001	0.801
2 dpi	1,72	132.199	< .001	0.647
4 dpi	1,72	385.23	< .001	0.843
6 dpi	1,72	242.466	< .001	0.771
8 dpi	1,72	968.849	< .001	0.931
10 dpi	1,72	1936.002	< .001	0.964
Treatment				
	df	f	p	η2
0 dpi	3,72	129.365	< .001	0.844
2 dpi	3,72	632.681	< .001	0.963
4 dpi	3,72	176.195	< .001	0.880
6 dpi	3,72	1060.458	< .001	0.978
8 dpi	3,72	1466.676	< .001	0.984
10 dpi	3,72	1152.819	< .001	0.980
Interaction				
	df	f	p	η2
0 dpi	3,72	15.102	< .001	0.386
2 dpi	3,72	45.319	< .001	0.654
4 dpi	3,72	25.286	< .001	0.513
6 dpi	3,72	32.683	> .05	0.577
8 dpi	3,72	208.866	< .05	0.897
10 dpi	3,72	16.488	< .001	0.407

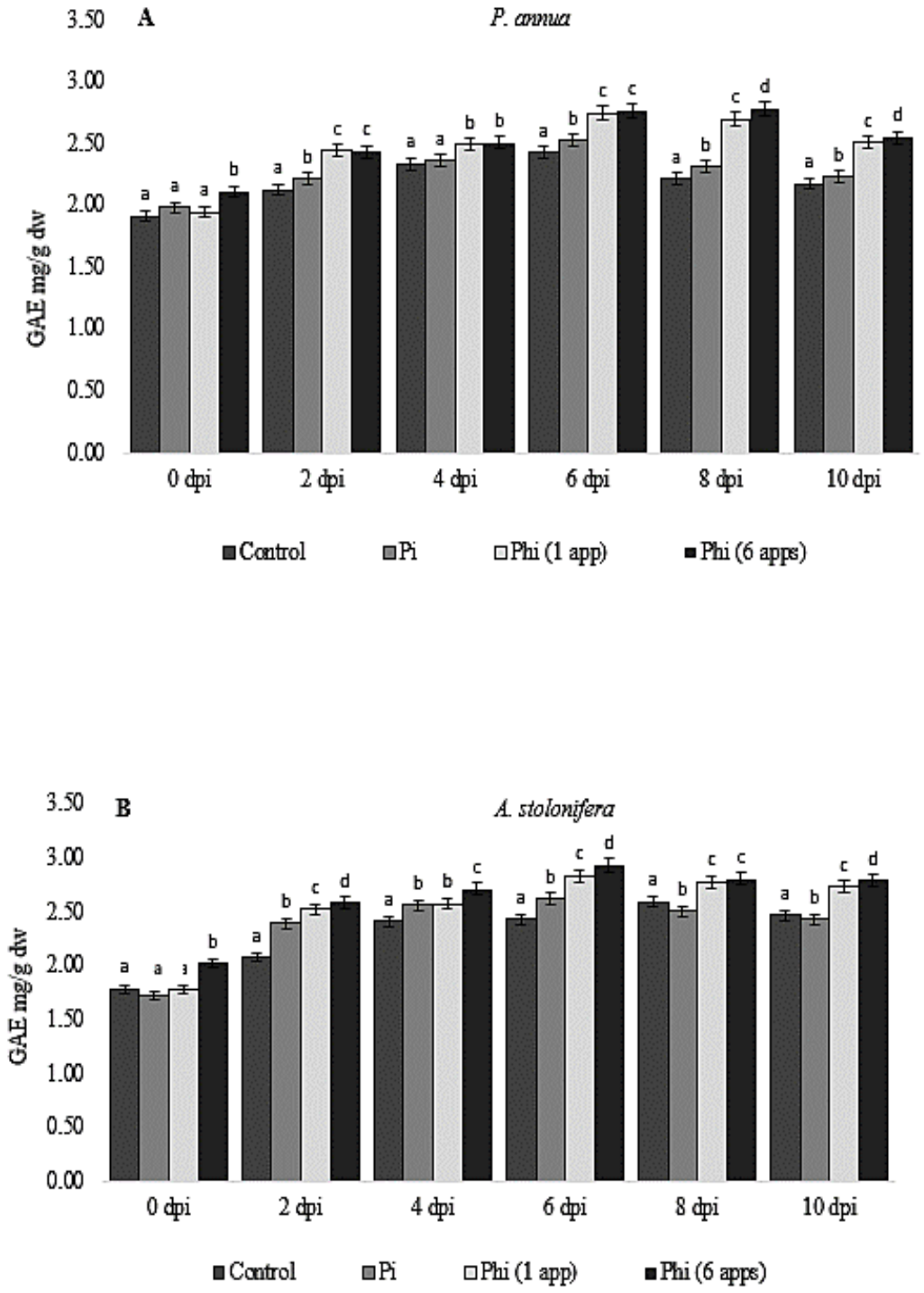


Figure 5-25 TPC as GAE mg/g dw, in *M. nivale* infected tissues over 10 dpi in greenhouse turfgrasses. TPC as GAE mg/g dw, in *M. nivale* infected tissues over 10 dpi in greenhouse turfgrasses treated with SDW (control), Pi, Phi (1 app) and Phi (6 apps). A: *P.annua*, B: *A. stolonifera*. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

5.5.2.4 Experiment 3, Effect of Phi treatment on H_2O_2 accumulation in infected and un-infected turfgrass

Generation of H_2O_2 was determined in leaf tissues of *P. annua* and *A. stolonifera*, collected from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment, descriptive statistics are shown in Table 5-12. Following two-way Anova, significant ($p < 0.05$) differences in H_2O_2 generation following treatments were determined at 1 and 6 hpa, and between turfgrasses at 0, 1, 6, 12, 24, 48 hpa, Table 5-13. The interaction effect between turfgrass species and treatment on H_2O_2 generation was not statistically significant ($p > 0.05$) at any time period post application, therefore, an analysis of the main effects was performed and pairwise comparisons run with 95% confidence intervals and p-values were Bonferroni-adjusted. There were significantly ($p < 0.05$) greater levels of H_2O_2 at 1, 6 and 24 hpa in both Phi and Pi treated tissues of *P. annua* and at 6 and 72 hpa in *A. stolonifera,* Fig. 5-26. Following treatment, over 72 hpa, there was a clear spike in H_2O_2 levels at 6 hpa in both turfgrass species with a second increase at 72 hpa in *A. stolonifera*. Over each time period, there were no significant ($p > 0.05$) difference in H_2O_2 levels between Pi and Phi treated tissues. Fig. 5-26.

Table 5-12 Descriptive statistics of H_2O_2 concentrations in leaf tissues of *P.annua* and *A.stolonifera*, collected from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua	0 hpa	Control	10	19.20	1.46	0.46	18.15	20.24	17.00	22.00
		Pi	10	20.12	1.25	0.39	19.22	21.01	18.40	22.00
		Phi	10	19.66	1.44	0.45	18.63	20.69	17.50	21.25
		Total	30	19.66	1.39	0.25	19.14	20.18	17.00	22.00
	1 hpa	Control	10	19.70	1.40	0.44	18.70	20.70	18.00	22.00
		Pi	10	22.65	1.25	0.40	21.75	23.54	20.50	24.40
		Phi	10	21.68	1.12	0.36	20.87	22.48	20.00	23.00
		Total	30	21.34	1.74	0.32	20.69	21.99	18.00	24.40
	6 hpa	Control	10	21.50	1.45	0.46	20.46	22.54	18.50	23.00
		Pi	10	25.58	1.15	0.36	24.75	26.41	24.00	27.50
		Phi	10	26.03	1.52	0.48	24.94	27.12	24.00	28.00
		Total	30	24.37	2.47	0.45	23.45	25.29	18.50	28.00
	12 hpa	Control	10	20.77	1.65	0.52	19.59	21.95	18.50	23.00
		Pi	10	21.77	1.04	0.33	21.03	22.51	20.40	23.00
		Phi	10	21.25	1.36	0.43	20.28	22.22	19.00	23.00
		Total	30	21.26	1.39	0.25	20.75	21.78	18.50	23.00
	24 hpa	Control	10	22.00	1.83	0.58	20.69	23.31	18.50	24.00
		Pi	10	22.52	1.22	0.39	21.65	23.39	20.50	24.00
		Phi	10	23.95	1.98	0.63	22.53	25.37	20.50	27.00
		Total	30	22.82	1.85	0.34	22.13	23.51	18.50	27.00
	48 hpa	Control	10	21.28	1.36	0.43	20.31	22.25	18.50	23.00
		Pi	10	19.85	1.47	0.47	18.80	20.90	18.00	22.00
		Phi	10	20.25	1.75	0.55	19.00	21.50	17.50	23.00
		Total	30	20.46	1.60	0.29	19.86	21.06	17.50	23.00
	72 hpa	Control	10	19.65	1.62	0.51	18.49	20.81	17.00	22.00
		Pi	10	20.25	1.75	0.55	19.00	21.50	17.50	23.00
		Phi	10	21.21	1.44	0.45	20.18	22.24	18.60	23.00
		Total	30	20.37	1.68	0.31	19.74	21.00	17.00	23.00
A.stolonifera	0 hpa	Control	10	18.80	1.40	0.44	17.80	19.80	16.50	20.50
		Pi	10	18.60	1.43	0.45	17.58	19.62	16.50	21.00
		Phi	10	17.99	1.16	0.37	17.16	18.82	16.50	20.00
		Total	30	18.46	1.33	0.24	17.97	18.96	16.50	21.00
	1 hpa	Control	10	18.73	1.53	0.48	17.63	19.82	16.50	21.00
		Pi	10	19.64	1.20	0.38	18.78	20.50	18.00	22.00
		Phi	10	19.18	1.02	0.32	18.45	19.91	17.50	20.50
		Total	30	19.18	1.28	0.23	18.70	19.66	16.50	22.00
	6 hpa	Control	10	20.16	1.61	0.51	19.01	21.31	17.50	22.00
		Pi	10	24.18	1.61	0.51	23.03	25.33	22.00	27.00
		Phi	10	22.78	1.57	0.50	21.66	23.90	20.50	26.00
		Total	30	22.37	2.29	0.42	21.52	23.23	17.50	27.00
	12 hpa	Control	10	15.30	1.23	0.39	14.42	16.18	13.00	17.00
		Pi	10	16.03	1.41	0.45	15.02	17.03	14.50	18.00
		Phi	10	15.66	0.99	0.31	14.95	16.37	14.00	17.00
		Total	30	15.66	1.22	0.22	15.21	16.12	13.00	18.00
	24 hpa	Control	10	18.81	1.43	0.45	17.79	19.83	17.00	21.00
		Pi	10	19.26	0.97	0.31	18.56	19.95	17.60	20.50
		Phi	10	19.72	1.41	0.45	18.71	20.72	17.80	22.00
		Total	30	19.26	1.30	0.24	18.77	19.75	17.00	22.00
	48 hpa	Control	10	21.75	1.25	0.40	20.85	22.65	19.50	23.00
		Pi	10	21.87	1.18	0.37	21.02	22.71	19.90	23.55
		Phi	10	20.99	1.54	0.49	19.89	22.09	18.50	23.00
		Total	30	21.54	1.34	0.25	21.03	22.04	18.50	23.55
	72 hpa	Control	10	18.44	1.69	0.53	17.23	19.65	16.40	21.00
		Pi	10	19.90	1.43	0.45	18.88	20.92	18.00	22.00
		Phi	10	20.99	1.82	0.58	19.68	22.29	18.50	23.50
		Total	30	19.78	1.92	0.35	19.06	20.49	16.40	23.50

Table 5-13 Two-way Anova of H_2O_2 concentrations in turfgrass leaf tissues collected from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment.

Turfgrass species				
	df	f	p	η2
0 hpa	1,54	11.568	0.001	0.176
1 hpa	1,54	43.762	< .001	0.448
6 hpa	1,54	26.813	< .001	0.332
12 hpa	1,54	278.67	< .001	0.838
24 hpa	1,54	83.276	< .001	0.607
48 hpa	1,54	8.394	0.005	0.135
72 hpa	1,54	1.997	0.163	0.036
Treatment				
	df	f	p	η2
0 hpa	2,54	0.800	0.455	0.029
1 hpa	2,54	19.046	< .001	0.306
6 hpa	2,54	43.955	< .001	0.619
12 hpa	2,54	2.203	0.120	0.075
24 hpa	2,54	4.611	0.014	0.146
48 hpa	2,54	2.082	0.135	0.072
72 hpa	2,54	7.923	0.001	0.227
Interaction				
	df	f	p	η2
0 hpa	2,54	1.311	0.278	0.046
1 hpa	2,54	3.492	0.037	0.115
6 hpa	2,54	2.643	0.080	0.089
12 hpa	2,54	0.056	0.945	0.002
24 hpa	2,54	0.743	0.481	0.027
48 hpa	2,54	1.649	0.202	0.058
72 hpa	2,54	0.541	0.585	0.02

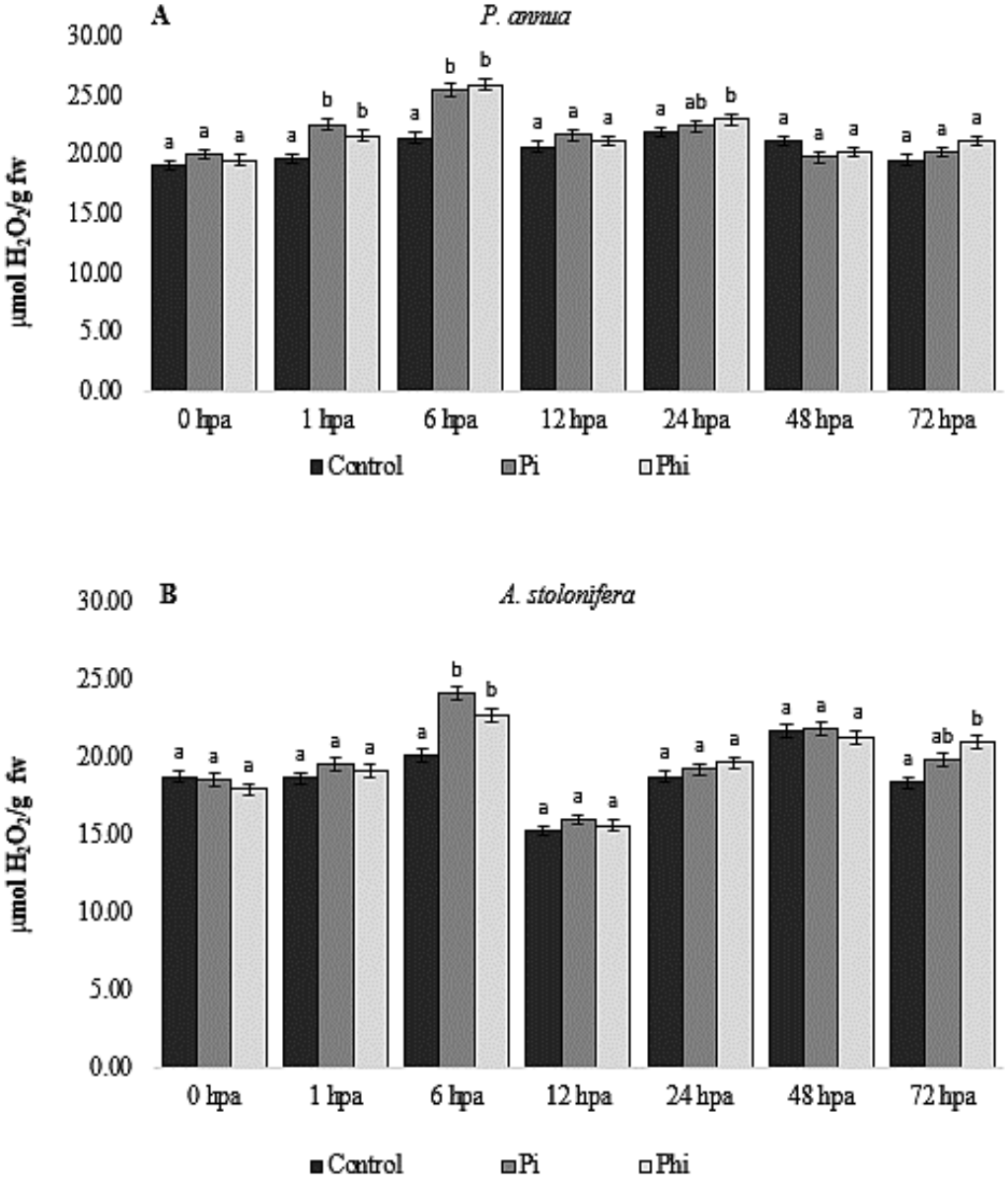


Figure 5-26 H_2O_2 concentrations in un-infected greenhouse turfgrass tissues. H_2O_2 concentrations as µmol H_2O_2/g fw, in turfgrass leaf tissues collected from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment. A: *P. annua*, B: *A. stolonifera*. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

5.5.2.5 Effect of Phi treatment on H_2O_2 accumulation in infected turfgrass

Descriptive statistics for the generation of H_2O_2 following SDW (control), Pi, Phi (1 app) and Phi (6 apps) treatment of *M. nivale* infected *P. annua* and *A.stolonifera* greenhouse plants are shown in Table 5-14.

Table 5-14 Descriptive statistics of H_2O_2 generation in SDW (control), Pi and Phi (1 app) and Phi (6 apps) treated tissues of *M. nivale* infected *P.annua* and *A. stolonifera* greenhouse plants over 10 days post inoculation.

Turfgrass species			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
P.annua		Control	10	19.20	0.96	0.30	18.51	19.89	18.00	21.00
		Pi	10	20.12	1.09	0.35	19.34	20.90	18.50	21.70
	0 dpi	Phi 1 app	10	19.66	0.85	0.27	19.06	20.27	18.20	21.00
		Phi 6 apps	10	19.66	0.85	0.27	19.06	20.27	18.20	21.00
		Total	40	19.66	0.96	0.15	19.35	19.97	18.00	21.70
		Control	10	29.00	2.09	0.66	27.50	30.50	27.00	32.50
		Pi	10	30.39	1.79	0.56	29.11	31.67	27.00	32.00
	2 dpi	Phi 1 app	10	29.69	1.95	0.62	28.30	31.08	27.00	32.00
		Phi 6 apps	10	29.69	2.59	0.82	27.84	31.54	27.00	34.00
		Total	40	29.69	2.10	0.33	29.02	30.36	27.00	34.00
		Control	10	22.50	1.37	0.43	21.52	23.49	19.50	24.00
		Pi	10	23.58	1.64	0.52	22.40	24.75	20.50	26.00
	4 dpi	Phi 1 app	10	23.03	1.47	0.47	21.97	24.08	20.50	25.50
		Phi 6 apps	10	23.03	1.47	0.47	21.97	24.08	20.50	25.50
		Total	40	23.03	1.49	0.23	22.56	23.51	19.50	26.00
		Control	10	18.00	1.73	0.55	16.76	19.24	16.00	21.00
		Pi	10	18.90	1.56	0.49	17.78	20.02	16.50	21.00
	6 dpi	Phi 1 app	10	18.43	1.29	0.41	17.50	19.35	16.50	20.50
		Phi 6 apps	10	18.43	1.29	0.41	17.50	19.35	16.50	20.50
		Total	40	18.44	1.46	0.23	17.97	18.90	16.00	21.00
		Control	10	23.78	1.57	0.50	22.66	24.89	21.50	26.75
		Pi	10	24.92	1.30	0.41	23.99	25.85	23.00	27.00
	8 dpi	Phi 1 app	10	24.35	1.26	0.40	23.45	25.25	22.33	25.80
		Phi 6 apps	10	24.36	1.26	0.40	23.45	25.26	22.33	25.80
		Total	40	24.35	1.36	0.22	23.91	24.79	21.50	27.00
		Control	10	19.66	1.09	0.35	18.88	20.44	18.20	21.40
		Pi	10	19.20	1.15	0.36	18.38	20.02	17.20	20.70
	10 dpi	Phi 1 app	10	19.66	0.85	0.27	19.06	20.27	18.20	21.00
		Phi 6 apps	10	20.12	1.39	0.44	19.13	21.12	18.00	22.40
		Total	40	19.66	1.14	0.18	19.30	20.03	17.20	22.40
A.stolonifera		Control	10	17.30	1.19	0.38	16.44	18.15	15.45	19.20
		Pi	10	20.12	1.09	0.35	19.34	20.90	18.50	21.70
	0 dpi	Phi 1 app	10	19.66	0.85	0.27	19.06	20.27	18.20	21.00
		Phi 6 apps	10	19.66	0.85	0.27	19.06	20.27	18.20	21.00
		Total	40	19.19	1.48	0.23	18.71	19.66	15.45	21.70
		Control	10	26.13	1.77	0.56	24.87	27.40	23.26	28.80
		Pi	10	28.39	1.33	0.42	27.44	29.35	26.45	30.00
	2 dpi	Phi 1 app	10	29.69	1.95	0.62	28.30	31.08	27.00	32.00
		Phi 6 apps	10	28.88	1.44	0.46	27.85	29.91	26.85	31.55
		Total	40	28.27	2.07	0.33	27.61	28.94	23.26	32.00
		Control	10	20.27	1.20	0.38	19.41	21.13	18.55	21.75
		Pi	10	23.58	1.80	0.57	22.29	24.87	20.50	26.50
	4 dpi	Phi 1 app	10	21.61	1.24	0.39	20.72	22.50	19.00	23.13
		Phi 6 apps	10	23.03	1.62	0.51	21.87	24.19	20.50	26.50
		Total	40	22.12	1.94	0.31	21.50	22.74	18.55	26.50
		Control	10	16.22	0.86	0.27	15.61	16.84	15.00	17.55
		Pi	10	18.87	1.42	0.45	17.85	19.89	16.70	21.00
	6 dpi	Phi 1 app	10	18.43	1.29	0.41	17.50	19.35	16.50	20.50
		Phi 6 apps	10	17.96	1.64	0.52	16.78	19.13	15.50	20.50
		Total	40	17.87	1.64	0.26	17.34	18.39	15.00	21.00
		Control	10	18.87	1.47	0.46	17.82	19.92	16.50	21.00
		Pi	10	16.22	1.26	0.40	15.31	17.12	14.50	18.00
	8 dpi	Phi 1 app	10	17.96	1.41	0.45	16.95	18.97	16.00	20.50
		Phi 6 apps	10	18.43	1.42	0.45	17.41	19.44	16.50	20.50
		Total	40	17.87	1.68	0.27	17.33	18.41	14.50	21.00
		Control	10	22.82	2.05	0.65	21.35	24.29	19.50	25.00
		Pi	10	25.91	1.77	0.56	24.65	27.17	23.28	28.20
	10 dpi	Phi 1 app	10	27.16	1.15	0.36	26.33	27.98	26.00	29.00
		Phi 6 apps	10	26.53	0.92	0.29	25.87	27.18	25.00	28.00
		Total	40	25.60	2.24	0.35	24.89	26.32	19.50	29.00

Figure 5-24 shows the course of infection in greenhouse inoculated turfgrasses, in a similar manner to TPC levels, it was determined that H_2O_2 generation increased in response to pathogen challenge. Analysis of leaf tissues determined that in infected plants, over the course of 10 dpi, H_2O_2 increased in both turfgrass species, with accumulation peaks at 2 dpi, Fig. 5-27. Levels at 4 and 6 dpi decreased to amounts similar to those prior to infection, with a second peak in *P. annua* at 8 dpi and at 10 dpi in *A. stolonifera.* Results of a two-way Anova are show in Table 5-15, post hoc analyses determined in *P. annua,* H_2O_2 generation did not vary significantly ($p > 0.05$) between treatments over the 10 day study period. But in *A. stolonifera* tissues, there were significantly ($p < 0.05$) greater levels in Pi, Phi (1 app) and Phi (6 apps) tissues, compared to controls over 10 dpi, with the exception of levels at 8 dpi, where in Pi treated tissues H_2O_2 levels were significantly ($p < 0.05$) lower that other treatments, including the controls.

Table 5-15 Two-way Anova of H_2O_2 concentrations in SDW (control), Pi, Phi (1 app) and Phi (6 apps) treated tissues of *M. nivale* infected *P.annua* and *A. stolonifera* greenhouse plants over 10 days post inoculation

Turfgrass species				
	df	f	p	η2
0 dpi	1,72	4.766	0.032	0.062
2 dpi	1,72	11.158	0.001	0.134
4 dpi	1,72	7.436	0.008	0.094
6 dpi	1,72	3.271	0.075	0.043
8 dpi	1,72	445.887	< .001	0.861
10 dpi	1,72	385.250	< .001	0.843
Treatment				
	df	f	p	η2
0 dpi	3,72	13.872	< .001	0.366
2 dpi	3,72	5.11	0.003	0.176
4 dpi	3,72	8.073	< .001	0.252
6 dpi	3,72	5.703	0.001	0.192
8 dpi	3,72	1.483	0.226	0.058
10 dpi	3,72	11.022	< .001	0.315
Interaction				
	df	f	p	η2
0 dpi	3,72	4.766	0.004	0.166
2 dpi	3,72	2.224	0.093	0.085
4 dpi	3,72	2.76	0.048	0.103
6 dpi	3,72	1.753	0.164	0.068
8 dpi	3,72	6.842	< .001	0.222
10 dpi	3,72	9.937	< .001	0.293

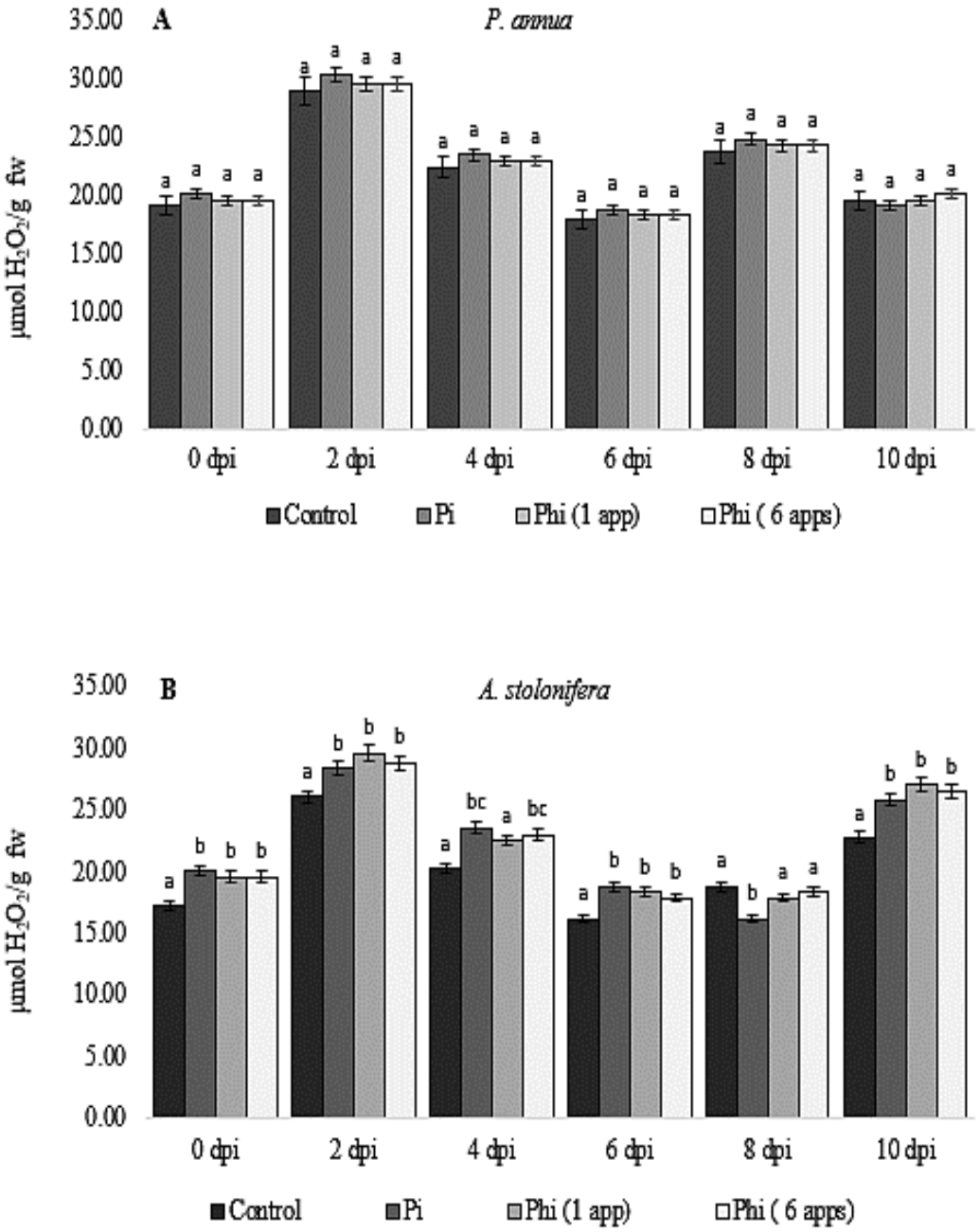


Figure 5-27 H_2O_2 concentrations in *M. nivale* infected greenhouse turfgrass tissues.H_2O_2 concentrations as µmol H_2O_2/g fw, in SDW (control), Pi, Phi (1 app) and Phi (6 apps) treated tissues of *M. nivale* infected *P.annua* and *A. stolonifera* greenhouse plants over 10 days post inoculation. Bars indicate 95% confidence limits, letters indicate significant differences at each time period determined by Post hoc comparisons using Bonferroni correction at $p < 0.05$, n=10.

5.5.2.6 Visualisations of TPC and H_2O_2

Analyses of infected leaf tissues using fluorescence microscopy, confirmed accumulations of H_2O_2 and TPC in response to *M. nivale* infection. H_2O_2 accumulations could be observed in close proximity to stomatal infection sites in response to pathogen ingression, Figs. 5-28 to 5-30. In Phi treated tissues TMB fluorescence was observed at penetration sites earlier than in Pi treated or control tissues, although eventual accumulations appeared similar at later stages of infection.

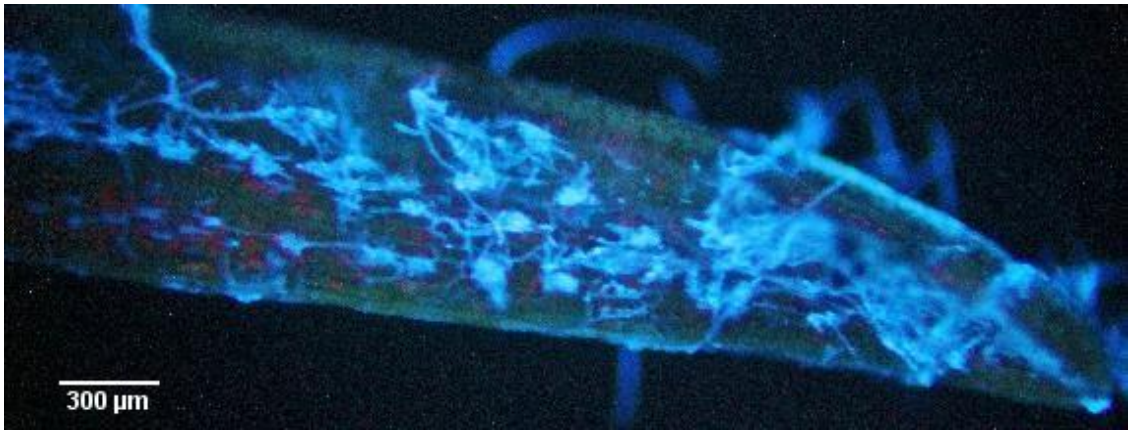


Figure 5-28 *M. nivale* infected *P.annua* leaf. *M. nivale* infected *P.annua* leaf, viewed under UV fluorescence using a Bresser L3001 epifluorescent microscope, following aniline blue and TMB staining. Blue hyphae are visible with H_2O_2 fluorescencing at stomatal infection sites.

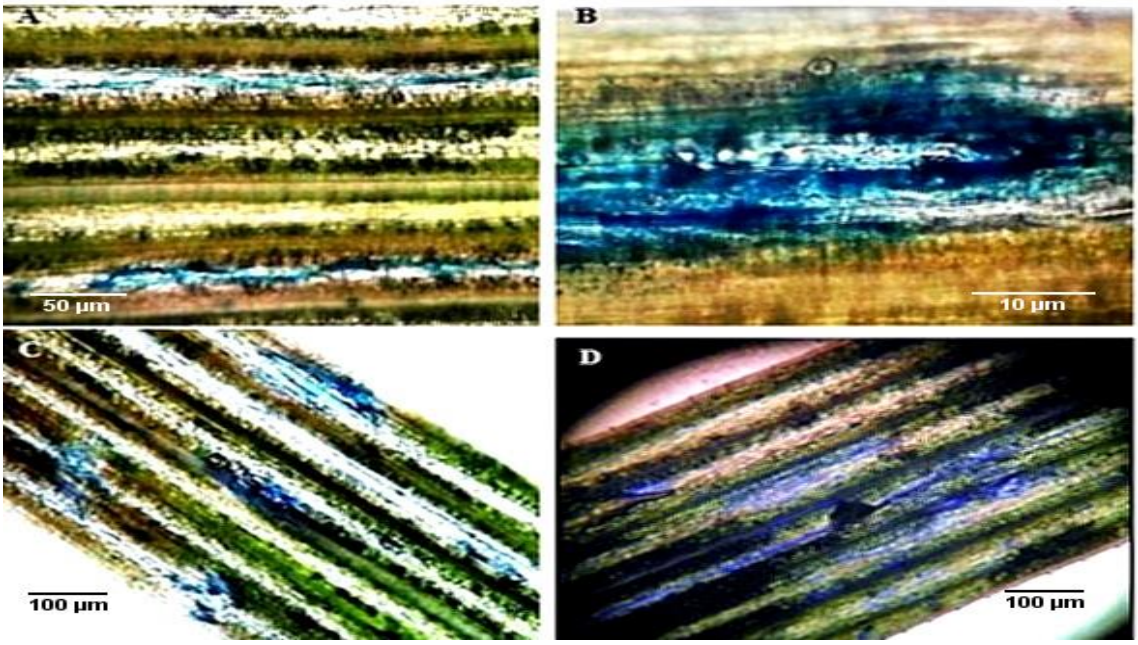


Figure 5-29 TMB stained leaf tissues showing H_2O_2 fluorescence. TMB stained turfgrass leaf tissues, viewed using a Bresser L3001 epifluorescent microscope, showing H_2O_2 fluorescence at *M. nivale* infection sites. A: *P. annua* leaf. B: H_2O_2 accumulation around site of infected stoma. C: *A. stolonifera* leaf showing H_2O_2 fluorescence around infection sites. D: *P. annua* leaf showing TMB fluorescence at infected stomata and red autofluorescence of chlorophyll.

Fig. 5-31 illustrates further examples of accumulations of H_2O_2 and TPC in response to *M. nivale* infection. Hyphal penetration of stomata produced blue TMB fluorescence indicating H_2O_2 accumulations. Also displayed in Fig.5-32 D are autofluorescence of phenolic compounds also in response to infection.

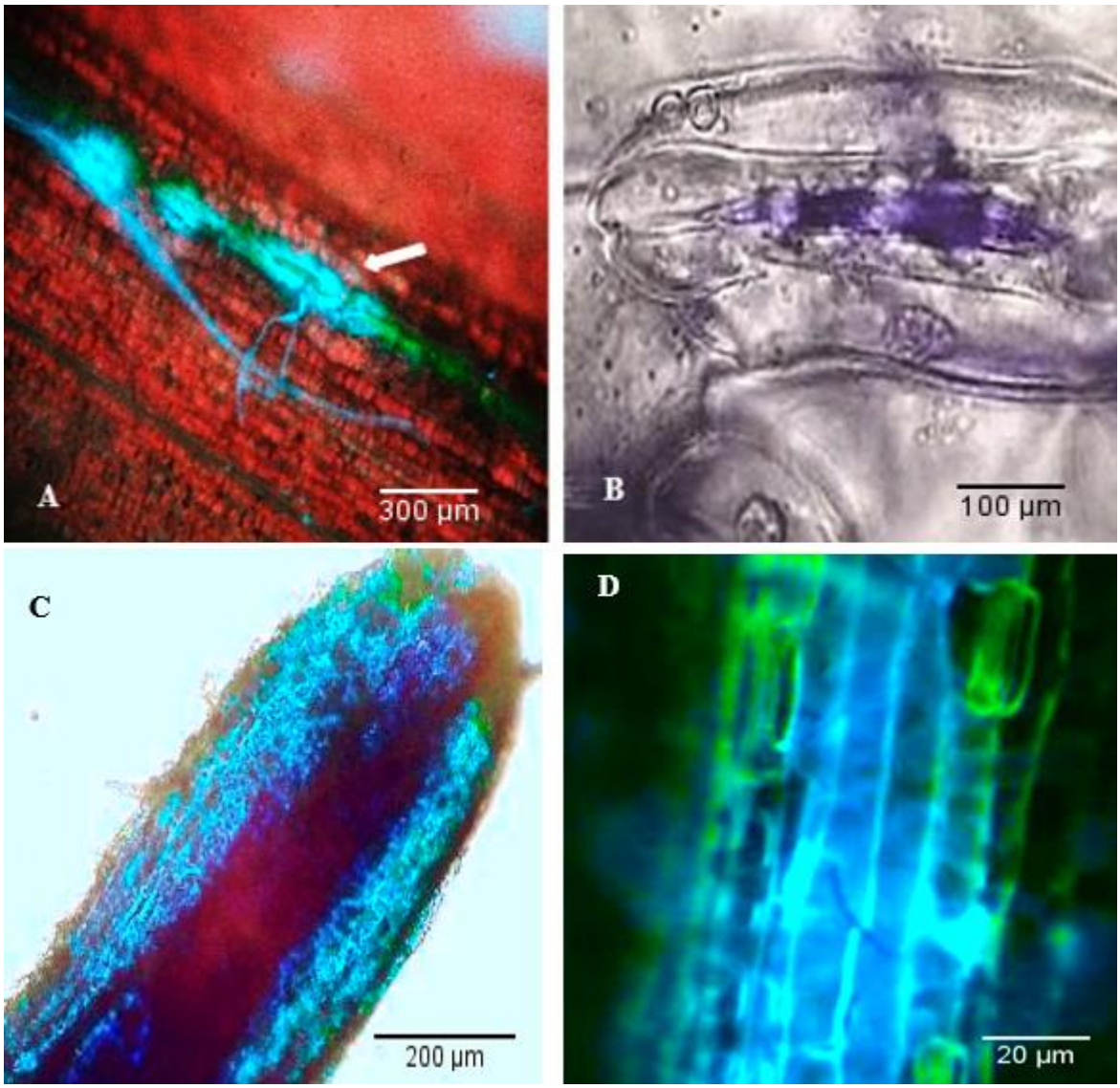


Figure 5-30 Accumulations of H_2O_2 and TPC in response to *M. nivale* infection in turfgrass leaves. Accumulations of H_2O_2 and TPC in response to *M. nivale* infection in turfgrass leaves, viewed using a Bresser L3001 epifluorescent microscope. A: *M. nivale* hyphae entering stoma, (arrow) with TMB fluorescence indicating H_2O_2 accumulation. B: view of infected stoma showing H_2O_2 accumulation. C: *P. annua* leaf following TMP staining showing H_2O_2 synthesis in response to infection (red autofluorescence of chlorophyll). D: Infected *A. stolonifera* leaf showing autofluorescence of phenolic compounds (light yellow).

5.6 Discussion

The goals of this section of the study were to determine the source of inoculum in field incidences, to plot the course of mycelial growth and host penetration in the field and following greenhouse inoculation and visualisation of the *in planta* infection process, reproduction and spore release. Following on from these assessments, turfgrass defence responses in regard to phenolic compound and H_2O_2 synthesis and the effect, if any, of Phi treatment on these compounds was determined.

5.6.1 *M. nivale* infection process

M. nivale incidence on intensely managed turfgrasses in the temperate climate of Ireland is very common, therefore, naturally infected plants in the field, to observe and to provide tissue samples were in abundance. Inoculated greenhouse pot samples were also a source of much data, as the controlled environment within the greenhouses provided excellent temperatures and levels of humidity for *M. nivale* incidence.

In the field, examination of the thatch and upper rootzone layers of golf greens and trial plots, showed that, in areas with prior history of *M. nivale* infections, sources of inoculum were in abundance throughout the year, evident as clearly identifiable and numerous hyphal fragments and larger amounts of mycelium. The observed levels of mycelium in the rootzones varied between the older golf greens (over ten years old), which had higher levels of semi-decomposed thatch layers, and more recently built sand based greens, with less organic layering. This would suggest that semi-decomposed organic matter does indeed provide a source of nutrition for the fungus. Furthermore, this indigenous organic layer, allows for a build-up of pathogenic fungal inoculum throughout the year, agreeing with the study by Domsch *et al*. (1980), who concluded that *M. nivale* can survive and proliferate for periods of up to a year in organic matter. This source of inoculum, readily available in the rootzones, is easily spread, and capable of remaining viable until favourable environmental conditions allow for re-growth and eventual colonisation of susceptible turfgrasses. This profusion of rootzone based inoculum enhances the current turfgrass management policy of limiting the amount of thatch and organic matter build-up in sports turf rootzones and also the theory that encouragement of a wide variety of soil micro-organisms would benefit disease suppression through competitive inhibition of *M. nivale* growth.

To date, there are no detailed published studies on the *M. nivale* infection process in turfgrasses. The general opinion is that sources of inoculum are mycelia, conidia or

ascospores, disseminated by wind, water, human or mechanical means, from infested soil or plant debris (Mann, 2004a; Mann, 2004b; Turgeon, 2005; Vargas, 2005) (Parry *et al.*, 1995; Mahuku *et al.*, 1998; Tronsmo *et al.*, 2001; Mann, 2004a). In many plant pathogenic fungi, conidia play a key role in causing new disease outbreaks, acting as the primary inoculum in the disease cycle (Agrios, 2005). *Colletotrichum graminicola,* for example, produce conidia which germinate on the host leaves producing germ tubes which grow either directly towards stomata, or form penetration appressoria in order to infect the plant (Khan and Hsiang, 2003). The role of conidia in the infection of wheat was reported by Kang *et al.* (2004) who showed that following germination, *M. nivale* var. *majus* conidia produced germ tubes and entered the cell wall directly via a penetration peg.

In this study, however, hyphal inoculum appeared to be the only source of infection. During microscopic analyses of golf green and trial plot rootzones, conidia were observed, but they did not lead to direct infection of the plants. Conidial amounts also varied through the year, with levels greatest during periods when climatic conditions favoured disease incidence and were more evident following infection and formation of aerial sporodochia. This lack of pathogenicity by conidia was also observed in other studies (Pronczuk and Messyasz; Jewell and Hsiang, 2013), conidial inoculum was much slower in producing disease symptoms on plants used in these experiments. It could be concluded from these data and from the profusion of conidial numbers following turfgrass infection, that their main function, is as a means of dispersal and propagation, rather than being a form of inoculum. The conidia may germinate in the thatch or on dead plant tissue and grow saprophytically, with the resulting mycelia being the source of inoculum.

Following analysis of numerous rootzone and turfgrass root systems, there were no observable incidences of *M. nivale* penetration or infection of the root tissues, despite the close proximity of fungal mycelium. In all observed cases in the field, hyphae were the primary source of infection, growing and extending from the plant/soil interface, to the crowns and lower sheaths of the turfgrasses. The initial sites of infection were the leaf sheaths and lower leaf blades, which were growing in contact with, or close to the infested soil. This initial infection process is similar to that reported in triticale (the cereal obtained by crossing *Triticum vulgare* with *Secale cereale*) by Dubas *et al*. (2010) and in *Secale cereale* by Zur *et al.* (2011), following soil borne inoculation. Both these studies

determined *M. nivale* infection began at ground level and progressed vertically up the plants, before entering the tissues via stomata.

In the disease conducive environment of the greenhouses, hyphal inoculation, via either infested wheat bran or hyphal suspension, produced rapid displays of disease incidence. Radial growths of mycelium were evident in abundance on the infected leaves 4 to 6 dpi. In all observable incidences, hyphae grew from the point of inoculation, and, in a similar manner as in field infections, entered the plants via the stomata. While there are no published studies on *M. nivale* infection of amenity turfgrasses in the field, there are reports of infections following inoculation in controlled environment conditions. Jewell and Hsiang (2013), reported the *M. nivale* infection process, following hyphal and conidial inoculations of Kentucky bluegrass (*Poa pratensis*), a species commonly used in amenity turf and closely related to *P. annua.* They concluded that following hyphal inoculation of detached leaf segments, penetration in all cases was via stomata and that no penetration appressoria were formed. Interestingly, they concluded that while conidial inoculum caused infection, it was at a much slower rate relative to the hyphal inoculum.

Once the pathogen had entered the plant, disease symptoms became rapidly evident. In all cases observed, either from field or greenhouse infections, following penetration, hyphae could be observed growing through the mesophyll, before entering the vascular tissues in the leaf. Dubas *et al*. (2010) and Zur *et al.* (2011) in their studies into *M. nivale* infection in cereals, reported formation of haustoria within the plant tissues following the initial penetration, these were not observed in this study. This is not to say that in some circumstances they are produced, the lack of observable haustoria in this research may be due to the prevalent environmental conditions or due to the different species of gramineae under study, Jewell and Hsiang (2013), in their study with *P. pratensis* also did not observe haustoria formation.

The effect of the pathogen infection on tissues was dramatic, outwardly, the leaves of the infected plants appeared discoloured and often wet, while internally, hyphae continued to extend, and entering cells, causing collapse. The mycelium continued to grow, lengthening and branching through the leaf tissues. As the infection progressed, the pathogen extracted nutrients and hyphae exited via the stomata, often producing sporodochia and conidia, thus completing the cycle if growth and reproduction..

5.6.2 Turfgrass defence responses

Plants produce a broad, complex array of induced defences and interconnected signaling pathways, which combine to combat invading micro-organisms. Two of the initial and important responses were assessed in this study, with a view to establishing how turfgrasses respond to *M. nivale* challenge and if Phi treatment enhanced these responses. The main focus of this study is the use of Phi to suppress *M. nivale* and this section of the research aimed to assess if Phi treatment enhanced turfgrass defence responses. Marketing of Phi products often indicates that Phi primes plants prior to disease challenge, leading to reduced susceptibility, by allowing the plant to respond more rapidly and vigorously than un-primed plants. Phi as a primer of plant defences has been researched and reported on previously. Numerous published studies, as detailed in the review of literature (Chapter One), have concluded that Phi can reduce pathogen challenge by enhancing synthesis of defence compounds (Saindrenan *et al.*, 1988; Jackson *et al.*, 2000; Daniel and Guest, 2005; Lobato *et al.*, 2011; Olivieri *et al.*, 2012). In these published studies however, it is unclear if the increased synthesis of defence compounds were as a result of interaction between the pathogen and Phi *in planta*, or whether Phi induced synthesis of defence related compounds prior to pathogen challenge.

5.6.2.1 Total phenolic content

TPC accumulation is an unspecific defence reaction commonly determined as an indicator of a plants response or reaction to exogenous stresses. Accumulation of TPC is a common response to pathogen challenge, the speed of synthesis following or accumulation of phenolics prior to infection, can influence the plants level of susceptibly or resistance to a particular pathogen. TPC accumulation is also a response to abiotic stresses in response to mechanical injury, drought, UV radiation and low temperature. It was therefore important to sample turfgrass tissues during a wide range of conditions and to ensure that treated turfgrasses and controls were harvested under identical situations. This ensured that TPC levels due to non-disease related pressures did not influence the assessments.

In this study, mean levels of TPC were assessed in infected and non-infected turfgrasses, in the field and from greenhouse samples. It was determined that *M. nivale* infection led to increased TPC accumulations in both situations and that levels in infected tissues were significantly higher ($p<0.01$), than non-infected plants. Overall, TPC levels in field samples, were greater than those samples from greenhouse tissues. This could be due to responses to the environmental conditions in the field. As stated, TPC accumulation is a

general response to many stresses including low temperatures. Increased levels of TPC have been demonstrated as a component of cold-hardening (Pociecha and Płażek, 2009) therefore, TPC levels during times of greatest disease risk (October to March) would have increased as a result of lower temperatures, so the response to pathogen challenge would be relatively less than in the moderate temperatures of the greenhouses. Despite these environmental pressures, TPC levels did increase in diseased plants relative to non-diseased, confirming the role of TPC accumulation as a defence response.

The accumulation of TPC in response to pathogen challenge and their importance in a plants resistance has been well documented in graminaceous species (Ishihara *et al.*, 1999; Jin and Yoshida, 2000; Okazaki *et al.*, 2004; Remusborel *et al.*, 2005). In barley for example, phenolic compound accumulation was determined in plants challenged with the pathogen *Erysiphe graminis* (powdery mildew) (Christensen *et al.*, 1998). Pociecha *et al.* (2009) concluded that increased levels of phenolic compounds gave rise to higher resistance to *M. nivale* in *Festulolium* spp. while Dubas *et al.* (2010) using fluorescence microscopy, concluded that phenolic compound accumulation at sites of *M. nivale* infection formed part of the defence response in triticale.

Results here determined that a single Phi treatment did influence TPC accumulations, leading to significantly higher levels, compared to controls in both field and greenhouse samples. Following Phi treatment TPC levels in field samples were significantly higher in *P. annua* from 12 to 72 hpa and from 1 to 72 hpa in *A. stolonifera* tissues, Fig. 5-21. The increases in TPC were similar in greenhouse turfgrasses with levels in *P. annua* significantly higher than controls at 1 hpa and from 12 to 72 hpa in *P. annua* and from 12 to 72 in *A. stolonifera,* Fig 5-22. While these data may indicate that a single Phi treatment enhances TPC accumulation, it should be noted that Pi treatment, both in field and in greenhouse turfgrasses also led to increased levels in a similar way to Phi, although the trend was for higher levels following Phi treatment.

A single Phi treatment led to significantly increased levels of TPC accumulations compared to controls, however, it can be argued that it was no different to the response elicited from the Pi treatments and therefore Phi has a similar effect as Pi treatments with regard to induced defence responses in plants. However, while a single treatment of Phi and Pi elicited similar responses in TPC levels, sequentially applied treatments, over a period of six months, gave rise to significantly higher levels, in Phi treated tissues compared with both Pi and control tissues, Figs 5-23 and 5-24. These data, therefore, would indicate that Phi does prime plants for defence prior to infection, and that Phi

mediated disease suppression to be successful, would require a number of applications prior to infection. This however, was not the case in the suppression of disease incidence in greenhouse inoculated plants. *M. nivale* infected greenhouse plants, following a single application of Phi, prior to inoculation, gave rise not only to significantly reduced disease incidence, but higher and more rapid accumulation of TPC, compared with Pi and untreated controls. This is not evidence of Phi priming the plant prior to pathogen challenge, but an enhancement of defence responses upon elicitation of infection. The presence of Phi in the plants tissues directly inhibited the growth of *M. nivale*, possibly stressing the pathogen, leading to increased production of elicitors, increasing both the time for the plants to respond and synthesis of defence compounds.

However, as stated, and as shown in Figs. 5-23 and 5-24, a series of sequential Phi applications, led to increased cumulative accumulations of TPC in treated turfgrasses, leading to the conclusion that Phi does indeed prime plants, prior to biotic stress. This conclusion that Phi primes plant defences prior to infection, is further strengthened by comparing disease diameters and TPC amounts in greenhouse turfgrasses following single or sequential Phi treatments, Figs. 5-24 and 5-25. Disease incidence in inoculated plants, as determined by infection diameters, were less in plants, following six sequential treatments of Phi, than those following a single Phi treatment. Furthermore, TPC accumulations in both *P.annua* and *A. stolonifera* following sequential Phi treatments, were significantly greater prior to inoculation and, as infection progressed, these TPC levels increased at a greater rate than those in untreated controls, Pi or single Phi treatments. The results here are significant, in that, not only did Phi treatment suppress disease symptoms and increased TPC following pathogen challenge, but sequential treatments primed the plants by increasing accumulations of TPC, thus allowing a more rapid and efficient response.

5.6.2.2 H_2O_2 accumulation

The determination of the speed of synthesis and accumulation of H_2O_2 at infection sites is one means to measure a plants level of resistance or susceptibility to a particular pathogen. H_2O_2 plays a major role in a plants response to pathogen challenge, as well as having direct antimicrobial properties, it is a component of the hypersensitive response, which produces a rapid, localised, transient, oxidative burst, directly impairing the pathogen. In this study, following hyphal inoculation, the time of first observation of disease incidences varied, this was due to a number of influencing factors, ambient

temperatures, humidity, turfgrass health status, being but some. Therefore, data regarding defence responses were pooled from 4 greenhouse studies and changes in the status of H_2O_2 synthesis were calculated from analyses of tissues following first elicitation of disease incidences. In greenhouse turfgrass samples, following applications of Phi and Pi, statistically significant differences in H_2O_2 generation were determined at 1 and 6 hpa, and between turfgrasses at 0, 1, 6, 12, 24, 48 hpa, Table 5-13. Following treatment, over 72 hpa, there was a clear spike in H_2O_2 levels at 6 hpa in both turfgrass species with a second increase at 72 hpa in *A. stolonifera,* Fig. 5-27, these however, could be attributed to stress, induced by the salt content of the nutrient solutions. Following post hoc analyses, there were significantly greater levels of H_2O_2, compared to controls, at 1, 6 and 24 hpa in both Phi and Pi treated tissues of *P. annua* and at 6 and 72 hpa in *A. stolonifera,* Fig. 5-27. Over each time period, however, there were no significant difference in H_2O_2 levels between Pi and Phi treated tissues. These data would indicate that while Phi treatment stimulates an increase in H_2O_2 generation, the response is no different to that in Pi treated tissues.

In *M. nivale* infected turfgrasses, it was shown that H_2O_2 production in response to pathogen challenge was rapid and appeared to have a twofold response, Fig. 5-28. Analysis determined that in infected tissues, over the course of 10 dpi, H_2O_2 increased in both turfgrass species, with accumulation peaks at 2 dpi, with levels decreasing at 4 and 6 dpi, with a second accumulation peak in *P. annua* at 8 dpi and at 10 dpi in *A. stolonifera*. Phi treatment did not appear to significantly influence H_2O_2 synthesis in response to infection, although Eshraghi *et al*. (2011), determined increased levels of H_2O_2 in response to pathogen challenge in *A. thaliana,* and that there were significant differences evident between the amount of H_2O_2 production between the Phi-treated and non-Phi-treated plants. The study here determined that statistically, the effect of Phi on H_2O_2 synthesis, did not differ from Pi or controls.

Accumulation of H_2O_2 is concentrated at sites of infection and sampling of whole leaves to determine H_2O_2 via extraction methodology, may not be the most efficient means to quantify changes in levels. Many published data on H_2O_2 production used fluorescence microscopy to visualise H_2O_2 at sites of infection. Huckelhoven *et al.* (1999) showed accumulations of H_2O_2 in barley leaves at sites of infection of powdery mildew. Dubas *et al.* (2010), used fluorescence staining techniques, to observe H_2O_2 accumulations in tritacle following penetration by *M. nivale* in close proximity to the infection sites. Here, as shown in Figs. 5-29 to 5-31, the *M. nivale* infection process was accompanied by

increased accumulations of TPC and H_2O_2. Following TMB staining of Phi treated and non-treated infected plants, H_2O_2 accumulation could be observed around areas of penetration and infection. This supports the results from the fluorescence microscopy showing the hyphae penetration into leaf tissues occurring via stomata and that H_2O_2 plays a significant role in defence responses. Significantly, in Phi treated plants, H_2O_2 fluorescence was observable earlier than that in Pi or control treated tissues. This is an important conclusion, which helps to supports the argument that Phi enhances responses following pathogen challenge.

In both the TPC and H_2O_2 studies there was a significant interaction effect between levels of these compounds produced, treatments applied and turfgrass species. It could be expected levels would vary between treatments, so the important result here is the effect turfgrass species had on the defence compounds. This result show species vary in their response to pathogen challenge, a factor which can be further studied

5.7 Conclusions

Assessment of numerous infection incidences in both the field and in greenhouses determined that hyphae are the main source of *M. nivale* inoculum and that infection was by means of stomatal penetration. Conidia produced via sporodochia following infection are the means of propagation and dispersal.

Phenolic compounds and H_2O_2 are a component of initial defence responses and Phi treatment led to enhanced responses in regard to TPC accumulation.

Results of H_2O_2 extractions indicated that Phi treatment did not appear to influence H_2O_2 responses, but fluorescence microscopy determined that Phi treatment did enhance this response.

Despite these results, there are many related areas which require further study. There are numerous questions which need resolving. The study here only touched on the role of *M. nivale* infection and the plants response in regard to synthesis of defence compounds, how Phi interacts with and influences these responses requires much further research.

6 Discussion

6.1 Introduction

This is the first major study to assess the ability of Phi suppress *M. nivale* in cool-season turfgrass and is a continuation of a preliminary study carried out by Dempsey and Owen (2010). In 2004, in the UK and Ireland, Phi based products began to be promoted by advisory turfgrass agronomists and nutrient producers, as a means to reduce disease incidence during the autumn winter season, in particular, disease caused by *M. nivale*. It was claimed that the inclusion of Phi in the nutrient programme at that time of year, would reduce disease incidence by enhancing turfgrass defence responses. This promotion of Phi as a means to reduce *M. nivale* disease was based not on published scientific data, but on the success of Phi as a means to control turfgrass diseases caused by oomycete pathogens. Phi had been used for many years in areas of different climactic conditions than those prevalent in the UK and Ireland, as a means to control Pythium. As already stated in the review of literature, prior to this research, there were no data published regarding the interaction between Phi and ascomycete pathogens in amenity turfgrasses. Therefore, at the start of this study, the questions which demanded answers were: can Phi suppress *M. nivale* incidences in amenity turfgrass, and if so, what was the mode of suppression? Supplementary to these questions were, what are the effects on turfgrass nutrition and quality, of long term sequential treatment with Phi.

6.2 *M. nivale* growth inhibition by Phi *in vitro* and *in vivo*

The study began with a twofold investigation, aimed to determine if Phi had any inhibitory effects on *M. nivale in vitro* and *in vivo. In vitro,* Phi, sourced from both reagent grade and commercial products, proved very successful in significantly inhibiting the mycelial growth of *M. nivale.* The level of *in vitro* suppression achieved here, across the full range of Phi amendments used was not expected. Prior to the start of this study, there was little evidence to support the premise that Phi had direct fungistatic properties against ascomycetes. The expected outcome was that there would be only limited growth reductions (due to the use of KOH to adjust the pH) or even no growth inhibition. The results therefore, while welcome, were unexpected, but were supported during the course of the study by publication of similar data by Hofgaard *et al.* (2010), who determined that Phi inhibited *M. majus* growth *in vitro.* The results also compare well with studies into Phi mediated suppression of oomycete pathogens. Phi has proven efficacy in inhibiting *in vitro* mycelial growth,

causing adverse hyphal morphology and reducing the percent germination of reproductive structures (Coffey and Bower, 1984; Fenn and Coffey, 1984; Coffey and Joseph, 1985; Darakis *et al*., 1997; Wilkinson *et al*., 2001; Daniel *et al*., 2005; Mccarren, 2006; Wong, 2006; Garbelotto *et al*., 2008; Mccarren *et al*., 2009). There are also published research into Phi and fungal inhibition (Reuveni *et al*., 2003; Mills *et al*., 2004; Burpee, 2005; Aguín *et al.,* 2006).

The *in vitro* studies and the field trials were carried out concurrently and it was interesting to observe and compare how Phi suppressed *M. nivale in vitro,* while at the same time in the field, Phi treatment consistently provided significantly reduced levels of disease incidence. Often, *in vitro* studies can produce significant and relevant data; however, these results do not always transfer to produce similar results in the field. This is due to the difference between the sterile environment of the laboratory and the influence of a wide range of biotic and abiotic factors in the field. It was important therefore, that the *in vitro* results showing Phi mediated *M. nivale* suppression were supported by similar results under natural conditions in the field. Overall in the field, Phi treatment to three turfgrass species, reduced disease incidence by 50% compared to controls. As with the *in vitro* research, the results of the field trials were bolstered by findings from similar trials which also reported Phi mediated suppression *of M. nivale* (Golembiewski *et al.,* 2010) and a more recent study by Mattox *et al.* (2014).

Despite the 50% percent reduction of disease incidence in the field, the level of disease damage remaining would not be considered acceptable to the majority of turfgrass managers, ensuring that chemical fungicides would still need to be employed. However, what was also evident from this study, was that the addition of Phi to standard turfgrass fungicides significantly enhanced their efficacy in suppressing of *M. nivale* and that the combination of Phi and fungicide in most cases fully suppressed disease incidence. Therefore, the use of Phi as part of a general nutrient package, as well as reducing disease incidence, would reduce the requirement of fungicide applications, leading to a significant cost savings.

When viewed in combination, the results of the *in vitro* and field trial studies produced significant, novel and relevant data, which is of great value to the turfgrass industry, however, while these data clearly demonstrated the efficacy of Phi in suppressing *M. nivale* growth and incidence, the mode of suppression needed to be determined.

6.3 Mode of suppression

It was clear from the *in vitro* study that Phi directly inhibited the hyphal growth of *M. nivale,* however, what was also clear was that Phi caused disruption of hyphal morphology and a reduction in conidial germination rates. These are important factors as these results have consequences in regard to not only the mode of suppression exerted by Phi, but also on the dissemination and dispersal of *M. nivale.* Published research as listed in Chapter one review of literature, shows Phi can suppress disease incidence in many plant systems by acting directly on the pathogen and indirectly via stimulation of host defences. The *in vitro* studies here determined that Phi, when interacting with *M. nivale*, has direct fungistatic properties, as it was shown to significantly reduce the hyphal growth rate. It was required however, to determine if this is the sole means of suppression or were there more complex interactions in regard to plant defences involved?

6.3.1 Direct mode of suppression

It was shown here that Phi directly inhibits the growth of *M. nivale* hyphae, but by what means does this occur? Published research has demonstrated the presence of Phi in growth media interferes with the uptake of Pi, as both these compounds are taken up via identical uptake mechanisms. The presence of Phi leads to disruption of P metabolism and inhibition of enzymes involved in the glycolytic and phosphogluconate pathways (Grant *et al.*, 1990; Niere *et al.*, 1994; Martin *et al.*, 1998; Stehmann and Grant, 2000; Mcdonald *et al.*, 2001). This interference and disruption to P uptake and metabolism was indicated here as relatively small amounts of Phi in the growth media caused significant reductions in hyphal growth. A further example of Phi's ability to reduce hyphal growth was determined by Niere *et al*. (1994), who concluded that the presence Phi interferes with Pi metabolism in pathogen cells, by causing accumulations of polyphosphate and pyrophosphate. This synthesis of poly and pyrophosphate requires energy, which is provided by ATP, which in turn, is not then available for other metabolic processes, such as hyphal extension or growth.

For Phi to suppress *M. nivale* via direct fungistatic means *in planta* there needs to be direct contact between Phi and the pathogen. As determined in Chapter 5, *M. nivale* infects turfgrass by entering the plant and extending hyphal growth to extract required nutrients. To suppress hyphal growth *in planta,* therefore, foliar applied Phi needs to be taken up and translocated throughout the plants tissues. The HPIC analyses carried out here, produced significant and novel data, until this study there were no data to support the foliar uptake of Phi in turfgrass, product manufacturers assumed it was taken into the plant in a similar manner as other foliar applied nutrients. It was determined here that following

foliar application to the leaf, Phi is rapidly taken up and translocated throughout the plant. Furthermore, following sequential Phi applications, turfgrass tissues displayed increasing cumulative accumulations in meristematic regions, such as roots and crowns. This established that foliar applied Phi was present in the turfgrass tissues and therefore could be taken up by the pathogen allowing Phi to interfere with its P metabolism.

While the HPIC data provided new insight in this area, there are many questions which can be further researched. For example, it was established that Phi accumulated rapidly in turfgrass leaf tissues, but the precise areas of accumulation need to be determined, whether these be in the vascular system or within the cell structures. Also, long-term Phi treatments can lead to cumulative accumulations in meristematic tissues and increases in soil P levels, this is a worrying factor as in many regions worldwide, the fate of applied P is strictly monitored and controlled.

As well as inhibiting *in vitro* mycelial growth, it has also been shown that Phi can cause adverse morphological changes, such as convolution and collapse of cell walls in the hyphae of oomycetes (Daniel *et al.*, 2005; Wong, 2006). Evidence of this disruption of normal morphology was also shown in this study, when hyphae, grown on Phi amended PDA appeared distorted and stunted, an important point with regard to disease suppression as it can be concluded that this stress would lead in increased production of elicitory compounds.

A further significant result from this research was the effect of Phi on conidial germination and growth. Conidia, as shown in Chapter 5, play a vital role in the dispersal of *M. nivale*, the results here determined significant reductions in conidial germination. It could be concluded from these data that if numbers of viable conidia are reduced then disease pressures would also reduce. Prior to this there were no published data on the effect Phi has on *M. nivale* conidial germination. The nearest relevant research being by Hofgaard *et al.* (2010), who demonstrated that increasing Phi concentrations correlated directly with delays in sporulation of *M. majus* on detached wheat leaves.

From these results it can be concluded that the significant suppression of *M. nivale* in the field is partly due to the presence of Phi in the plant causing a direct inhibition of hyphal growth and development, however other factors in areas of enhanced plant defence measures need also to be taken into consideration.

6.3.2 Indirect mode of suppression

The general consensus of published research is that while Phi can act directly to inhibit many pathogens, the mode of disease suppression also includes an enhancement of defences in treated plants. Therefore, in order to determine if Phi enhanced constitutive or inducible defences, research into the responses of *M. nivale* infected turfgrasses was required. Following from the research into the infection process of *M. nivale,* it was confirmed that in turfgrasses, TPC and H_2O_2 are components of initial defence responses and that Phi treatment led to an enhancement of these following single and sequential treatments.

Plant phenolics are secondary metabolites and a vital component of the defence mechanisms of plants. The synthesis and accumulation of both constitutive and induced phenolic compounds prior to, and in response to pathogen challenge has been well documented (Ishihara *et al.*, 1999; Jin and Yoshida, 2000; Okazaki *et al.*, 2004; Remusborel *et al.*, 2005). Enhancement of these plant defence mechanisms by Phi has also been documented (Saindrenan *et al.*, 1988; Jackson *et al.*, 2000; Daniel and Guest, 2006). It was determined that while a single Phi treatment influenced TPC accumulations in non-infected plants, sequential applications over a period of six months, gave rise to increased levels of accumulations. This can be interpreted as Phi priming the plants prior to infection. Pre-formed phenolic compounds are ubiquitous in plants and play an important role in resistance to pathogenic fungi. Some are stored in plant cells as inactive bound forms, phytoanticipins, which are enzymatically converted into biologically active compounds in response to pathogen attack. Here it was determined that there were increased accumulations of available phytoanticipins following sequential Phi treatments, thus increasing the defence response level. As well as phytoanticipins, antifungal phenolic compounds are formed upon elicitation of pathogen challenge. This enhancement of defence responses was determined in treated plants where, following a single Phi treatment, plants, upon infection, displayed more rapid accumulations of TPC, compared with Pi and untreated controls. These data indicate the Phi not only primes the plant prior to infection but can also enhance phenolic defence response following a single application.

A second defence compound studied here was H_2O_2. Analysis of the extractions from infected and Phi treated plants indicated that Phi treatment did not appear to significantly influence H_2O_2 synthesis in response to infection. Statistically, the effect of Phi on H_2O_2 synthesis, did not differ from Pi or controls. However, following TMB staining of Phi treated and non-treated infected plants, H_2O_2 accumulations, around areas of penetration and

infection, were observed at a faster rate and with greater accumulations that in non-treated plants, leading to the conclusion that Phi treatment did enhance significantly this response.

It can be concluded from these data therefore, that Phi suppression of *M. nivale* is as a result of a dual process of direct and indirect means. The presence of Phi in the plant tissues directly inhibited the growth of *M. nivale*, slowing the rate of infection. The disruption of fungal metabolism due to the interference of Phi in the uptake and metabolism of Pi, stressing the pathogen, leading to increased production of elicitors. This combination of reduced hyphal growth and increased release of elicitors, allows for a more rapid and effective response in treated plants. This enhancement of defence responses was determined in treated plants where, following a single Phi treatment, treated plants, upon infection, displayed more rapid accumulations of TPC, compared with Pi and untreated controls and further strengthened by data showing that sequential Phi treatments gave rise to increased levels of phenolic phytoanticipins.

Despite these results, there are numerous questions which need resolving. In particular, how the presence of Pi and the balance between Phi and Pi in the growth media can affect the proven inhibitory properties of Phi. These questions have been studied in oomycetes with a range of outcomes, some research concluded that the inhibitory properties of Phi are restricted by and are dependent of the levels of Pi concentration in the growth media (Smillie *et al.*, 1989; Griffith *et al.*, 1993; Darakis *et al.*, 1997), while others report the concentration of Pi has no significant effect (Fenn and Coffey, 1984). Furthermore, the study here only touched on the role of *M. nivale* infection and the plants response in regard to synthesis of defence compounds, how Phi interacts with and influences these responses requires much further research.

Further to these questions is the contentious issue of the use of Phi as a source of P nutrition and of the effect on the plant and growth environment of long term use of Phi.

6.4 Effects of Phi turfgrass growth and the environment

The main focus of this research is the suppression of *M. nivale* infection in turfgrasses and the means by which this comes about. However, a major factor which had to be considered was that no treatment or maintenance operation, carried out in the management of fine turf surfaces, can be viewed as a single entity. All form part of the overall procedures which combine to produce the high specification playing surfaces required for amenity sports. Any treatments must be assessed as part of the overall effect it could have on the playing qualities, aesthetic appearance and sustainability within the sports environment. If, for

example, a fungicidal treatment fully inhibited a pathogen, but detrimentally affected the turfgrass colour or density, then the treatment would be deemed unacceptable. For Phi to be acceptable as a means to suppress *M. nivale* it was necessary to assess any effects, both detrimental and beneficial, Phi treatment may have on amenity turfgrasses, in areas of turfgrass quality, growth and sustainability of the growth environment.

In a plants response to pathogen challenge, be it resistant or susceptible, the level of resistance can be crucially affected by its overall health and nutritional status, as the expression of large numbers of defence related compounds requires a substantial commitment of resources. To obtain optimum playing surfaces, amenity turfgrasses are maintained using minimal nutritional inputs. Turfgrasses growing under balanced nutritional regime are better able to produce the resources required to synthesise the wide range of compounds required for defence. It is clear also, that nutritionally deficient plants are more susceptible to disease. The use of Phi as source of nutrition has been described in the review of literature. The arguments for and against its use are not decisive, with Phi's use in many instances being detrimental to the treated plants, while in others, it was concluded to be beneficial.

6.4.1 Phi in the plant and effect on growth

Here, the properties of Phi as a nutritional input provided useful, significant and in some areas, novel data. The study into Phi as a source of P nutrition, determined significant differences in growth responses following treatment. It was shown from the HPIC analyses that Phi is rapidly taken up by turfgrass, but it was clearly demonstrated that Phi does not supply a usable form of P and furthermore, in plants growing under limited P availability, deficiency responses were repressed. Interestingly, in plants growing under non-limiting P levels, foliar-applied Phi increased biomass in all plants. The effect Phi has on plants in limit P situations is well documented and the results here were not unexpected. But the beneficial effect to plants where there were adequate availability of P was surprising. Some product producers claim that Phi can be used as a source of P nutrition as following take up Phi is converted *in planta* to planted metabolisable forms of P. These claims were refuted here as the results were conclusive, foliar application of Phi did not affect the mean level of Pi in any of the turfgrass tissues.

6.4.2 Turf quality

A disease free and aesthetically pleasing turfgrass surface, with highly specified playing qualities, is of the utmost priority for the turfgrass manager. For Phi to be useful in suppressing disease on fine turfgrass, its effects on the overall properties of the sward must

be assessed. A disease free surface which suffers from nutrient deficiency and is phytotoxicity damaged by Phi would be of little use the turfgrass manager. In this research, as well as reducing disease incidence, an important conclusion from the field trials, was the determination that Phi treatment gave rise to significantly improved greater density than the untreated controls. Factors which influenced the improvement were discussed in Chapter 3, but the conclusion that Phi can enhance the growth or vigour of turfgrass growing under non P limited conditions could also be a factor in the significant improvement in turfgrass quality.

6.5 Recommendations for Phi use in turfgrass

It is concluded from this research, that Phi can be valuable addition to a turfgrass management programme. Results from the field trials would indicate that Phi, applied sequentially on a 3 to 4 week cycle, at a rate of 0.35g/m^{-1} of PO_3^{3-} will not only suppress *M. nivale*, but also increase the efficacy of turfgrass fungicides. Sequentially applied Phi will also provide enhanced turfgrass quality and density, which will positively affect the aesthetic appearance of the sports surface but also improve the playing qualities.

In the northern hemisphere, September to March is the period of highest *M. nivale* disease pressure and this would be the major period for Phi applications. However, as determined here, sequential treatments during other seasons would also enhance turfgrass quality and given the mode of suppression Phi should also provide protection against other turfgrass pathogens such as Anthracnose (*Colletotrichum cereale*) and *Pythium*.

What also needs to be taken into consideration however, are the negative aspects of long term sequential Phi applications such as its effect on soil P levels, further research in this area is required as the data here indicated that there could be cumulative increases in soil P.

6.6 Conclusions

This study has produced significant and novel data which is relevant to turfgrass disease prevention and control. The main conclusions of this study are that Phi:

- Suppresses *M. nivale* mycelial growth *in vitro.*
- Disrupts P metabolism in *M. nivale.*
- Inhibits conidial germination.
- Suppresses *M. nivale* incidence in the field.
- Enhances turfgrass growth and quality.
- Does not provide a source of plant usable P.
- Limits P deficiency responses.
- Enhances synthesis of phenolics and H_2O_2 as turfgrass defence responses.

Phi suppressed the *in vitro* mycelial growth, led to disruption of hyphal morphology and inhibited conidial germination.

Field trials determined that Phi significantly reduced the incidence and severity of *M. nivale* infection and significantly enhanced the efficacy of turfgrass fungicides. Phi also gave rise to significantly improved turfgrass quality.

Phi is rapidly taken up and translocated by turfgrass but does not supply a usable form of P and furthermore in P deficient situations, deficiency responses were repressed. In P sufficient rootzones foliar-applied Phi increased biomass in treated plants.

Long-term Phi treatment maintains leaf tissue accumulations, but can lead to cumulative increases in meristematic tissues and can cause increases in soil P levels.

Assessment of infection incidences determined that hyphae are the main source of *M. nivale* inoculum and that infection is by means of stomatal penetration. Conidia produced via sporodochia following infection are the means of propagation and dispersal.

Synthesis of phenolic compounds and H_2O_2 are components of the initial defences and Phi treatment led to enhanced responses in this area.

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Appendices to the Thesis

Suppression of *Microdochium nivale* by Phosphite in Cool-season Amenity Turfgrasses

John Dempsey

Please note, that the full appendix is not included in the printed version of the thesis due to the large volume of pages

Faculty of Applied Sciences, University of the West of England, Bristol
May 2016

Contents

Chapter 2 Statistics

Appendix 1: 2.6.1 Mean daily growth rates of *M. nivale* on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA, descriptive statistics.

Descriptive Statistics

Compound	Concentration		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
H3PO3	0 µg	Mean daily growth	6	10.81	10.98	10.8470	.02716	.06653	.004
		Residual for Mean daily	6	-.04	.13	.0000	.02716	.06653	.004
		Valid N (listwise)	6						
	10 µg	Mean daily growth	6	8.72	8.86	8.7920	.02555	.06259	.004
		Residual for Mean daily	6	-.07	.07	.0000	.02555	.06259	.004
		Valid N (listwise)	6						
	50 µg	Mean daily growth	6	4.47	4.76	4.6063	.04727	.11579	.013
		Residual for Mean daily	6	-.14	.15	.0000	.04727	.11579	.013
		Valid N (listwise)	6						
	100 µg	Mean daily growth	6	1.11	1.26	1.1415	.02325	.05696	.003
		Residual for Mean daily	6	-.03	.12	.0000	.02325	.05696	.003
		Valid N (listwise)	6						
	250 µg	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
H3PO4	0 µg	Mean daily growth	6	10.78	10.93	10.8602	.02416	.05917	.004
		Residual for Mean daily	6	-.08	.07	.0000	.02416	.05917	.004
		Valid N (listwise)	6						
	10 µg	Mean daily growth	6	10.74	11.27	10.9955	.09094	.22276	.050
		Residual for Mean daily	6	-.26	.28	.0000	.09094	.22276	.050
		Valid N (listwise)	6						
	50 µg	Mean daily growth	6	10.17	10.60	10.3981	.06854	.16788	.028
		Residual for Mean daily	6	-.22	.20	.0000	.06854	.16788	.028
		Valid N (listwise)	6						
	100 µg	Mean daily growth	6	9.86	10.10	9.9808	.04665	.11426	.013
		Residual for Mean daily	6	-.13	.12	.0000	.04665	.11426	.013
		Valid N (listwise)	6						
	250 µg	Mean daily growth	6	9.22	9.71	9.5540	.06856	.16794	.028
		Residual for Mean daily	6	-.33	.15	.0000	.06856	.16794	.028
		Valid N (listwise)	6						

	0 µg	Mean daily growth	6	10.70	10.94	10.8286	.04872	.11934	.014
		Residual for Mean daily	6	-.13	.11	.0000	.04872	.11934	.014
		Valid N (listwise)	6						
	10 µg	Mean daily growth	6	7.68	8.02	7.8712	.06460	.15823	.025
		Residual for Mean daily	6	-.19	.15	.0000	.06460	.15823	.025
		Valid N (listwise)	6						
	50 µg	Mean daily growth	6	3.71	3.87	3.7979	.02853	.06987	.005
KH2PO3		Residual for Mean daily	6	-.09	.08	.0000	.02853	.06987	.005
		Valid N (listwise)	6						
	100 µg	Mean daily growth	6	.81	.91	.8727	.01868	.04575	.002
		Residual for Mean daily	6	-.06	.03	.0000	.01868	.04575	.002
		Valid N (listwise)	6						
	250 µg	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	0 µg	Mean daily growth	6	10.81	11.13	10.9522	.05166	.12654	.016
		Residual for Mean daily	6	-.14	.18	.0000	.05166	.12654	.016
		Valid N (listwise)	6						
	10 µg	Mean daily growth	6	10.85	11.00	10.9166	.02861	.07007	.005
		Residual for Mean daily	6	-.06	.09	.0000	.02861	.07007	.005
		Valid N (listwise)	6						
	50 µg	Mean daily growth	6	9.90	10.35	10.1128	.08254	.20219	.041
KH2PO4		Residual for Mean daily	6	-.21	.24	.0000	.08254	.20219	.041
		Valid N (listwise)	6						
	100 µg	Mean daily growth	6	9.84	10.24	10.0206	.07387	.18093	.033
		Residual for Mean daily	6	-.18	.22	.0000	.07387	.18093	.033
		Valid N (listwise)	6						
	250 µg	Mean daily growth	6	9.77	9.88	9.8187	.02180	.05341	.003
		Residual for Mean daily	6	-.05	.06	.0000	.02180	.05341	.003
		Valid N (listwise)	6						
	0 µg	Mean daily growth	6	10.73	10.97	10.8826	.04734	.11595	.013
		Residual for Mean daily	6	-.15	.09	.0000	.04734	.11595	.013
		Valid N (listwise)	6						
	10 µg	Mean daily growth	6	10.60	11.07	10.9420	.07403	.18133	.033
		Residual for Mean daily	6	-.34	.13	.0000	.07403	.18133	.033
		Valid N (listwise)	6						
	50 µg	Mean daily growth	6	9.84	10.15	9.9755	.04897	.11995	.014
KOH		Residual for Mean daily	6	-.14	.18	.0000	.04897	.11995	.014
		Valid N (listwise)	6						
	100 µg	Mean daily growth	6	9.04	9.38	9.1521	.06902	.16906	.029
		Residual for Mean daily	6	-.11	.23	.0000	.06902	.16906	.029
		Valid N (listwise)	6						
	250 µg	Mean daily growth	6	8.49	8.92	8.7170	.07814	.19139	.037
		Residual for Mean daily	6	-.22	.20	.0000	.07814	.19139	.037
		Valid N (listwise)	6						

Appendix 2: Tukey pairwise comparisons for Fig. 2-3, mean daily growth rates on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA.

Multiple Comparisons

Dependent Variable: Mean daily growth

Tukey HSD

Concentration	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
0 µg	H3PO3	H3PO4	-.0074	.07349	1.000	-.2343	.2196
		KH2PO3	.0312	.07349	.992	-.1957	.2582
		KH2PO4	-.1218	.07349	.486	-.3488	.1051
		KOH	-.0255	.07349	.997	-.2524	.2015
	H3PO4	H3PO3	.0074	.07349	1.000	-.2196	.2343
		KH2PO3	.0386	.07349	.983	-.1884	.2655
		KH2PO4	-.1145	.07349	.544	-.3414	.1125
		KOH	-.0181	.07349	.999	-.2451	.2088
	KH2PO3	H3PO3	-.0312	.07349	.992	-.2582	.1957
		H3PO4	-.0386	.07349	.983	-.2655	.1884
		KH2PO4	-.1531	.07349	.277	-.3800	.0739
		KOH	-.0567	.07349	.935	-.2836	.1702
	KH2PO4	H3PO3	.1218	.07349	.486	-.1051	.3488
		H3PO4	.1145	.07349	.544	-.1125	.3414
		KH2PO3	.1531	.07349	.277	-.0739	.3800
		KOH	.0964	.07349	.689	-.1306	.3233
	KOH	H3PO3	.0255	.07349	.997	-.2015	.2524
		H3PO4	.0181	.07349	.999	-.2088	.2451
		KH2PO3	.0567	.07349	.935	-.1702	.2836
		KH2PO4	-.0964	.07349	.689	-.3233	.1306
10 µg	H3PO3	H3PO4	-2.1734*	.11436	.000	-2.5266	-1.8203
		KH2PO3	.8946*	.11436	.000	.5415	1.2478
		KH2PO4	-2.1354*	.11436	.000	-2.4885	-1.7823
		KOH	-2.1398*	.11436	.000	-2.4929	-1.7867
	H3PO4	H3PO3	2.1734*	.11436	.000	1.8203	2.5266
		KH2PO3	3.0681*	.11436	.000	2.7150	3.4212
		KH2PO4	.0380	.11436	.997	-.3151	.3912
		KOH	.0336	.11436	.998	-.3195	.3868
	KH2PO3	H3PO3	-.8946*	.11436	.000	-1.2478	-.5415
		H3PO4	-3.0681*	.11436	.000	-3.4212	-2.7150
		KH2PO4	-3.0300*	.11436	.000	-3.3832	-2.6769
		KOH	-3.0344*	.11436	.000	-3.3876	-2.6813
	KH2PO4	H3PO3	2.1354*	.11436	.000	1.7823	2.4885
		H3PO4	-.0380	.11436	.997	-.3912	.3151
		KH2PO3	3.0300*	.11436	.000	2.6769	3.3832
		KOH	-.0044	.11436	1.000	-.3575	.3487
	KOH	H3PO3	2.1398*	.11436	.000	1.7867	2.4929
		H3PO4	-.0336	.11436	.998	-.3868	.3195
		KH2PO3	3.0344*	.11436	.000	2.6813	3.3876
		KH2PO4	.0044	.11436	1.000	-.3487	.3575

50 µg	H3PO3	H3PO4	-5.7248*	.10079	.000	-6.0361	-5.4136
		KH2PO3	.8238*	.10079	.000	.5126	1.1350
		KH2PO4	-5.4735*	.10079	.000	-5.7847	-5.1623
		KOH	-5.3677*	.10079	.000	-5.6789	-5.0565
	H3PO4	H3PO3	5.7248*	.10079	.000	5.4136	6.0361
		KH2PO3	6.5486*	.10079	.000	6.2374	6.8599
		KH2PO4	.2514	.10079	.144	-.0599	.5626
		KOH	.3571*	.10079	.021	.0459	.6684
	KH2PO3	H3PO3	-.8238*	.10079	.000	-1.1350	-.5126
		H3PO4	-6.5486*	.10079	.000	-6.8599	-6.2374
		KH2PO4	-6.2973*	.10079	.000	-6.6085	-5.9860
		KOH	-6.1915*	.10079	.000	-6.5027	-5.8803
	KH2PO4	H3PO3	5.4735*	.10079	.000	5.1623	5.7847
		H3PO4	-.2514	.10079	.144	-.5626	.0599
		KH2PO3	6.2973*	.10079	.000	5.9860	6.6085
		KOH	.1058	.10079	.829	-.2055	.4170
	KOH	H3PO3	5.3677*	.10079	.000	5.0565	5.6789
		H3PO4	-.3571*	.10079	.021	-.6684	-.0459
		KH2PO3	6.1915*	.10079	.000	5.8803	6.5027
		KH2PO4	-.1058	.10079	.829	-.4170	.2055
100 µg	H3PO3	H3PO4	-8.8296*	.09285	.000	-9.1163	-8.5429
		KH2PO3	.2725	.09285	.066	-.0142	.5592
		KH2PO4	-8.8476*	.09285	.000	-9.1343	-8.5609
		KOH	-8.0543*	.09285	.000	-8.3410	-7.7676
	H3PO4	H3PO3	8.8296*	.09285	.000	8.5429	9.1163
		KH2PO3	9.1021*	.09285	.000	8.8154	9.3888
		KH2PO4	-.0180	.09285	1.000	-.3047	.2687
		KOH	.7753*	.09285	.000	.4886	1.0620
	KH2PO3	H3PO3	-.2725	.09285	.066	-.5592	.0142
		H3PO4	-9.1021*	.09285	.000	-9.3888	-8.8154
		KH2PO4	-9.1201*	.09285	.000	-9.4068	-8.8334
		KOH	-8.3268*	.09285	.000	-8.6135	-8.0401
	KH2PO4	H3PO3	8.8476*	.09285	.000	8.5609	9.1343
		H3PO4	.0180	.09285	1.000	-.2687	.3047
		KH2PO3	9.1201*	.09285	.000	8.8334	9.4068
		KOH	.7933*	.09285	.000	.5066	1.0800
	KOH	H3PO3	8.0543*	.09285	.000	7.7676	8.3410
		H3PO4	-.7753*	.09285	.000	-1.0620	-.4886
		KH2PO3	8.3268*	.09285	.000	8.0401	8.6135
		KH2PO4	-.7933*	.09285	.000	-1.0800	-.5066
250 µg	H3PO3	H3PO4	-9.5320*	.08864	.000	-9.8057	-9.2583
		KH2PO3	0.0000	.08864	1.000	-.2737	.2737
		KH2PO4	-9.8145*	.08864	.000	-10.0882	-9.5408
		KOH	-8.7219*	.08864	.000	-8.9957	-8.4482
	H3PO4	H3PO3	9.5320*	.08864	.000	9.2583	9.8057
		KH2PO3	9.5320*	.08864	.000	9.2583	9.8057
		KH2PO4	-.2825*	.08864	.042	-.5562	-.0088
		KOH	.8101*	.08864	.000	.5363	1.0838
	KH2PO3	H3PO3	0.0000	.08864	1.000	-.2737	.2737
		H3PO4	-9.5320*	.08864	.000	-9.8057	-9.2583
		KH2PO4	-9.8145*	.08864	.000	-10.0882	-9.5408
		KOH	-8.7219*	.08864	.000	-8.9957	-8.4482
	KH2PO4	H3PO3	9.8145*	.08864	.000	9.5408	10.0882
		H3PO4	.2825*	.08864	.042	.0088	.5562
		KH2PO3	9.8145*	.08864	.000	9.5408	10.0882
		KOH	1.0926*	.08864	.000	.8189	1.3663
	KOH	H3PO3	8.7219*	.08864	.000	8.4482	8.9957
		H3PO4	-.8101*	.08864	.000	-1.0838	-.5363
		KH2PO3	8.7219*	.08864	.000	8.4482	8.9957
		KH2PO4	-1.0926*	.08864	.000	-1.3663	-.8189

Based on observed means.

*. The mean difference is significant at the .05 level.

Appendix 3: 2.6.2 Mean daily growth on commercial Phi amended PDA. Descriptive statistics for mean daily growth of *M. nivale* on PDA amended with concentrations of phosphite derived from the commercial compounds TKO, Naturfos, PK Fight, Turfite and PK Plus.

Descriptive Statistics

Concentration	Compound		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	TKO	Mean daily growth	6	10.29	10.45	10.3272	.02655	.06504	.004
		Residual for Mean daily	6	-.03	.13	.0000	.02655	.06504	.004
		Valid N (listwise)	6						
	Naturfos	Mean daily growth	6	10.26	10.41	10.3415	.02350	.05757	.003
		Residual for Mean daily	6	-.08	.07	.0000	.02350	.05757	.003
		Valid N (listwise)	6						
	PK Fight	Mean daily growth	6	10.19	10.42	10.3082	.04623	.11324	.013
		Residual for Mean daily	6	-.12	.11	.0000	.04623	.11324	.013
		Valid N (listwise)	6						
	Turfite	Mean daily growth	6	10.29	10.60	10.4272	.05029	.12317	.015
		Residual for Mean daily	6	-.13	.17	.0000	.05029	.12317	.015
		Valid N (listwise)	6						
	PK Plus	Mean daily growth	6	10.22	10.44	10.3590	.04546	.11136	.012
		Residual for Mean daily	6	-.14	.09	.0000	.04546	.11136	.012
		Valid N (listwise)	6						
10 µg	TKO	Mean daily growth	6	9.11	9.26	9.1873	.02580	.06319	.004
		Residual for Mean daily	6	-.07	.07	.0000	.02580	.06319	.004
		Valid N (listwise)	6						
	Naturfos	Mean daily growth	6	8.72	9.15	8.9293	.07322	.17935	.032
		Residual for Mean daily	6	-.21	.22	.0000	.07322	.17935	.032
		Valid N (listwise)	6						
	PK Fight	Mean daily growth	6	8.86	9.25	9.0767	.07403	.18133	.033
		Residual for Mean daily	6	-.22	.17	.0000	.07403	.18133	.033
		Valid N (listwise)	6						
	Turfite	Mean daily growth	6	9.46	9.59	9.5204	.02475	.06062	.004
		Residual for Mean daily	6	-.06	.07	.0000	.02475	.06062	.004
		Valid N (listwise)	6						
	PK Plus	Mean daily growth	6	8.96	9.36	9.2545	.06296	.15423	.024
		Residual for Mean daily	6	-.29	.11	.0000	.06296	.15423	.024
		Valid N (listwise)	6						

50 µg	TKO	Mean daily growth	6	4.62	4.92	4.7570	.04843	.11864	.014
		Residual for Mean daily	6	-.14	.16	.0000	.04843	.11864	.014
		Valid N (listwise)	6						
	Naturfos	Mean daily growth	6	4.12	4.39	4.2467	.04324	.10591	.011
		Residual for Mean daily	6	-.12	.14	.0000	.04324	.10591	.011
		Valid N (listwise)	6						
	PK Fight	Mean daily growth	6	3.90	4.16	4.0202	.04093	.10026	.010
		Residual for Mean daily	6	-.12	.14	.0000	.04093	.10026	.010
		Valid N (listwise)	6						
	Turfite	Mean daily growth	6	4.64	4.94	4.7805	.04867	.11922	.014
		Residual for Mean daily	6	-.14	.16	.0000	.04867	.11922	.014
		Valid N (listwise)	6						
	PK Plus	Mean daily growth	6	4.87	5.19	5.0208	.05112	.12522	.016
		Residual for Mean daily	6	-.15	.17	.0000	.05112	.12522	.016
		Valid N (listwise)	6						
100 µg	TKO	Mean daily growth	6	1.17	1.33	1.2013	.02526	.06186	.004
		Residual for Mean daily	6	-.03	.12	.0000	.02526	.06186	.004
		Valid N (listwise)	6						
	Naturfos	Mean daily growth	6	1.32	1.50	1.3586	.02856	.06996	.005
		Residual for Mean daily	6	-.04	.14	.0000	.02856	.06996	.005
		Valid N (listwise)	6						
	PK Fight	Mean daily growth	6	1.04	1.18	1.0716	.02253	.05518	.003
		Residual for Mean daily	6	-.03	.11	.0000	.02253	.05518	.003
		Valid N (listwise)	6						
	Turfite	Mean daily growth	6	1.01	1.14	1.0373	.02181	.05342	.003
		Residual for Mean daily	6	-.03	.11	.0000	.02181	.05342	.003
		Valid N (listwise)	6						
	PK Plus	Mean daily growth	6	1.20	1.36	1.2315	.02589	.06342	.004
		Residual for Mean daily	6	-.03	.13	.0000	.02589	.06342	.004
		Valid N (listwise)	6						
250 µg	TKO	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Naturfos	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Fight	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Turfite	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Plus	Mean daily growth	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Mean daily	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

Appendix 4: Tukey pairwise comparisons for Fig. 2-4, mean daily growth rates on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA.

Multiple Comparisons

Dependent Variable: Mean daily growth

Tukey HSD

Concentration	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
0 µg	TKO	Naturfos	-.0143	.05653	.999	-.1803	.1518
		PK Fight	.0190	.05653	.997	-.1470	.1851
		Turfite	-.1000	.05653	.413	-.2660	.0661
		PK Plus	-.0317	.05653	.979	-.1978	.1343
	Naturfos	TKO	.0143	.05653	.999	-.1518	.1803
		PK Fight	.0333	.05653	.975	-.1327	.1994
		Turfite	-.0857	.05653	.562	-.2517	.0803
		PK Plus	-.0175	.05653	.998	-.1835	.1486
	PK Fight	TKO	-.0190	.05653	.997	-.1851	.1470
		Naturfos	-.0333	.05653	.975	-.1994	.1327
		Turfite	-.1190	.05653	.249	-.2850	.0470
		PK Plus	-.0508	.05653	.895	-.2168	.1153
	Turfite	TKO	.1000	.05653	.413	-.0661	.2660
		Naturfos	.0857	.05653	.562	-.0803	.2517
		PK Fight	.1190	.05653	.249	-.0470	.2850
		PK Plus	.0682	.05653	.747	-.0978	.2343
	PK Plus	TKO	.0317	.05653	.979	-.1343	.1978
		Naturfos	.0175	.05653	.998	-.1486	.1835
		PK Fight	.0508	.05653	.895	-.1153	.2168
		Turfite	-.0682	.05653	.747	-.2343	.0978
10 µg	TKO	Naturfos	.2580*	.08021	.027	.0224	.4936
		PK Fight	.1106	.08021	.646	-.1250	.3461
		Turfite	-.3331*	.08021	.003	-.5687	-.0975
		PK Plus	-.0673	.08021	.916	-.3028	.1683
	Naturfos	TKO	-.2580*	.08021	.027	-.4936	-.0224
		PK Fight	-.1474	.08021	.375	-.3830	.0881
		Turfite	-.5911*	.08021	.000	-.8267	-.3555
		PK Plus	-.3253*	.08021	.004	-.5608	-.0897
	PK Fight	TKO	-.1106	.08021	.646	-.3461	.1250
		Naturfos	.1474	.08021	.375	-.0881	.3830
		Turfite	-.4437*	.08021	.000	-.6792	-.2081
		PK Plus	-.1778	.08021	.206	-.4134	.0577
	Turfite	TKO	.3331*	.08021	.003	.0975	.5687
		Naturfos	.5911*	.08021	.000	.3555	.8267
		PK Fight	.4437*	.08021	.000	.2081	.6792
		PK Plus	.2658*	.08021	.021	.0303	.5014
	PK Plus	TKO	.0673	.08021	.916	-.1683	.3028
		Naturfos	.3253*	.08021	.004	.0897	.5608
		PK Fight	.1778	.08021	.206	-.0577	.4134
		Turfite	-.2658*	.08021	.021	-.5014	-.0303

50 µg	TKO	Naturfos	.5102*	.06595	.000	.3166	.7039
		PK Fight	.7368*	.06595	.000	.5431	.9305
		Turfite	-.0235	.06595	.996	-.2172	.1702
		PK Plus	-.2639*	.06595	.004	-.4575	-.0702
	Naturfos	TKO	-.5102*	.06595	.000	-.7039	-.3166
		PK Fight	.2266*	.06595	.016	.0329	.4202
		Turfite	-.5337*	.06595	.000	-.7274	-.3400
		PK Plus	-.7741*	.06595	.000	-.9678	-.5804
	PK Fight	TKO	-.7368*	.06595	.000	-.9305	-.5431
		Naturfos	-.2266*	.06595	.016	-.4202	-.0329
		Turfite	-.7603*	.06595	.000	-.9540	-.5666
		PK Plus	-1.0007*	.06595	.000	-1.1943	-.8070
	Turfite	TKO	.0235	.06595	.996	-.1702	.2172
		Naturfos	.5337*	.06595	.000	.3400	.7274
		PK Fight	.7603*	.06595	.000	.5666	.9540
		PK Plus	-.2404*	.06595	.010	-.4341	-.0467
	PK Plus	TKO	.2639*	.06595	.004	.0702	.4575
		Naturfos	.7741*	.06595	.000	.5804	.9678
		PK Fight	1.0007*	.06595	.000	.8070	1.1943
		Turfite	.2404*	.06595	.010	.0467	.4341
100 µg	TKO	Naturfos	-.1573*	.03525	.001	-.2609	-.0538
		PK Fight	.1297*	.03525	.009	.0262	.2332
		Turfite	.1639*	.03525	.001	.0604	.2675
		PK Plus	-.0303	.03525	.909	-.1338	.0733
	Naturfos	TKO	.1573*	.03525	.001	.0538	.2609
		PK Fight	.2870*	.03525	.000	.1835	.3906
		Turfite	.3213*	.03525	.000	.2177	.4248
		PK Plus	.1271*	.03525	.011	.0235	.2306
	PK Fight	TKO	-.1297*	.03525	.009	-.2332	-.0262
		Naturfos	-.2870*	.03525	.000	-.3906	-.1835
		Turfite	.0343	.03525	.865	-.0693	.1378
		PK Plus	-.1599*	.03525	.001	-.2635	-.0564
	Turfite	TKO	-.1639*	.03525	.001	-.2675	-.0604
		Naturfos	-.3213*	.03525	.000	-.4248	-.2177
		PK Fight	-.0343	.03525	.865	-.1378	.0693
		PK Plus	-.1942*	.03525	.000	-.2977	-.0907
	PK Plus	TKO	.0303	.03525	.909	-.0733	.1338
		Naturfos	-.1271*	.03525	.011	-.2306	-.0235
		PK Fight	.1599*	.03525	.001	.0564	.2635
		Turfite	.1942*	.03525	.000	.0907	.2977

Based on observed means.

*. The mean difference is significant at the .05 level.

Appendix 5: 2.6.3.1 Percent inhibition of *M. nivale in vitro* growth by H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH, descriptive statistics.

Descriptive Statistics

Concentration	Compound		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	H3PO3	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
10 µg	H3PO3	Percent inhibition	6	19.14	19.31	19.2300	.02309	.05657	.003
		Arcsine transformed percent inhibition	6	.91	.91	.9079	.00059	.00144	.000
		Residual for Percent_inhibition	6	-.09	.08	.0000	.02309	.05657	.003
		Valid N (listwise)	6						
	H3PO4	Percent inhibition	6	.56	.99	.7850	.06212	.15215	.023
		Arcsine transformed percent inhibition	6	.15	.20	.1767	.00717	.01757	.000
		Residual for Percent_inhibition	6	-.23	.21	.0000	.06212	.15215	.023
		Valid N (listwise)	6						
	KH2PO3	Percent inhibition	6	27.03	27.37	27.2150	.04515	.11059	.012
		Arcsine transformed percent inhibition	6	1.09	1.10	1.0976	.00101	.00249	.000
		Residual for Percent_inhibition	6	-.19	.15	.0000	.04515	.11059	.012
		Valid N (listwise)	6						
	KH2PO4	Percent inhibition	6	.28	.42	.3450	.02217	.05431	.003
		Arcsine transformed percent inhibition	6	.11	.13	.1172	.00375	.00919	.000
		Residual for Percent_inhibition	6	-.07	.08	.0000	.02217	.05431	.003
		Valid N (listwise)	6						
	KOH	Percent inhibition	6	.13	.36	.2200	.03194	.07823	.006
		Arcsine transformed percent inhibition	6	.07	.12	.0927	.00657	.01608	.000
		Residual for Percent_inhibition	6	-.09	.14	.0000	.03194	.07823	.006
		Valid N (listwise)	6						

50 µg	H3PO3	Percent inhibition	6	57.30	57.78	57.5033	.07961	.19500	.038
		Arcsine transformed percent inhibition	6	1.72	1.73	1.7214	.00161	.00395	.000
		Residual for Percent_inhibition	6	-.20	.28	.0000	.07961	.19500	.038
		Valid N (listwise)	6						
	H3PO4	Percent inhibition	6	4.44	4.94	4.7217	.08542	.20923	.044
		Arcsine transformed percent inhibition	6	.42	.45	.4380	.00404	.00989	.000
		Residual for Percent_inhibition	6	-.28	.22	.0000	.08542	.20923	.044
		Valid N (listwise)	6						
	KH2PO3	Percent inhibition	6	64.64	64.91	64.7750	.04595	.11256	.013
		Arcsine transformed percent inhibition	6	1.87	1.87	1.8708	.00096	.00236	.000
		Residual for Percent_inhibition	6	-.13	.14	.0000	.04595	.11256	.013
		Valid N (listwise)	6						
	KH2PO4	Percent inhibition	6	7.99	8.14	8.0650	.02172	.05320	.003
		Arcsine transformed percent inhibition	6	.57	.58	.5759	.00080	.00195	.000
		Residual for Percent_inhibition	6	-.07	.08	.0000	.02172	.05320	.003
		Valid N (listwise)	6						
	KOH	Percent inhibition	6	7.84	8.34	8.1683	.08961	.21949	.048
		Arcsine transformed percent inhibition	6	.57	.59	.5796	.00329	.00805	.000
		Residual for Percent_inhibition	6	-.33	.17	.0000	.08961	.21949	.048
		Valid N (listwise)	6						
100 µg	H3PO3	Percent inhibition	6	89.29	89.64	89.4483	.06096	.14932	.022
		Arcsine transformed percent inhibition	6	2.47	2.49	2.4799	.00199	.00487	.000
		Residual for Percent_inhibition	6	-.16	.19	.0000	.06096	.14932	.022
		Valid N (listwise)	6						
	H3PO4	Percent inhibition	6	8.03	8.35	8.1983	.05724	.14020	.020
		Arcsine transformed percent inhibition	6	.57	.59	.5808	.00209	.00511	.000
		Residual for Percent_inhibition	6	-.17	.15	.0000	.05724	.14020	.020
		Valid N (listwise)	6						
	KH2PO3	Percent inhibition	6	91.50	91.99	91.6717	.07481	.18324	.034
		Arcsine transformed percent inhibition	6	2.55	2.57	2.5561	.00272	.00666	.000
		Residual for Percent_inhibition	6	-.17	.32	.0000	.07481	.18324	.034
		Valid N (listwise)	6						
	KH2PO4	Percent inhibition	6	8.92	9.19	9.0283	.05082	.12449	.015
		Arcsine transformed percent inhibition	6	.61	.62	.6104	.00177	.00434	.000
		Residual for Percent_inhibition	6	-.11	.16	.0000	.05082	.12449	.015
		Valid N (listwise)	6						
	KOH	Percent inhibition	6	15.16	15.65	15.4183	.08056	.19732	.039
		Arcsine transformed percent inhibition	6	.80	.81	.8070	.00223	.00546	.000
		Residual for Percent_inhibition	6	-.26	.23	.0000	.08056	.19732	.039
		Valid N (listwise)	6						
250 µg	H3PO3	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Percent inhibition	6	12.09	12.53	12.3033	.07473	.18305	.034
		Arcsine transformed percent inhibition	6	.71	.72	.7168	.00227	.00557	.000
		Residual for Percent_inhibition	6	-.21	.23	.0000	.07473	.18305	.034
		Valid N (listwise)	6						
	KH2PO3	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent_inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Percent inhibition	6	10.54	10.90	10.7017	.05935	.14538	.021
		Arcsine transformed percent inhibition	6	.66	.67	.6665	.00192	.00470	.000
		Residual for Percent_inhibition	6	-.16	.20	.0000	.05935	.14538	.021
		Valid N (listwise)	6						
	KOH	Percent inhibition	6	19.76	20.09	19.9433	.05057	.12388	.015
		Arcsine transformed percent inhibition	6	.92	.93	.9259	.00127	.00310	.000
		Residual for Percent_inhibition	6	-.18	.15	.0000	.05057	.12388	.015
		Valid N (listwise)	6						

Appendix 6: Tukey pairwise comparisons for Fig. 2-5, percent inhibition of *M. nivale* mycelial growth on H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH amended PDA. Data were arcsine transformed prior to analysis and back transformed for the graph.

Multiple Comparisons

Dependent Variable: arcsine

Tukey HSD

Concentration	Compound		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
10 µg	H3PO3	H3PO4	.73119*	.00663	.000	.7117	.7507
		KH2PO3	-.18973*	.00663	.000	-.2092	-.1702
		KH2PO4	.79066*	.00663	.000	.7712	.8101
		KOH	.81521*	.00663	.000	.7957	.8347
	H3PO4	H3PO3	-.73119*	.00663	.000	-.7507	-.7117
		KH2PO3	-.92092*	.00663	.000	-.9404	-.9014
		KH2PO4	.05947*	.00663	.000	.0400	.0790
		KOH	.08402*	.00663	.000	.0645	.1035
	KH2PO3	H3PO3	.18973*	.00663	.000	.1702	.2092
		H3PO4	.92092*	.00663	.000	.9014	.9404
		KH2PO4	.98039*	.00663	.000	.9609	.9999
		KOH	1.00495*	.00663	.000	.9855	1.0244
	KH2PO4	H3PO3	-.79066*	.00663	.000	-.8101	-.7712
		H3PO4	-.05947*	.00663	.000	-.0790	-.0400
		KH2PO3	-.98039*	.00663	.000	-.9999	-.9609
		KOH	.02455*	.00663	.587	.0051	.0440
	KOH	H3PO3	-.81521*	.00663	.000	-.8347	-.7957
		H3PO4	-.08402*	.00663	.000	-.1035	-.0645
		KH2PO3	-1.00495*	.00663	.000	-1.0244	-.9855
		KH2PO4	-.02455*	.00663	.587	-.0440	-.0051
50 µg	H3PO3	H3PO4	1.28344*	.00354	.000	1.2731	1.2938
		KH2PO3	-.14934*	.00354	.000	-.1597	-.1390
		KH2PO4	1.14553*	.00354	.000	1.1351	1.1559
		KOH	1.14179*	.00354	.000	1.1314	1.1522
	H3PO4	H3PO3	-1.28344*	.00354	.000	-1.2938	-1.2731
		KH2PO3	-1.43278*	.00354	.000	-1.4432	-1.4224
		KH2PO4	-.13791*	.00354	.000	-.1483	-.1275
		KOH	-.14165*	.00354	.000	-.1520	-.1313
	KH2PO3	H3PO3	.14934*	.00354	.000	.1390	.1597
		H3PO4	1.43278*	.00354	.000	1.4224	1.4432
		KH2PO4	1.29487*	.00354	.000	1.2845	1.3053
		KOH	1.29113*	.00354	.000	1.2807	1.3015
	KH2PO4	H3PO3	-1.14553*	.00354	.000	-1.1559	-1.1351
		H3PO4	.13791*	.00354	.000	.1275	.1483
		KH2PO3	-1.29487*	.00354	.000	-1.3053	-1.2845
		KOH	-.00375	.00354	.825	-.0141	.0066
	KOH	H3PO3	-1.14179*	.00354	.000	-1.1522	-1.1314
		H3PO4	.14165*	.00354	.000	.1313	.1520
		KH2PO3	-1.29113*	.00354	.000	-1.3015	-1.2807
		KH2PO4	.00375	.00354	.825	-.0066	.0141

100 µg	H3PO3	H3PO4	1.89917*	.00309	.000	1.8901	1.9082
		KH2PO3	-.07618*	.00309	.000	-.0852	-.0671
		KH2PO4	1.86957*	.00309	.000	1.8605	1.8786
		KOH	1.67290*	.00309	.000	1.6638	1.6820
	H3PO4	H3PO3	-1.89917*	.00309	.000	-1.9082	-1.8901
		KH2PO3	-1.97535*	.00309	.000	-1.9844	-1.9663
		KH2PO4	-.02960*	.00309	.602	-.0387	-.0205
		KOH	-.22627*	.00309	.000	-.2353	-.2172
	KH2PO3	H3PO3	.07618*	.00309	.000	.0671	.0852
		H3PO4	1.97535*	.00309	.000	1.9663	1.9844
		KH2PO4	1.94575*	.00309	.000	1.9367	1.9548
		KOH	1.74908*	.00309	.602	1.7400	1.7581
	KH2PO4	H3PO3	-1.86957*	.00309	.000	-1.8786	-1.8605
		H3PO4	.02960*	.00309	.000	.0205	.0387
		KH2PO3	-1.94575*	.00309	.000	-1.9548	-1.9367
		KOH	-.19667*	.00309	.000	-.2057	-.1876
	KOH	H3PO3	-1.67290*	.00309	.000	-1.6820	-1.6638
		H3PO4	.22627*	.00309	.000	.2172	.2353
		KH2PO3	-1.74908*	.00309	.000	-1.7581	-1.7400
		KH2PO4	.19667*	.00309	.000	.1876	.2057
250 µg	H3PO3	H3PO4	2.42484*	.00204	.000	2.4188	2.4308
		KH2PO3	0.00000	.00204	1.000	-.0060	.0060
		KH2PO4	2.47507*	.00204	.000	2.4691	2.4811
		KOH	2.21572*	.00204	.000	2.2097	2.2217
	H3PO4	H3PO3	-2.42484*	.00204	.000	-2.4308	-2.4188
		KH2PO3	-2.42484*	.00204	.000	-2.4308	-2.4188
		KH2PO4	.05023*	.00204	.000	.0442	.0562
		KOH	-.20912*	.00204	.000	-.2151	-.2031
	KH2PO3	H3PO3	0.00000	.00204	1.000	-.0060	.0060
		H3PO4	2.42484*	.00204	.000	2.4188	2.4308
		KH2PO4	2.47507*	.00204	.000	2.4691	2.4811
		KOH	2.21572*	.00204	.000	2.2097	2.2217
	KH2PO4	H3PO3	-2.47507*	.00204	.000	-2.4811	-2.4691
		H3PO4	-.05023*	.00204	.000	-.0562	-.0442
		KH2PO3	-2.47507*	.00204	.000	-2.4811	-2.4691
		KOH	-.25935*	.00204	.000	-.2654	-.2533
	KOH	H3PO3	-2.21572*	.00204	.000	-2.2217	-2.2097
		H3PO4	.20912*	.00204	.000	.2031	.2151
		KH2PO3	-2.21572*	.00204	.000	-2.2217	-2.2097
		KH2PO4	.25935*	.00204	.000	.2533	.2654

*. The mean difference is significant at the 0.05 level.

Appendix 7: Percent inhibition of *M. nivale* on PDA amended with concentrations of phosphite derived from the commercial compounds TKO, Naturfos, PK Fight, Turfite and PK Plus, descriptive statistics.

Descriptive Statistics

Concentration	Compound		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	TKO	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Naturfos	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Fight	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Turfite	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Plus	Percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
10 µg	TKO	Percent inhibition	6	10.87	11.30	11.0783	.06695	.16400	.027
		Arcsine transformed percent inhibition	6	.67	.69	.6786	.00213	.00523	.000
		Residual for Percent inhibition	6	-.21	.22	.0000	.06695	.16400	.027
		Valid N (listwise)	6						
	Naturfos	Percent inhibition	6	13.55	13.84	13.6650	.05071	.12422	.015
		Arcsine transformed percent inhibition	6	.75	.76	.7573	.00147	.00361	.000
		Residual for Percent inhibition	6	-.11	.18	.0000	.05071	.12422	.015
		Valid N (listwise)	6						
	PK Fight	Percent inhibition	6	11.81	12.10	11.9683	.04722	.11566	.013
		Arcsine transformed percent inhibition	6	.70	.71	.7065	.00145	.00356	.000
		Residual for Percent inhibition	6	-.16	.13	.0000	.04722	.11566	.013
		Valid N (listwise)	6						
	Turfite	Percent inhibition	6	8.54	8.99	8.7167	.07911	.19377	.038
		Arcsine transformed percent inhibition	6	.59	.61	.5994	.00280	.00686	.000
		Residual for Percent inhibition	6	-.18	.27	.0000	.07911	.19377	.038
		Valid N (listwise)	6						
	PK Plus	Percent inhibition	6	10.48	10.82	10.6533	.05766	.14123	.020
		Arcsine transformed percent inhibition	6	.66	.67	.6650	.00187	.00458	.000
		Residual for Percent inhibition	6	-.17	.17	.0000	.05766	.14123	.020
		Valid N (listwise)	6						

50 µg	TKO	Percent inhibition	6	53.81	54.11	53.9133	.05044	.12356	.015
		Arcsine transformed percent inhibition	6	1.65	1.65	1.6491	.00101	.00248	.000
		Residual for Percent inhibition	6	-.10	.20	.0000	.05044	.12356	.015
		Valid N (listwise)	6						
	Naturfos	Percent inhibition	6	58.70	59.11	58.9100	.06728	.16480	.027
		Arcsine transformed percent inhibition	6	1.75	1.75	1.7500	.00137	.00335	.000
		Residual for Percent inhibition	6	-.21	.20	.0000	.06728	.16480	.027
		Valid N (listwise)	6						
	PK Fight	Percent inhibition	6	60.89	61.20	61.0367	.04984	.12209	.015
		Arcsine transformed percent inhibition	6	1.79	1.80	1.7934	.00102	.00250	.000
		Residual for Percent inhibition	6	-.15	.16	.0000	.04984	.12209	.015
		Valid N (listwise)	6						
	Turfite	Percent inhibition	6	54.17	54.27	54.2033	.01626	.03983	.002
		Arcsine transformed percent inhibition	6	1.65	1.66	1.6550	.00033	.00080	.000
		Residual for Percent inhibition	6	-.03	.07	.0000	.01626	.03983	.002
		Valid N (listwise)	6						
	PK Plus	Percent inhibition	6	51.44	51.73	51.6433	.04455	.10912	.012
		Arcsine transformed percent inhibition	6	1.60	1.61	1.6037	.00089	.00218	.000
		Residual for Percent inhibition	6	-.20	.09	.0000	.04455	.10912	.012
		Valid N (listwise)	6						
100 µg	TKO	Percent inhibition	6	88.24	88.59	88.3917	.04976	.12189	.015
		Arcsine transformed percent inhibition	6	2.44	2.45	2.4463	.00155	.00381	.000
		Residual for Percent inhibition	6	-.15	.20	.0000	.04976	.12189	.015
		Valid N (listwise)	6						
	Naturfos	Percent inhibition	6	86.63	86.94	86.8200	.04782	.11713	.014
		Arcsine transformed percent inhibition	6	2.39	2.40	2.3985	.00141	.00346	.000
		Residual for Percent inhibition	6	-.19	.12	.0000	.04782	.11713	.014
		Valid N (listwise)	6						
	PK Fight	Percent inhibition	6	89.43	89.88	89.5950	.06781	.16610	.028
		Arcsine transformed percent inhibition	6	2.48	2.49	2.4847	.00223	.00545	.000
		Residual for Percent inhibition	6	-.16	.29	.0000	.06781	.16610	.028
		Valid N (listwise)	6						
	Turfite	Percent inhibition	6	89.90	90.17	90.0783	.04586	.11232	.013
		Arcsine transformed percent inhibition	6	2.49	2.50	2.5007	.00153	.00375	.000
		Residual for Percent inhibition	6	-.18	.09	.0000	.04586	.11232	.013
		Valid N (listwise)	6						
	PK Plus	Percent inhibition	6	88.04	88.40	88.2200	.05348	.13100	.017
		Arcsine transformed percent inhibition	6	2.44	2.45	2.4409	.00166	.00406	.000
		Residual for Percent inhibition	6	-.18	.18	.0000	.05348	.13100	.017
		Valid N (listwise)	6						
250 µg	TKO	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Naturfos	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Fight	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Turfite	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Plus	Percent inhibition	6	100.00	100.00	100.0000	0.00000	0.00000	0.000
		Arcsine transformed percent inhibition	6	3.14	3.14	3.1416	0.00000	0.00000	0.000
		Residual for Percent inhibition	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

Appendix 8: Tukey pairwise comparisons for figure 2-6, Percent inhibition of *M. nivale* mycelial growth on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA. Data were arcsine transformed prior to analysis and back transformed for the graph.

Multiple Comparisons

Dependent Variable: arcsine

Tukey HSD

Concentration	Compound		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
10 µg	TKO	Naturfos	-.0787*	.00284	.000	-.0870	-.0703
		PK Fight	-.0279*	.00284	.000	-.0362	-.0195
		Turfite	.0792*	.00284	.000	.0709	.0876
		PK Plus	.0137*	.00284	.001	.0053	.0220
	Naturfos	TKO	.0787*	.00284	.000	.0703	.0870
		PK Fight	.0508*	.00284	.000	.0424	.0591
		Turfite	.1579*	.00284	.000	.1496	.1662
		PK Plus	.0923*	.00284	.000	.0840	.1007
	PK Fight	TKO	.0279*	.00284	.000	.0195	.0362
		Naturfos	-.0508*	.00284	.000	-.0591	-.0424
		Turfite	.1071*	.00284	.000	.0988	.1155
		PK Plus	.0415*	.00284	.000	.0332	.0499
	Turfite	TKO	-.0792*	.00284	.000	-.0876	-.0709
		Naturfos	-.1579*	.00284	.000	-.1662	-.1496
		PK Fight	-.1071*	.00284	.000	-.1155	-.0988
		PK Plus	-.0656*	.00284	.000	-.0739	-.0572
	PK Plus	TKO	-.0137*	.00284	.001	-.0220	-.0053
		Naturfos	-.0923*	.00284	.000	-.1007	-.0840
		PK Fight	-.0415*	.00284	.000	-.0499	-.0332
		Turfite	.0656*	.00284	.000	.0572	.0739

50 µg	TKO	Naturfos	-.1008*	.00139	.000	-.1049	-.0967
		PK Fight	-.1442*	.00139	.000	-.1483	-.1401
		Turfite	-.0058*	.00139	.003	-.0099	-.0017
		PK Plus	.0455*	.00139	.000	.0414	.0496
	Naturfos	TKO	.1008*	.00139	.000	.0967	.1049
		PK Fight	-.0434*	.00139	.000	-.0475	-.0393
		Turfite	.0950*	.00139	.000	.0909	.0991
		PK Plus	.1463*	.00139	.000	.1422	.1504
	PK Fight	TKO	.1442*	.00139	.000	.1401	.1483
		Naturfos	.0434*	.00139	.000	.0393	.0475
		Turfite	.1384*	.00139	.000	.1343	.1425
		PK Plus	.1897*	.00139	.000	.1856	.1938
	Turfite	TKO	.0058*	.00139	.003	.0017	.0099
		Naturfos	-.0950*	.00139	.000	-.0991	-.0909
		PK Fight	-.1384*	.00139	.000	-.1425	-.1343
		PK Plus	.0513*	.00139	.000	.0472	.0554
	PK Plus	TKO	-.0455*	.00139	.000	-.0496	-.0414
		Naturfos	-.1463*	.00139	.000	-.1504	-.1422
		PK Fight	-.1897*	.00139	.000	-.1938	-.1856
		Turfite	-.0513*	.00139	.000	-.0554	-.0472
100 µg	TKO	Naturfos	.0477*	.00241	.000	.0407	.0548
		PK Fight	-.0385*	.00241	.000	-.0455	-.0314
		Turfite	-.0545*	.00241	.000	-.0615	-.0474
		PK Plus	.0053	.00241	.205	-.0017	.0124
	Naturfos	TKO	-.0477*	.00241	.000	-.0548	-.0407
		PK Fight	-.0862*	.00241	.000	-.0933	-.0791
		Turfite	-.1022*	.00241	.000	-.1092	-.0951
		PK Plus	-.0424*	.00241	.000	-.0494	-.0353
	PK Fight	TKO	.0385*	.00241	.000	.0314	.0455
		Naturfos	.0862*	.00241	.000	.0791	.0933
		Turfite	-.0160*	.00241	.000	-.0231	-.0089
		PK Plus	.0438*	.00241	.000	.0367	.0509
	Turfite	TKO	.0545*	.00241	.000	.0474	.0615
		Naturfos	.1022*	.00241	.000	.0951	.1092
		PK Fight	.0160*	.00241	.000	.0089	.0231
		PK Plus	.0598*	.00241	.000	.0527	.0669
	PK Plus	TKO	-.0053	.00241	.205	-.0124	.0017
		Naturfos	.0424*	.00241	.000	.0353	.0494
		PK Fight	-.0438*	.00241	.000	-.0509	-.0367
		Turfite	-.0598*	.00241	.000	-.0669	-.0527

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

Appendix 9: 2.6.6, descriptive statistics for EC_{50} and EC_{90} values.

Compound		N	Minimum	Maximum	Mean		Std. Deviation	Variance
		Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
H3PO3	EC50	6	40.33	41.86	40.9883	.23955	.58677	.344
	Residual for EC50	6	-.66	.87	.0000	.23955	.58677	.344
	Valid N (listwise)	6						
KH2PO3	EC50	6	35.04	36.79	35.9467	.28016	.68626	.471
	Residual for EC50	6	-.91	.84	.0000	.28016	.68626	.471
	Valid N (listwise)	6						
TKO	EC50	6	47.41	47.96	47.6383	.09421	.23077	.053
	Residual for EC50	6	-.23	.32	.0000	.09421	.23077	.053
	Valid N (listwise)	6						
Naturfos	EC50	6	44.01	44.97	44.5850	.17231	.42208	.178
	Residual for EC50	6	-.58	.38	.0000	.17231	.42208	.178
	Valid N (listwise)	6						
PK Fight	EC50	6	45.18	45.98	45.6667	.12115	.29676	.088
	Residual for EC50	6	-.49	.31	.0000	.12115	.29676	.088
	Valid N (listwise)	6						
Turfite	EC50	6	47.17	49.00	48.2200	.33708	.82566	.682
	Residual for EC50	6	-1.05	.78	.0000	.33708	.82566	.682
	Valid N (listwise)	6						
PK Plus	EC50	6	47.13	47.98	47.4383	.14559	.35662	.127
	Residual for EC50	6	-.31	.54	.0000	.14559	.35662	.127
	Valid N (listwise)	6						

Compound		N	Minimum	Maximum	Mean		Std. Deviation	Variance
		Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
H3PO3	EC90	6	80.02	81.61	80.9017	.24118	.59078	.349
	Residual for EC90	6	-.88	.71	.0000	.24118	.59078	.349
	Valid N (listwise)	6						
KH2PO3	EC90	6	77.10	77.93	77.6783	.14246	.34897	.122
	Residual for EC90	6	-.58	.25	.0000	.14246	.34897	.122
	Valid N (listwise)	6						
TKO	EC90	6	87.09	87.94	87.5667	.12795	.31341	.098
	Residual for EC90	6	-.48	.37	.0000	.12795	.31341	.098
	Valid N (listwise)	6						
Naturfos	EC90	6	84.06	84.74	84.3600	.10636	.26054	.068
	Residual for EC90	6	-.30	.38	.0000	.10636	.26054	.068
	Valid N (listwise)	6						
PK Fight	EC90	6	85.05	85.93	85.6683	.14293	.35011	.123
	Residual for EC90	6	-.62	.26	.0000	.14293	.35011	.123
	Valid N (listwise)	6						
Turfite	EC90	6	88.07	88.78	88.3733	.11313	.27710	.077
	Residual for EC90	6	-.30	.41	.0000	.11313	.27710	.077
	Valid N (listwise)	6						
PK Plus	EC90	6	87.23	87.93	87.6750	.11383	.27884	.078
	Residual for EC90	6	-.44	.26	.0000	.11383	.27884	.078
	Valid N (listwise)	6						

Appendix 10: 2.6.6 EC_{50} and EC_{90} values, Tukey pairwise comparisons for figure 2-8, EC_{50} and EC_{90} values of Phi sourced from reagent grade and commercial compounds.

Tukey HSD

Multiple Comparisons

Dependent Variable	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EC50	H3PO3	KH2PO3	5.04167*	.30423	.000	4.0907	5.9927
		TKO	-6.65000*	.30423	.000	-7.6010	-5.6990
		Naturfos	-3.59667*	.30423	.000	-4.5477	-2.6457
		PK Fight	-4.67833*	.30423	.000	-5.6293	-3.7273
		Turfite	-7.23167*	.30423	.000	-8.1827	-6.2807
		PK Plus	-6.45000*	.30423	.000	-7.4010	-5.4990
	KH2PO3	H3PO3	-5.04167*	.30423	.000	-5.9927	-4.0907
		TKO	-11.69167*	.30423	.000	-12.6427	-10.7407
		Naturfos	-8.63833*	.30423	.000	-9.5893	-7.6873
		PK Fight	-9.72000*	.30423	.000	-10.6710	-8.7690
		Turfite	-12.27333*	.30423	.000	-13.2243	-11.3223
		PK Plus	-11.49167*	.30423	.000	-12.4427	-10.5407
	TKO	H3PO3	6.65000*	.30423	.000	5.6990	7.6010
		KH2PO3	11.69167*	.30423	.000	10.7407	12.6427
		Naturfos	3.05333*	.30423	.000	2.1023	4.0043
		PK Fight	1.97167*	.30423	.000	1.0207	2.9227
		Turfite	-.58167	.30423	.486	-1.5327	.3693
		PK Plus	.20000	.30423	.994	-.7510	1.1510
	Naturfos	H3PO3	3.59667*	.30423	.000	2.6457	4.5477
		KH2PO3	8.63833*	.30423	.000	7.6873	9.5893
		TKO	-3.05333*	.30423	.000	-4.0043	-2.1023
		PK Fight	-1.08167*	.30423	.017	-2.0327	-.1307
		Turfite	-3.63500*	.30423	.000	-4.5860	-2.6840
		PK Plus	-2.85333*	.30423	.000	-3.8043	-1.9023
	PK Fight	H3PO3	4.67833*	.30423	.000	3.7273	5.6293
		KH2PO3	9.72000*	.30423	.000	8.7690	10.6710
		TKO	-1.97167*	.30423	.000	-2.9227	-1.0207
		Naturfos	1.08167*	.30423	.017	.1307	2.0327
		Turfite	-2.55333*	.30423	.000	-3.5043	-1.6023
		PK Plus	-1.77167*	.30423	.000	-2.7227	-.8207
	Turfite	H3PO3	7.23167*	.30423	.000	6.2807	8.1827
		KH2PO3	12.27333*	.30423	.000	11.3223	13.2243
		TKO	.58167	.30423	.486	-.3693	1.5327
		Naturfos	3.63500*	.30423	.000	2.6840	4.5860
		PK Fight	2.55333*	.30423	.000	1.6023	3.5043
		PK Plus	.78167	.30423	.167	-.1693	1.7327
	PK Plus	H3PO3	6.45000*	.30423	.000	5.4990	7.4010
		KH2PO3	11.49167*	.30423	.000	10.5407	12.4427
		TKO	-.20000	.30423	.994	-1.1510	.7510
		Naturfos	2.85333*	.30423	.000	1.9023	3.8043
		PK Fight	1.77167*	.30423	.000	.8207	2.7227
		Turfite	-.78167	.30423	.167	-1.7327	.1693

EC90	H3PO3	KH2PO3	3.22333*	.20863	.000	2.5712	3.8755
		TKO	-6.66500*	.20863	.000	-7.3171	-6.0129
		Naturfos	-3.45833*	.20863	.000	-4.1105	-2.8062
		PK Fight	-4.76667*	.20863	.000	-5.4188	-4.1145
		Turfite	-7.47167*	.20863	.000	-8.1238	-6.8195
		PK Plus	-6.77333*	.20863	.000	-7.4255	-6.1212
	KH2PO3	H3PO3	-3.22333*	.20863	.000	-3.8755	-2.5712
		TKO	-9.88833*	.20863	.000	-10.5405	-9.2362
		Naturfos	-6.68167*	.20863	.000	-7.3338	-6.0295
		PK Fight	-7.99000*	.20863	.000	-8.6421	-7.3379
		Turfite	-10.69500*	.20863	.000	-11.3471	-10.0429
		PK Plus	-9.99667*	.20863	.000	-10.6488	-9.3445
	TKO	H3PO3	6.66500*	.20863	.000	6.0129	7.3171
		KH2PO3	9.88833*	.20863	.000	9.2362	10.5405
		Naturfos	3.20667*	.20863	.000	2.5545	3.8588
		PK Fight	1.89833*	.20863	.000	1.2462	2.5505
		Turfite	-.80667*	.20863	.008	-1.4588	-.1545
		PK Plus	-.10833	.20863	.998	-.7605	.5438
	Naturfos	H3PO3	3.45833*	.20863	.000	2.8062	4.1105
		KH2PO3	6.68167*	.20863	.000	6.0295	7.3338
		TKO	-3.20667*	.20863	.000	-3.8588	-2.5545
		PK Fight	-1.30833*	.20863	.000	-1.9605	-.6562
		Turfite	-4.01333*	.20863	.000	-4.6655	-3.3612
		PK Plus	-3.31500*	.20863	.000	-3.9671	-2.6629
	PK Fight	H3PO3	4.76667*	.20863	.000	4.1145	5.4188
		KH2PO3	7.99000*	.20863	.000	7.3379	8.6421
		TKO	-1.89833*	.20863	.000	-2.5505	-1.2462
		Naturfos	1.30833*	.20863	.000	.6562	1.9605
		Turfite	-2.70500*	.20863	.000	-3.3571	-2.0529
		PK Plus	-2.00667*	.20863	.000	-2.6588	-1.3545
	Turfite	H3PO3	7.47167*	.20863	.000	6.8195	8.1238
		KH2PO3	10.69500*	.20863	.000	10.0429	11.3471
		TKO	.80667*	.20863	.008	.1545	1.4588
		Naturfos	4.01333*	.20863	.000	3.3612	4.6655
		PK Fight	2.70500*	.20863	.000	2.0529	3.3571
		PK Plus	.69833*	.20863	.029	.0462	1.3505
	PK Plus	H3PO3	6.77333*	.20863	.000	6.1212	7.4255
		KH2PO3	9.99667*	.20863	.000	9.3445	10.6488
		TKO	.10833	.20863	.998	-.5438	.7605
		Naturfos	3.31500*	.20863	.000	2.6629	3.9671
		PK Fight	2.00667*	.20863	.000	1.3545	2.6588
		Turfite	-.69833*	.20863	.029	-1.3505	-.0462

*. The mean difference is significant at the 0.05 level.

Appendix 11: 2.6.5 Fungicide or fungistatic properties of Phi. *M. nivale* colony diameters in mm, 5 days post inoculation, following immersion for 10 days in solutions of KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH, descriptive statistics.

Descriptive Statistics

Concentration	Compound		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	H3PO3	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
10 µg	H3PO3	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony_diameters	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
50 µg	H3PO3	Colony diameters	6	85.32	86.85	86.1750	.25442	.62321	.388
		Residual for Colony_diameters	6	-.86	.67	.0000	.25442	.62321	.388
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	85.16	86.98	86.1300	.30441	.74565	.556
		Residual for Colony_diameters	6	-.97	.85	.0000	.30441	.74565	.556
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	83.18	84.58	83.7950	.22975	.56277	.317
		Residual for Colony_diameters	6	-.61	.78	.0000	.22975	.56277	.317
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	86.10	87.92	87.1933	.31941	.78240	.612
		Residual for Colony_diameters	6	-1.09	.73	.0000	.31941	.78240	.612
		Valid N (listwise)	6						
	KOH	Colony diameters	6	85.57	87.34	86.3150	.32949	.80709	.651
		Residual for Colony_diameters	6	-.75	1.02	.0000	.32949	.80709	.651
		Valid N (listwise)	6						

100 µg	H3PO3	Colony diameters	6	81.20	83.89	82.0333	.38349	.93935	.882
		Residual for Colony_diameters	6	-.83	1.86	.0000	.38349	.93935	.882
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	84.10	86.93	85.6883	.52506	1.28613	1.654
		Residual for Colony_diameters	6	-1.59	1.24	.0000	.52506	1.28613	1.654
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	75.21	77.31	76.3600	.38095	.93315	.871
		Residual for Colony_diameters	6	-1.15	.95	.0000	.38095	.93315	.871
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	83.04	85.55	84.2550	.51902	1.27133	1.616
		Residual for Colony_diameters	6	-1.22	1.29	.0000	.51902	1.27133	1.616
		Valid N (listwise)	6						
	KOH	Colony diameters	6	85.39	88.00	86.6900	.41772	1.02319	1.047
		Residual for Colony_diameters	6	-1.30	1.31	.0000	.41772	1.02319	1.047
		Valid N (listwise)	6						
250 µg	H3PO3	Colony diameters	6	70.31	71.92	71.0600	.24439	.59863	.358
		Residual for Colony_diameters	6	-.75	.86	.0000	.24439	.59863	.358
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	80.00	82.03	81.0617	.36145	.88538	.784
		Residual for Colony_diameters	6	-1.06	.97	.0000	.36145	.88538	.784
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	68.01	70.69	69.2200	.52703	1.29095	1.667
		Residual for Colony_diameters	6	-1.21	1.47	.0000	.52703	1.29095	1.667
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	83.71	85.65	84.4783	.27328	.66940	.448
		Residual for Colony_diameters	6	-.77	1.17	.0000	.27328	.66940	.448
		Valid N (listwise)	6						
	KOH	Colony diameters	6	73.23	75.55	74.2717	.40077	.98168	.964
		Residual for Colony_diameters	6	-1.04	1.28	.0000	.40077	.98168	.964
		Valid N (listwise)	6						
500 µg	H3PO3	Colony diameters	6	53.12	56.51	54.6200	.59987	1.46938	2.159
		Residual for Colony_diameters	6	-1.50	1.89	.0000	.59987	1.46938	2.159
		Valid N (listwise)	6						
	H3PO4	Colony diameters	6	68.34	70.15	69.2217	.27146	.66493	.442
		Residual for Colony_diameters	6	-.88	.93	.0000	.27146	.66493	.442
		Valid N (listwise)	6						
	KH2PO3	Colony diameters	6	57.56	60.86	58.7533	.53379	1.30751	1.710
		Residual for Colony_diameters	6	-1.19	2.11	.0000	.53379	1.30751	1.710
		Valid N (listwise)	6						
	KH2PO4	Colony diameters	6	72.35	75.61	73.8383	.56976	1.39561	1.948
		Residual for Colony_diameters	6	-1.49	1.77	.0000	.56976	1.39561	1.948
		Valid N (listwise)	6						
	KOH	Colony diameters	6	64.63	68.00	66.4717	.52464	1.28511	1.651
		Residual for Colony_diameters	6	-1.84	1.53	.0000	.52464	1.28511	1.651
		Valid N (listwise)	6						

Appendix 12: Tukey pairwise comparisons for figure 2-9, *M. nivale* colony diameters, following immersion in solutions of H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH.

Multiple Comparisons

Dependent Variable: Colony diameters

Tukey HSD

Concentration	Compound		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
50 µg	H3PO3	H3PO4	.0450	.41025	1.000	-1.1599	1.2499
		KH2PO3	2.3800*	.41025	.000	1.1751	3.5849
		KH2PO4	-1.0183	.41025	.127	-2.2232	.1865
		KOH	-.1400	.41025	.997	-1.3449	1.0649
	H3PO4	H3PO3	-.0450	.41025	1.000	-1.2499	1.1599
		KH2PO3	2.3350*	.41025	.000	1.1301	3.5399
		KH2PO4	-1.0633	.41025	.102	-2.2682	.1415
		KOH	-.1850	.41025	.991	-1.3899	1.0199
	KH2PO3	H3PO3	-2.3800*	.41025	.000	-3.5849	-1.1751
		H3PO4	-2.3350*	.41025	.000	-3.5399	-1.1301
		KH2PO4	-3.3983*	.41025	.000	-4.6032	-2.1935
		KOH	-2.5200*	.41025	.000	-3.7249	-1.3151
	KH2PO4	H3PO3	1.0183	.41025	.127	-.1865	2.2232
		H3PO4	1.0633	.41025	.102	-.1415	2.2682
		KH2PO3	3.3983*	.41025	.000	2.1935	4.6032
		KOH	.8783	.41025	.235	-.3265	2.0832
	KOH	H3PO3	.1400	.41025	.997	-1.0649	1.3449
		H3PO4	.1850	.41025	.991	-1.0199	1.3899
		KH2PO3	2.5200*	.41025	.000	1.3151	3.7249
		KH2PO4	-.8783	.41025	.235	-2.0832	.3265
100 µg	H3PO3	H3PO4	-3.6550*	.63616	.000	-5.5233	-1.7867
		KH2PO3	5.6733*	.63616	.000	3.8050	7.5417
		KH2PO4	-2.2217*	.63616	.014	-4.0900	-.3533
		KOH	-4.6567*	.63616	.000	-6.5250	-2.7883
	H3PO4	H3PO3	3.6550*	.63616	.000	1.7867	5.5233
		KH2PO3	9.3283*	.63616	.000	7.4600	11.1967
		KH2PO4	1.4333	.63616	.194	-.4350	3.3017
		KOH	-1.0017	.63616	.526	-2.8700	.8667
	KH2PO3	H3PO3	-5.6733*	.63616	.000	-7.5417	-3.8050
		H3PO4	-9.3283*	.63616	.000	-11.1967	-7.4600
		KH2PO4	-7.8950*	.63616	.000	-9.7633	-6.0267
		KOH	-10.3300*	.63616	.000	-12.1983	-8.4617
	KH2PO4	H3PO3	2.2217*	.63616	.014	.3533	4.0900
		H3PO4	-1.4333	.63616	.194	-3.3017	.4350
		KH2PO3	7.8950*	.63616	.000	6.0267	9.7633
		KOH	-2.4350*	.63616	.006	-4.3033	-.5667
	KOH	H3PO3	4.6567*	.63616	.000	2.7883	6.5250
		H3PO4	1.0017	.63616	.526	-.8667	2.8700
		KH2PO3	10.3300*	.63616	.000	8.4617	12.1983
		KH2PO4	2.4350*	.63616	.006	.5667	4.3033

250 µg	H3PO3	H3PO4	-10.0017*	.53045	.000	-11.5595	-8.4438
		KH2PO3	1.8400*	.53045	.015	.2821	3.3979
		KH2PO4	-13.4183*	.53045	.000	-14.9762	-11.8605
		KOH	-3.2117*	.53045	.000	-4.7695	-1.6538
	H3PO4	H3PO3	10.0017*	.53045	.000	8.4438	11.5595
		KH2PO3	11.8417*	.53045	.000	10.2838	13.3995
		KH2PO4	-3.4167*	.53045	.000	-4.9745	-1.8588
		KOH	6.7900*	.53045	.000	5.2321	8.3479
	KH2PO3	H3PO3	-1.8400*	.53045	.015	-3.3979	-.2821
		H3PO4	-11.8417*	.53045	.000	-13.3995	-10.2838
		KH2PO4	-15.2583*	.53045	.000	-16.8162	-13.7005
		KOH	-5.0517*	.53045	.000	-6.6095	-3.4938
	KH2PO4	H3PO3	13.4183*	.53045	.000	11.8605	14.9762
		H3PO4	3.4167*	.53045	.000	1.8588	4.9745
		KH2PO3	15.2583*	.53045	.000	13.7005	16.8162
		KOH	10.2067*	.53045	.000	8.6488	11.7645
	KOH	H3PO3	3.2117*	.53045	.000	1.6538	4.7695
		H3PO4	-6.7900*	.53045	.000	-8.3479	-5.2321
		KH2PO3	5.0517*	.53045	.000	3.4938	6.6095
		KH2PO4	-10.2067*	.53045	.000	-11.7645	-8.6488
500 µg	H3PO3	H3PO4	-14.6017*	.72618	.000	-16.7344	-12.4690
		KH2PO3	-4.1333*	.72618	.000	-6.2660	-2.0006
		KH2PO4	-19.2183*	.72618	.000	-21.3510	-17.0856
		KOH	-11.8517*	.72618	.000	-13.9844	-9.7190
	H3PO4	H3PO3	14.6017*	.72618	.000	12.4690	16.7344
		KH2PO3	10.4683*	.72618	.000	8.3356	12.6010
		KH2PO4	-4.6167*	.72618	.000	-6.7494	-2.4840
		KOH	2.7500*	.72618	.007	.6173	4.8827
	KH2PO3	H3PO3	4.1333*	.72618	.000	2.0006	6.2660
		H3PO4	-10.4683*	.72618	.000	-12.6010	-8.3356
		KH2PO4	-15.0850*	.72618	.000	-17.2177	-12.9523
		KOH	-7.7183*	.72618	.000	-9.8510	-5.5856
	KH2PO4	H3PO3	19.2183*	.72618	.000	17.0856	21.3510
		H3PO4	4.6167*	.72618	.000	2.4840	6.7494
		KH2PO3	15.0850*	.72618	.000	12.9523	17.2177
		KOH	7.3667*	.72618	.000	5.2340	9.4994
	KOH	H3PO3	11.8517*	.72618	.000	9.7190	13.9844
		H3PO4	-2.7500*	.72618	.007	-4.8827	-.6173
		KH2PO3	7.7183*	.72618	.000	5.5856	9.8510
		KH2PO4	-7.3667*	.72618	.000	-9.4994	-5.2340

Based on observed means.
The error term is Mean Square(Error) = 1.582.
*. The mean difference is significant at the .05 level.

Appendix 13: 2.6.6 Colony diameters on H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4 and KOH amended PDA, descriptive statistics at 5 and 10 dpi.

Descriptive Statistics

Concentration	Compound		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	H3PO3	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
10 µg	H3PO3	Colony diameters 5 dpi	6	82.13	83.15	82.7750	.17433	.42702	.182
		Residual for colony diameters 5dpi	6	-.65	.38	.0000	.17433	.42702	.182
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	H3PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters 5 dpi	6	78.43	79.91	79.2817	.27410	.67140	.451
		Residual for colony diameters 5dpi	6	-.85	.63	.0000	.27410	.67140	.451
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

50 µg	H3PO3	Colony diameters 5 dpi	6	39.30	40.96	39.8833	.23600	.57809	.334
		Residual for colony diameters 5dpi	6	-.58	1.08	.0000	.23600	.57809	.334
		Colony diameters 10 dpi	6	77.37	79.39	78.2533	.29805	.73006	.533
		Residual for colony diameters 10dpi	6	-.88	1.14	.0000	.29805	.73006	.533
		Valid N (listwise)	6						
	H3PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters 5 dpi	6	36.05	37.40	36.6483	.18472	.45248	.205
		Residual for colony diameters 5dpi	6	-.60	.75	.0000	.18472	.45248	.205
		Colony diameters 10 dpi	6	73.16	74.40	73.7200	.20637	.50549	.256
		Residual for colony diameters 10dpi	6	-.56	.68	.0000	.20637	.50549	.256
		Valid N (listwise)	6						
	KH2PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
100 µg	H3PO3	Colony diameters 5 dpi	6	8.09	8.99	8.4633	.14428	.35342	.125
		Residual for colony diameters 5dpi	6	-.37	.53	.0000	.14428	.35342	.125
		Colony diameters 10 dpi	6	13.85	14.80	14.4800	.14133	.34618	.120
		Residual for colony diameters 10dpi	6	-.63	.32	.0000	.14133	.34618	.120
		Valid N (listwise)	6						
	H3PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters 5 dpi	6	7.32	7.88	7.6167	.08812	.21584	.047
		Residual for colony diameters 5dpi	6	-.30	.26	.0000	.08812	.21584	.047
		Colony diameters 10 dpi	6	12.19	12.81	12.4100	.10221	.25036	.063
		Residual for colony diameters 10dpi	6	-.22	.40	.0000	.10221	.25036	.063
		Valid N (listwise)	6						
	KH2PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

250 µg	H3PO3	Colony diameters 5 dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	2.22	2.40	2.3150	.02997	.07342	.005
		Residual for colony diameters 10dpi	6	-.09	.09	.0000	.02997	.07342	.005
		Valid N (listwise)	6						
	H3PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KH2PO3	Colony diameters 5 dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	1.80	1.94	1.8583	.02386	.05845	.003
		Residual for colony diameters 10dpi	6	-.06	.08	.0000	.02386	.05845	.003
		Valid N (listwise)	6						
	KH2PO4	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	KOH	Colony diameters 5 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10 dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

Appendix 14: Figs 2-10 and 2-11. Tukey pairwise comparisons for *M. nivale* colony diameters 5 dpi on H_3PO_3, KH_2PO_3, H_3PO_4, KH_2PO_4 and KOH amended PDA.

Multiple Comparisons

Dependent Variable: Colony_diameters_5dpi

Tukey HSD

Concentration	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
10 µg	H3PO3	H3PO4	-7.2250*	.20545	.000	-7.8284	-6.6216
		KH2PO3	3.4933*	.20545	.000	2.8900	4.0967
		KH2PO4	-7.2250*	.20545	.000	-7.8284	-6.6216
		KOH	-7.2250*	.20545	.000	-7.8284	-6.6216
	H3PO4	H3PO3	7.2250*	.20545	.000	6.6216	7.8284
		KH2PO3	10.7183*	.20545	.000	10.1150	11.3217
		KH2PO4	0.0000	.20545	1.000	-.6034	.6034
		KOH	0.0000	.20545	1.000	-.6034	.6034
	KH2PO3	H3PO3	-3.4933*	.20545	.000	-4.0967	-2.8900
		H3PO4	-10.7183*	.20545	.000	-11.3217	-10.1150
		KH2PO4	-10.7183*	.20545	.000	-11.3217	-10.1150
		KOH	-10.7183*	.20545	.000	-11.3217	-10.1150
	KH2PO4	H3PO3	7.2250*	.20545	.000	6.6216	7.8284
		H3PO4	0.0000	.20545	1.000	-.6034	.6034
		KH2PO3	10.7183*	.20545	.000	10.1150	11.3217
		KOH	0.0000	.20545	1.000	-.6034	.6034
	KOH	H3PO3	7.2250*	.20545	.000	6.6216	7.8284
		H3PO4	0.0000	.20545	1.000	-.6034	.6034
		KH2PO3	10.7183*	.20545	.000	10.1150	11.3217
		KH2PO4	0.0000	.20545	1.000	-.6034	.6034
50 µg	H3PO3	H3PO4	-50.1167*	.18955	.000	-50.6733	-49.5600
		KH2PO3	3.2350*	.18955	.000	2.6783	3.7917
		KH2PO4	-50.1167*	.18955	.000	-50.6733	-49.5600
		KOH	-50.1167*	.18955	.000	-50.6733	-49.5600
	H3PO4	H3PO3	50.1167*	.18955	.000	49.5600	50.6733
		KH2PO3	53.3517*	.18955	.000	52.7950	53.9083
		KH2PO4	0.0000	.18955	1.000	-.5567	.5567
		KOH	0.0000	.18955	1.000	-.5567	.5567
	KH2PO3	H3PO3	-3.2350*	.18955	.000	-3.7917	-2.6783
		H3PO4	-53.3517*	.18955	.000	-53.9083	-52.7950
		KH2PO4	-53.3517*	.18955	.000	-53.9083	-52.7950
		KOH	-53.3517*	.18955	.000	-53.9083	-52.7950
	KH2PO4	H3PO3	50.1167*	.18955	.000	49.5600	50.6733
		H3PO4	0.0000	.18955	1.000	-.5567	.5567
		KH2PO3	53.3517*	.18955	.000	52.7950	53.9083
		KOH	0.0000	.18955	1.000	-.5567	.5567
	KOH	H3PO3	50.1167*	.18955	.000	49.5600	50.6733
		H3PO4	0.0000	.18955	1.000	-.5567	.5567
		KH2PO3	53.3517*	.18955	.000	52.7950	53.9083
		KH2PO4	0.0000	.18955	1.000	-.5567	.5567

100 µg	H3PO3	H3PO4	-815367*	.10692	.000	-818507	-812226
		KH2PO3	.8467*	.10692	.000	.5326	1.1607
		KH2PO4	-815367*	.10692	.000	-818507	-812226
		KOH	-815367*	.10692	.000	-818507	-812226
	H3PO4	H3PO3	815367*	.10692	.000	812226	818507
		KH2PO3	82.3833*	.10692	.000	82.0693	82.6974
		KH2PO4	0.0000	.10692	1.000	-.3140	.3140
		KOH	0.0000	.10692	1.000	-.3140	.3140
	KH2PO3	H3PO3	-.8467*	.10692	.000	-1.1607	-.5326
		H3PO4	-82.3833*	.10692	.000	-82.6974	-82.0693
		KH2PO4	-82.3833*	.10692	.000	-82.6974	-82.0693
		KOH	-82.3833*	.10692	.000	-82.6974	-82.0693
	KH2PO4	H3PO3	815367*	.10692	.000	812226	818507
		H3PO4	0.0000	.10692	1.000	-.3140	.3140
		KH2PO3	82.3833*	.10692	.000	82.0693	82.6974
		KOH	0.0000	.10692	1.000	-.3140	.3140
	KOH	H3PO3	815367*	.10692	.000	812226	818507
		H3PO4	0.0000	.10692	1.000	-.3140	.3140
		KH2PO3	82.3833*	.10692	.000	82.0693	82.6974
		KH2PO4	0.0000	.10692	1.000	-.3140	.3140
250 µg	H3PO3	H3PO4	-90.0000*	.00000	.000	-90.0000	-90.0000
		KH2PO3	0.0000	.00000	1.000	.0000	.0000
		KH2PO4	-90.0000*	.00000	.000	-90.0000	-90.0000
		KOH	-90.0000*	.00000	.000	-90.0000	-90.0000
	H3PO4	H3PO3	90.0000*	.00000	.000	90.0000	90.0000
		KH2PO3	90.0000*	.00000	.000	90.0000	90.0000
		KH2PO4	0.0000	.00000	1.000	.0000	.0000
		KOH	0.0000	.00000	1.000	.0000	.0000
	KH2PO3	H3PO3	0.0000	.00000	1.000	.0000	.0000
		H3PO4	-90.0000*	.00000	.000	-90.0000	-90.0000
		KH2PO4	-90.0000*	.00000	.000	-90.0000	-90.0000
		KOH	-90.0000*	.00000	.000	-90.0000	-90.0000
	KH2PO4	H3PO3	90.0000*	.00000	.000	90.0000	90.0000
		H3PO4	0.0000	.00000	1.000	.0000	.0000
		KH2PO3	90.0000*	.00000	.000	90.0000	90.0000
		KOH	0.0000	.00000	1.000	.0000	.0000
	KOH	H3PO3	90.0000*	.00000	.000	90.0000	90.0000
		H3PO4	0.0000	.00000	1.000	.0000	.0000
		KH2PO3	90.0000*	.00000	.000	90.0000	90.0000
		KH2PO4	0.0000	.00000	1.000	.0000	.0000

Based on observed means.
The error term is Mean Square(Error) = 4.85E-029.
*. The mean difference is significant at the .05 level.

Appendix 15: 2.6.6.3 Colony diameters on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA, descriptive statistics at 5 and 10 dpi.

Descriptive Statistics

Concentration	Compounds		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	TKO	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Naturfos	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Fight	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Turfite	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Plus	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
10 µg	TKO	Colony diameters 5dpi	6	82.13	83.15	82.7750	.17433	.42702	.182
		Residual for Colony diameters 5dpi	6	-.65	.37	.0000	.17433	.42702	.182
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Naturfos	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Fight	Colony diameters 5dpi	6	84.61	89.47	87.6200	.79072	1.93686	3.751
		Residual for Colony diameters 5dpi	6	-3.01	1.85	.0000	.79072	1.93686	3.751
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	Turfite	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						
	PK Plus	Colony diameters 5dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	90.00	90.00	90.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 10dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	6						

100 µg	TKO	Colony diameters 5dpi	6	11.65	12.59	12.2233	.15275	.37415	.140
		Residual for Colony diameters 5dpi	6	-.57	.37	.0000	.15275	.37415	.140
		Colony diameters 10dpi	6	17.10	20.47	18.5500	.54487	1.33466	1.781
		Residual for Colony diameters 10dpi	6	-1.45	1.92	.0000	.54487	1.33466	1.781
		Valid N (listwise)	6						
	Naturfos	Colony diameters 5dpi	6	9.92	12.22	11.6967	.36002	.88188	.778
		Residual for Colony diameters 5dpi	6	-1.78	.52	.0000	.36002	.88188	.778
		Colony diameters 10dpi	6	18.76	21.48	19.7883	.47355	1.15996	1.345
		Residual for Colony diameters 10dpi	6	-1.03	1.69	.0000	.47355	1.15996	1.345
		Valid N (listwise)	6						
	PK Fight	Colony diameters 5dpi	6	10.11	13.70	11.5417	.54019	1.32320	1.751
		Residual for Colony diameters 5dpi	6	-1.43	2.16	.0000	.54019	1.32320	1.751
		Colony diameters 10dpi	6	17.30	21.41	18.9317	.63156	1.54699	2.393
		Residual for Colony diameters 10dpi	6	-1.63	2.48	.0000	.63156	1.54699	2.393
		Valid N (listwise)	6						
	Turfite	Colony diameters 5dpi	6	9.82	13.96	12.5017	.65034	1.59300	2.538
		Residual for Colony diameters 5dpi	6	-2.68	1.46	.0000	.65034	1.59300	2.538
		Colony diameters 10dpi	6	17.56	20.78	18.9133	.53137	1.30159	1.694
		Residual for Colony diameters 10dpi	6	-1.35	1.87	.0000	.53137	1.30159	1.694
		Valid N (listwise)	6						
	PK Plus	Colony diameters 5dpi	6	10.37	12.98	11.3717	.44774	1.09673	1.203
		Residual for Colony diameters 5dpi	6	-1.00	1.61	.0000	.44774	1.09673	1.203
		Colony diameters 10dpi	6	17.88	21.31	19.9083	.55891	1.36905	1.874
		Residual for Colony diameters 10dpi	6	-2.03	1.40	.0000	.55891	1.36905	1.874
		Valid N (listwise)	6						
250 µg	TKO	Colony diameters 5dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	3.68	5.47	4.8117	.26325	.64484	.416
		Residual for Colony diameters 10dpi	6	-1.13	.66	.0000	.26325	.64484	.416
		Valid N (listwise)	6						
	Naturfos	Colony diameters 5dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	3.57	4.46	4.1067	.15398	.37718	.142
		Residual for Colony diameters 10dpi	6	-.54	.35	.0000	.15398	.37718	.142
		Valid N (listwise)	6						
	PK Fight	Colony diameters 5dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	3.86	4.92	4.4117	.15281	.37429	.140
		Residual for Colony diameters 10dpi	6	-.55	.51	.0000	.15281	.37429	.140
		Valid N (listwise)	6						
	Turfite	Colony diameters 5dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	3.69	5.41	4.9783	.27710	.67875	.461
		Residual for Colony diameters 10dpi	6	-1.29	.43	.0000	.27710	.67875	.461
		Valid N (listwise)	6						
	PK Plus	Colony diameters 5dpi	6	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Colony diameters 5dpi	6	.00	.00	.0000	0.00000	0.00000	0.000
		Colony diameters 10dpi	6	3.46	4.47	4.0567	.16514	.40451	.164
		Residual for Colony diameters 10dpi	6	-.60	.41	.0000	.16514	.40451	.164
		Valid N (listwise)	6						

Appendix 16: Fig 2-13, Tukey pairwise comparisons for *M. nivale* colony diameters 5 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA.

Multiple Comparisons

Dependent Variable: Colony diameters 5dpi
Tukey HSD

Concentration	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
10 µg	TKO	Naturfos	-7.2250*	.51211	.000	-8.7290	-5.7210
		PK Fight	-4.8450*	.51211	.000	-6.3490	-3.3410
		Turfite	-7.2250*	.51211	.000	-8.7290	-5.7210
		PK Plus	-7.2250*	.51211	.000	-8.7290	-5.7210
	Naturfos	TKO	7.2250*	.51211	.000	5.7210	8.7290
		PK Fight	2.3800*	.51211	.001	.8760	3.8840
		Turfite	0.0000	.51211	1.000	-1.5040	1.5040
		PK Plus	0.0000	.51211	1.000	-1.5040	1.5040
	PK Fight	TKO	4.8450*	.51211	.000	3.3410	6.3490
		Naturfos	-2.3800*	.51211	.001	-3.8840	-.8760
		Turfite	-2.3800*	.51211	.001	-3.8840	-.8760
		PK Plus	-2.3800*	.51211	.001	-3.8840	-.8760
	Turfite	TKO	7.2250*	.51211	.000	5.7210	8.7290
		Naturfos	0.0000	.51211	1.000	-1.5040	1.5040
		PK Fight	2.3800*	.51211	.001	.8760	3.8840
		PK Plus	0.0000	.51211	1.000	-1.5040	1.5040
	PK Plus	TKO	7.2250*	.51211	.000	5.7210	8.7290
		Naturfos	0.0000	.51211	1.000	-1.5040	1.5040
		PK Fight	2.3800*	.51211	.001	.8760	3.8840
		Turfite	0.0000	.51211	1.000	-1.5040	1.5040
50 µg	TKO	Naturfos	-2.8367	1.02663	.072	-5.8518	.1784
		PK Fight	-.8617	1.02663	.916	-3.8768	2.1534
		Turfite	-1.1800	1.02663	.779	-4.1951	1.8351
		PK Plus	-.5967	1.02663	.977	-3.6118	2.4184
	Naturfos	TKO	2.8367	1.02663	.072	-.1784	5.8518
		PK Fight	1.9750	1.02663	.331	-1.0401	4.9901
		Turfite	1.6567	1.02663	.503	-1.3584	4.6718
		PK Plus	2.2400	1.02663	.219	-.7751	5.2551
	PK Fight	TKO	.8617	1.02663	.916	-2.1534	3.8768
		Naturfos	-1.9750	1.02663	.331	-4.9901	1.0401
		Turfite	-.3183	1.02663	.998	-3.3334	2.6968
		PK Plus	.2650	1.02663	.999	-2.7501	3.2801
	Turfite	TKO	1.1800	1.02663	.779	-1.8351	4.1951
		Naturfos	-1.6567	1.02663	.503	-4.6718	1.3584
		PK Fight	.3183	1.02663	.998	-2.6968	3.3334
		PK Plus	.5833	1.02663	.979	-2.4318	3.5984
	PK Plus	TKO	.5967	1.02663	.977	-2.4184	3.6118
		Naturfos	-2.2400	1.02663	.219	-5.2551	.7751
		PK Fight	-.2650	1.02663	.999	-3.2801	2.7501
		Turfite	-.5833	1.02663	.979	-3.5984	2.4318
100 µg	TKO	Naturfos	.5267	.65366	.926	-1.3930	2.4464
		PK Fight	.6817	.65366	.833	-1.2380	2.6014
		Turfite	-.2783	.65366	.993	-2.1980	1.6414
		PK Plus	.8517	.65366	.692	-1.0680	2.7714
	Naturfos	TKO	-.5267	.65366	.926	-2.4464	1.3930
		PK Fight	.1550	.65366	.999	-1.7647	2.0747
		Turfite	-.8050	.65366	.734	-2.7247	1.1147
		PK Plus	.3250	.65366	.987	-1.5947	2.2447
	PK Fight	TKO	-.6817	.65366	.833	-2.6014	1.2380
		Naturfos	-.1550	.65366	.999	-2.0747	1.7647
		Turfite	-.9600	.65366	.591	-2.8797	.9597
		PK Plus	.1700	.65366	.999	-1.7497	2.0897
	Turfite	TKO	.2783	.65366	.993	-1.6414	2.1980
		Naturfos	.8050	.65366	.734	-1.1147	2.7247
		PK Fight	.9600	.65366	.591	-.9597	2.8797
		PK Plus	1.1300	.65366	.435	-.7897	3.0497
	PK Plus	TKO	-.8517	.65366	.692	-2.7714	1.0680
		Naturfos	-.3250	.65366	.987	-2.2447	1.5947
		PK Fight	-.1700	.65366	.999	-2.0897	1.7497
		Turfite	-1.1300	.65366	.435	-3.0497	.7897

Appendix 17: Fig 2-13, Tukey pairwise comparisons for *M. nivale* colony diameters 10 dpi on TKO, Naturfos, PK Fight, Turfite and PK Plus amended PDA.

Multiple Comparisons

Dependent Variable: Colony_diameters_10dpi

Tukey HSD

Concentration			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
50 µg	TKO	Naturfos	.5950	2.10373	.999	-5.5834	6.7734
		PK Fight	-2.4600	2.10373	.768	-8.6384	3.7184
		Turfite	-1.1200	2.10373	.983	-7.2984	5.0584
		PK Plus	-1.3517	2.10373	.966	-7.5300	4.8267
	Naturfos	TKO	-.5950	2.10373	.999	-6.7734	5.5834
		PK Fight	-3.0550	2.10373	.601	-9.2334	3.1234
		Turfite	-1.7150	2.10373	.923	-7.8934	4.4634
		PK Plus	-1.9467	2.10373	.884	-8.1250	4.2317
	PK Fight	TKO	2.4600	2.10373	.768	-3.7184	8.6384
		Naturfos	3.0550	2.10373	.601	-3.1234	9.2334
		Turfite	1.3400	2.10373	.967	-4.8384	7.5184
		PK Plus	1.1083	2.10373	.984	-5.0700	7.2867
	Turfite	TKO	1.1200	2.10373	.983	-5.0584	7.2984
		Naturfos	1.7150	2.10373	.923	-4.4634	7.8934
		PK Fight	-1.3400	2.10373	.967	-7.5184	4.8384
		PK Plus	-.2317	2.10373	1.000	-6.4100	5.9467
	PK Plus	TKO	1.3517	2.10373	.966	-4.8267	7.5300
		Naturfos	1.9467	2.10373	.884	-4.2317	8.1250
		PK Fight	-1.1083	2.10373	.984	-7.2867	5.0700
		Turfite	.2317	2.10373	1.000	-5.9467	6.4100
100 µg	TKO	Naturfos	-1.2383	.77839	.517	-3.5244	1.0477
		PK Fight	-.3817	.77839	.988	-2.6677	1.9044
		Turfite	-.3633	.77839	.990	-2.6494	1.9227
		PK Plus	-1.3583	.77839	.426	-3.6444	.9277
	Naturfos	TKO	1.2383	.77839	.517	-1.0477	3.5244
		PK Fight	.8567	.77839	.805	-1.4294	3.1427
		Turfite	.8750	.77839	.792	-1.4110	3.1610
		PK Plus	-.1200	.77839	1.000	-2.4060	2.1660
	PK Fight	TKO	.3817	.77839	.988	-1.9044	2.6677
		Naturfos	-.8567	.77839	.805	-3.1427	1.4294
		Turfite	.0183	.77839	1.000	-2.2677	2.3044
		PK Plus	-.9767	.77839	.720	-3.2627	1.3094
	Turfite	TKO	.3633	.77839	.990	-1.9227	2.6494
		Naturfos	-.8750	.77839	.792	-3.1610	1.4110
		PK Fight	-.0183	.77839	1.000	-2.3044	2.2677
		PK Plus	-.9950	.77839	.706	-3.2810	1.2910
	PK Plus	TKO	1.3583	.77839	.426	-.9277	3.6444
		Naturfos	.1200	.77839	1.000	-2.1660	2.4060
		PK Fight	.9767	.77839	.720	-1.3094	3.2627
		Turfite	.9950	.77839	.706	-1.2910	3.2810
250 µg	TKO	Naturfos	.7050	.29693	.156	-.1670	1.5770
		PK Fight	.4000	.29693	.665	-.4720	1.2720
		Turfite	-.1667	.29693	.979	-1.0387	.7054
		PK Plus	.7550	.29693	.113	-.1170	1.6270
	Naturfos	TKO	-.7050	.29693	.156	-1.5770	.1670
		PK Fight	-.3050	.29693	.840	-1.1770	.5670
		Turfite	-.8717	.29693	.050	-1.7437	.0004
		PK Plus	.0500	.29693	1.000	-.8220	.9220
	PK Fight	TKO	-.4000	.29693	.665	-1.2720	.4720
		Naturfos	.3050	.29693	.840	-.5670	1.1770
		Turfite	-.5667	.29693	.339	-1.4387	.3054
		PK Plus	.3550	.29693	.754	-.5170	1.2270
	Turfite	TKO	.1667	.29693	.979	-.7054	1.0387
		Naturfos	.8717	.29693	.050	-.0004	1.7437
		PK Fight	.5667	.29693	.339	-.3054	1.4387
		PK Plus	.9217*	.29693	.035	.0496	1.7937
	PK Plus	TKO	-.7550	.29693	.113	-1.6270	.1170
		Naturfos	-.0500	.29693	1.000	-.9220	.8220
		PK Fight	-.3550	.29693	.754	-1.2270	.5170
		Turfite	-.9217*	.29693	.035	-1.7937	-.0496

Based on observed means.

Appendix 18: 2.6.8 Effects on conidial germination, descriptive statistics.

Descriptive Statistics

Concentration	Compounds		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
0 µg	H3PO3	Percent germination	6	83.58	86.96	85.6017	.58806	1.44045	2.075
		Arcsine transformed percent germination	6	2.31	2.40	2.3639	.01666	.04081	.002
		Residual for arcsine transformed percent	6	-.06	.04	.0000	.01666	.04081	.002
		Valid N (listwise)	6						
	H3PO4	Percent germination	6	84.19	85.55	85.0933	.24477	.59956	.359
		Arcsine transformed percent germination	6	2.32	2.36	2.3489	.00684	.01675	.000
		Residual for arcsine transformed percent	6	-.03	.01	.0000	.00684	.01675	.000
		Valid N (listwise)	6						
	KH2PO3	Percent germination	6	84.26	85.62	84.7417	.19433	.47600	.227
		Arcsine transformed percent germination	6	2.33	2.36	2.3391	.00544	.01332	.000
		Residual for arcsine transformed percent	6	-.01	.02	.0000	.00544	.01332	.000
		Valid N (listwise)	6						
	KH2PO4	Percent germination	6	84.17	85.74	85.0533	.24773	.60682	.368
		Arcsine transformed percent germination	6	2.32	2.37	2.3478	.00694	.01700	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00694	.01700	.000
		Valid N (listwise)	6						
	KOH	Percent germination	6	83.20	84.63	83.8750	.21531	.52740	.278
		Arcsine transformed percent germination	6	2.30	2.34	2.3152	.00586	.01436	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00586	.01436	.000
		Valid N (listwise)	6						
10 µg	H3PO3	Percent germination	6	82.19	84.57	83.8617	.35287	.86435	.747
		Arcsine transformed percent germination	6	2.27	2.33	2.3150	.00947	.02319	.001
		Residual for arcsine transformed percent	6	-.04	.02	.0000	.00947	.02319	.001
		Valid N (listwise)	6						
	H3PO4	Percent germination	6	83.29	85.00	84.1217	.26653	.65285	.426
		Arcsine transformed percent germination	6	2.30	2.35	2.3220	.00730	.01788	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00730	.01788	.000
		Valid N (listwise)	6						
	KH2PO3	Percent germination	6	84.56	85.95	85.2467	.26538	.65004	.423
		Arcsine transformed percent germination	6	2.33	2.37	2.3533	.00748	.01833	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00748	.01833	.000
		Valid N (listwise)	6						
	KH2PO4	Percent germination	6	84.32	85.88	85.0383	.27357	.67012	.449
		Arcsine transformed percent germination	6	2.33	2.37	2.3474	.00770	.01887	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00770	.01887	.000
		Valid N (listwise)	6						
	KOH	Percent germination	6	82.10	83.72	82.9550	.25758	.63093	.398
		Arcsine transformed percent germination	6	2.27	2.31	2.2905	.00685	.01677	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00685	.01677	.000
		Valid N (listwise)	6						

50 µg	H3PO3	Percent germination	6	72.36	74.34	73.3567	.30659	.75099	.564
		Arcsine transformed percent germination	6	2.03	2.08	2.0569	.00694	.01700	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00694	.01700	.000
		Valid N (listwise)	6						
	H3PO4	Percent germination	6	82.29	83.65	83.0167	.23114	.56617	.321
		Arcsine transformed percent germination	6	2.27	2.31	2.2921	.00615	.01507	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00615	.01507	.000
		Valid N (listwise)	6						
	KH2PO3	Percent germination	6	71.35	73.66	72.5583	.36247	.88786	.788
		Arcsine transformed percent germination	6	2.01	2.06	2.0390	.00812	.01989	.000
		Residual for arcsine transformed percent	6	-.03	.02	.0000	.00812	.01989	.000
		Valid N (listwise)	6						
	KH2PO4	Percent germination	6	82.00	83.93	82.8817	.30391	.74443	.554
		Arcsine transformed percent germination	6	2.27	2.32	2.2886	.00808	.01979	.000
		Residual for arcsine transformed percent	6	-.02	.03	.0000	.00808	.01979	.000
		Valid N (listwise)	6						
	KOH	Percent germination	6	85.53	86.26	85.8950	.13368	.32746	.107
		Arcsine transformed percent germination	6	2.36	2.38	2.3716	.00384	.00941	.000
		Residual for arcsine transformed percent	6	-.01	.01	.0000	.00384	.00941	.000
		Valid N (listwise)	6						
100 µg	H3PO3	Percent germination	6	44.34	45.93	45.2583	.30032	.73562	.541
		Arcsine transformed percent germination	6	1.46	1.49	1.4758	.00604	.01478	.000
		Residual for arcsine transformed percent	6	-.02	.01	.0000	.00604	.01478	.000
		Valid N (listwise)	6						
	H3PO4	Percent germination	6	84.34	85.63	84.9000	.22994	.56324	.317
		Arcsine transformed percent germination	6	2.33	2.36	2.3435	.00644	.01579	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00644	.01579	.000
		Valid N (listwise)	6						
	KH2PO3	Percent germination	6	45.00	46.02	45.5567	.16550	.40540	.164
		Arcsine transformed percent germination	6	1.47	1.49	1.4818	.00332	.00814	.000
		Residual for arcsine transformed percent	6	-.01	.01	.0000	.00332	.00814	.000
		Valid N (listwise)	6						
	KH2PO4	Percent germination	6	82.08	84.97	83.6850	.52671	1.29016	1.665
		Arcsine transformed percent germination	6	2.27	2.35	2.3105	.01421	.03480	.001
		Residual for arcsine transformed percent	6	-.04	.03	.0000	.01421	.03480	.001
		Valid N (listwise)	6						
	KOH	Percent germination	6	79.32	81.19	80.6217	.30085	.73692	.543
		Arcsine transformed percent germination	6	2.20	2.24	2.2300	.00756	.01853	.000
		Residual for arcsine transformed percent	6	-.03	.01	.0000	.00756	.01853	.000
		Valid N (listwise)	6						
250 µg	H3PO3	Percent germination	6	10.14	10.71	10.4483	.10410	.25498	.065
		Arcsine transformed percent germination	6	.65	.67	.6583	.00341	.00834	.000
		Residual for arcsine transformed percent	6	-.01	.01	.0000	.00341	.00834	.000
		Valid N (listwise)	6						
	H3PO4	Percent germination	6	73.26	74.74	73.9650	.23059	.56483	.319
		Arcsine transformed percent germination	6	2.05	2.09	2.0707	.00526	.01288	.000
		Residual for arcsine transformed percent	6	-.02	.02	.0000	.00526	.01288	.000
		Valid N (listwise)	6						
	KH2PO3	Percent germination	6	9.60	11.00	10.5717	.21960	.53790	.289
		Arcsine transformed percent germination	6	.63	.68	.6622	.00723	.01770	.000
		Residual for arcsine transformed percent	6	-.03	.01	.0000	.00723	.01770	.000
		Valid N (listwise)	6						
	KH2PO4	Percent germination	6	73.28	74.47	73.7500	.18173	.44515	.198
		Arcsine transformed percent germination	6	2.06	2.08	2.0658	.00414	.01013	.000
		Residual for arcsine transformed percent	6	-.01	.02	.0000	.00414	.01013	.000
		Valid N (listwise)	6						
	KOH	Percent germination	6	69.01	71.30	69.8650	.44932	1.10060	1.211
		Arcsine transformed percent germination	6	1.96	2.01	1.9795	.00983	.02407	.001
		Residual for arcsine transformed percent	6	-.02	.03	.0000	.00983	.02407	.001
		Valid N (listwise)	6						

Appendix 19: Figure 2-20 Percent germination of *M. nivale* conidia following immersion in solutions of 0 (control), 10, 50, 100 and 250 µg/ml^{-1} concentrations of H_3PO_3, H_3PO_4, KH_2PO_3, KH_2PO_4, and KOH and re-plating on PDA, Tukey pairwise comparisons.

Multiple Comparisons

Dependent Variable: Arcsine transformed percent germination

Tukey HSD

Concentration	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
0 µg	H3PO3	H3PO4	.01496	.01321	.788	-.0238	.0538
		KH2PO3	.02483	.01321	.354	-.0140	.0636
		KH2PO4	.01608	.01321	.742	-.0227	.0549
		KOH	.04865*	.01321	.009	.0098	.0875
	H3PO4	H3PO3	-.01496	.01321	.788	-.0538	.0238
		KH2PO3	.00987	.01321	.943	-.0289	.0487
		KH2PO4	.00112	.01321	1.000	-.0377	.0399
		KOH	.03369	.01321	.111	-.0051	.0725
	KH2PO3	H3PO3	-.02483	.01321	.354	-.0636	.0140
		H3PO4	-.00987	.01321	.943	-.0487	.0289
		KH2PO4	-.00875	.01321	.963	-.0476	.0301
		KOH	.02382	.01321	.394	-.0150	.0626
	KH2PO4	H3PO3	-.01608	.01321	.742	-.0549	.0227
		H3PO4	-.00112	.01321	1.000	-.0399	.0377
		KH2PO3	.00875	.01321	.963	-.0301	.0476
		KOH	.03257	.01321	.131	-.0062	.0714
	KOH	H3PO3	-.04865*	.01321	.009	-.0875	-.0098
		H3PO4	-.03369	.01321	.111	-.0725	.0051
		KH2PO3	-.02382	.01321	.394	-.0626	.0150
		KH2PO4	-.03257	.01321	.131	-.0714	.0062
10 µg	H3PO3	H3PO4	-.00701	.01105	.968	-.0395	.0254
		KH2PO3	-.03827*	.01105	.015	-.0707	-.0058
		KH2PO4	-.03242	.01105	.050	-.0649	.0000
		KOH	.02448	.01105	.207	-.0080	.0569
	H3PO4	H3PO3	.00701	.01105	.968	-.0254	.0395
		KH2PO3	-.03126	.01105	.063	-.0637	.0012
		KH2PO4	-.02541	.01105	.178	-.0579	.0070
		KOH	.03149	.01105	.060	-.0010	.0639
	KH2PO3	H3PO3	.03827*	.01105	.015	.0058	.0707
		H3PO4	.03126	.01105	.063	-.0012	.0637
		KH2PO4	.00585	.01105	.983	-.0266	.0383
		KOH	.06274*	.01105	.000	.0303	.0952
	KH2PO4	H3PO3	.03242	.01105	.050	.0000	.0649
		H3PO4	.02541	.01105	.178	-.0070	.0579
		KH2PO3	-.00585	.01105	.983	-.0383	.0266
		KOH	.05689*	.01105	.000	.0244	.0893
	KOH	H3PO3	-.02448	.01105	.207	-.0569	.0080
		H3PO4	-.03149	.01105	.060	-.0639	.0010
		KH2PO3	-.06274*	.01105	.000	-.0952	-.0303
		KH2PO4	-.05689*	.01105	.000	-.0893	-.0244

50 µg	H3PO3	H3PO4	-.23524*	.00963	.000	-.2635	-.2069
		KH2PO3	.01795	.00963	.362	-.0103	.0462
		KH2PO4	-.23171*	.00963	.000	-.2600	-.2034
		KOH	-.31471*	.00963	.000	-.3430	-.2864
	H3PO4	H3PO3	.23524*	.00963	.000	.2069	.2635
		KH2PO3	.25319*	.00963	.000	.2249	.2815
		KH2PO4	.00353	.00963	.996	-.0248	.0318
		KOH	-.07947*	.00963	.000	-.1078	-.0512
	KH2PO3	H3PO3	-.01795	.00963	.362	-.0462	.0103
		H3PO4	-.25319*	.00963	.000	-.2815	-.2249
		KH2PO4	-.24966*	.00963	.000	-.2780	-.2214
		KOH	-.33266*	.00963	.000	-.3610	-.3044
	KH2PO4	H3PO3	.23171*	.00963	.000	.2034	.2600
		H3PO4	-.00353	.00963	.996	-.0318	.0248
		KH2PO3	.24966*	.00963	.000	.2214	.2780
		KOH	-.08300*	.00963	.000	-.1113	-.0547
	KOH	H3PO3	.31471*	.00963	.000	.2864	.3430
		H3PO4	.07947*	.00963	.000	.0512	.1078
		KH2PO3	.33266*	.00963	.000	.3044	.3610
		KH2PO4	.08300*	.00963	.000	.0547	.1113
100 µg	H3PO3	H3PO4	-.86769*	.01180	.000	-.9023	-.8330
		KH2PO3	-.00600	.01180	.986	-.0407	.0287
		KH2PO4	-.83465*	.01180	.000	-.8693	-.8000
		KOH	-.75423*	.01180	.000	-.7889	-.7196
	H3PO4	H3PO3	.86769*	.01180	.000	.8330	.9023
		KH2PO3	.86169*	.01180	.000	.8270	.8963
		KH2PO4	.03304	.01180	.067	-.0016	.0677
		KOH	.11346*	.01180	.000	.0788	.1481
	KH2PO3	H3PO3	.00600	.01180	.986	-.0287	.0407
		H3PO4	-.86169*	.01180	.000	-.8963	-.8270
		KH2PO4	-.82865*	.01180	.000	-.8633	-.7940
		KOH	-.74823*	.01180	.000	-.7829	-.7136
	KH2PO4	H3PO3	.83465*	.01180	.000	.8000	.8693
		H3PO4	-.03304	.01180	.067	-.0677	.0016
		KH2PO3	.82865*	.01180	.000	.7940	.8633
		KOH	.08042*	.01180	.000	.0458	.1151
	KOH	H3PO3	.75423*	.01180	.000	.7196	.7889
		H3PO4	-.11346*	.01180	.000	-.1481	-.0788
		KH2PO3	.74823*	.01180	.000	.7136	.7829
		KH2PO4	-.08042*	.01180	.000	-.1151	-.0458
250 µg	H3PO3	H3PO4	-1.41243*	.00906	.000	-1.4390	-1.3858
		KH2PO3	-.00389	.00906	.992	-.0305	.0227
		KH2PO4	-1.40752*	.00906	.000	-1.4341	-1.3809
		KOH	-1.32121*	.00906	.000	-1.3478	-1.2946
	H3PO4	H3PO3	1.41243*	.00906	.000	1.3858	1.4390
		KH2PO3	1.40854*	.00906	.000	1.3819	1.4351
		KH2PO4	.00491	.00906	.982	-.0217	.0315
		KOH	.09122*	.00906	.000	.0646	.1178
	KH2PO3	H3PO3	.00389	.00906	.992	-.0227	.0305
		H3PO4	-1.40854*	.00906	.000	-1.4351	-1.3819
		KH2PO4	-1.40363*	.00906	.000	-1.4302	-1.3770
		KOH	-1.31732*	.00906	.000	-1.3439	-1.2907
	KH2PO4	H3PO3	1.40752*	.00906	.000	1.3809	1.4341
		H3PO4	-.00491	.00906	.982	-.0315	.0217
		KH2PO3	1.40363*	.00906	.000	1.3770	1.4302
		KOH	.08631*	.00906	.000	.0597	.1129
	KOH	H3PO3	1.32121*	.00906	.000	1.2946	1.3478
		H3PO4	-.09122*	.00906	.000	-.1178	-.0646
		KH2PO3	1.31732*	.00906	.000	1.2907	1.3439
		KH2PO4	-.08631*	.00906	.000	-.1129	-.0597

*. The mean difference is significant at the 0.05 level.

Chapter 3 Statistics

Appendix 20: 3.4.1 Disease incidence – year 1, descriptive statistics.

Descriptive Statistics

Turfgrass species	Compounds		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
P.annua	Phi	Percent disease year 1	5	11.25	15.95	13.9500	.78867	1.76352	3.110
		Percent disease arcsine transformed	5	.68	.82	.7644	.02316	.05178	.003
		Residual for arcsine transformed	5	-.08	.06	.0000	.02316	.05178	.003
		Valid N (listwise)	5						
	Phi/Biostimulant	Percent disease year 1	5	11.85	15.25	13.3500	.60663	1.35647	1.840
		Percent disease arcsine transformed	5	.70	.80	.7474	.01778	.03976	.002
		Residual for arcsine transformed	5	-.04	.05	.0000	.01778	.03976	.002
		Valid N (listwise)	5						
	Iprodione	Percent disease year 1	5	1.70	3.55	2.5100	.39224	.87707	.769
		Percent disease arcsine transformed	5	.26	.38	.3144	.02482	.05550	.003
		Residual for arcsine transformed	5	-.05	.06	.0000	.02482	.05550	.003
		Valid N (listwise)	5						
	Phi/Iprodione	Percent disease year 1	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Percent disease arcsine transformed	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for arcsine transformed	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	NPK control	Percent disease year 1	5	30.45	39.70	34.7700	1.80434	4.03463	16.278
		Percent disease arcsine transformed	5	1.17	1.36	1.2604	.03783	.08459	.007
		Residual for arcsine transformed	5	-.09	.10	.0000	.03783	.08459	.007
		Valid N (listwise)	5						
	Control	Percent disease year 1	5	33.45	39.65	35.8000	1.13952	2.54804	6.493
		Percent disease arcsine transformed	5	1.23	1.36	1.2825	.02370	.05299	.003
		Residual for arcsine transformed	5	-.05	.08	.0000	.02370	.05299	.003
		Valid N (listwise)	5						
A. canina	Phi	Percent disease year 1	5	15.95	20.20	17.6400	.79111	1.76897	3.129
		Percent disease arcsine transformed	5	.82	.93	.8662	.02060	.04605	.002
		Residual for arcsine transformed	5	-.04	.07	.0000	.02060	.04605	.002
		Valid N (listwise)	5						
	Phi/Biostimulant	Percent disease year 1	5	15.75	19.20	17.1500	.57380	1.28306	1.646
		Percent disease arcsine transformed	5	.82	.91	.8536	.01509	.03374	.001
		Residual for arcsine transformed	5	-.04	.05	.0000	.01509	.03374	.001
		Valid N (listwise)	5						
	Iprodione	Percent disease year 1	5	3.75	5.45	4.7400	.28827	.64459	.416
		Percent disease arcsine transformed	5	.39	.47	.4381	.01385	.03098	.001
		Residual for arcsine transformed	5	-.05	.03	.0000	.01385	.03098	.001
		Valid N (listwise)	5						
	Phi/Iprodione	Percent disease year 1	5	2.35	3.85	2.9800	.27459	.61400	.377
		Percent disease arcsine transformed	5	.31	.39	.3456	.01603	.03584	.001
		Residual for arcsine transformed	5	-.04	.05	.0000	.01603	.03584	.001
		Valid N (listwise)	5						
	NPK control	Percent disease year 1	5	44.15	49.15	45.6700	.96042	2.14756	4.612
		Percent disease arcsine transformed	5	1.45	1.55	1.4840	.01926	.04307	.002
		Residual for arcsine transformed	5	-.03	.07	.0000	.01926	.04307	.002
		Valid N (listwise)	5						
	Control	Percent disease year 1	5	42.75	50.85	46.6100	1.64061	3.66851	13.458
		Percent disease arcsine transformed	5	1.43	1.59	1.5028	.03291	.07358	.005
		Residual for arcsine transformed	5	-.08	.08	.0000	.03291	.07358	.005
		Valid N (listwise)	5						

Appendix 21: Figure 3-2 monthly disease incidence, *P. annua*, January 2011 (year 1), Tukey pairwise comparisons.

Tukey HSD Arcsine transformed yr 1 **Multiple Comparisons**

Turfgrass species	Treatments			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	Jan-11	Phi	Phi/Biostimulant	.0099	.01493	.986	-.0338	.0536
			Iprodione	.2927*	.01493	.000	.2490	.3364
			Phi/Iprodione	.4283*	.01493	.000	.3846	.4720
			NPK control	-.3713*	.01493	.000	-.4150	-.3276
			Control	-.3849*	.01493	.000	-.4286	-.3412
		Phi/Biostimulant	Phi	-.0099	.01493	.986	-.0536	.0338
			Iprodione	.2828*	.01493	.000	.2391	.3265
			Phi/Iprodione	.4185*	.01493	.000	.3748	.4622
			NPK control	-.3811*	.01493	.000	-.4249	-.3374
			Control	-.3948*	.01493	.000	-.4385	-.3511
		Iprodione	Phi	-.2927*	.01493	.000	-.3364	-.2490
			Phi/Biostimulant	-.2828*	.01493	.000	-.3265	-.2391
			Phi/Iprodione	.1357*	.01493	.000	.0920	.1794
			NPK control	-.6639*	.01493	.000	-.7077	-.6202
			Control	-.6776*	.01493	.000	-.7213	-.6339
		Phi/Iprodione	Phi	-.4283*	.01493	.000	-.4720	-.3846
			Phi/Biostimulant	-.4185*	.01493	.000	-.4622	-.3748
			Iprodione	-.1357*	.01493	.000	-.1794	-.0920
			NPK control	-.7996*	.01493	.000	-.8433	-.7559
			Control	-.8132*	.01493	.000	-.8570	-.7695
		NPK control	Phi	.3713*	.01493	.000	.3276	.4150
			Phi/Biostimulant	.3811*	.01493	.000	.3374	.4249
			Iprodione	.6639*	.01493	.000	.6202	.7077
			Phi/Iprodione	.7996*	.01493	.000	.7559	.8433
			Control	-.0136	.01493	.942	-.0573	.0301
		Control	Phi	.3849*	.01493	.000	.3412	.4286
			Phi/Biostimulant	.3948*	.01493	.000	.3511	.4385
			Iprodione	.6776*	.01493	.000	.6339	.7213
			Phi/Iprodione	.8132*	.01493	.000	.7695	.8570
			NPK control	.0136	.01493	.942	-.0301	.0573

Appendix 22: Figure 3-3 monthly disease incidence, *A. canina* December 2010 (year 1) Tukey pairwise comparisons.

Tukey HSD Arcsine transformed yr 1 **Multiple Comparisons**

Turfgrass species	Treatments			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
A. canina	Dec-10	Phi	Phi/Biostimulant	.01534	.02200	.980	-.0527	.0834
			Iprodione	.45106*	.02200	.000	.3830	.5191
			Phi/Iprodione	.86063*	.02200	.000	.7926	.9287
			NPK control	-.42119*	.02200	.000	-.4892	-.3532
			Control	-.43758*	.02200	.000	-.5056	-.3696
		Phi/Biostimulant	Phi	-.01534	.02200	.980	-.0834	.0527
			Iprodione	.43572*	.02200	.000	.3677	.5037
			Phi/Iprodione	.84529*	.02200	.000	.7773	.9133
			NPK control	-.43653*	.02200	.000	-.5046	-.3685
			Control	-.45292*	.02200	.000	-.5209	-.3849
		Iprodione	Phi	-.45106*	.02200	.000	-.5191	-.3830
			Phi/Biostimulant	-.43572*	.02200	.000	-.5037	-.3677
			Phi/Iprodione	.40958*	.02200	.000	.3416	.4776
			NPK control	-.87225*	.02200	.000	-.9403	-.8042
			Control	-.88864*	.02200	.000	-.9567	-.8206
		Phi/Iprodione	Phi	-.86063*	.02200	.000	-.9287	-.7926
			Phi/Biostimulant	-.84529*	.02200	.000	-.9133	-.7773
			Iprodione	-.40958*	.02200	.000	-.4776	-.3416
			NPK control	-1.28183*	.02200	.000	-1.3498	-1.2138
			Control	-1.29822*	.02200	.000	-1.3662	-1.2302
		NPK control	Phi	.42119*	.02200	.000	.3532	.4892
			Phi/Biostimulant	.43653*	.02200	.000	.3685	.5046
			Iprodione	.87225*	.02200	.000	.8042	.9403
			Phi/Iprodione	1.28183*	.02200	.000	1.2138	1.3498
			Control	-.01639	.02200	.974	-.0844	.0516
		Control	Phi	.43758*	.02200	.000	.3696	.5056
			Phi/Biostimulant	.45292*	.02200	.000	.3849	.5209
			Iprodione	.88864*	.02200	.000	.8206	.9567
			Phi/Iprodione	1.29822*	.02200	.000	1.2302	1.3662
			NPK control	.01639	.02200	.974	-.0516	.0844

*. The mean difference is significant at the 0.05 level.

Appendix 23: Figure 3-4 Mean disease incidence, *P. annua* and *A. canina*, from September 2010 to March 2011 (year 1), Tukey pairwise comparisons.

Multiple Comparisons

Tukey HSD Arcsine transformed yr 1

Turfgrass species	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P.annua	Phi	Phi/Biostimulant	.01703	.03397	.894	-.0880	.1220
		Iprodione	.44998*	.03397	.000	.3450	.5550
		Phi/Iprodione	.76442*	.03397	.000	.6594	.8694
		NPK control	-.49595*	.03397	.000	-.6010	-.3909
		Control	-.51809*	.03397	.000	-.6231	-.4131
	Phi/Biostimulant	Phi	-.01703	.03397	.894	-.1220	.0880
		Iprodione	.43295*	.03397	.000	.3279	.5380
		Phi/Iprodione	.74739*	.03397	.000	.6424	.8524
		NPK control	-.51298*	.03397	.000	-.6180	-.4080
		Control	-.53512*	.03397	.000	-.6401	-.4301
	Iprodione	Phi	-.44998*	.03397	.000	-.5550	-.3450
		Phi/Biostimulant	-.43295*	.03397	.000	-.5380	-.3279
		Phi/Iprodione	.31444*	.03397	.000	.2094	.4195
		NPK control	-.94594*	.03397	.000	-1.0510	-.8409
		Control	-.96807*	.03397	.000	-1.0731	-.8631
	Phi/Iprodione	Phi	-.76442*	.03397	.000	-.8694	-.6594
		Phi/Biostimulant	-.74739*	.03397	.000	-.8524	-.6424
		Iprodione	-.31444*	.03397	.000	-.4195	-.2094
		NPK control	-1.26038*	.03397	.000	-1.3654	-1.1554
		Control	-1.28251*	.03397	.000	-1.3875	-1.1775
	NPK control	Phi	.49595*	.03397	.000	.3909	.6010
		Phi/Biostimulant	.51298*	.03397	.000	.4080	.6180
		Iprodione	.94594*	.03397	.000	.8409	1.0510
		Phi/Iprodione	1.26038*	.03397	.000	1.1554	1.3654
		Control	-.02213	.03397	.986	-.1271	.0829
	Control	Phi	.51809*	.03397	.000	.4131	.6231
		Phi/Biostimulant	.53512*	.03397	.000	.4301	.6401
		Iprodione	.96807*	.03397	.000	.8631	1.0731
		Phi/Iprodione	1.28251*	.03397	.000	1.1775	1.3875
		NPK control	.02213	.03397	.986	-.0829	.1271
A. canina	Phi	Phi/Biostimulant	.01261	.02918	.998	-.0776	.1028
		Iprodione	.42805*	.02918	.000	.3378	.5183
		Phi/Iprodione	.52061*	.02918	.000	.4304	.6108
		NPK control	-.61786*	.02918	.000	-.7081	-.5276
		Control	-.63663*	.02918	.000	-.7269	-.5464
	Phi/Biostimulant	Phi	-.01261	.02918	.998	-.1028	.0776
		Iprodione	.41544*	.02918	.000	.3252	.5057
		Phi/Iprodione	.50800*	.02918	.000	.4178	.5982
		NPK control	-.63046*	.02918	.000	-.7207	-.5402
		Control	-.64923*	.02918	.000	-.7395	-.5590
	Iprodione	Phi	-.42805*	.02918	.000	-.5183	-.3378
		Phi/Biostimulant	-.41544*	.02918	.000	-.5057	-.3252
		Phi/Iprodione	.09256*	.02918	.042	.0023	.1828
		NPK control	-1.04591*	.02918	.000	-1.1361	-.9557
		Control	-1.06468*	.02918	.000	-1.1549	-.9745
	Phi/Iprodione	Phi	-.52061*	.02918	.000	-.6108	-.4304
		Phi/Biostimulant	-.50800*	.02918	.000	-.5982	-.4178
		Iprodione	-.09256*	.02918	.042	-.1828	-.0023
		NPK control	-1.13846*	.02918	.000	-1.2287	-1.0482
		Control	-1.15724*	.02918	.000	-1.2475	-1.0670
	NPK control	Phi	.61786*	.02918	.000	.5276	.7081
		Phi/Biostimulant	.63046*	.02918	.000	.5402	.7207
		Iprodione	1.04591*	.02918	.000	.9557	1.1361
		Phi/Iprodione	1.13846*	.02918	.000	1.0482	1.2287
		Control	-.01877	.02918	.835	-.1090	.0715
	Control	Phi	.63663*	.02918	.000	.5464	.7269
		Phi/Biostimulant	.64923*	.02918	.000	.5590	.7395
		Iprodione	1.06468*	.02918	.000	.9745	1.1549
		Phi/Iprodione	1.15724*	.02918	.000	1.0670	1.2475
		NPK control	.01877	.02918	.835	-.0715	.1090

Appendix 24: 3.4.1.3 Disease incidence – year 2, descriptive statistics.

Descriptive Statistics

Turfgrass species	Compounds		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
P.annua	Phi	Percent disease year 2	5	15.95	19.20	17.4200	.62861	1.40561	1.976
		Percent disease arcsine transformed yr 2	5	.82	.91	.8606	.01656	.03702	.001
		Residual for arcsine transformed percent disease yr 2	5	-.04	.05	.0000	.01656	.03702	.001
		Valid N (listwise)	5						
	Phi/Biostimulant	Percent disease year 2	5	14.95	18.25	16.8400	.56798	1.27004	1.613
		Percent disease arcsine transformed yr 2	5	.79	.88	.8453	.01529	.03419	.001
		Residual for arcsine transformed percent disease yr 2	5	-.05	.04	.0000	.01529	.03419	.001
		Valid N (listwise)	5						
	Iprodione	Percent disease year 2	5	3.15	5.10	4.1600	.32187	.71972	.518
		Percent disease arcsine transformed yr 2	5	.36	.46	.4096	.01633	.03653	.001
		Residual for arcsine transformed percent disease yr 2	5	-.05	.05	.0000	.01633	.03653	.001
		Valid N (listwise)	5						
	Phi/Iprodione	Percent disease year 2	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Percent disease arcsine transformed yr 2	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for arcsine transformed percent disease yr 2	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	NPK control	Percent disease year 2	5	33.45	38.15	35.7600	.81093	1.81328	3.288
		Percent disease arcsine transformed yr 2	5	1.23	1.33	1.2818	.01693	.03785	.001
		Residual for arcsine transformed percent disease yr 2	5	-.05	.05	.0000	.01693	.03785	.001
		Valid N (listwise)	5						
	Control	Percent disease year 2	5	33.25	39.15	36.5500	.94816	2.12014	4.495
		Percent disease arcsine transformed yr 2	5	1.23	1.35	1.2982	.01976	.04419	.002
		Residual for arcsine transformed percent disease yr 2	5	-.07	.05	.0000	.01976	.04419	.002
		Valid N (listwise)	5						
A. canina	Phi	Percent disease year 2	5	16.80	22.65	19.7600	.98899	2.21145	4.891
		Percent disease arcsine transformed yr 2	5	.84	.99	.9203	.02491	.05570	.003
		Residual for arcsine transformed percent disease yr 2	5	-.08	.07	.0000	.02491	.05570	.003
		Valid N (listwise)	5						
	Phi/Biostimulant	Percent disease year 2	5	15.85	18.65	17.1700	.44961	1.00536	1.011
		Percent disease arcsine transformed yr 2	5	.82	.89	.8542	.01190	.02661	.001
		Residual for arcsine transformed percent disease yr 2	5	-.04	.04	.0000	.01190	.02661	.001
		Valid N (listwise)	5						
	Iprodione	Percent disease year 2	5	4.15	6.40	5.1300	.38942	.87078	.758
		Percent disease arcsine transformed yr 2	5	.41	.51	.4557	.01747	.03906	.002
		Residual for arcsine transformed percent disease yr 2	5	-.05	.06	.0000	.01747	.03906	.002
		Valid N (listwise)	5						
	Phi/Iprodione	Percent disease year 2	5	2.45	4.15	3.1600	.27946	.62490	.391
		Percent disease arcsine transformed yr 2	5	.31	.41	.3561	.01566	.03502	.001
		Residual for arcsine transformed percent disease yr 2	5	-.04	.05	.0000	.01566	.03502	.001
		Valid N (listwise)	5						
	NPK control	Percent disease year 2	5	46.30	53.50	50.5700	1.32718	2.96766	8.807
		Percent disease arcsine transformed yr 2	5	1.50	1.64	1.5822	.02657	.05940	.004
		Residual for arcsine transformed percent disease yr 2	5	-.09	.06	.0000	.02657	.05940	.004
		Valid N (listwise)	5						
	Control	Percent disease year 2	5	46.85	54.20	50.3100	1.23869	2.76979	7.672
		Percent disease arcsine transformed yr 2	5	1.51	1.65	1.5770	.02480	.05544	.003
		Residual for arcsine transformed percent disease yr 2	5	-.07	.08	.0000	.02480	.05544	.003
		Valid N (listwise)	5						
A. stolonifera	Phi	Percent disease year 2	5	13.45	16.95	15.0900	.56798	1.27004	1.613
		Percent disease arcsine transformed yr 2	5	.75	.85	.7974	.01583	.03539	.001
		Residual for arcsine transformed percent disease yr 2	5	-.05	.05	.0000	.01583	.03539	.001
		Valid N (listwise)	5						
	Phi/Biostimulant	Percent disease year 2	5	13.30	17.45	15.6600	.83448	1.86594	3.482
		Percent disease arcsine transformed yr 2	5	.75	.86	.8127	.02316	.05179	.003
		Residual for arcsine transformed percent disease yr 2	5	-.07	.05	.0000	.02316	.05179	.003
		Valid N (listwise)	5						
	Iprodione	Percent disease year 2	5	3.65	5.45	4.3900	.34871	.77974	.608
		Percent disease arcsine transformed yr 2	5	.38	.47	.4209	.01693	.03785	.001
		Residual for arcsine transformed percent disease yr 2	5	-.04	.05	.0000	.01693	.03785	.001
		Valid N (listwise)	5						
	Phi/Iprodione	Percent disease year 2	5	0.00	1.00	.4700	.20833	.46583	.217
		Percent disease arcsine transformed yr 2	5	0.00	.20	.1053	.04406	.09851	.010
		Residual for arcsine transformed percent disease yr 2	5	-.11	.10	.0000	.04406	.09851	.010
		Valid N (listwise)	5						
	NPK control	Percent disease year 2	5	26.80	33.20	29.6300	1.36827	3.05953	9.361
		Percent disease arcsine transformed yr 2	5	1.09	1.23	1.1504	.02987	.06678	.004
		Residual for arcsine transformed percent disease yr 2	5	-.06	.08	.0000	.02987	.06678	.004
		Valid N (listwise)	5						
	Control	Percent disease year 2	5	25.80	34.95	29.9400	1.84020	4.11482	16.932
		Percent disease arcsine transformed yr 2	5	1.07	1.27	1.1566	.04011	.08968	.008
		Residual for arcsine transformed percent disease yr 2	5	-.09	.11	.0000	.04011	.08968	.008
		Valid N (listwise)	5						

Appendix 25: Figures 3-5, 3-6 and 3-7. Monthly disease incidence, *P. annua, A. canina* and *A. stolonifera,* November 2011 (year 2), Tukey pairwise comparisons.

Tukey HSD Acrsine transformed yr 2 **Multiple Comparisons**

Turfgrass species	Compounds			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	Nov-11	Phi	Phi/Biostimulant	.01534	.02200	.980	-.0527	.0834
			Iprodione	.45106*	.02200	.000	.3830	.5191
			Phi/Iprodione	.86063*	.02200	.000	.7926	.9287
			NPK control	-.42119*	.02200	.000	-.4892	-.3532
			Control	-.43758*	.02200	.000	-.5056	-.3696
		Phi/Biostimulant	Phi	-.01534	.02200	.980	-.0834	.0527
			Iprodione	.43572*	.02200	.000	.3677	.5037
			Phi/Iprodione	.84529*	.02200	.000	.7773	.9133
			NPK control	-.43653*	.02200	.000	-.5046	-.3685
			Control	-.45292*	.02200	.000	-.5209	-.3849
		Iprodione	Phi	-.45106*	.02200	.000	-.5191	-.3830
			Phi/Biostimulant	-.43572*	.02200	.000	-.5037	-.3677
			Phi/Iprodione	.40958*	.02200	.000	.3416	.4776
			NPK control	-.87225*	.02200	.000	-.9403	-.8042
			Control	-.88864*	.02200	.000	-.9567	-.8206
		Phi/Iprodione	Phi	-.86063*	.02200	.000	-.9287	-.7926
			Phi/Biostimulant	-.84529*	.02200	.000	-.9133	-.7773
			Iprodione	-.40958*	.02200	.000	-.4776	-.3416
			NPK control	-1.28183*	.02200	.000	-1.3498	-1.2138
			Control	-1.29822*	.02200	.000	-1.3662	-1.2302
		NPK control	Phi	.42119*	.02200	.000	.3532	.4892
			Phi/Biostimulant	.43653*	.02200	.000	.3685	.5046
			Iprodione	.87225*	.02200	.000	.8042	.9403
			Phi/Iprodione	1.28183*	.02200	.000	1.2138	1.3498
			Control	-.01639	.02200	.974	-.0844	.0516
		Control	Phi	.43758*	.02200	.000	.3696	.5056
			Phi/Biostimulant	.45292*	.02200	.000	.3849	.5209
			Iprodione	.88864*	.02200	.000	.8206	.9567
			Phi/Iprodione	1.29822*	.02200	.000	1.2302	1.3662
			NPK control	.01639	.02200	.974	-.0516	.0844
A. canina	Nov 2011	Phi	Phi/Biostimulant	.06609	.02963	.261	-.0255	.1577
			Iprodione	0.46462	.02963	.000	.3730	.5562
			Phi/Iprodione	0.56422	.02963	.000	.4726	.6558
			NPK control	-0.66187	.02963	.000	-.7535	-.5703
			Control	-0.65667	.02963	.000	-.7483	-.5651
		Phi/Biostimulant	Phi	-.06609	.02963	.261	-.1577	.0255
			Iprodione	0.39853	.02963	.000	.3069	.4901
			Phi/Iprodione	0.49813	.02963	.000	.4065	.5897
			NPK control	-0.72795	.02963	.000	-.8196	-.6364
			Control	-0.72276	.02963	.000	-.8144	-.6312
		Iprodione	Phi	-0.46462	.02963	.000	-.5562	-.3730
			Phi/Biostimulant	-0.39853	.02963	.000	-.4901	-.3069
			Phi/Iprodione	0.0996	.02963	.028	.0080	.1912
			NPK control	-1.12649	.02963	.000	-1.2181	-1.0349
			Control	-1.12129	.02963	.000	-1.2129	-1.0297
		Phi/Iprodione	Phi	-0.56422	.02963	.000	-.6558	-.4726
			Phi/Biostimulant	-0.49813	.02963	.000	-.5897	-.4065
			Iprodione	-0.0996	.02963	.028	-.1912	-.0080
			NPK control	-1.22609	.02963	.000	-1.3177	-1.1345
			Control	-1.22089	.02963	.000	-1.3125	-1.1293
		NPK control	Phi	0.66187	.02963	.000	.5703	.7535
			Phi/Biostimulant	0.72795	.02963	.000	.6364	.8196
			Iprodione	1.12649	.02963	.000	1.0349	1.2181
			Phi/Iprodione	1.22609	.02963	.000	1.1345	1.3177
			Control	.00519	.02963	1.000	-.0864	.0968
		Control	Phi	0.65667	.02963	.000	.5651	.7483
			Phi/Biostimulant	0.72276	.02963	.000	.6312	.8144
			Iprodione	1.12129	.02963	.000	1.0297	1.2129
			Phi/Iprodione	1.22089	.02963	.000	1.1293	1.3125
			NPK control	-.00519	.02963	1.000	-.0968	.0864

A. stolonifera	Nov-11	Phi	Phi/Biostimulant	-.01527	.04287	.999	-0.1478	0.1173
			Iprodione	0.37653	.04287	.000	0.2440	0.5091
			Phi/Iprodione	0.69212	.04287	.000	0.5596	0.8247
			NPK control	-0.35298	.04287	.000	-0.4855	-0.2204
			Control	-0.35915	.04287	.000	-0.4917	-0.2266
		Phi/Biostimulant	Phi	.01527	.04287	.999	-0.1173	0.1478
			Iprodione	0.3918	.04287	.000	0.2592	0.5244
			Phi/Iprodione	0.70739	.04287	.000	0.5748	0.8400
			NPK control	-0.33771	.04287	.000	-0.4703	-0.2051
			Control	-0.34388	.04287	.000	-0.4764	-0.2113
		Iprodione	Phi	-0.37653	.04287	.000	-0.5091	-0.2440
			Phi/Biostimulant	-0.3918	.04287	.000	-0.5244	-0.2592
			Phi/Iprodione	0.31559	.04287	.000	0.1830	0.4482
			NPK control	-0.72951	.04287	.000	-0.8621	-0.5969
			Control	-0.73568	.04287	.000	-0.8682	-0.6031
		Phi/Iprodione	Phi	-0.69212	.04287	.000	-0.8247	-0.5596
			Phi/Biostimulant	-0.70739	.04287	.000	-0.8400	-0.5748
			Iprodione	-0.31559	.04287	.000	-0.4482	-0.1830
			NPK control	-1.0451	.04287	.000	-1.1777	-0.9125
			Control	-1.05127	.04287	.000	-1.1838	-0.9187
		NPK control	Phi	0.35298	.04287	.000	0.2204	0.4855
			Phi/Biostimulant	0.33771	.04287	.000	0.2051	0.4703
			Iprodione	0.72951	.04287	.000	0.5969	0.8621
			Phi/Iprodione	1.0451	.04287	.000	0.9125	1.1777
			Control	-.00617	.04287	1.000	-.1387	0.1264
		Control	Phi	0.35915	.04287	.000	0.2266	0.4917
			Phi/Biostimulant	0.34388	.04287	.000	0.2113	0.4764
			Iprodione	0.73568	.04287	.000	0.6031	0.8682
			Phi/Iprodione	1.05127	.04287	.000	0.9187	1.1838
			NPK control	.00617	.04287	1.000	-0.1264	.1387

Appendix 26: Figure 3-8 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera,* from September 2011 to March 2012, Tukey pairwise comparisons.

Tukey HSD Acrsine transformed yr 2 **Multiple Comparisons**

Turfgrass species	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P.annua	Phi	Phi/Biostimulant	.01362	.02700	.785	-.0527	.0834
		Iprodione	.40036	.02700	.000	.3830	.5191
		Phi/Iprodione	.76390	.02700	.000	.7926	.9287
		NPK control	-.37385	.02700	.000	-.4892	-.3532
		Control	-.38840	.02700	.000	-.5056	-.3696
	Phi/Biostimulant	Phi	-.01362	.02700	.785	-.0834	.0527
		Iprodione	.38674	.02700	.000	.3677	.5037
		Phi/Iprodione	.75028	.02700	.000	.7773	.9133
		NPK control	-.38747	.02700	.000	-.5046	-.3685
		Control	-.40202	.02700	.000	-.5209	-.3849
	Iprodione	Phi	-.40036	.02700	.000	-.5191	-.3830
		Phi/Biostimulant	-.38674	.02700	.000	-.5037	-.3677
		Phi/Iprodione	.36354	.02700	.000	.3416	.4776
		NPK control	-.77421	.02700	.000	-.9403	-.8042
		Control	-.78876	.02700	.000	-.9567	-.8206
	Phi/Iprodione	Phi	-.76390	.02700	.000	-.9287	-.7926
		Phi/Biostimulant	-.75028	.02700	.000	-.9133	-.7773
		Iprodione	-.36354	.02700	.000	-.4776	-.3416
		NPK control	-1.13775	.02700	.000	-1.3498	-1.2138
		Control	-1.15230	.02700	.000	-1.3662	-1.2302
	NPK control	Phi	.37385	.02700	.000	.3532	.4892
		Phi/Biostimulant	.38747	.02700	.000	.3685	.5046
		Iprodione	.77421	.02700	.000	.8042	.9403
		Phi/Iprodione	1.13775	.02700	.000	1.2138	1.3498
		Control	-.01455	.02700	.924	-.0844	.0516
	Control	Phi	.38840	.02700	.000	.3696	.5056
		Phi/Biostimulant	.40202	.02700	.000	.3849	.5209
		Iprodione	.78876	.02700	.000	.8206	.9567
		Phi/Iprodione	1.15230	.02700	.000	1.2302	1.3662
		NPK control	.01455	.02700	.924	-.0516	.0844
A. canina	Phi	Phi/Biostimulant	.05867	.02263	.367	-.0255	.1577
		Iprodione	.41238	.02263	.000	.3730	.5562
		Phi/Iprodione	.50078	.02263	.000	.4726	.6558
		NPK control	-.58750	.02263	.000	-.7535	-.5703
		Control	-.58289	.02263	.000	-.7483	-.5651
	Phi/Biostimulant	Phi	-.05867	.02263	.367	-.1577	.0255
		Iprodione	.35371	.02263	.000	.3069	.4901
		Phi/Iprodione	.44211	.02263	.000	.4065	.5897
		NPK control	-.64617	.02263	.000	-.8196	-.6364
		Control	-.64156	.02263	.000	-.8144	-.6312
	Iprodione	Phi	-.41238	.02263	.000	-.5562	-.3730
		Phi/Biostimulant	-.35371	.02263	.000	-.4901	-.3069
		Phi/Iprodione	.08840	.02263	.037	.0080	.1912
		NPK control	-.99988	.02263	.000	-1.2181	-1.0349
		Control	-.99527	.02263	.000	-1.2129	-1.0297
	Phi/Iprodione	Phi	-.50078	.02263	.000	-.6558	-.4726
		Phi/Biostimulant	-.44211	.02263	.000	-.5897	-.4065
		Iprodione	-.08840	.02263	.037	-.1912	-.0080
		NPK control	-1.08829	.02263	.000	-1.3177	-1.1345
		Control	-1.08367	.02263	.000	-1.3125	-1.1293
	NPK control	Phi	.58750	.02263	.000	.5703	.7535
		Phi/Biostimulant	.64617	.02263	.000	.6364	.8196
		Iprodione	.99988	.02263	.000	1.0349	1.2181
		Phi/Iprodione	1.08829	.02263	.000	1.1345	1.3177
		Control	.00462	.02263	1.000	-.0864	.0968
	Control	Phi	.58289	.02263	.000	.5651	.7483
		Phi/Biostimulant	.64156	.02263	.000	.6312	.8144
		Iprodione	.99527	.02263	.000	1.0297	1.2129
		Phi/Iprodione	1.08367	.02263	.000	1.1293	1.3125
		NPK control	-.00462	.02263	1.000	-.0968	.0864

A. stolonifera	Phi	Phi/Biostimulant	-.01354	.04487	.878	-0.1478	0.1173
		Iprodione	.33423	.04487	.000	0.2440	0.5091
		Phi/Iprodione	.61435	.04487	.000	0.5596	0.8247
		NPK control	-.31328	.04487	.000	-0.4855	-0.2204
		Control	-.31878	.04487	.000	-0.4917	-0.2266
	Phi/Biostimulant	Phi	.01354	.04487	.878	-0.1173	0.1478
		Iprodione	.34776	.04487	.000	0.2592	0.5244
		Phi/Iprodione	.62789	.04487	.000	0.5748	0.8400
		NPK control	-.29974	.04487	.000	-0.4703	-0.2051
		Control	-.30520	.04487	.000	-0.4764	-0.2113
	Iprodione	Phi	-.33423	.04487	.000	-0.5091	-0.2440
		Phi/Biostimulant	-.34776	.04487	.000	-0.5244	-0.2592
		Phi/Iprodione	.28013	.04487	.000	0.1830	0.4482
		NPK control	-.64750	.04487	.000	-0.8621	-0.5969
		Control	-.65296	.04487	.000	-0.8682	-0.6031
	Phi/Iprodione	Phi	-.61435	.04487	.000	-0.8247	-0.5596
		Phi/Biostimulant	-.62789	.04487	.000	-0.8400	-0.5748
		Iprodione	-.28013	.04487	.000	-0.4482	-0.1830
		NPK control	-.92763	.04487	.000	-1.1777	-0.9125
		Control	-.93309	.04487	.000	-1.1838	-0.9187
	NPK control	Phi	.31328	.04487	.000	0.2204	0.4855
		Phi/Biostimulant	.29974	.04487	.000	0.2051	0.4703
		Iprodione	.64750	.04487	.000	0.5969	0.8621
		Phi/Iprodione	.92763	.04487	.000	0.9125	1.1777
		Control	-.00546	.04487	1.000	-.1387	0.1264
	Control	Phi	.31878	.04487	.000	0.2266	0.4917
		Phi/Biostimulant	.30520	.04487	.000	0.2113	0.4764
		Iprodione	.65296	.04487	.000	0.6031	0.8682
		Phi/Iprodione	.93309	.04487	.000	0.9187	1.1838
		NPK control	.00546	.04487	1.000	-0.1264	.1387

Appendix 27: 3.4.2 Disease incidence – year 3, descriptive statistics.

Descriptive Statistics

Turfgrass species	Treatments		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
P.annua	Phi bi weekly	Percent disease year 3	5	14.85	17.90	16.3800	.56824	1.27063	1.615
		Arcsine transformed yr 3	5	.79	.87	.8329	.01537	.03436	.001
		Residual for Arcsine_yr3	5	-.04	.04	.0000	.01537	.03436	.001
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 3	5	17.10	22.30	19.2000	.86299	1.92970	3.724
		Arcsine transformed yr 3	5	.85	.98	.9064	.02167	.04845	.002
		Residual for Arcsine_yr3	5	-.05	.08	.0000	.02167	.04845	.002
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 3	5	16.85	22.10	19.4500	.88077	1.96945	3.879
		Arcsine transformed yr 3	5	.85	.98	.9127	.02229	.04984	.002
		Residual for Arcsine_yr3	5	-.07	.07	.0000	.02229	.04984	.002
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 3	5	.90	2.10	1.4700	.23431	.52393	.275
		Arcsine transformed yr 3	5	.19	.29	.2400	.01941	.04341	.002
		Residual for Arcsine_yr3	5	-.05	.05	.0000	.01941	.04341	.002
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine_yr3	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 3	5	25.75	33.50	29.9800	1.30063	2.90831	8.458
		Arcsine transformed yr 3	5	1.06	1.23	1.1581	.02856	.06387	.004
		Residual for Arcsine_yr3	5	-.09	.08	.0000	.02856	.06387	.004
		Valid N (listwise)	5						
A. canina	Phi bi weekly	Percent disease year 3	5	19.75	25.00	22.5200	1.05221	2.35282	5.536
		Arcsine transformed yr 3	5	.92	1.05	.9881	.02518	.05629	.003
		Residual for Arcsine_yr3	5	-.07	.06	.0000	.02518	.05629	.003
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 3	5	22.60	30.20	26.4900	1.25722	2.81123	7.903
		Arcsine transformed yr 3	5	.99	1.16	1.0804	.02859	.06393	.004
		Residual for Arcsine_yr3	5	-.09	.08	.0000	.02859	.06393	.004
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 3	5	22.35	30.30	26.7500	1.27681	2.85504	8.151
		Arcsine transformed yr 3	5	.98	1.17	1.0863	.02914	.06515	.004
		Residual for Arcsine_yr3	5	-.10	.08	.0000	.02914	.06515	.004
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 3	5	1.55	3.25	2.0300	.31409	.70232	.493
		Arcsine transformed yr 3	5	.25	.36	.2830	.02067	.04622	.002
		Residual for Arcsine_yr3	5	-.03	.08	.0000	.02067	.04622	.002
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine_yr3	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 3	5	38.55	45.30	41.2700	1.10664	2.47452	6.123
		Arcsine transformed yr 3	5	1.34	1.48	1.3951	.02243	.05015	.003
		Residual for Arcsine_yr3	5	-.06	.08	.0000	.02243	.05015	.003
		Valid N (listwise)	5						
A. stolonifera	Phi bi weekly	Percent disease year 3	5	8.65	15.20	12.1300	1.33983	2.99596	8.976
		Arcsine transformed yr 3	5	.60	.80	.7074	.04188	.09365	.009
		Residual for Arcsine_yr3	5	-.11	.09	.0000	.04188	.09365	.009
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 3	5	11.45	17.10	14.5400	1.00541	2.24817	5.054
		Arcsine transformed yr 3	5	.69	.85	.7807	.02892	.06466	.004
		Residual for Arcsine_yr3	5	-.09	.07	.0000	.02892	.06466	.004
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 3	5	11.45	17.35	14.4900	1.08136	2.41801	5.847
		Arcsine transformed yr 3	5	.69	.86	.7791	.03092	.06913	.005
		Residual for Arcsine_yr3	5	-.09	.08	.0000	.03092	.06913	.005
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 3	5	.35	1.00	.7400	.12490	.27928	.078
		Arcsine transformed yr 3	5	.12	.20	.1694	.01565	.03499	.001
		Residual for Arcsine_yr3	5	-.05	.03	.0000	.01565	.03499	.001
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 3	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine_yr3	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 3	5	19.25	23.65	22.0400	.78492	1.75514	3.081
		Arcsine transformed yr 3	5	.91	1.02	.9769	.01915	.04282	.002
		Residual for Arcsine_yr3	5	-.07	.04	.0000	.01915	.04282	.002
		Valid N (listwise)	5						

Appendix 28: Figs 3-9, 3-10 and 3-11. Monthly disease incidence, *P. annua, A. canina* and *A. stolonifera,* November 2012 (year 3), Tukey pairwise comparisons.

Tukey HSD Acrsine transformed yr 3 **Multiple Comparisons**

Turfgrass species	Compounds		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P.annua	Nov-12	Phi bi weekly	Phi monthly	-0.05119	.01053	.084	-.0837	-.0186
			Phi bi weekly 6 apps	-0.05076	.01053	.061	-.0833	-.0182
			Chlorothalonil	.67998*	.01053	.000	.6474	.7125
			Chlorothalonil + Phi	.99134*	.01053	.000	.9588	1.0239
			Control	-.48220*	.01053	.000	-.5148	-.4497
		Phi monthly	Phi bi weekly	0.05119	.01053	.084	.0186	.0837
			Phi bi weekly 6 apps	.00043	.01053	1.000	-.0321	.0330
			Chlorothalonil	.73117*	.01053	.000	.6986	.7637
			Chlorothalonil + Phi	1.04253*	.01053	.000	1.0100	1.0751
			Control	-.43101*	.01053	.000	-.4636	-.3985
		Phi bi weekly 6 apps	Phi bi weekly	0.05076	.01053	.061	.0182	.0833
			Phi monthly	-.00043	.01053	1.000	-.0330	.0321
			Chlorothalonil	.73074*	.01053	.000	.6982	.7633
			Chlorothalonil + Phi	1.04210*	.01053	.000	1.0096	1.0747
			Control	-.43144*	.01053	.000	-.4640	-.3989
		Chlorothalonil	Phi bi weekly	-.67998*	.01053	.000	-.7125	-.6474
			Phi monthly	-.73117*	.01053	.000	-.7637	-.6986
			Phi bi weekly 6 apps	-.73074*	.01053	.000	-.7633	-.6982
			Chlorothalonil + Phi	.31136*	.01053	.000	.2788	.3439
			Control	-1.16218*	.01053	.000	-1.1947	-1.1296
		Chlorothalonil + Phi	Phi bi weekly	-.99134*	.01053	.000	-1.0239	-.9588
			Phi monthly	-1.04253*	.01053	.000	-1.0751	-1.0100
			Phi bi weekly 6 apps	-1.04210*	.01053	.000	-1.0747	-1.0096
			Chlorothalonil	-.31136*	.01053	.000	-.3439	-.2788
			Control	-1.47354*	.01053	.000	-1.5061	-1.4410
		Control	Phi bi weekly	.48220*	.01053	.000	.4497	.5148
			Phi monthly	.43101*	.01053	.000	.3985	.4636
			Phi bi weekly 6 apps	.43144*	.01053	.000	.3989	.4640
			Chlorothalonil	1.16218*	.01053	.000	1.1296	1.1947
			Chlorothalonil + Phi	1.47354*	.01053	.000	1.4410	1.5061
A. canina	Nov-12	Phi bi weekly	Phi monthly	-0.07986	.01280	.063	-.1194	-.0403
			Phi bi weekly 6 apps	-0.05599	.01280	.052	-.0956	-.0164
			Chlorothalonil	.82548*	.01280	.000	.7859	.8650
			Chlorothalonil + Phi	1.19886*	.01280	.000	1.1593	1.2384
			Control	-.64624*	.01280	.000	-.6858	-.6067
		Phi monthly	Phi bi weekly	0.07986	.01280	.053	.0403	.1194
			Phi bi weekly 6 apps	.02387	.01280	.446	-.0157	.0634
			Chlorothalonil	.90534*	.01280	.000	.8658	.9449
			Chlorothalonil + Phi	1.27872*	.01280	.000	1.2392	1.3183
			Control	-.56638*	.01280	.000	-.6059	-.5268
		Phi bi weekly 6 apps	Phi bi weekly	0.05599	.01280	.052	.0164	.0956
			Phi monthly	-.02387	.01280	.446	-.0634	.0157
			Chlorothalonil	.88147*	.01280	.000	.8419	.9210
			Chlorothalonil + Phi	1.25485*	.01280	.000	1.2153	1.2944
			Control	-.59025*	.01280	.000	-0.6298	-.5507
		Chlorothalonil	Phi bi weekly	-.82548*	.01280	.000	-.8650	-.7859
			Phi monthly	-.90534*	.01280	.000	-.9449	-.8658
			Phi bi weekly 6 apps	-.88147*	.01280	.000	-.9210	-.8419
			Chlorothalonil + Phi	0.37338	.01280	.069	0.3338	0.4129
			Control	-1.47172*	.01280	.000	-1.5113	-1.4322
		Chlorothalonil + Phi	Phi bi weekly	-1.19886*	.01280	.000	-1.2384	-1.1593
			Phi monthly	-1.27872*	.01280	.000	-1.3183	-1.2392
			Phi bi weekly 6 apps	-1.25485*	.01280	.000	-1.2944	-1.2153
			Chlorothalonil	-0.37338	.01280	.069	-0.4129	-0.3338
			Control	-1.84510*	.01280	.000	-1.8847	-1.8055
		Control	Phi bi weekly	.64624*	.01280	.000	.6067	.6858
			Phi monthly	.56638*	.01280	.000	.5268	.6059
			Phi bi weekly 6 apps	.59025*	.01280	.000	.5507	0.6298
			Chlorothalonil	1.47172*	.01280	.000	1.4322	1.5113
			Chlorothalonil + Phi	1.84510*	.01280	.000	1.8055	1.8847

A. stolonifera	Nov-12	Phi bi weekly	Phi monthly	-0.03677	.00959	.079	-0.0664	-0.0071
			Phi bi weekly 6 apps	-.02034	.00959	.310	-0.0500	0.0093
			Chlorothalonil	.59647*	.00959	.000	0.5668	0.6261
			Chlorothalonil + Phi	.86520*	.00959	.000	0.8356	0.8948
			Control	-.36927*	.00959	.000	-0.3989	-0.3396
		Phi monthly	Phi bi weekly	0.03677	.00959	.079	0.0071	0.0664
			Phi bi weekly 6 apps	.01643	.00959	.536	-0.0132	0.0461
			Chlorothalonil	.63324*	.00959	.000	0.6036	0.6629
			Chlorothalonil + Phi	.90197*	.00959	.000	0.8723	0.9316
			Control	-.33250*	.00959	.000	-0.3621	-0.3029
		Phi bi weekly 6 apps	Phi bi weekly	.02034	.00959	.310	-0.0093	0.0500
			Phi monthly	-.01643	.00959	.536	-0.0461	0.0132
			Chlorothalonil	.61682*	.00959	.000	0.5872	0.6465
			Chlorothalonil + Phi	.88554*	.00959	.000	0.8559	0.9152
			Control	-.34893*	.00959	.000	-0.3786	-0.3193
		Chlorothalonil	Phi bi weekly	-.59647*	.00959	.000	-0.6261	-0.5668
			Phi monthly	-.63324*	.00959	.000	-0.6629	-0.6036
			Phi bi weekly 6 apps	-.61682*	.00959	.000	-0.6465	-0.5872
			Chlorothalonil + Phi	0.26872	.00959	.086	0.2391	0.2984
			Control	-.96575*	.00959	.000	-0.9954	-0.9361
		Chlorothalonil + Phi	Phi bi weekly	-.86520*	.00959	.000	-0.8948	-0.8356
			Phi monthly	-.90197*	.00959	.000	-0.9316	-0.8723
			Phi bi weekly 6 apps	-.88554*	.00959	.000	-0.9152	-0.8559
			Chlorothalonil	-0.26872	.00959	.086	-0.2984	-0.2391
			Control	-1.23447*	.00959	.000	-1.2641	-1.2048
		Control	Phi bi weekly	.36927*	.00959	.000	0.3396	0.3989
			Phi monthly	.33250*	.00959	.000	0.3029	0.3621
			Phi bi weekly 6 apps	.34893*	.00959	.000	0.3193	0.3786
			Chlorothalonil	.96575*	.00959	.000	0.9361	0.9954
			Chlorothalonil + Phi	1.23447*	.00959	.000	1.2048	1.2641

Appendix 29: Figure 3-12 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera*, from September 2012 to March 2013, Tukey pairwise comparisons.

Tukey HSD Arcsine transformed yr 3 **Multiple Comparisons**

Turfgrass species	Treatments		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P.annua	Phi bi weekly	Phi monthly	-.07350	.02825	.136	-.1609	.0139
		Phi bi weekly 6 apps	-.07978	.02825	.088	-.1671	.0076
		Chlorothalonil	.59289*	.02825	.000	.5055	.6803
		Chlorothalonil + Phi	.83292*	.02825	.000	.7456	.9203
		Control	-.32520*	.02825	.000	-.4126	-.2378
	Phi monthly	Phi bi weekly	.07350	.02825	.136	-.0139	.1609
		Phi bi weekly 6 apps	-.00629	.02825	1.000	-.0937	.0811
		Chlorothalonil	.66639*	.02825	.000	.5790	.7538
		Chlorothalonil + Phi	.90642*	.02825	.000	.8191	.9938
		Control	-.25170*	.02825	.000	-.3391	-.1643
	Phi bi weekly 6 apps	Phi bi weekly	.07978	.02825	.088	-.0076	.1671
		Phi monthly	.00629	.02825	1.000	-.0811	.0937
		Chlorothalonil	.67268*	.02825	.000	.5853	.7600
		Chlorothalonil + Phi	.91271*	.02825	.000	.8253	1.0001
		Control	-.24541*	.02825	.000	-.3328	-.1581
	Chlorothalonil	Phi bi weekly	-.59289*	.02825	.000	-.6803	-.5055
		Phi monthly	-.66639*	.02825	.000	-.7538	-.5790
		Phi bi weekly 6 apps	-.67268*	.02825	.000	-.7600	-.5853
		Chlorothalonil + Phi	0.24003	.02825	.068	.1527	.3274
		Control	-.91809*	.02825	.000	-1.0055	-.8307
	Chlorothalonil + Phi	Phi bi weekly	-.83292*	.02825	.000	-.9203	-.7456
		Phi monthly	-.90642*	.02825	.000	-.9938	-.8191
		Phi bi weekly 6 apps	-.91271*	.02825	.000	-1.0001	-.8253
		Chlorothalonil	-0.24003	.02825	.068	-.3274	-.1527
		Control	-1.15812*	.02825	.000	-1.2455	-1.0708
	Control	Phi bi weekly	.32520*	.02825	.000	.2378	.4126
		Phi monthly	.25170*	.02825	.000	.1643	.3391
		Phi bi weekly 6 apps	.24541*	.02825	.000	.1581	.3328
		Chlorothalonil	.91809*	.02825	.000	.8307	1.0055
		Chlorothalonil + Phi	1.15812*	.02825	.000	1.0708	1.2455
A. canina	Phi bi weekly	Phi monthly	-.09232	.03281	.089	-.1938	.0091
		Phi bi weekly 6 apps	-.09817	.03281	.062	-.1996	.0033
		Chlorothalonil	.70506*	.03281	.000	.6036	.8065
		Chlorothalonil + Phi	.98808*	.03281	.000	.8866	1.0895
		Control	-.40705*	.03281	.000	-.5085	-.3056
	Phi monthly	Phi bi weekly	.09232	.03281	.089	-.0091	.1938
		Phi bi weekly 6 apps	-.00585	.03281	1.000	-.1073	.0956
		Chlorothalonil	.79738*	.03281	.000	.6959	.8988
		Chlorothalonil + Phi	1.08040*	.03281	.000	.9789	1.1819
		Control	-.31473*	.03281	.000	-.4162	-.2133
	Phi bi weekly 6 apps	Phi bi weekly	.09817	.03281	.062	-.0033	.1996
		Phi monthly	.00585	.03281	1.000	-.0956	.1073
		Chlorothalonil	.80323*	.03281	.000	.7018	.9047
		Chlorothalonil + Phi	1.08625*	.03281	.000	.9848	1.1877
		Control	-.30888*	.03281	.000	-.4103	-.2074
	Chlorothalonil	Phi bi weekly	-.70506*	.03281	.000	-.8065	-.6036
		Phi monthly	-.79738*	.03281	.000	-.8988	-.6959
		Phi bi weekly 6 apps	-.80323*	.03281	.000	-.9047	-.7018
		Chlorothalonil + Phi	0.28302	.03281	.096	.1816	.3845
		Control	-1.11211*	.03281	.000	-1.2136	-1.0106
	Chlorothalonil + Phi	Phi bi weekly	-.98808*	.03281	.000	-1.0895	-.8866
		Phi monthly	-1.08040*	.03281	.000	-1.1819	-.9789
		Phi bi weekly 6 apps	-1.08625*	.03281	.000	-1.1877	-.9848
		Chlorothalonil	-0.28302	.03281	.096	-.3845	-.1816
		Control	-1.39513*	.03281	.000	-1.4966	-1.2937
	Control	Phi bi weekly	.40705*	.03281	.000	.3056	.5085
		Phi monthly	.31473*	.03281	.000	.2133	.4162
		Phi bi weekly 6 apps	.30888*	.03281	.000	.2074	.4103
		Chlorothalonil	1.11211*	.03281	.000	1.0106	1.2136
		Chlorothalonil + Phi	1.39513*	.03281	.000	1.2937	1.4966

A. stolonifera	Phi bi weekly	Phi monthly	-.07338	.03723	.387	-.1885	.0417
		Phi bi weekly 6 apps	-.07172	.03723	.411	-.1868	.0434
		Chlorothalonil	.53794*	.03723	.000	.4228	.6530
		Chlorothalonil + Phi	.70736*	.03723	.000	.5923	.8225
		Control	-.26952*	.03723	.000	-.3846	-.1544
	Phi monthly	Phi bi weekly	.07338	.03723	.387	-.0417	.1885
		Phi bi weekly 6 apps	.00166	.03723	1.000	-.1134	.1168
		Chlorothalonil	.61132*	.03723	.000	.4962	.7264
		Chlorothalonil + Phi	.78074*	.03723	.000	.6656	.8958
		Control	-.19614*	.03723	.000	-.3112	-.0810
	Phi bi weekly 6 apps	Phi bi weekly	.07172	.03723	.411	-.0434	.1868
		Phi monthly	-.00166	.03723	1.000	-.1168	.1134
		Chlorothalonil	.60966*	.03723	.000	.4946	.7248
		Chlorothalonil + Phi	.77908*	.03723	.000	.6640	.8942
		Control	-.19780*	.03723	.000	-.3129	-.0827
	Chlorothalonil	Phi bi weekly	-.53794*	.03723	.000	-.6530	-.4228
		Phi monthly	-.61132*	.03723	.000	-.7264	-.4962
		Phi bi weekly 6 apps	-.60966*	.03723	.000	-.7248	-.4946
		Chlorothalonil + Phi	0.16942	.03723	.063	.0543	.2845
		Control	-.80746*	.03723	.000	-.9226	-.6924
	Chlorothalonil + Phi	Phi bi weekly	-.70736*	.03723	.000	-.8225	-.5923
		Phi monthly	-.78074*	.03723	.000	-.8958	-.6656
		Phi bi weekly 6 apps	-.77908*	.03723	.000	-.8942	-.6640
		Chlorothalonil	-0.16942	.03723	.063	-.2845	-.0543
		Control	-.97687*	.03723	.000	-1.0920	-.8618
	Control	Phi bi weekly	.26952*	.03723	.000	.1544	.3846
		Phi monthly	.19614*	.03723	.000	.0810	.3112
		Phi bi weekly 6 apps	.19780*	.03723	.000	.0827	.3129
		Chlorothalonil	.80746*	.03723	.000	.6924	.9226
		Chlorothalonil + Phi	.97687*	.03723	.000	.8618	1.0920

Appendix 30: 3.4.2.3 Disease incidence – year 4, descriptive statistics.

Descriptive Statistics

Turfgrass species	Treatments		N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
P.annua	Phi bi weekly	Percent disease year 4	5	7.40	10.40	8.7000	.63384	1.41730	2.009
		Arcsine transformed yr 4	5	.55	.66	.5974	.02235	.04998	.002
		Residual for Arcsine yr4	5	-.05	.06	.0000	.02235	.04998	.002
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 4	5	7.90	12.15	10.0800	.75343	1.68471	2.838
		Arcsine transformed yr 4	5	.57	.71	.6445	.02521	.05637	.003
		Residual for Arcsine yr4	5	-.07	.07	.0000	.02521	.05637	.003
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 4	5	7.15	13.20	9.4600	1.10084	2.46155	6.059
		Arcsine transformed yr 4	5	.85	1.01	.9126	.02750	.06149	.004
		Residual for Arcsine yr4	5	-.06	.09	.0000	.02750	.06149	.004
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine yr4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine yr4	5	.00	.00	.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 4	5	16.15	22.00	18.5500	1.18121	2.64126	6.976
		Arcsine transformed yr 4	5	.83	.98	.8891	.03016	.06745	.005
		Residual for Arcsine yr4	5	-.06	.09	.0000	.03016	.06745	.005
		Valid N (listwise)	5						
A. canina	Phi bi weekly	Percent disease year 4	5	8.70	13.80	11.5700	1.01311	2.26539	5.132
		Arcsine transformed yr 4	5	.60	.76	.6916	.03218	.07195	.005
		Residual for Arcsine yr4	5	-.09	.07	.0000	.03218	.07195	.005
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 4	5	11.20	15.95	13.4600	.76720	1.71552	2.943
		Arcsine transformed yr 4	5	.68	.82	.7502	.02245	.05021	.003
		Residual for Arcsine yr4	5	-.07	.07	.0000	.02245	.05021	.003
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 4	5	12.40	17.40	15.0000	.80296	1.79548	3.224
		Arcsine transformed yr 4	5	.72	.86	.7944	.02266	.05066	.003
		Residual for Arcsine yr4	5	-.07	.07	.0000	.02266	.05066	.003
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 4	5	.70	1.15	.9200	.09566	.21389	.046
		Arcsine transformed yr 4	5	.17	.21	.1911	.01014	.02268	.001
		Residual for Arcsine yr4	5	-.02	.02	.0000	.01014	.02268	.001
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine yr4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 4	5	21.45	28.35	24.5500	1.13270	2.53279	6.415
		Arcsine transformed yr 4	5	.96	1.12	1.0360	.02621	.05860	.003
		Residual for Arcsine yr4	5	-.07	.09	.0000	.02621	.05860	.003
		Valid N (listwise)	5						

A. stolonifera	Phi bi weekly	Percent disease year 4	5	4.50	7.35	6.0400	.55281	1.23612	1.528
		Arcsine transformed yr 4	5	.43	.55	.4946	.02336	.05223	.003
		Residual for Arcsine yr4	5	-.07	.05	.0000	.02336	.05223	.003
		Valid N (listwise)	5						
	Phi monthly	Percent disease year 4	5	5.25	8.65	6.9400	.55866	1.24920	1.561
		Arcsine transformed yr 4	5	.46	.60	.5315	.02213	.04947	.002
		Residual for Arcsine yr4	5	-.07	.07	.0000	.02213	.04947	.002
		Valid N (listwise)	5						
	Phi bi weekly 6 apps	Percent disease year 4	5	4.85	8.20	6.7200	.56383	1.26075	1.590
		Arcsine transformed yr 4	5	.44	.58	.5226	.02308	.05161	.003
		Residual for Arcsine yr4	5	-.08	.06	.0000	.02308	.05161	.003
		Valid N (listwise)	5						
	Chlorothalonil	Percent disease year 4	5	0.00	1.10	.4800	.22170	.49573	.246
		Arcsine transformed yr 4	5	0.00	.21	.1058	.04489	.10038	.010
		Residual for Arcsine yr4	5	-.11	.10	.0000	.04489	.10038	.010
		Valid N (listwise)	5						
	Chlorothalonil + Phi	Percent disease year 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Arcsine transformed yr 4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Residual for Arcsine yr4	5	0.00	0.00	0.0000	0.00000	0.00000	0.000
		Valid N (listwise)	5						
	Control	Percent disease year 4	5	9.85	15.40	12.3800	1.07292	2.39911	5.756
		Arcsine transformed yr 4	5	.64	.81	.7167	.03258	.07285	.005
		Residual for Arcsine yr4	5	-.08	.09	.0000	.03258	.07285	.005
		Valid N (listwise)	5						

Appendix 31: Figures 3-13, 3-14 and 3-15. Monthly disease incidence, *P. annua, A. canina* and *A. stolonifera,* November 2013 (year 4), Tukey pairwise comparisons.

Tukey HSD Acrsine transformed yr 4 **Multiple Comparisons**

Turfgrass species	Compounds			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	Nov-12	Phi bi weekly	Phi monthly	-1.57327	.47034	.059	-3.0275	-.1190
			Phi bi weekly 6 apps	-1.61155	.47034	.054	-3.0658	-.1573
			Chlorothalonil	22.50049*	.47034	.000	21.0462	23.9548
			Chlorothalonil + Phi	22.50049*	.47034	.000	21.0462	23.9548
			Control	-22.74257*	.47034	.000	-24.1968	-21.2883
		Phi monthly	Phi bi weekly	1.57327	.47034	.059	.1190	3.0275
			Phi bi weekly 6 apps	-.03828	.47034	1.000	-1.4925	1.4160
			Chlorothalonil	24.07376*	.47034	.000	22.6195	25.5280
			Chlorothalonil + Phi	24.07376*	.47034	.000	22.6195	25.5280
			Control	-21.16930*	.47034	.000	-22.6236	-19.7150
		Phi bi weekly 6 apps	Phi bi weekly	1.61155	.47034	.054	.1573	3.0658
			Phi monthly	.03828	.47034	1.000	-1.4160	1.4925
			Chlorothalonil	24.11203*	.47034	.000	22.6578	25.5663
			Chlorothalonil + Phi	24.11203*	.47034	.000	22.6578	25.5663
			Control	-21.13103*	.47034	.000	-22.5853	-19.6768
		Chlorothalonil	Phi bi weekly	-22.50049*	.47034	.000	-23.9548	-21.0462
			Phi monthly	-24.07376*	.47034	.000	-25.5280	-22.6195
			Phi bi weekly 6 apps	-24.11203*	.47034	.000	-25.5663	-22.6578
			Chlorothalonil + Phi	0.00000	.47034	1.000	-1.4543	1.4543
			Control	-45.24306*	.47034	.000	-46.6973	-43.7888
		Chlorothalonil + Phi	Phi bi weekly	-22.50049*	.47034	.000	-23.9548	-21.0462
			Phi monthly	-24.07376*	.47034	.000	-25.5280	-22.6195
			Phi bi weekly 6 apps	-24.11203*	.47034	.000	-25.5663	-22.6578
			Chlorothalonil	0.00000	.47034	1.000	-1.4543	1.4543
			Control	-45.24306*	.47034	.000	-46.6973	-43.7888
		Control	Phi bi weekly	22.74257*	.47034	.000	21.2883	24.1968
			Phi monthly	21.16930*	.47034	.000	19.7150	22.6236
			Phi bi weekly 6 apps	21.13103*	.47034	.000	19.6768	22.5853
			Chlorothalonil	45.24306*	.47034	.000	43.7888	46.6973
			Chlorothalonil + Phi	45.24306*	.47034	.000	43.7888	46.6973
A. canina	Nov-12	Phi bi weekly	Phi monthly	-1.68246	.65819	.148	-3.7175	.3526
			Phi bi weekly 6 apps	-1.78458	.65819	.110	-3.8196	.2505
			Chlorothalonil	25.36779*	.65819	.000	23.3327	27.4028
			Chlorothalonil + Phi	28.31147*	.65819	.000	26.2764	30.3465
			Control	-25.53173*	.65819	.000	-27.5668	-23.4967
		Phi monthly	Phi bi weekly	1.68246	.65819	.148	-.3526	3.7175
			Phi bi weekly 6 apps	-.10213	.65819	1.000	-2.1372	1.9329
			Chlorothalonil	27.05024*	.65819	.000	25.0152	29.0853
			Chlorothalonil + Phi	29.99393*	.65819	.000	27.9589	32.0290
			Control	-23.84928*	.65819	.000	-25.8843	-21.8142
		Phi bi weekly 6 apps	Phi bi weekly	1.78458	.65819	.110	-.2505	3.8196
			Phi monthly	.10213	.65819	1.000	-1.9329	2.1372
			Chlorothalonil	27.15237*	.65819	.000	25.1173	29.1874
			Chlorothalonil + Phi	30.09606*	.65819	.000	28.0610	32.1311
			Control	-23.74715*	.65819	.000	-25.7822	-21.7121
		Chlorothalonil	Phi bi weekly	-25.36779*	.65819	.000	-27.4028	-23.3327
			Phi monthly	-27.05024*	.65819	.000	-29.0853	-25.0152
			Phi bi weekly 6 apps	-27.15237*	.65819	.000	-29.1874	-25.1173
			Chlorothalonil + Phi	2.94369	.65819	.082	.9086	4.9787
			Control	-50.89952*	.65819	.000	-52.9346	-48.8645
		Chlorothalonil + Phi	Phi bi weekly	-28.31147*	.65819	.000	-30.3465	-26.2764
			Phi monthly	-29.99393*	.65819	.000	-32.0290	-27.9589
			Phi bi weekly 6 apps	-30.09606*	.65819	.000	-32.1311	-28.0610
			Chlorothalonil	-2.94369	.65819	.082	-4.9787	.9086
			Control	-53.84320*	.65819	.000	-55.8783	-51.8081
		Control	Phi bi weekly	25.53173*	.65819	.000	23.4967	27.5668
			Phi monthly	23.84928*	.65819	.000	21.8142	25.8843
			Phi bi weekly 6 apps	23.74715*	.65819	.000	21.7121	25.7822
			Chlorothalonil	50.89952*	.65819	.000	48.8645	52.9346
			Chlorothalonil + Phi	53.84320*	.65819	.000	51.8081	55.8783

A. stolonifera	Nov-12	Phi bi weekly	Phi monthly	-.81622	.37009	.272	-1.9605	.3281
			Phi bi weekly 6 apps	-.55619	.37009	.666	-1.7005	.5881
			Chlorothalonil	10.78282*	.37009	.000	9.6385	11.9271
			Chlorothalonil + Phi	11.98467*	.37009	.000	10.8404	13.1290
			Control	-10.81268*	.37009	.000	-11.9570	-9.6684
		Phi monthly	Phi bi weekly	.81622	.37009	.272	-.3281	1.9605
			Phi bi weekly 6 apps	.26003	.37009	.980	-.8843	1.4043
			Chlorothalonil	11.59904*	.37009	.000	10.4547	12.7433
			Chlorothalonil + Phi	12.80089*	.37009	.000	11.6566	13.9452
			Control	-9.99646*	.37009	.000	-11.1408	-8.8522
		Phi bi weekly 6 apps	Phi bi weekly	.55619	.37009	.666	-.5881	1.7005
			Phi monthly	-.26003	.37009	.980	-1.4043	.8843
			Chlorothalonil	11.33901*	.37009	.000	10.1947	12.4833
			Chlorothalonil + Phi	12.54086*	.37009	.000	11.3966	13.6852
			Control	-10.25650*	.37009	.000	-11.4008	-9.1122
		Chlorothalonil	Phi bi weekly	-10.78282*	.37009	.000	-11.9271	-9.6385
			Phi monthly	-11.59904*	.37009	.000	-12.7433	-10.4547
			Phi bi weekly 6 apps	-11.33901*	.37009	.000	-12.4833	-10.1947
			Chlorothalonil + Phi	1.20185	.37009	.066	.0576	2.3462
			Control	-21.59550*	.37009	.000	-22.7398	-20.4512
		Chlorothalonil + Phi	Phi bi weekly	-11.98467*	.37009	.000	-13.1290	-10.8404
			Phi monthly	-12.80089*	.37009	.000	-13.9452	-11.6566
			Phi bi weekly 6 apps	-12.54086*	.37009	.000	-13.6852	-11.3966
			Chlorothalonil	-1.20185	.37009	.066	-2.3462	-.0576
			Control	-22.79736*	.37009	.000	-23.9417	-21.6531
		Control	Phi bi weekly	10.81268*	.37009	.000	9.6684	11.9570
			Phi monthly	9.99646*	.37009	.000	8.8522	11.1408
			Phi bi weekly 6 apps	10.25650*	.37009	.000	9.1122	11.4008
			Chlorothalonil	21.59550*	.37009	.000	20.4512	22.7398
			Chlorothalonil + Phi	22.79736*	.37009	.000	21.6531	23.9417

Appendix 32: Figure 3-16 Mean disease incidence, *P.annua, A. canina* and *A. stolonifera*, from September 2013 to March 2014, Tukey pairwise comparisons.

Turfgrass species	Treatments		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P.annua	Phi bi weekly	Phi monthly	-.04711	.03056	.642	-.1416	.0474
		Phi bi weekly 6 apps	-0.31522	.03056	.724	-.4097	-.2207
		Chlorothalonil	.59736*	.03056	.000	.5029	.6918
		Chlorothalonil + Phi	.59736*	.03056	.000	.5029	.6918
		Control	-.29171*	.03056	.000	-.3862	-.1972
	Phi monthly	Phi bi weekly	.04711	.03056	.642	-.0474	.1416
		Phi bi weekly 6 apps	-0.2681	.03056	.112	-.3626	-.1736
		Chlorothalonil	.64447*	.03056	.000	.5500	.7390
		Chlorothalonil + Phi	.64447*	.03056	.000	.5500	.7390
		Control	-.24460*	.03056	.000	-.3391	-.1501
	Phi bi weekly 6 apps	Phi bi weekly	0.31522	.03056	.724	.2207	.4097
		Phi monthly	0.2681	.03056	.112	.1736	.3626
		Chlorothalonil	.91257*	.03056	.000	.8181	1.0071
		Chlorothalonil + Phi	.91257*	.03056	.000	.8181	1.0071
		Control	.02351*	.03056	.040	-.0710	.1180
	Chlorothalonil	Phi bi weekly	-.59736*	.03056	.000	-.6918	-.5029
		Phi monthly	-.64447*	.03056	.000	-.7390	-.5500
		Phi bi weekly 6 apps	-.91257*	.03056	.000	-1.0071	-.8181
		Chlorothalonil + Phi	0.00000	.03056	1.000	-.0945	.0945
		Control	-.88907*	.03056	.000	-.9835	-.7946
	Chlorothalonil + Phi	Phi bi weekly	-.59736*	.03056	.000	-.6918	-.5029
		Phi monthly	-.64447*	.03056	.000	-.7390	-.5500
		Phi bi weekly 6 apps	-.91257*	.03056	.000	-1.0071	-.8181
		Chlorothalonil	0.00000	.03056	1.000	-.0945	.0945
		Control	-.88907*	.03056	.000	-.9835	-.7946
	Control	Phi bi weekly	.29171*	.03056	.000	.1972	.3862
		Phi monthly	.24460*	.03056	.000	.1501	.3391
		Phi bi weekly 6 apps	.02351*	.03056	.040	-.1180	.0710
		Chlorothalonil	.88907*	.03056	.000	.7946	.9835
		Chlorothalonil + Phi	.88907*	.03056	.000	.7946	.9835
A. canina	Phi bi weekly	Phi monthly	-.05859	.03078	.424	-.1538	.0366
		Phi bi weekly 6 apps	-.10275*	.03078	.029	-.1979	-.0076
		Chlorothalonil	.50057*	.03078	.000	.4054	.5957
		Chlorothalonil + Phi	.69164*	.03078	.000	.5965	.7868
		Control	-.34433*	.03078	.000	-.4395	-.2492
	Phi monthly	Phi bi weekly	.05859	.03078	.424	-.0366	.1538
		Phi bi weekly 6 apps	-.04416	.03078	.706	-.1393	.0510
		Chlorothalonil	.55916*	.03078	.000	.4640	.6543
		Chlorothalonil + Phi	.75022*	.03078	.000	.6550	.8454
		Control	-.28575*	.03078	.000	-.3809	-.1906
	Phi bi weekly 6 apps	Phi bi weekly	.10275*	.03078	.029	.0076	.1979
		Phi monthly	.04416	.03078	.706	-.0510	.1393
		Chlorothalonil	.60332*	.03078	.000	.5081	.6985
		Chlorothalonil + Phi	.79439*	.03078	.000	.6992	.8896
		Control	-.24158*	.03078	.000	-.3368	-.1464
	Chlorothalonil	Phi bi weekly	-.50057*	.03078	.000	-.5957	-.4054
		Phi monthly	-.55916*	.03078	.000	-.6543	-.4640
		Phi bi weekly 6 apps	-.60332*	.03078	.000	-.6985	-.5081
		Chlorothalonil + Phi	.19107*	.03078	.000	.0959	.2862
		Control	-.84490*	.03078	.000	-.9401	-.7497
	Chlorothalonil + Phi	Phi bi weekly	-.69164*	.03078	.000	-.7868	-.5965
		Phi monthly	-.75022*	.03078	.000	-.8454	-.6550
		Phi bi weekly 6 apps	-.79439*	.03078	.000	-.8896	-.6992
		Chlorothalonil	-.19107*	.03078	.000	-.2862	-.0959
		Control	-1.03597*	.03078	.000	-1.1311	-.9408
	Control	Phi bi weekly	.34433*	.03078	.000	.2492	.4395
		Phi monthly	.28575*	.03078	.000	.1906	.3809
		Phi bi weekly 6 apps	.24158*	.03078	.000	.1464	.3368
		Chlorothalonil	.84490*	.03078	.000	.7497	.9401
		Chlorothalonil + Phi	1.03597*	.03078	.000	.9408	1.1311

A. stolonifera	Phi bi weekly	Phi monthly	-.03691	.03935	.932	-.1586	.0847
		Phi bi weekly 6 apps	-.02800	.03935	.979	-.1497	.0937
		Chlorothalonil	.38878*	.03935	.000	.2671	.5104
		Chlorothalonil + Phi	.49460*	.03935	.000	.3729	.6163
		Control	-.22207*	.03935	.000	-.3437	-.1004
	Phi monthly	Phi bi weekly	.03691	.03935	.932	-.0847	.1586
		Phi bi weekly 6 apps	.00891	.03935	1.000	-.1127	.1306
		Chlorothalonil	.42569*	.03935	.000	.3040	.5474
		Chlorothalonil + Phi	.53151*	.03935	.000	.4098	.6532
		Control	-.18516*	.03935	.001	-.3068	-.0635
	Phi bi weekly 6 apps	Phi bi weekly	.02800	.03935	.979	-.0937	.1497
		Phi monthly	-.00891	.03935	1.000	-.1306	.1127
		Chlorothalonil	.41678*	.03935	.000	.2951	.5384
		Chlorothalonil + Phi	.52259*	.03935	.000	.4009	.6443
		Control	-.19408*	.03935	.001	-.3157	-.0724
	Chlorothalonil	Phi bi weekly	-.38878*	.03935	.000	-.5104	-.2671
		Phi monthly	-.42569*	.03935	.000	-.5474	-.3040
		Phi bi weekly 6 apps	-.41678*	.03935	.000	-.5384	-.2951
		Chlorothalonil + Phi	.10581	.03935	.114	-.0158	.2275
		Control	-.61086*	.03935	.000	-.7325	-.4892
	Chlorothalonil + Phi	Phi bi weekly	-.49460*	.03935	.000	-.6163	-.3729
		Phi monthly	-.53151*	.03935	.000	-.6532	-.4098
		Phi bi weekly 6 apps	-.52259*	.03935	.000	-.6443	-.4009
		Chlorothalonil	-.10581	.03935	.114	-.2275	.0158
		Control	-.71667*	.03935	.000	-.8383	-.5950
	Control	Phi bi weekly	.22207*	.03935	.000	.1004	.3437
		Phi monthly	.18516*	.03935	.001	.0635	.3068
		Phi bi weekly 6 apps	.19408*	.03935	.001	.0724	.3157
		Chlorothalonil	.61086*	.03935	.000	.4892	.7325
		Chlorothalonil + Phi	.71667*	.03935	.000	.5950	.8383

*. The mean difference is significant at the 0.05 level.

Appendix 33: Figure 3-18 Monthly disease incidence all turfgrass species, February 2013 (year 3), Tukey pairwise comparisons.

Tukey HSD

Multible comparisons

Turfgrass species	Treatments		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P. annua February 2013	Phi bi weekly	Phi monthly	-4.57340*	.69256	.000	-6.7147	-2.4321
		Phi bi weekly 6 apps	-9.45840*	.69256	.000	-11.5997	-7.3171
		Chlorothalonil	21.56140*	.69256	.000	19.4201	23.7027
		Chlorothalonil + Phi	24.84820*	.69256	.000	22.7069	26.9895
		Control	-16.91500*	.69256	.000	-19.0563	-14.7737
	Phi monthly	Phi bi weekly	4.57340*	.69256	.000	2.4321	6.7147
		Phi bi weekly 6 apps	-4.88500*	.69256	.000	-7.0263	-2.7437
		Chlorothalonil	26.13480*	.69256	.000	23.9935	28.2761
		Chlorothalonil + Phi	29.42160*	.69256	.000	27.2803	31.5629
		Control	-12.34160*	.69256	.000	-14.4829	-10.2003
	Phi bi weekly 6 apps	Phi bi weekly	9.45840*	.69256	.000	7.3171	11.5997
		Phi monthly	4.88500*	.69256	.000	2.7437	7.0263
		Chlorothalonil	31.01980*	.69256	.000	28.8785	33.1611
		Chlorothalonil + Phi	34.30660*	.69256	.000	32.1653	36.4479
		Control	-7.45660*	.69256	.000	-9.5979	-5.3153
A. canina February 2013	Phi bi weekly	Phi monthly	-8.22342*	1.14921	.000	-11.7767	-4.6701
		Phi bi weekly 6 apps	-14.40242*	1.14921	.000	-17.9557	-10.8491
		Chlorothalonil	30.97365*	1.14921	.000	27.4204	34.5269
		Chlorothalonil + Phi	35.72761*	1.14921	.000	32.1743	39.2809
		Control	-26.65070*	1.14921	.000	-30.2040	-23.0974
	Phi monthly	Phi bi weekly	8.22342*	1.14921	.000	4.6701	11.7767
		Phi bi weekly 6 apps	-6.17900*	1.14921	.000	-9.7323	-2.6257
		Chlorothalonil	39.19707*	1.14921	.000	35.6438	42.7503
		Chlorothalonil + Phi	43.95103*	1.14921	.000	40.3978	47.5043
		Control	-18.42728*	1.14921	.000	-21.9806	-14.8740
	Phi bi weekly 6 apps	Phi bi weekly	14.40242*	1.14921	.000	10.8491	17.9557
		Phi monthly	6.17900*	1.14921	.000	2.6257	9.7323
		Chlorothalonil	45.37607*	1.14921	.000	41.8228	48.9293
		Chlorothalonil + Phi	50.13003*	1.14921	.000	46.5768	53.6833
		Control	-12.24828*	1.14921	.000	-15.8015	-8.6950
A. stolonifera February 2013	Phi bi weekly	Phi monthly	-4.47132*	.38512	.000	-5.6621	-3.2806
		Phi bi weekly 6 apps	-8.32246*	.38512	.000	-9.5132	-7.1317
		Chlorothalonil	16.71943*	.38512	.000	15.5287	17.9102
		Chlorothalonil + Phi	19.40232*	.38512	.000	18.2116	20.5931
		Control	-14.82481*	.38512	.000	-16.0156	-13.6341
	Phi monthly	Phi bi weekly	4.47132*	.38512	.000	3.2806	5.6621
		Phi bi weekly 6 apps	-3.85114*	.38512	.000	-5.0419	-2.6604
		Chlorothalonil	21.19075*	.38512	.000	20.0000	22.3815
		Chlorothalonil + Phi	23.87363*	.38512	.000	22.6829	25.0644
		Control	-10.35350*	.38512	.000	-11.5442	-9.1627
	Phi bi weekly 6 apps	Phi bi weekly	8.32246*	.38512	.000	7.1317	9.5132
		Phi monthly	3.85114*	.38512	.000	2.6604	5.0419
		Chlorothalonil	25.04189*	.38512	.000	23.8511	26.2326
		Chlorothalonil + Phi	27.72478*	.38512	.000	26.5340	28.9155
		Control	-6.50235*	.38512	.000	-7.6931	-5.3116

*. The mean difference is significant at the 0.05 level.

Appendix 34: Figure 3-20 Monthly disease incidence all turfgrass species, February 2014 (year 4), Tukey pairwise comparisons.

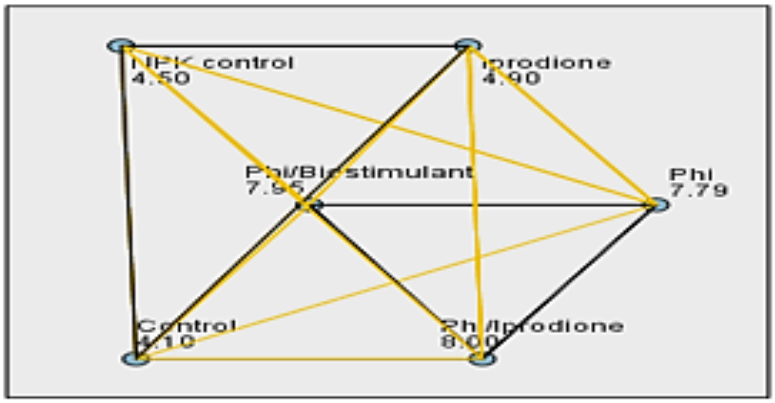
Tukey HSD

Multible comparisons

Turfgrass species	Treatments		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
P. annua February 2013	Phi bi weekly	Phi monthly	-2.13134*	.20810	.000	-2.7748	-1.4879
		Phi bi weekly 6 apps	-3.19025*	.20810	.000	-3.8337	-2.5468
		Chlorothalonil	7.24570*	.20810	.000	6.6023	7.8891
		Chlorothalonil + Phi	8.37605*	.20810	.000	7.7326	9.0195
		Control	-5.90054*	.20810	.000	-6.5440	-5.2571
	Phi monthly	Phi bi weekly	2.13134*	.20810	.000	1.4879	2.7748
		Phi bi weekly 6 apps	-1.05890*	.20810	.000	-1.7023	-.4155
		Chlorothalonil	9.37705*	.20810	.000	8.7336	10.0205
		Chlorothalonil + Phi	10.50740*	.20810	.000	9.8640	11.1508
		Control	-3.76920*	.20810	.000	-4.4126	-3.1258
	Phi bi weekly 6 apps	Phi bi weekly	3.19025*	.20810	.000	2.5468	3.8337
		Phi monthly	1.05890*	.20810	.000	.4155	1.7023
		Chlorothalonil	10.43595*	.20810	.000	9.7925	11.0794
		Chlorothalonil + Phi	11.56630*	.20810	.000	10.9229	12.2097
		Control	-2.71029*	.20810	.000	-3.3537	-2.0668
A. canina February 2013	Phi bi weekly	Phi monthly	-2.53112*	.29584	.000	-3.4458	-1.6164
		Phi bi weekly 6 apps	-5.07014*	.29584	.000	-5.9849	-4.1554
		Chlorothalonil	11.01492*	.29584	.000	10.1002	11.9296
		Chlorothalonil + Phi	12.76100*	.29584	.000	11.8463	13.6757
		Control	-9.29587*	.29584	.000	-10.2106	-8.3812
	Phi monthly	Phi bi weekly	2.53112*	.29584	.000	1.6164	3.4458
		Phi bi weekly 6 apps	-2.53902*	.29584	.000	-3.4537	-1.6243
		Chlorothalonil	13.54604*	.29584	.000	12.6313	14.4608
		Chlorothalonil + Phi	15.29211*	.29584	.000	14.3774	16.2068
		Control	-6.76476*	.29584	.000	-7.6795	-5.8500
	Phi bi weekly 6 apps	Phi bi weekly	5.07014*	.29584	.000	4.1554	5.9849
		Phi monthly	2.53902*	.29584	.000	1.6243	3.4537
		Chlorothalonil	16.08506*	.29584	.000	15.1703	16.9998
		Chlorothalonil + Phi	17.83113*	.29584	.000	16.9164	18.7458
		Control	-4.22574*	.29584	.000	-5.1405	-3.3110
A. stolonifera February 2013	Phi bi weekly	Phi monthly	-2.13134*	.20810	.000	-2.7748	-1.4879
		Phi bi weekly 6 apps	-3.19025*	.20810	.000	-3.8337	-2.5468
		Chlorothalonil	7.24570*	.20810	.000	6.6023	7.8891
		Chlorothalonil + Phi	8.37605*	.20810	.000	7.7326	9.0195
		Control	-5.90054*	.20810	.000	-6.5440	-5.2571
	Phi monthly	Phi bi weekly	2.13134*	.20810	.000	1.4879	2.7748
		Phi bi weekly 6 apps	-1.05890*	.20810	.000	-1.7023	-.4155
		Chlorothalonil	9.37705*	.20810	.000	8.7336	10.0205
		Chlorothalonil + Phi	10.50740*	.20810	.000	9.8640	11.1508
		Control	-3.76920*	.20810	.000	-4.4126	-3.1258
	Phi bi weekly 6 apps	Phi bi weekly	3.19025*	.20810	.000	2.5468	3.8337
		Phi monthly	1.05890*	.20810	.000	.4155	1.7023
		Chlorothalonil	10.43595*	.20810	.000	9.7925	11.0794
		Chlorothalonil + Phi	11.56630*	.20810	.000	10.9229	12.2097
		Control	-2.71029*	.20810	.000	-3.3537	-2.0668

Appendix 35: Figure 3-21 Turfgrass quality, *P. annua* and *A. canina,* from September 2010 to March 2011 (year 1). Treatment effect on median levels of turfgrass quality on *P.annua* and *A. canina* trial plots. Pairwise comparisons using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

***P. annua* median turf quality, year 1**

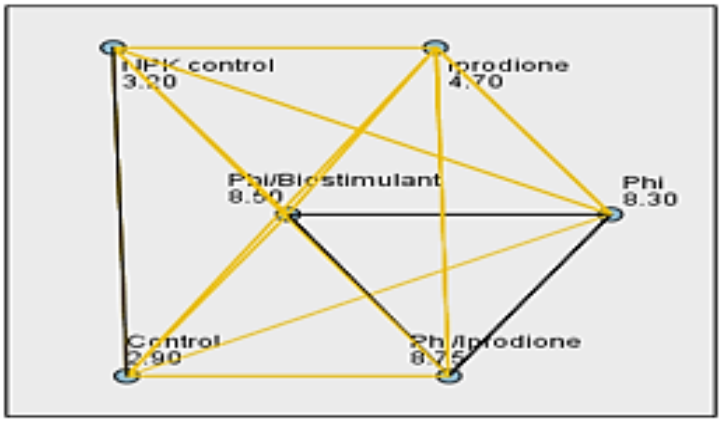


Each node shows the sample median of Treatments years 1 and 2.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-NPK control	1.667	.197	1.000
Control-Iprodione	3.600	.058	.867
Control-Phi	10.000	.002	.023
Control-Phi/Biostimulant	10.000	.002	.023
Control-Phi/Iprodione	10.000	.002	.023
NPK control-Iprodione	3.600	.058	.867
NPK control-Phi	10.000	.002	.023
NPK control-Phi/Biostimulant	10.000	.002	.023
NPK control-Phi/Iprodione	10.000	.002	.023
Iprodione-Phi	10.000	.002	.023
Iprodione-Phi/Biostimulant	10.000	.002	.023
Iprodione-Phi/Iprodione	10.000	.002	.023
Phi-Phi/Biostimulant	.400	.527	1.000
Phi-Phi/Iprodione	.400	.527	1.000
Phi/Biostimulant-Phi/Iprodione	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

A. canina median turf quality, year 1



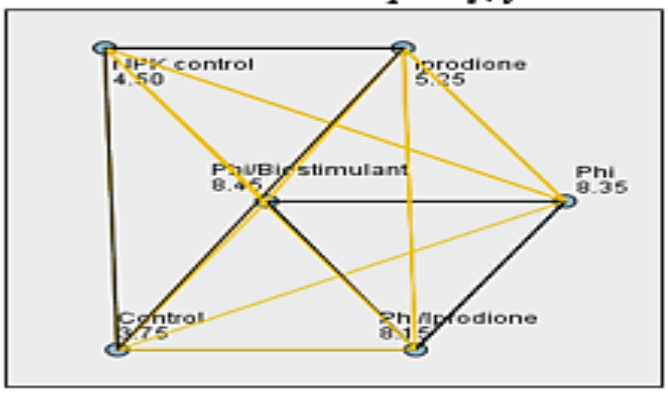
Each node shows the sample median of Treatments years 1 and 2.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-NPK control	.400	.527	1.000
Control-Iprodione	10.000	.002	.023
Control-Phi	10.000	.002	.023
Control-Phi/Biostimulant	10.000	.002	.023
Control-Phi/Iprodione	10.000	.002	.023
NPK control-Iprodione	10.000	.002	.023
NPK control-Phi	10.000	.002	.023
NPK control-Phi/Biostimulant	10.000	.002	.023
NPK control-Phi/Iprodione	10.000	.002	.023
Iprodione-Phi	10.000	.002	.023
Iprodione-Phi/Biostimulant	10.000	.002	.023
Iprodione-Phi/Iprodione	10.000	.002	.023
Phi-Phi/Biostimulant	.400	.527	1.000
Phi-Phi/Iprodione	.400	.527	1.000
Phi/Biostimulant-Phi/Iprodione	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Appendix 36: Figure 3-22 Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2011 to March 2012 (year 2).Treatment effect on median levels of turfgrass quality on *P.annua, A. canina* and *A. stolonifera* trial plots. Pairwise comparisons using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

***P. annua* median turf quality, year 2**

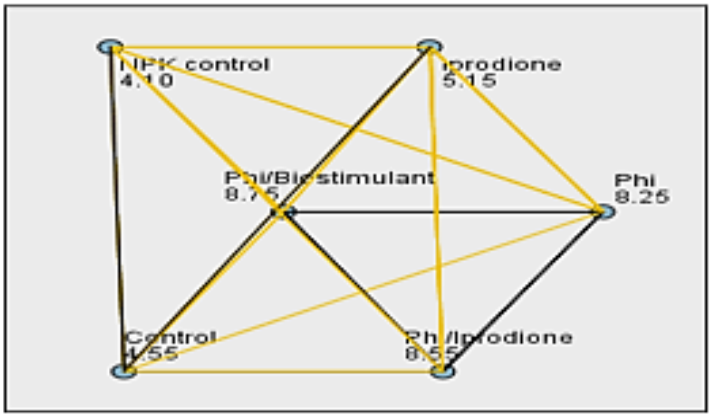


Each node shows the sample median of Treatments years 1 and 2.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-NPK control	.400	.527	1.000
Control-Iprodione	6.667	.010	.147
Control-Phi/Iprodione	10.000	.002	.023
Control-Phi	10.000	.002	.023
Control-Phi/Biostimulant	10.000	.002	.023
NPK control-Iprodione	3.600	.058	.867
NPK control-Phi/Iprodione	10.000	.002	.023
NPK control-Phi	10.000	.002	.023
NPK control-Phi/Biostimulant	10.000	.002	.023
Iprodione-Phi/Iprodione	10.000	.002	.023
Iprodione-Phi	10.000	.002	.023
Iprodione-Phi/Biostimulant	10.000	.002	.023
Phi/Iprodione-Phi	.400	.527	1.000
Phi/Iprodione-Phi/Biostimulant	.400	.527	1.000
Phi-Phi/Biostimulant	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

***A. canina* median turf quality, year 2**

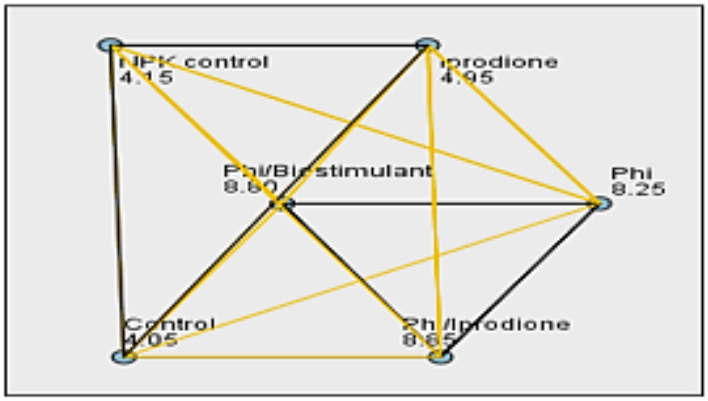


Each node shows the sample median of Treatments years 1 and 2.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
NPK control-Control	.400	.527	1.000
NPK control-Iprodione	10.000	.002	.023
NPK control-Phi	10.000	.002	.023
NPK control-Phi/Iprodione	10.000	.002	.023
NPK control-Phi/Biostimulant	10.000	.002	.023
Control-Iprodione	3.600	.058	.867
Control-Phi	10.000	.002	.023
Control-Phi/Iprodione	10.000	.002	.023
Control-Phi/Biostimulant	10.000	.002	.023
Iprodione-Phi	10.000	.002	.023
Iprodione-Phi/Iprodione	10.000	.002	.023
Iprodione-Phi/Biostimulant	10.000	.002	.023
Phi-Phi/Iprodione	.400	.527	1.000
Phi-Phi/Biostimulant	.400	.527	1.000
Phi/Iprodione-Phi/Biostimulant	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

A. stolonifera median turf quality, year 2



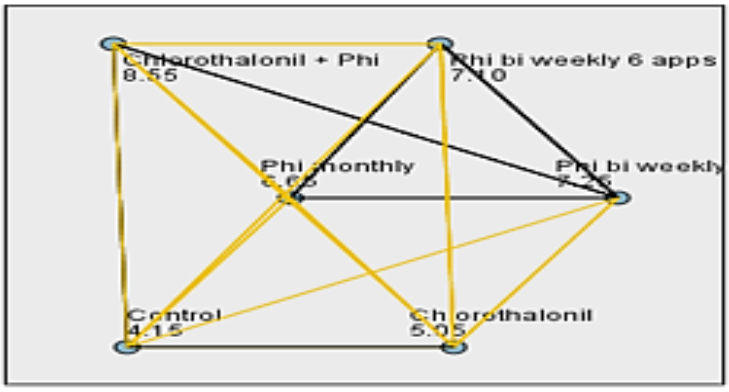
Each node shows the sample median of Treatments years 1 and 2.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-NPK control	.400	.527	1.000
Control-Iprodione	1.667	.197	1.000
Control-Phi	10.000	.002	.023
Control-Phi/Biostimulant	10.000	.002	.023
Control-Phi/Iprodione	10.000	.002	.023
NPK control-Iprodione	1.667	.197	1.000
NPK control-Phi	10.000	.002	.023
NPK control-Phi/Biostimulant	10.000	.002	.023
NPK control-Phi/Iprodione	10.000	.002	.023
Iprodione-Phi	10.000	.002	.023
Iprodione-Phi/Biostimulant	10.000	.002	.023
Iprodione-Phi/Iprodione	10.000	.002	.023
Phi-Phi/Biostimulant	.400	.527	1.000
Phi-Phi/Iprodione	.400	.527	1.000
Phi/Biostimulant-Phi/Iprodione	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Appendix 37: Figure 3-25 Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2012 to March 2013 (year 3).Treatment effect on median levels of turfgrass quality on *P.annua, A. canina* and *A. stolonifera* trial plots. Pairwise comparisons using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

***P. annua* median turf quality, year 3**

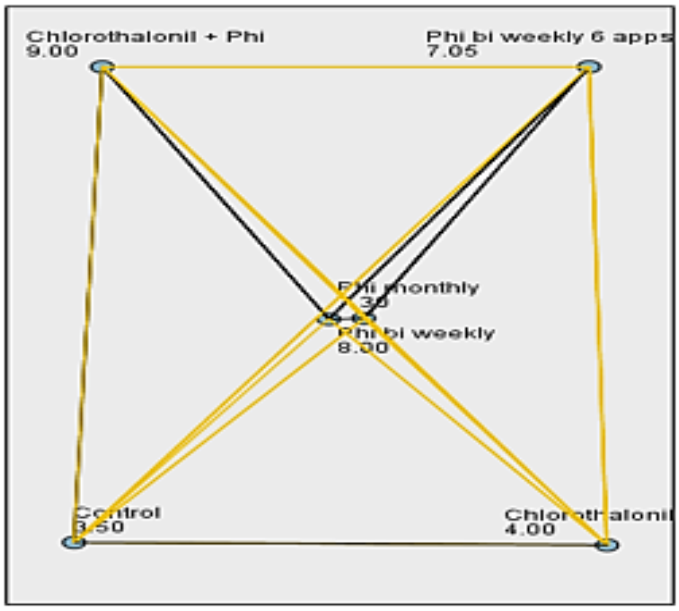


Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-Chlorothalonil	.400	.527	1.000
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly 6 apps	.400	.527	1.000
Phi monthly-Phi bi weekly	.400	.527	1.000
Phi monthly-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi bi weekly	.400	.527	1.000
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly-Chlorothalonil + Phi	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

A. canina median turf quality, year 3

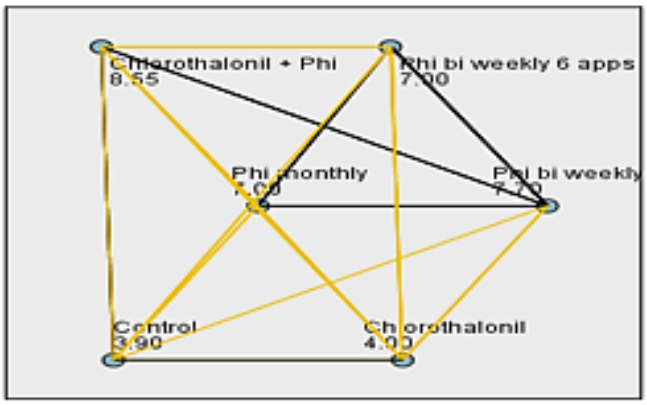


Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-Chlorothalonil	3.600	.058	.867
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi monthly	3.600	.058	.867
Phi bi weekly 6 apps-Phi bi weekly	3.600	.058	.867
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly	.400	.527	1.000
Phi monthly-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly-Chlorothalonil + Phi	3.600	.058	.867

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

***A. stolonifera* median turf quality, year 3**



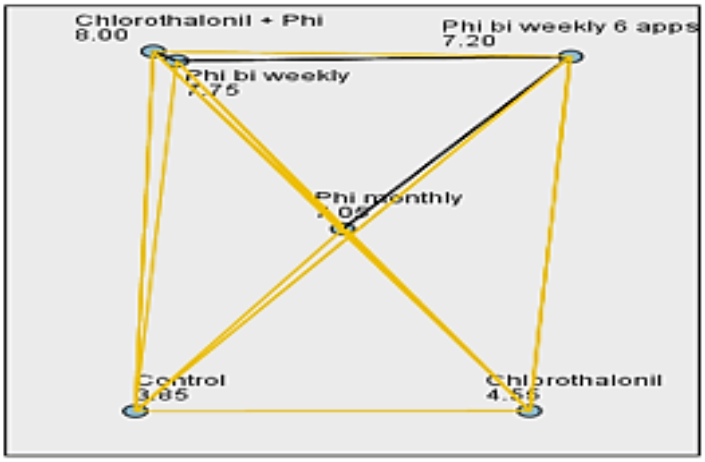
Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-Chlorothalonil	.476	.490	1.000
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly 6 apps	.000	1.000	1.000
Phi monthly-Phi bi weekly	3.600	.058	.867
Phi monthly-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi bi weekly	3.600	.058	.867
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly-Chlorothalonil + Phi	3.600	.058	.867

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Appendix 38: Figure 3-26, Turfgrass quality, *P.annua, A. canina* and *A. stolonifera,* from September 2013 to March 2014 (year 4).Treatment effect on median levels of turfgrass quality on *P.annua, A. canina* and *A. stolonifera* trial plots. Pairwise comparisons using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

***P. annua* median turf quality, year 4**

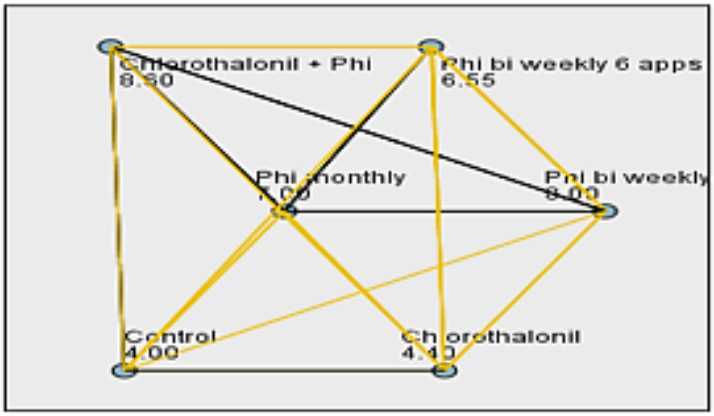


Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-Chlorothalonil	10.000	.002	.023
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly 6 apps	3.600	.058	.867
Phi monthly-Phi bi weekly	10.000	.002	.023
Phi monthly-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi bi weekly	4.286	.038	.577
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly-Chlorothalonil + Phi	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

A. canina median turf quality, year 4

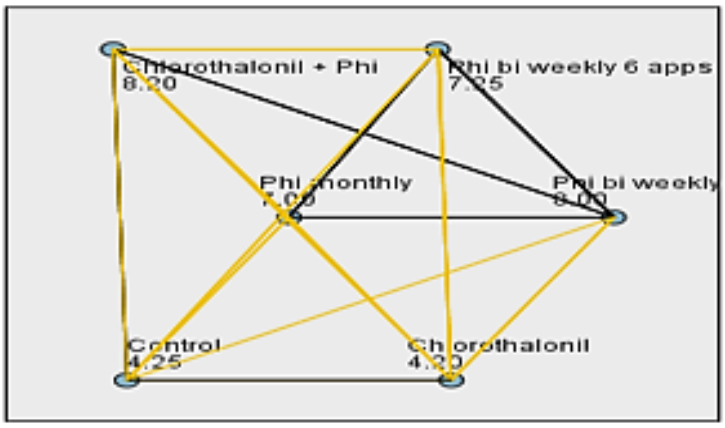


Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Control-Chlorothalonil	.400	.527	1.000
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi monthly	.400	.527	1.000
Phi bi weekly 6 apps-Phi bi weekly	10.000	.002	.023
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly	4.286	.038	.577
Phi monthly-Chlorothalonil + Phi	6.667	.010	.147
Phi bi weekly-Chlorothalonil + Phi	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

***A. stolonifera* median turf quality, year 4**



Each node shows the sample median of Treatments years 3 and 4.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Chlorothalonil-Control	.400	.527	1.000
Chlorothalonil-Phi monthly	10.000	.002	.023
Chlorothalonil-Phi bi weekly 6 apps	10.000	.002	.023
Chlorothalonil-Phi bi weekly	10.000	.002	.023
Chlorothalonil-Chlorothalonil + Phi	10.000	.002	.023
Control-Phi monthly	10.000	.002	.023
Control-Phi bi weekly 6 apps	10.000	.002	.023
Control-Phi bi weekly	10.000	.002	.023
Control-Chlorothalonil + Phi	10.000	.002	.023
Phi monthly-Phi bi weekly 6 apps	.400	.527	1.000
Phi monthly-Phi bi weekly	3.600	.058	.867
Phi monthly-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly 6 apps-Phi bi weekly	.400	.527	1.000
Phi bi weekly 6 apps-Chlorothalonil + Phi	10.000	.002	.023
Phi bi weekly-Chlorothalonil + Phi	3.600	.058	.867

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Chapter 4 statistics

Appendix 39: Figs 4-13 and 4-14, Phi accumulations in *A. stolonifera* leaf and root tissues between July 2012 and July 2014, Tukey pairwise comparisons.

Multiple Comparisons

Tukey HSD

Tissues				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
leaf	Phi amount pre treatment app	Jan 13	July 13	349.50000*	3.76976	.000	339.7082	359.2918
			July 14	268.50000*	3.76976	.000	258.7082	278.2918
		July 13	Jan 13	-349.50000*	3.76976	.000	-359.2918	-339.7082
			July 14	-81.00000*	3.76976	.000	-90.7918	-71.2082
		July 14	Jan 13	-268.50000*	3.76976	.000	-278.2918	-258.7082
			July 13	81.00000*	3.76976	.000	71.2082	90.7918
	Phi amount post treatment app	Jan 13	July 13	318.16667*	2.87131	.000	310.7085	325.6248
			July 14	121.50000*	2.87131	.000	114.0418	128.9582
		July 13	Jan 13	-318.16667*	2.87131	.000	-325.6248	-310.7085
			July 14	-196.66667*	2.87131	.000	-204.1248	-189.2085
		July 14	Jan 13	-121.50000*	2.87131	.000	-128.9582	-114.0418
			July 13	196.66667*	2.87131	.000	189.2085	204.1248
Root	Phi amount pre treatment app	Jan 13	July 13	-234.66667*	3.32165	.000	-243.2945	-226.0388
			July 14	-328.00000*	3.32165	.000	-336.6279	-319.3721
		July 13	Jan 13	234.66667*	3.32165	.000	226.0388	243.2945
			July 14	-93.33333*	3.32165	.000	-101.9612	-84.7055
		July 14	Jan 13	328.00000*	3.32165	.000	319.3721	336.6279
			July 13	93.33333*	3.32165	.000	84.7055	101.9612
	Phi amount post treatment app	Jan 13	July 13	-263.33333*	1.61475	.000	-267.5276	-259.1391
			July 14	-345.00000*	1.61475	.000	-349.1943	-340.8057
		July 13	Jan 13	263.33333*	1.61475	.000	259.1391	267.5276
			July 14	-81.66667*	1.61475	.000	-85.8609	-77.4724
		July 14	Jan 13	345.00000*	1.61475	.000	340.8057	349.1943
			July 13	81.66667*	1.61475	.000	77.4724	85.8609

*. The mean difference is significant at the 0.05 level.

Appendix 40: Figs 4-15 and 4-16, Phi accumulations in *P. annua* leaf and root tissues between July 2012 and July 2014, Tukey pairwise comparisons.

Multiple Comparisons

Tukey HSD

Tissue				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
leaf	Phi amount pre-treatment app	Jan 13	July 13	248.33333*	3.11270	.000	240.2482	256.4185
			July 14	167.33333*	3.11270	.000	159.2482	175.4185
		July 13	Jan 13	-248.33333*	3.11270	.000	-256.4185	-240.2482
			July 14	-81.00000*	3.11270	.000	-89.0851	-72.9149
		July 14	Jan 13	-167.33333*	3.11270	.000	-175.4185	-159.2482
			July 13	81.00000*	3.11270	.000	72.9149	89.0851
	Phi amount 1 wk post-treatment app	Jan 13	July 13	504.50000*	3.29646	.000	495.9375	513.0625
			July 14	366.00000*	3.29646	.000	357.4375	374.5625
		July 13	Jan 13	-504.50000*	3.29646	.000	-513.0625	-495.9375
			July 14	-138.50000*	3.29646	.000	-147.0625	-129.9375
		July 14	Jan 13	-366.00000*	3.29646	.000	-374.5625	-357.4375
			July 13	138.50000*	3.29646	.000	129.9375	147.0625
Root	Phi amount pre-treatment app	Jan 13	July 13	-60.50000*	2.04124	.000	-65.8021	-55.1979
			July 14	-45.50000*	2.04124	.000	-50.8021	-40.1979
		July 13	Jan 13	60.50000*	2.04124	.000	55.1979	65.8021
			July 14	15.00000*	2.04124	.000	9.6979	20.3021
		July 14	Jan 13	45.50000*	2.04124	.000	40.1979	50.8021
			July 13	-15.00000*	2.04124	.000	-20.3021	-9.6979
	Phi amount 1 wk post-treatment app	Jan 13	July 13	37.33333*	3.19084	.000	29.0452	45.6215
			July 14	30.83333*	3.19084	.000	22.5452	39.1215
		July 13	Jan 13	-37.33333*	3.19084	.000	-45.6215	-29.0452
			July 14	-6.50000	3.19084	.138	-14.7881	1.7881
		July 14	Jan 13	-30.83333*	3.19084	.000	-39.1215	-22.5452
			July 13	6.50000	3.19084	.138	-1.7881	14.7881

*. The mean difference is significant at the 0.05 level.

Appendix 38: 4-17 Treatment effect on the growth *L. perenne* in a P sufficient rootzone.
Effect on the growth of leaf, crown and root tissues of *L. perenne*, growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Tukey pairwise comparisons.

Multiple Comparisons

Dependent Vari Weight P sufficient rootzone

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
leaf	KCl	Pi	-.02667	.03916	.778	-.1284	.0750
		Phi	-.49667*	.03916	.000	-.5984	-.3950
	Pi	KCl	.02667	.03916	.778	-.0750	.1284
		Phi	-.47000*	.03916	.000	-.5717	-.3683
	Phi	KCl	.49667*	.03916	.000	.3950	.5984
		Pi	.47000*	.03916	.000	.3683	.5717
Crown	KCl	Pi	-.45833*	.03976	.000	-.5616	-.3550
		Phi	-3.94500*	.03976	.000	-4.0483	-3.8417
	Pi	KCl	.45833*	.03976	.000	.3550	.5616
		Phi	-3.48667*	.03976	.000	-3.5900	-3.3834
	Phi	KCl	3.94500*	.03976	.000	3.8417	4.0483
		Pi	3.48667*	.03976	.000	3.3834	3.5900
Root	KCl	Pi	.80833*	.03428	.000	.7193	.8974
		Phi	-.49167*	.03428	.000	-.5807	-.4026
	Pi	KCl	-.80833*	.03428	.000	-.8974	-.7193
		Phi	-1.30000*	.03428	.000	-1.3890	-1.2110
	Phi	KCl	.49167*	.03428	.000	.4026	.5807
		Pi	1.30000*	.03428	.000	1.2110	1.3890

*. The mean difference is significant at the 0.05 level.

Appendix 39: 4-18 Treatment effect on the growth *P. annua* in a P sufficient rootzone. Effect on the growth of leaf, crown and root tissues of *P. annua*, growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Tukey pairwise comparisons.

Multiple Comparisons

Dependent Vari Weight P sufficient rootzone

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
leaf	KCl	Pi	-.62667*	.04343	.000	-.7395	-.5139
		Phi	-.96333*	.04343	.000	-1.0761	-.8505
	Pi	KCl	.62667*	.04343	.000	.5139	.7395
		Phi	-.33667*	.04343	.000	-.4495	-.2239
	Phi	KCl	.96333*	.04343	.000	.8505	1.0761
		Pi	.33667*	.04343	.000	.2239	.4495
Crown	KCl	Pi	-1.07833*	.03860	.000	-1.1786	-.9781
		Phi	-4.22500*	.03860	.000	-4.3253	-4.1247
	Pi	KCl	1.07833*	.03860	.000	.9781	1.1786
		Phi	-3.14667*	.03860	.000	-3.2469	-3.0464
	Phi	KCl	4.22500*	.03860	.000	4.1247	4.3253
		Pi	3.14667*	.03860	.000	3.0464	3.2469
Root	KCl	Pi	.57000*	.03801	.000	.4713	.6687
		Phi	-.33000*	.03801	.000	-.4287	-.2313
	Pi	KCl	-.57000*	.03801	.000	-.6687	-.4713
		Phi	-.90000*	.03801	.000	-.9987	-.8013
	Phi	KCl	.33000*	.03801	.000	.2313	.4287
		Pi	.90000*	.03801	.000	.8013	.9987

*. The mean difference is significant at the 0.05 level.

Appendix 40: 4-19 Treatment effect on the growth *L. perenne* in a P deficient rootzone. Effect on the growth of leaf, crown and root tissues of *L. perenne,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control).Tukey pairwise comparisons.

Multiple Comparisons

Dependent Vari Weight P deficient rootzone

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
leaf	KCl	Pi	-.64500*	.02188	.000	-.7018	-.5882
		Phi	.64667*	.02188	.000	.5898	.7035
	Pi	KCl	.64500*	.02188	.000	.5882	.7018
		Phi	1.29167*	.02188	.000	1.2348	1.3485
	Phi	KCl	-.64667*	.02188	.000	-.7035	-.5898
		Pi	-1.29167*	.02188	.000	-1.3485	-1.2348
Crown	KCl	Pi	-.67000*	.02434	.000	-.7332	-.6068
		Phi	1.22667*	.02434	.000	1.1635	1.2899
	Pi	KCl	.67000*	.02434	.000	.6068	.7332
		Phi	1.89667*	.02434	.000	1.8335	1.9599
	Phi	KCl	-1.22667*	.02434	.000	-1.2899	-1.1635
		Pi	-1.89667*	.02434	.000	-1.9599	-1.8335
Root	KCl	Pi	-.65833*	.01189	.000	-.6892	-.6274
		Phi	1.75667*	.01189	.000	1.7258	1.7876
	Pi	KCl	.65833*	.01189	.000	.6274	.6892
		Phi	2.41500*	.01189	.000	2.3841	2.4459
	Phi	KCl	-1.75667*	.01189	.000	-1.7876	-1.7258
		Pi	-2.41500*	.01189	.000	-2.4459	-2.3841

*. The mean difference is significant at the 0.05 level.

Appendix 41: 4-20 Treatment effect on the growth *P. annua* in a P deficient rootzone. Effect on the growth of leaf, crown and root tissues of *P. annua*, growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control).Tukey pairwise comparisons.

Multiple Comparisons

Dependent Vari Weight P deficient rootzone

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
leaf	KCl	Pi	-.78833*	.01679	.000	-.8319	-.7447
		Phi	.69167*	.01679	.000	.6481	.7353
	Pi	KCl	.78833*	.01679	.000	.7447	.8319
		Phi	1.48000*	.01679	.000	1.4364	1.5236
	Phi	KCl	-.69167*	.01679	.000	-.7353	-.6481
		Pi	-1.48000*	.01679	.000	-1.5236	-1.4364
Crown	KCl	Pi	-.63000*	.02790	.000	-.7025	-.5575
		Phi	1.54167*	.02790	.000	1.4692	1.6141
	Pi	KCl	.63000*	.02790	.000	.5575	.7025
		Phi	2.17167*	.02790	.000	2.0992	2.2441
	Phi	KCl	-1.54167*	.02790	.000	-1.6141	-1.4692
		Pi	-2.17167*	.02790	.000	-2.2441	-2.0992
Root	KCl	Pi	-.76833*	.02017	.000	-.8207	-.7160
		Phi	1.20000*	.02017	.000	1.1476	1.2524
	Pi	KCl	.76833*	.02017	.000	.7160	.8207
		Phi	1.96833*	.02017	.000	1.9160	2.0207
	Phi	KCl	-1.20000*	.02017	.000	-1.2524	-1.1476
		Pi	-1.96833*	.02017	.000	-2.0207	-1.9160

*. The mean difference is significant at the 0.05 level.

Appendix 42: Figs 4-21 and 4-22, Treatment effect on root to shoot ratios of *L. perenne* and *P. annua* growing in P sufficient and P deficient rootzones. Effect on root to shoot ratios *of L. perenne* and *P. annua* growing in a P sufficient and deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Tukey pairwise comparisons.

Multiple Comparisons

Dependent Vari root to shoot ratio

Tukey HSD

rootzone				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
P sufficient	L. perenne	KCl	Pi	.07833*	.00355	.000	.0691	.0876
			Phi	.11167*	.00355	.000	.1024	.1209
		Pi	KCl	-.07833*	.00355	.000	-.0876	-.0691
			Phi	.03333*	.00355	.000	.0241	.0426
		Phi	KCl	-.11167*	.00355	.000	-.1209	-.1024
			Pi	-.03333*	.00355	.000	-.0426	-.0241
	P. annua	KCl	Pi	.09833*	.00463	.000	.0863	.1104
			Phi	.13167*	.00463	.000	.1196	.1437
		Pi	KCl	-.09833*	.00463	.000	-.1104	-.0863
			Phi	.03333*	.00463	.000	.0213	.0454
		Phi	KCl	-.13167*	.00463	.000	-.1437	-.1196
			Pi	-.03333*	.00463	.000	-.0454	-.0213
P deficient	L. perenne	KCl	Pi	-.01167*	.00355	.013	-.0209	-.0024
			Phi	.09667*	.00355	.000	.0874	.1059
		Pi	KCl	.01167*	.00355	.013	.0024	.0209
			Phi	.10833*	.00355	.000	.0991	.1176
		Phi	KCl	-.09667*	.00355	.000	-.1059	-.0874
			Pi	-.10833*	.00355	.000	-.1176	-.0991
	P. annua	KCl	Pi	-.04333*	.00471	.000	-.0556	-.0311
			Phi	.02667*	.00471	.000	.0144	.0389
		Pi	KCl	.04333*	.00471	.000	.0311	.0556
			Phi	.07000*	.00471	.000	.0578	.0822
		Phi	KCl	-.02667*	.00471	.000	-.0389	-.0144
			Pi	-.07000*	.00471	.000	-.0822	-.0578

*. The mean difference is significant at the 0.05 level.

Appendix 43: Fig. 4-23 Treatment effect on P levels of *L. perenne* growing in a P sufficient rootzone. Effect on leaf, crown and root P levels of *L. perenne*, growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control). Tukey pairwise comparisons.

Multiple Comparisons

Dependent Variable: Tissue P levels

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Leaf	KCl	Pi	-1185.50000*	6.49701	.000	-1202.3758	-1168.6242
		Phi	-910.50000*	6.49701	.000	-927.3758	-893.6242
	Pi	KCl	1185.50000*	6.49701	.000	1168.6242	1202.3758
		Phi	275.00000*	6.49701	.000	258.1242	291.8758
	Phi	KCl	910.50000*	6.49701	.000	893.6242	927.3758
		Pi	-275.00000*	6.49701	.000	-291.8758	-258.1242
Crown	KCl	Pi	-915.33333*	6.67694	.000	-932.6765	-897.9902
		Phi	-1505.16667*	6.67694	.000	-1522.5098	-1487.8235
	Pi	KCl	915.33333*	6.67694	.000	897.9902	932.6765
		Phi	-589.83333*	6.67694	.000	-607.1765	-572.4902
	Phi	KCl	1505.16667*	6.67694	.000	1487.8235	1522.5098
		Pi	589.83333*	6.67694	.000	572.4902	607.1765
Root	KCl	Pi	-156.16667*	6.08124	.000	-171.9625	-140.3708
		Phi	-653.33333*	6.08124	.000	-669.1292	-637.5375
	Pi	KCl	156.16667*	6.08124	.000	140.3708	171.9625
		Phi	-497.16667*	6.08124	.000	-512.9625	-481.3708
	Phi	KCl	653.33333*	6.08124	.000	637.5375	669.1292
		Pi	497.16667*	6.08124	.000	481.3708	512.9625

*. The mean difference is significant at the 0.05 level.

Appendix 44: Treatment effect on P levels of *P. annua* growing in a P sufficient rootzone. Effect on leaf, crown and root P levels of P. annua growing in a P sufficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control), Tukey pairwise comparisons.

Multiple Comparisons

Dependent Variable: Tissue P levels

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Leaf	KCl	Pi	-844.16667*	7.42094	.000	-863.4423	-824.8910
		Phi	92.00000*	7.42094	.000	72.7243	111.2757
	Pi	KCl	844.16667*	7.42094	.000	824.8910	863.4423
		Phi	936.16667*	7.42094	.000	916.8910	955.4423
	Phi	KCl	-92.00000*	7.42094	.000	-111.2757	-72.7243
		Pi	-936.16667*	7.42094	.000	-955.4423	-916.8910
Crown	KCl	Pi	-411.16667*	6.48217	.000	-428.0039	-394.3294
		Phi	-889.66667*	6.48217	.000	-906.5039	-872.8294
	Pi	KCl	411.16667*	6.48217	.000	394.3294	428.0039
		Phi	-478.50000*	6.48217	.000	-495.3372	-461.6628
	Phi	KCl	889.66667*	6.48217	.000	872.8294	906.5039
		Pi	478.50000*	6.48217	.000	461.6628	495.3372
Root	KCl	Pi	-229.50000*	7.41695	.000	-248.7653	-210.2347
		Phi	-790.00000*	7.41695	.000	-809.2653	-770.7347
	Pi	KCl	229.50000*	7.41695	.000	210.2347	248.7653
		Phi	-560.50000*	7.41695	.000	-579.7653	-541.2347
	Phi	KCl	790.00000*	7.41695	.000	770.7347	809.2653
		Pi	560.50000*	7.41695	.000	541.2347	579.7653

*. The mean difference is significant at the 0.05 level.

Appendix 45: Figure 4-25 Treatment effect on P levels of *L. perenne* growing in a P deficient rootzone. Effect on leaf, crown and root P levels of *L. perenne,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control), Tukey pairwise comparisons.

Multiple Comparisons

Dependent Variable: Tissue P levels

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Leaf	KCl	Pi	-1128.50000*	7.38718	.000	-1147.6880	-1109.3120
		Phi	-413.33333*	7.38718	.000	-432.5213	-394.1454
	Pi	KCl	1128.50000*	7.38718	.000	1109.3120	1147.6880
		Phi	715.16667*	7.38718	.000	695.9787	734.3546
	Phi	KCl	413.33333*	7.38718	.000	394.1454	432.5213
		Pi	-715.16667*	7.38718	.000	-734.3546	-695.9787
Crown	KCl	Pi	-725.50000*	8.15612	.000	-746.6853	-704.3147
		Phi	-2542.33333*	8.15612	.000	-2563.5186	-2521.1481
	Pi	KCl	725.50000*	8.15612	.000	704.3147	746.6853
		Phi	-1816.83333*	8.15612	.000	-1838.0186	-1795.6481
	Phi	KCl	2542.33333*	8.15612	.000	2521.1481	2563.5186
		Pi	1816.83333*	8.15612	.000	1795.6481	1838.0186
Root	KCl	Pi	-136.00000*	6.30960	.000	-152.3890	-119.6110
		Phi	-1026.50000*	6.30960	.000	-1042.8890	-1010.1110
	Pi	KCl	136.00000*	6.30960	.000	119.6110	152.3890
		Phi	-890.50000*	6.30960	.000	-906.8890	-874.1110
	Phi	KCl	1026.50000*	6.30960	.000	1010.1110	1042.8890
		Pi	890.50000*	6.30960	.000	874.1110	906.8890

*. The mean difference is significant at the 0.05 level.

Appendix 46: Figure 4-26 Treatment effect on P levels of *P. annua* growing in a P deficient rootzone. Effect on leaf, crown and root P levels of *P. annua,* growing in a P deficient rootzone, following sequential treatments over a six month period, of Pi, Phi and KCl (control).Tukey pairwise comparisons.

Multiple Comparisons

Dependent Variable: Tissue P levels

Tukey HSD

Tissues			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Leaf	KCl	Pi	-999.00000*	6.26572	.000	-1015.2750	-982.7250
		Phi	-211.66667*	6.26572	.000	-227.9417	-195.3916
	Pi	KCl	999.00000*	6.26572	.000	982.7250	1015.2750
		Phi	787.33333*	6.26572	.000	771.0583	803.6084
	Phi	KCl	211.66667*	6.26572	.000	195.3916	227.9417
		Pi	-787.33333*	6.26572	.000	-803.6084	-771.0583
Crown	KCl	Pi	-903.66667*	7.61091	.000	-923.4358	-883.8976
		Phi	-1725.00000*	7.61091	.000	-1744.7691	-1705.2309
	Pi	KCl	903.66667*	7.61091	.000	883.8976	923.4358
		Phi	-821.33333*	7.61091	.000	-841.1024	-801.5642
	Phi	KCl	1725.00000*	7.61091	.000	1705.2309	1744.7691
		Pi	821.33333*	7.61091	.000	801.5642	841.1024
Root	KCl	Pi	-416.83333*	5.58072	.000	-431.3291	-402.3376
		Phi	-1031.00000*	5.58072	.000	-1045.4958	-1016.5042
	Pi	KCl	416.83333*	5.58072	.000	402.3376	431.3291
		Phi	-614.16667*	5.58072	.000	-628.6624	-599.6709
	Phi	KCl	1031.00000*	5.58072	.000	1016.5042	1045.4958
		Pi	614.16667*	5.58072	.000	599.6709	628.6624

*. The mean difference is significant at the 0.05 level.

Chapter 5 statistics

Appendix 47: Fig 5-18 TPC as GAE mg/g dw, in infected and non-infected field trial plots. TPC as GAE mg/g dw, in infected and non-infected turfgrass leaf tissues, sampled from field trial plots over three years. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Pairwise Comparisons

Dependent Variable: TPC greens 2012

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.338*	.016	.000	.305	.371
	control	Infected	-.338*	.016	.000	-.371	-.305
A.stolonifera	Infected	control	.436*	.016	.000	.403	.469
	control	Infected	-.436*	.016	.000	-.469	-.403

Dependent Variable: TPC greens 2013

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.279*	.018	.000	.242	.316
	control	Infected	-.279*	.018	.000	-.316	-.242
A.stolonifera	Infected	control	.340*	.013	.000	.313	.367
	control	Infected	-.340*	.013	.000	-.367	-.313

Dependent Variable: TPC greens 2014

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.347*	.016	.000	.313	.381
	control	Infected	-.347*	.016	.000	-.381	-.313
A.stolonifera	Infected	control	.214*	.019	.000	.175	.253
	control	Infected	-.214*	.019	.000	-.253	-.175

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 48: Fig 5-19 TPC as GAE mg/g dw, in infected and non-infected greenhouse turfgrasses. TPC as GAE mg/g dw, in infected and non-infected turfgrass leaf tissues, sampled from control and M. nivale inoculated greenhouse plants over three years. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Pairwise Comparisons

Dependent Variable: TPC greenhouse 2012

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.419*	.015	.000	.387	.451
	control	Infected	-.419*	.015	.000	-.451	-.387
A.stolonifera	Infected	control	.403*	.016	.000	.370	.436
	control	Infected	-.403*	.016	.000	-.436	-.370

Dependent Variable: TPC greenhouse 2013

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.530*	.012	.000	.504	.556
	control	Infected	-.530*	.012	.000	-.556	-.504
A.stolonifera	Infected	control	.394*	.015	.000	.362	.426
	control	Infected	-.394*	.015	.000	-.426	-.362

Dependent Variable: TPC greenhouse 2014

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Infected	control	.520*	.017	.000	.484	.556
	control	Infected	-.520*	.017	.000	-.556	-.484
A.stolonifera	Infected	control	.603*	.019	.000	.563	.643
	control	Infected	-.603*	.019	.000	-.643	-.563

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 49: Figure 5-20 TPC as GAE mg/g dw in turfgrass tissues sampled from trial plots (greens) over 72 hours post treatment. TPC as GAE mg/g dw, of turfgrass leaf tissues, sampled from trial plots over 72 hours following SDW (control), Pi and Phi treatment. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Multiple Comparisons

Bonferonni

Turfgrass_species				Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	TPC_0hr_greens	Control	Pi	-.00600	.01681	.932	-.0477	.0357
			Phi	.03600	.01681	.100	-.0057	.0777
		Pi	Control	.00600	.01681	.932	-.0357	.0477
			Phi	.04200*	.01681	.048	.0003	.0837
		Phi	Control	-.03600	.01681	.100	-.0777	.0057
			Pi	-.04200*	.01681	.048	-.0837	-.0003
	TPC_1hr_greens	Control	Pi	-.03100	.01448	.100	-.0669	.0049
			Phi	-.03100	.01448	.100	-.0669	.0049
		Pi	Control	.03100	.01448	.100	-.0049	.0669
			Phi	0.00000	.01448	1.000	-.0359	.0359
		Phi	Control	.03100	.01448	.100	-.0049	.0669
			Pi	0.00000	.01448	1.000	-.0359	.0359
	TPC_6hr_greens	Control	Pi	.03912	.01587	.052	-.0002	.0785
			Phi	.01400	.01587	.656	-.0254	.0534
		Pi	Control	-.03912	.01587	.052	-.0785	.0002
			Phi	-.02512	.01587	.270	-.0645	.0142
		Phi	Control	-.01400	.01587	.656	-.0534	.0254
			Pi	.02512	.01587	.270	-.0142	.0645
	TPC_12hr_greens	Control	Pi	-.18980*	.01513	.000	-.2273	-.1523
			Phi	-.28876*	.01513	.000	-.3263	-.2512
		Pi	Control	.18980*	.01513	.000	.1523	.2273
			Phi	-.09896*	.01513	.000	-.1365	-.0614
		Phi	Control	.28876*	.01513	.000	.2512	.3263
			Pi	.09896*	.01513	.000	.0614	.1365
	TPC_24hr_greens	Control	Pi	-.22196*	.01351	.000	-.2555	-.1885
			Phi	-.15096*	.01351	.000	-.1845	-.1175
		Pi	Control	.22196*	.01351	.000	.1885	.2555
			Phi	.07100*	.01351	.000	.0375	.1045
		Phi	Control	.15096*	.01351	.000	.1175	.1845
			Pi	-.07100*	.01351	.000	-.1045	-.0375
	TPC_48hr_greens	Control	Pi	-.18944*	.01757	.000	-.2330	-.1459
			Phi	-.18944*	.01757	.000	-.2330	-.1459
		Pi	Control	.18944*	.01757	.000	.1459	.2330
			Phi	.00000	.01757	1.000	-.0436	.0436
		Phi	Control	.18944*	.01757	.000	.1459	.2330
			Pi	.00000	.01757	1.000	-.0436	.0436
	TPC_72hr_greens	Control	Pi	-.17696*	.01500	.000	-.2142	-.1398
			Phi	-.22428*	.01500	.000	-.2615	-.1871
		Pi	Control	.17696*	.01500	.000	.1398	.2142
			Phi	-.04732*	.01500	.011	-.0845	-.0101
		Phi	Control	.22428*	.01500	.000	.1871	.2615
			Pi	.04732*	.01500	.011	.0101	.0845

A.stolonifera	TPC_0hr_greens	Control	Pi	-.00800	.01457	.848	-.0441	.0281
			Phi	.05100*	.01457	.004	.0149	.0871
		Pi	Control	.00800	.01457	.848	-.0281	.0441
			Phi	.05900*	.01457	.001	.0229	.0951
		Phi	Control	-.05100*	.01457	.004	-.0871	-.0149
			Pi	-.05900*	.01457	.001	-.0951	-.0229
	TPC_1hr_greens	Control	Pi	-.03000	.01909	.275	-.0773	.0173
			Phi	-.13400*	.01909	.000	-.1813	-.0867
		Pi	Control	.03000	.01909	.275	-.0173	.0773
			Phi	-.10400*	.01909	.000	-.1513	-.0567
		Phi	Control	.13400*	.01909	.000	.0867	.1813
			Pi	.10400*	.01909	.000	.0567	.1513
	TPC_6hr_greens	Control	Pi	-.05700*	.01395	.001	-.0916	-.0224
			Phi	-.18600*	.01395	.000	-.2206	-.1514
		Pi	Control	.05700*	.01395	.001	.0224	.0916
			Phi	-.12900*	.01395	.000	-.1636	-.0944
		Phi	Control	.18600*	.01395	.000	.1514	.2206
			Pi	.12900*	.01395	.000	.0944	.1636
	TPC_12hr_greens	Control	Pi	-.20000*	.01613	.000	-.2400	-.1600
			Phi	-.26700*	.01613	.000	-.3070	-.2270
		Pi	Control	.20000*	.01613	.000	.1600	.2400
			Phi	-.06700*	.01613	.001	-.1070	-.0270
		Phi	Control	.26700*	.01613	.000	.2270	.3070
			Pi	.06700*	.01613	.001	.0270	.1070
	TPC_24hr_greens	Control	Pi	-.19400*	.01560	.000	-.2327	-.1553
			Phi	-.17500*	.01560	.000	-.2137	-.1363
		Pi	Control	.19400*	.01560	.000	.1553	.2327
			Phi	.01900	.01560	.453	-.0197	.0577
		Phi	Control	.17500*	.01560	.000	.1363	.2137
			Pi	-.01900	.01560	.453	-.0577	.0197
	TPC_48hr_greens	Control	Pi	-.18700*	.01689	.000	-.2289	-.1451
			Phi	-.19800*	.01689	.000	-.2399	-.1561
		Pi	Control	.18700*	.01689	.000	.1451	.2289
			Phi	-.01100	.01689	.793	-.0529	.0309
		Phi	Control	.19800*	.01689	.000	.1561	.2399
			Pi	.01100	.01689	.793	-.0309	.0529
	TPC_72hr_greens	Control	Pi	-.13800*	.01560	.000	-.1767	-.0993
			Phi	-.16700*	.01560	.000	-.2057	-.1283
		Pi	Control	.13800*	.01560	.000	.0993	.1767
			Phi	-.02900	.01560	.170	-.0677	.0097
		Phi	Control	.16700*	.01560	.000	.1283	.2057
			Pi	.02900	.01560	.170	-.0097	.0677

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 50: Figure 5-21 TPC as GAE mg/g dw in turfgrass tissues sampled from greenhouse turfgrasses over 72 hours post treatment. TPC as GAE mg/g dw, of turfgrass leaf tissues from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Multiple Comparisons

Bonferonni

Turfgrass_species				Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	TPC_0hr_greenhouse	Control	Pi	.14600*	.01597	.000	.1064	.1856
			Phi	.03600	.01597	.080	-.0036	.0756
		Pi	Control	-.14600*	.01597	.000	-.1856	-.1064
			Phi	-.11000*	.01597	.000	-.1496	-.0704
		Phi	Control	-.03600	.01597	.080	-.0756	.0036
			Pi	.11000*	.01597	.000	.0704	.1496
	TPC_1hr_greenhouse	Control	Pi	.02700	.01551	.209	-.0115	.0655
			Phi	-.10000*	.01551	.000	-.1385	-.0615
		Pi	Control	-.02700	.01551	.209	-.0655	.0115
			Phi	-.12700*	.01551	.000	-.1655	-.0885
		Phi	Control	.10000*	.01551	.000	.0615	.1385
			Pi	.12700*	.01551	.000	.0885	.1655
	TPC_6hr_greenhouse	Control	Pi	-.00500	.01374	.930	-.0391	.0291
			Phi	.01100	.01374	.706	-.0231	.0451
		Pi	Control	.00500	.01374	.930	-.0291	.0391
			Phi	.01600	.01374	.484	-.0181	.0501
		Phi	Control	-.01100	.01374	.706	-.0451	.0231
			Pi	-.01600	.01374	.484	-.0501	.0181
	TPC_12hr_greenhouse	Control	Pi	-.07800*	.01601	.000	-.1177	-.0383
			Phi	-.12600*	.01601	.000	-.1657	-.0863
		Pi	Control	.07800*	.01601	.000	.0383	.1177
			Phi	-.04800*	.01601	.015	-.0877	-.0083
		Phi	Control	.12600*	.01601	.000	.0863	.1657
			Pi	.04800*	.01601	.015	.0083	.0877
	TPC_24hr_greenhouse	Control	Pi	-.09000*	.01428	.000	-.1254	-.0546
			Phi	-.17200*	.01428	.000	-.2074	-.1366
		Pi	Control	.09000*	.01428	.000	.0546	.1254
			Phi	-.08200*	.01428	.000	-.1174	-.0466
		Phi	Control	.17200*	.01428	.000	.1366	.2074
			Pi	.08200*	.01428	.000	.0466	.1174
	TPC_48hr_greenhouse	Control	Pi	-.25700*	.01546	.000	-.2953	-.2187
			Phi	-.30800*	.01546	.000	-.3463	-.2697
		Pi	Control	.25700*	.01546	.000	.2187	.2953
			Phi	-.05100*	.01546	.007	-.0893	-.0127
		Phi	Control	.30800*	.01546	.000	.2697	.3463
			Pi	.05100*	.01546	.007	.0127	.0893
	TPC_72hr_greenhouse	Control	Pi	-.20200*	.01411	.000	-.2370	-.1670
			Phi	-.17500*	.01411	.000	-.2100	-.1400
		Pi	Control	.20200*	.01411	.000	.1670	.2370
			Phi	.02700	.01411	.154	-.0080	.0620
		Phi	Control	.17500*	.01411	.000	.1400	.2100
			Pi	-.02700	.01411	.154	-.0620	.0080

A.stolonifera	TPC_0hr_greenhouse	Control	Pi	.03700*	.01479	.048	.0003	.0737
			Phi	-.04500*	.01479	.014	-.0817	-.0083
		Pi	Control	-.03700*	.01479	.048	-.0737	-.0003
			Phi	-.08200*	.01479	.000	-.1187	-.0453
		Phi	Control	.04500*	.01479	.014	.0083	.0817
			Pi	.08200*	.01479	.000	.0453	.1187
	TPC_1hr_greenhouse	Control	Pi	-.00300	.01497	.978	-.0401	.0341
			Phi	-.03700	.01497	.051	-.0741	.0001
		Pi	Control	.00300	.01497	.978	-.0341	.0401
			Phi	-.03400	.01497	.077	-.0711	.0031
		Phi	Control	.03700	.01497	.051	-.0001	.0741
			Pi	.03400	.01497	.077	-.0031	.0711
	TPC_6hr_greenhouse	Control	Pi	-.05300*	.01632	.008	-.0935	-.0125
			Phi	-.03700	.01632	.078	-.0775	.0035
		Pi	Control	.05300*	.01632	.008	.0125	.0935
			Phi	.01600	.01632	.595	-.0245	.0565
		Phi	Control	.03700	.01632	.078	-.0035	.0775
			Pi	-.01600	.01632	.595	-.0565	.0245
	TPC_12hr_greenhouse	Control	Pi	-.12800*	.01731	.000	-.1709	-.0851
			Phi	-.16700*	.01731	.000	-.2099	-.1241
		Pi	Control	.12800*	.01731	.000	.0851	.1709
			Phi	-.03900	.01731	.080	-.0819	.0039
		Phi	Control	.16700*	.01731	.000	.1241	.2099
			Pi	.03900	.01731	.080	-.0039	.0819
	TPC_24hr_greenhouse	Control	Pi	-.15200*	.01593	.000	-.1915	-.1125
			Phi	-.09100*	.01593	.000	-.1305	-.0515
		Pi	Control	.15200*	.01593	.000	.1125	.1915
			Phi	.06100*	.01593	.002	.0215	.1005
		Phi	Control	.09100*	.01593	.000	.0515	.1305
			Pi	-.06100*	.01593	.002	-.1005	-.0215
	TPC_48hr_greenhouse	Control	Pi	-.05700*	.01724	.007	-.0998	-.0142
			Phi	-.11800*	.01724	.000	-.1608	-.0752
		Pi	Control	.05700*	.01724	.007	.0142	.0998
			Phi	-.06100*	.01724	.004	-.1038	-.0182
		Phi	Control	.11800*	.01724	.000	.0752	.1608
			Pi	.06100*	.01724	.004	.0182	.1038
	TPC_72hr_greenhouse	Control	Pi	-.11700*	.01487	.000	-.1539	-.0801
			Phi	-.09100*	.01487	.000	-.1279	-.0541
		Pi	Control	.11700*	.01487	.000	.0801	.1539
			Phi	.02600	.01487	.206	-.0109	.0629
		Phi	Control	.09100*	.01487	.000	.0541	.1279
			Pi	-.02600	.01487	.206	-.0629	.0109

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 51: Figs 5-22 and 5-23, TPC as GAE mg/g dw, in turfgrass tissues sampled from field trial plots and greenhouse plants. TPC as GAE mg/g dw, in turfgrass tissues sampled from field trial plots and greenhouse plants following six, monthly applications of SDW (control), Pi and Phi. Pairwise comparisons using Bonferroni correction at $p < 0.05$

Pairwise Comparisons

Dependent Variable: Trial plot samples

Turfgrass species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Control	Pi	-.071*	.018	.001	-.116	-.026
		Phi	-.700*	.018	.000	-.745	-.655
	Pi	Control	.071*	.018	.001	.026	.116
		Phi	-.629*	.018	.000	-.674	-.584
	Phi	Control	.700*	.018	.000	.655	.745
		Pi	.629*	.018	.000	.584	.674
A.stolonifera	Control	Pi	-.280*	.016	.000	-.320	-.240
		Phi	-.932*	.016	.000	-.972	-.892
	Pi	Control	.280*	.016	.000	.240	.320
		Phi	-.652*	.016	.000	-.692	-.612
	Phi	Control	.932*	.016	.000	.892	.972
		Pi	.652*	.016	.000	.612	.692

Dependent Variable: Greenhouse samples

Turfgrass_species			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
P.annua	Control	Pi	-.243*	.016	.000	-.284	-.202
		Phi	-1.106*	.016	.000	-1.147	-1.065
	Pi	Control	.243*	.016	.000	.202	.284
		Phi	-.863*	.016	.000	-.904	-.822
	Phi	Control	1.106*	.016	.000	1.065	1.147
		Pi	.863*	.016	.000	.822	.904
A.stolonifera	Control	Pi	-.289*	.016	.000	-.329	-.249
		Phi	-.847*	.016	.000	-.887	-.807
	Pi	Control	.289*	.016	.000	.249	.329
		Phi	-.558*	.016	.000	-.598	-.518
	Phi	Control	.847*	.016	.000	.807	.887
		Pi	.558*	.016	.000	.518	.598

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 52: Figure 5-25 TPC as GAE mg/g dw, in *M. nivale* infected tissues over 10 dpi in greenhouse turfgrasses. TPC as GAE mg/g dw, in *M. nivale* infected tissues over 10 dpi in greenhouse turfgrasses treated with SDW (control), Pi, Phi (1 app) and Phi (6 apps). Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Multiple Comparisons

Bonferroni

Turfgrass_species				Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval Lower	95% Confidence Interval Upper
P.annua	dpi0	Control	Pi	-.06600*	.02215	.031	-.1278	-.0042
			Phi 1 app	-.03000	.02215	1.000	-.0918	.0318
			Phi 6 apps	-.19400*	.02215	.000	-.2558	-.1322
		Pi	Control	.06600*	.02215	.031	.0042	.1278
			Phi 1 app	.03600	.02215	.677	-.0258	.0978
			Phi 6 apps	-.12800*	.02215	.000	-.1898	-.0662
		Phi 1 app	Control	.03000	.02215	1.000	-.0318	.0918
			Pi	-.03600	.02215	.677	-.0978	.0258
			Phi 6 apps	-.16400*	.02215	.000	-.2258	-.1022
		Phi 6 apps	Control	.19400*	.02215	.000	.1322	.2558
			Pi	.12800*	.02215	.000	.0662	.1898
			Phi 1 app	.16400*	.02215	.000	.1022	.2258
	dpi2	Control	Pi	-.09700*	.01389	.000	-.1358	-.0582
			Phi 1 app	-.32900*	.01389	.000	-.3678	-.2902
			Phi 6 apps	-.31400*	.01389	.000	-.3528	-.2752
		Pi	Control	.09700*	.01389	.000	.0582	.1358
			Phi 1 app	-.23200*	.01389	.000	-.2708	-.1932
			Phi 6 apps	-.21700*	.01389	.000	-.2558	-.1782
		Phi 1 app	Control	.32900*	.01389	.000	.2902	.3678
			Pi	.23200*	.01389	.000	.1932	.2708
			Phi 6 apps	.01500	.01389	1.000	-.0238	.0538
		Phi 6 apps	Control	.31400*	.01389	.000	.2752	.3528
			Pi	.21700*	.01389	.000	.1782	.2558
			Phi 1 app	-.01500	.01389	1.000	-.0538	.0238
	dpi4	Control	Pi	0.00000	.01499	1.000	-.0419	.0419
			Phi 1 app	-.15500*	.01499	.000	-.1969	-.1131
			Phi 6 apps	-.16600*	.01499	.000	-.2079	-.1241
		Pi	Control	0.00000	.01499	1.000	-.0419	.0419
			Phi 1 app	-.15500*	.01499	.000	-.1969	-.1131
			Phi 6 apps	-.16600*	.01499	.000	-.2079	-.1241
		Phi 1 app	Control	.15500*	.01499	.000	.1131	.1969
			Pi	.15500*	.01499	.000	.1131	.1969
			Phi 6 apps	-.01100	.01499	1.000	-.0529	.0309
		Phi 6 apps	Control	.16600*	.01499	.000	.1241	.2079
			Pi	.16600*	.01499	.000	.1241	.2079
			Phi 1 app	.01100	.01499	1.000	-.0309	.0529
	dpi6	Control	Pi	-.10100*	.01205	.000	-.1346	-.0674
			Phi 1 app	-.32500*	.01205	.000	-.3586	-.2914
			Phi 6 apps	-.34000*	.01205	.000	-.3736	-.3064
		Pi	Control	.10100*	.01205	.000	.0674	.1346
			Phi 1 app	-.22400*	.01205	.000	-.2576	-.1904
			Phi 6 apps	-.23900*	.01205	.000	-.2726	-.2054
		Phi 1 app	Control	.32500*	.01205	.000	.2914	.3586
			Pi	.22400*	.01205	.000	.1904	.2576
			Phi 6 apps	-.01500	.01205	1.000	-.0486	.0186
		Phi 6 apps	Control	.34000*	.01205	.000	.3064	.3736
			Pi	.23900*	.01205	.000	.2054	.2726
			Phi 1 app	.01500	.01205	1.000	-.0186	.0486
	dpi8	Control	Pi	-.10200*	.01150	.000	-.1341	-.0699
			Phi 1 app	-.48000*	.01150	.000	-.5121	-.4479
			Phi 6 apps	-.56300*	.01150	.000	-.5951	-.5309
		Pi	Control	.10200*	.01150	.000	.0699	.1341
			Phi 1 app	-.37800*	.01150	.000	-.4101	-.3459
			Phi 6 apps	-.46100*	.01150	.000	-.4931	-.4289
		Phi 1 app	Control	.48000*	.01150	.000	.4479	.5121
			Pi	.37800*	.01150	.000	.3459	.4101
			Phi 6 apps	-.08300*	.01150	.000	-.1151	-.0509
		Phi 6 apps	Control	.56300*	.01150	.000	.5309	.5951
			Pi	.46100*	.01150	.000	.4289	.4931
			Phi 1 app	.08300*	.01150	.000	.0509	.1151
	dpi10	Control	Pi	-.05900*	.01109	.000	-.0900	-.0280
			Phi 1 app	-.33700*	.01109	.000	-.3680	-.3060
			Phi 6 apps	-.36900*	.01109	.000	-.4000	-.3380
		Pi	Control	.05900*	.01109	.000	.0280	.0900
			Phi 1 app	-.27800*	.01109	.000	-.3090	-.2470
			Phi 6 apps	-.31000*	.01109	.000	-.3410	-.2790
		Phi 1 app	Control	.33700*	.01109	.000	.3060	.3680
			Pi	.27800*	.01109	.000	.2470	.3090
			Phi 6 apps	-.03200*	.01109	.039	-.0630	-.0010
		Phi 6 apps	Control	.36900*	.01109	.000	.3380	.4000
			Pi	.31000*	.01109	.000	.2790	.3410
			Phi 1 app	.03200*	.01109	.039	.0010	.0630

A.stolonifera	dpi0	Control	Pi	.05900*	.01475	.002	.0178	.1002
			Phi 1 app	.01900	.01475	1.000	-.0222	.0602
			Phi 6 apps	-.24000*	.01475	.000	-.2812	-.1988
		Pi	Control	-.05900*	.01475	.002	-.1002	-.0178
			Phi 1 app	-.04000	.01475	.061	-.0812	.0012
			Phi 6 apps	-.29900*	.01475	.000	-.3402	-.2578
		Phi 1 app	Control	-.01900	.01475	1.000	-.0602	.0222
			Pi	.04000	.01475	.061	-.0012	.0812
			Phi 6 apps	-.25900*	.01475	.000	-.3002	-.2178
		Phi 6 apps	Control	.24000*	.01475	.000	.1988	.2812
			Pi	.29900*	.01475	.000	.2578	.3402
			Phi 1 app	.25900*	.01475	.000	.2178	.3002
	dpi2	Control	Pi	-.32860*	.01641	.000	-.3744	-.2828
			Phi 1 app	-.45100*	.01641	.000	-.4968	-.4052
			Phi 6 apps	-.51000*	.01641	.000	-.5558	-.4642
		Pi	Control	.32860*	.01641	.000	.2828	.3744
			Phi 1 app	-.12240*	.01641	.000	-.1682	-.0766
			Phi 6 apps	-.18140*	.01641	.000	-.2272	-.1356
		Phi 1 app	Control	.45100*	.01641	.000	.4052	.4968
			Pi	.12240*	.01641	.000	.0766	.1682
			Phi 6 apps	-.05900*	.01641	.006	-.1048	-.0132
		Phi 6 apps	Control	.51000*	.01641	.000	.4642	.5558
			Pi	.18140*	.01641	.000	.1356	.2272
			Phi 1 app	.05900*	.01641	.006	.0132	.1048
	dpi4	Control	Pi	-.13300*	.01501	.000	-.1749	-.0911
			Phi 1 app	-.15100*	.01501	.000	-.1929	-.1091
			Phi 6 apps	-.29000*	.01501	.000	-.3319	-.2481
		Pi	Control	.13300*	.01501	.000	.0911	.1749
			Phi 1 app	-.01800	.01501	1.000	-.0599	.0239
			Phi 6 apps	-.15700*	.01501	.000	-.1989	-.1151
		Phi 1 app	Control	.15100*	.01501	.000	.1091	.1929
			Pi	.01800	.01501	1.000	-.0239	.0599
			Phi 6 apps	-.13900*	.01501	.000	-.1809	-.0971
		Phi 6 apps	Control	.29000*	.01501	.000	.2481	.3319
			Pi	.15700*	.01501	.000	.1151	.1989
			Phi 1 app	.13900*	.01501	.000	.0971	.1809
	dpi6	Control	Pi	-.19800*	.01197	.000	-.2314	-.1646
			Phi 1 app	-.40300*	.01197	.000	-.4364	-.3696
			Phi 6 apps	-.50700*	.01197	.000	-.5404	-.4736
		Pi	Control	.19800*	.01197	.000	.1646	.2314
			Phi 1 app	-.20500*	.01197	.000	-.2384	-.1716
			Phi 6 apps	-.30900*	.01197	.000	-.3424	-.2756
		Phi 1 app	Control	.40300*	.01197	.000	.3696	.4364
			Pi	.20500*	.01197	.000	.1716	.2384
			Phi 6 apps	-.10400*	.01197	.000	-.1374	-.0706
		Phi 6 apps	Control	.50700*	.01197	.000	.4736	.5404
			Pi	.30900*	.01197	.000	.2756	.3424
			Phi 1 app	.10400*	.01197	.000	.0706	.1374
	dpi8	Control	Pi	.08400*	.00997	.000	.0562	.1118
			Phi 1 app	-.18700*	.00997	.000	-.2148	-.1592
			Phi 6 apps	-.20800*	.00997	.000	-.2358	-.1802
		Pi	Control	-.08400*	.00997	.000	-.1118	-.0562
			Phi 1 app	-.27100*	.00997	.000	-.2988	-.2432
			Phi 6 apps	-.29200*	.00997	.000	-.3198	-.2642
		Phi 1 app	Control	.18700*	.00997	.000	.1592	.2148
			Pi	.27100*	.00997	.000	.2432	.2988
			Phi 6 apps	-.02100	.00997	.253	-.0488	.0068
		Phi 6 apps	Control	.20800*	.00997	.000	.1802	.2358
			Pi	.29200*	.00997	.000	.2642	.3198
			Phi 1 app	.02100	.00997	.253	-.0068	.0488
	dpi10	Control	Pi	.04500*	.01089	.001	.0146	.0754
			Phi 1 app	-.26400*	.01089	.000	-.2944	-.2336
			Phi 6 apps	-.32900*	.01089	.000	-.3594	-.2986
		Pi	Control	-.04500*	.01089	.001	-.0754	-.0146
			Phi 1 app	-.30900*	.01089	.000	-.3394	-.2786
			Phi 6 apps	-.37400*	.01089	.000	-.4044	-.3436
		Phi 1 app	Control	.26400*	.01089	.000	.2336	.2944
			Pi	.30900*	.01089	.000	.2786	.3394
			Phi 6 apps	-.06500*	.01089	.000	-.0954	-.0346
		Phi 6 apps	Control	.32900*	.01089	.000	.2986	.3594
			Pi	.37400*	.01089	.000	.3436	.4044
			Phi 1 app	.06500*	.01089	.000	.0346	.0954

*. The mean difference is significant at the 0.05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Appendix 53: Figure 5-26 H_2O_2 concentrations in un-infected greenhouse turfgrass tissues. H_2O_2 concentrations as µmol H_2O_2/g fw, in turfgrass leaf tissues collected from greenhouse samples over 72 hours following SDW (control), Pi and Phi treatment. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Multiple Comparisons

Bonferonni

Turfgrass_species				Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	0 hpa	Control	Pi	-.92000	.61885	.313	-2.4544	.6144
			Phi	-.46500	.61885	.735	-1.9994	1.0694
		Pi	Control	.92000	.61885	.313	-.6144	2.4544
			Phi	.45500	.61885	.745	-1.0794	1.9894
		Phi	Control	.46500	.61885	.735	-1.0694	1.9994
			Pi	-.45500	.61885	.745	-1.9894	1.0794
	1 hpa	Control	Pi	-2.94500*	.56456	.000	-4.3448	-1.5452
			Phi	-1.97800*	.56456	.004	-3.3778	-.5782
		Pi	Control	2.94500*	.56456	.000	1.5452	4.3448
			Phi	.96700	.56456	.219	-.4328	2.3668
		Phi	Control	1.97800*	.56456	.004	.5782	3.3778
			Pi	-.96700	.56456	.219	-2.3668	.4328
	6 hpa	Control	Pi	-4.08000*	.61978	.000	-5.6167	-2.5433
			Phi	-4.53000*	.61978	.000	-6.0667	-2.9933
		Pi	Control	4.08000*	.61978	.000	2.5433	5.6167
			Phi	-.45000	.61978	.750	-1.9867	1.0867
		Phi	Control	4.53000*	.61978	.000	2.9933	6.0667
			Pi	.45000	.61978	.750	-1.0867	1.9867
	12 hpa	Control	Pi	-1.00000	.61347	.251	-2.5211	.5211
			Phi	-.48000	.61347	.717	-2.0011	1.0411
		Pi	Control	1.00000	.61347	.251	-.5211	2.5211
			Phi	.52000	.61347	.677	-1.0011	2.0411
		Phi	Control	.48000	.61347	.717	-1.0411	2.0011
			Pi	-.52000	.61347	.677	-2.0411	1.0011
	24 hpa	Control	Pi	-.52000	.76333	.776	-2.4126	1.3726
			Phi	-1.95000*	.76333	.042	-3.8426	-.0574
		Pi	Control	.52000	.76333	.776	-1.3726	2.4126
			Phi	-1.43000	.76333	.166	-3.3226	.4626
		Phi	Control	1.95000*	.76333	.042	.0574	3.8426
			Pi	1.43000	.76333	.166	-.4626	3.3226
	48 hpa	Control	Pi	1.43000	.68695	.113	-.2732	3.1332
			Phi	1.03000	.68695	.307	-.6732	2.7332
		Pi	Control	-1.43000	.68695	.113	-3.1332	.2732
			Phi	-.40000	.68695	.831	-2.1032	1.3032
		Phi	Control	-1.03000	.68695	.307	-2.7332	.6732
			Pi	.40000	.68695	.831	-1.3032	2.1032
	72 hpa	Control	Pi	-.60000	.71864	.685	-2.3818	1.1818
			Phi	-1.56000	.71864	.095	-3.3418	.2218
		Pi	Control	.60000	.71864	.685	-1.1818	2.3818
			Phi	-.96000	.71864	.388	-2.7418	.8218
		Phi	Control	1.56000	.71864	.095	-.2218	3.3418
			Pi	.96000	.71864	.388	-.8218	2.7418

A.stolonifera	0 hpa	Control	Pi	.20000	.59634	.940	-1.2786	1.6786
			Phi	.81000	.59634	.376	-.6686	2.2886
		Pi	Control	-.20000	.59634	.940	-1.6786	1.2786
			Phi	.61000	.59634	.569	-.8686	2.0886
		Phi	Control	-.81000	.59634	.376	-2.2886	.6686
			Pi	-.61000	.59634	.569	-2.0886	.8686
	1 hpa	Control	Pi	-.91500	.56618	.256	-2.3188	.4888
			Phi	-.45500	.56618	.704	-1.8588	.9488
		Pi	Control	.91500	.56618	.256	-.4888	2.3188
			Phi	.46000	.56618	.699	-.9438	1.8638
		Phi	Control	.45500	.56618	.704	-.9488	1.8588
			Pi	-.46000	.56618	.699	-1.8638	.9438
	6 hpa	Control	Pi	-4.02000*	.71272	.000	-5.7871	-2.2529
			Phi	-2.62000*	.71272	.003	-4.3871	-.8529
		Pi	Control	4.02000*	.71272	.000	2.2529	5.7871
			Phi	1.40000	.71272	.141	-.3671	3.1671
		Phi	Control	2.62000*	.71272	.003	.8529	4.3871
			Pi	-1.40000	.71272	.141	-3.1671	.3671
	12 hpa	Control	Pi	-.72500	.54705	.394	-2.0814	.6314
			Phi	-.36000	.54705	.789	-1.7164	.9964
		Pi	Control	.72500	.54705	.394	-.6314	2.0814
			Phi	.36500	.54705	.784	-.9914	1.7214
		Phi	Control	.36000	.54705	.789	-.9964	1.7164
			Pi	-.36500	.54705	.784	-1.7214	.9914
	24 hpa	Control	Pi	-.44500	.57633	.723	-1.8740	.9840
			Phi	-.90500	.57633	.275	-2.3340	.5240
		Pi	Control	.44500	.57633	.723	-.9840	1.8740
			Phi	-.46000	.57633	.707	-1.8890	.9690
		Phi	Control	.90500	.57633	.275	-.5240	2.3340
			Pi	.46000	.57633	.707	-.9690	1.8890
	48 hpa	Control	Pi	-.11500	.59507	.980	-1.5904	1.3604
			Phi	.76000	.59507	.420	-.7154	2.2354
		Pi	Control	.11500	.59507	.980	-1.3604	1.5904
			Phi	.87500	.59507	.321	-.6004	2.3504
		Phi	Control	-.76000	.59507	.420	-2.2354	.7154
			Pi	-.87500	.59507	.321	-2.3504	.6004
	72 hpa	Control	Pi	-1.46000	.73959	.138	-3.2938	.3738
			Phi	-2.54500*	.73959	.005	-4.3788	-.7112
		Pi	Control	1.46000	.73959	.138	-.3738	3.2938
			Phi	-1.08500	.73959	.322	-2.9188	.7488
		Phi	Control	2.54500*	.73959	.005	.7112	4.3788
			Pi	1.08500	.73959	.322	-.7488	2.9188

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 54: Figure 5-27 H_2O_2 concentrations in *M. nivale* infected greenhouse turfgrass tissues. H_2O_2 concentrations as µmol H_2O_2/g fw, in SDW (control), Pi, Phi (1 app) and Phi (6 apps) treated tissues of *M. nivale* infected *P.annua* and *A. stolonifera* greenhouse plants over 10 days post inoculation. Pairwise comparisons using Bonferroni correction at $p < 0.05$.

Multiple Comparisons

Bonferonni

Turfgrass_species				Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval	
							Lower Bound	Upper Bound
P.annua	dpi0	Control	Pi	-.06600*	.02215	.025	-.1257	-.0063
			Phi 1 app	-.03000	.02215	.535	-.0897	.0297
			Phi 6 apps	-.19400*	.02215	.000	-.2537	-.1343
		Pi	Control	.06600*	.02215	.025	.0063	.1257
			Phi 1 app	.03600	.02215	.378	-.0237	.0957
			Phi 6 apps	-.12800*	.02215	.000	-.1877	-.0683
		Phi 1 app	Control	.03000	.02215	.535	-.0297	.0897
			Pi	-.03600	.02215	.378	-.0957	.0237
			Phi 6 apps	-.16400*	.02215	.000	-.2237	-.1043
		Phi 6 apps	Control	.19400*	.02215	.000	.1343	.2537
			Pi	.12800*	.02215	.000	.0683	.1877
			Phi 1 app	.16400*	.02215	.000	.1043	.2237
	dpi2	Control	Pi	-.09700*	.01389	.000	-.1344	-.0596
			Phi 1 app	-.32900*	.01389	.000	-.3664	-.2916
			Phi 6 apps	-.31400*	.01389	.000	-.3514	-.2766
		Pi	Control	.09700*	.01389	.000	.0596	.1344
			Phi 1 app	-.23200*	.01389	.000	-.2694	-.1946
			Phi 6 apps	-.21700*	.01389	.000	-.2544	-.1796
		Phi 1 app	Control	.32900*	.01389	.000	.2916	.3664
			Pi	.23200*	.01389	.000	.1946	.2694
			Phi 6 apps	.01500	.01389	.704	-.0224	.0524
		Phi 6 apps	Control	.31400*	.01389	.000	.2766	.3514
			Pi	.21700*	.01389	.000	.1796	.2544
			Phi 1 app	-.01500	.01389	.704	-.0524	.0224
	dpi4	Control	Pi	0.00000	.01499	1.000	-.0404	.0404
			Phi 1 app	-.15500*	.01499	.000	-.1954	-.1146
			Phi 6 apps	-.16600*	.01499	.000	-.2064	-.1256
		Pi	Control	0.00000	.01499	1.000	-.0404	.0404
			Phi 1 app	-.15500*	.01499	.000	-.1954	-.1146
			Phi 6 apps	-.16600*	.01499	.000	-.2064	-.1256
		Phi 1 app	Control	.15500*	.01499	.000	.1146	.1954
			Pi	.15500*	.01499	.000	.1146	.1954
			Phi 6 apps	-.01100	.01499	.883	-.0514	.0294
		Phi 6 apps	Control	.16600*	.01499	.000	.1256	.2064
			Pi	.16600*	.01499	.000	.1256	.2064
			Phi 1 app	.01100	.01499	.883	-.0294	.0514
	dpi6	Control	Pi	-.10100*	.01205	.000	-.1335	-.0685
			Phi 1 app	-.32500*	.01205	.000	-.3575	-.2925
			Phi 6 apps	-.34000*	.01205	.000	-.3725	-.3075
		Pi	Control	.10100*	.01205	.000	.0685	.1335
			Phi 1 app	-.22400*	.01205	.000	-.2565	-.1915
			Phi 6 apps	-.23900*	.01205	.000	-.2715	-.2065
		Phi 1 app	Control	.32500*	.01205	.000	.2925	.3575
			Pi	.22400*	.01205	.000	.1915	.2565
			Phi 6 apps	-.01500	.01205	.603	-.0475	.0175
		Phi 6 apps	Control	.34000*	.01205	.000	.3075	.3725
			Pi	.23900*	.01205	.000	.2065	.2715
			Phi 1 app	.01500	.01205	.603	-.0175	.0475
	dpi8	Control	Pi	-.10200*	.01150	.000	-.1330	-.0710
			Phi 1 app	-.48000*	.01150	.000	-.5110	-.4490
			Phi 6 apps	-.56300*	.01150	.000	-.5940	-.5320
		Pi	Control	.10200*	.01150	.000	.0710	.1330
			Phi 1 app	-.37800*	.01150	.000	-.4090	-.3470
			Phi 6 apps	-.46100*	.01150	.000	-.4920	-.4300
		Phi 1 app	Control	.48000*	.01150	.000	.4490	.5110
			Pi	.37800*	.01150	.000	.3470	.4090
			Phi 6 apps	-.08300*	.01150	.000	-.1140	-.0520
		Phi 6 apps	Control	.56300*	.01150	.000	.5320	.5940
			Pi	.46100*	.01150	.000	.4300	.4920
			Phi 1 app	.08300*	.01150	.000	.0520	.1140
	dpi10	Control	Pi	-.05900*	.01109	.000	-.0889	-.0291
			Phi 1 app	-.33700*	.01109	.000	-.3669	-.3071
			Phi 6 apps	-.36900*	.01109	.000	-.3989	-.3391
		Pi	Control	.05900*	.01109	.000	.0291	.0889
			Phi 1 app	-.27800*	.01109	.000	-.3079	-.2481
			Phi 6 apps	-.31000*	.01109	.000	-.3399	-.2801
		Phi 1 app	Control	.33700*	.01109	.000	.3071	.3669
			Pi	.27800*	.01109	.000	.2481	.3079
			Phi 6 apps	-.03200*	.01109	.032	-.0619	-.0021
		Phi 6 apps	Control	.36900*	.01109	.000	.3391	.3989
			Pi	.31000*	.01109	.000	.2801	.3399
			Phi 1 app	.03200*	.01109	.032	.0021	.0619

A.stolonifera	dpi0	Control	Pi	.05900*	.01475	.002	.0193	.0987
			Phi 1 app	.01900	.01475	.576	-.0207	.0587
			Phi 6 apps	-.24000*	.01475	.000	-.2797	-.2003
		Pi	Control	-.05900*	.01475	.002	-.0987	-.0193
			Phi 1 app	-.04000*	.01475	.048	-.0797	-.0003
			Phi 6 apps	-.29900*	.01475	.000	-.3387	-.2593
		Phi 1 app	Control	-.01900	.01475	.576	-.0587	.0207
			Pi	.04000*	.01475	.048	.0003	.0797
			Phi 6 apps	-.25900*	.01475	.000	-.2987	-.2193
		Phi 6 apps	Control	.24000*	.01475	.000	.2003	.2797
			Pi	.29900*	.01475	.000	.2593	.3387
			Phi 1 app	.25900*	.01475	.000	.2193	.2987
	dpi2	Control	Pi	-.32860*	.01641	.000	-.3728	-.2844
			Phi 1 app	-.45100*	.01641	.000	-.4952	-.4068
			Phi 6 apps	-.51000*	.01641	.000	-.5542	-.4658
		Pi	Control	.32860*	.01641	.000	.2844	.3728
			Phi 1 app	-.12240*	.01641	.000	-.1666	-.0782
			Phi 6 apps	-.18140*	.01641	.000	-.2256	-.1372
		Phi 1 app	Control	.45100*	.01641	.000	.4068	.4952
			Pi	.12240*	.01641	.000	.0782	.1666
			Phi 6 apps	-.05900*	.01641	.005	-.1032	-.0148
		Phi 6 apps	Control	.51000*	.01641	.000	.4658	.5542
			Pi	.18140*	.01641	.000	.1372	.2256
			Phi 1 app	.05900*	.01641	.005	.0148	.1032
	dpi4	Control	Pi	-.13300*	.01501	.000	-.1734	-.0926
			Phi 1 app	-.15100*	.01501	.000	-.1914	-.1106
			Phi 6 apps	-.29000*	.01501	.000	-.3304	-.2496
		Pi	Control	.13300*	.01501	.000	.0926	.1734
			Phi 1 app	-.01800	.01501	.632	-.0584	.0224
			Phi 6 apps	-.15700*	.01501	.000	-.1974	-.1166
		Phi 1 app	Control	.15100*	.01501	.000	.1106	.1914
			Pi	.01800	.01501	.632	-.0224	.0584
			Phi 6 apps	-.13900*	.01501	.000	-.1794	-.0986
		Phi 6 apps	Control	.29000*	.01501	.000	.2496	.3304
			Pi	.15700*	.01501	.000	.1166	.1974
			Phi 1 app	.13900*	.01501	.000	.0986	.1794
	dpi6	Control	Pi	-.19800*	.01197	.000	-.2302	-.1658
			Phi 1 app	-.40300*	.01197	.000	-.4352	-.3708
			Phi 6 apps	-.50700*	.01197	.000	-.5392	-.4748
		Pi	Control	.19800*	.01197	.000	.1658	.2302
			Phi 1 app	-.20500*	.01197	.000	-.2372	-.1728
			Phi 6 apps	-.30900*	.01197	.000	-.3412	-.2768
		Phi 1 app	Control	.40300*	.01197	.000	.3708	.4352
			Pi	.20500*	.01197	.000	.1728	.2372
			Phi 6 apps	-.10400*	.01197	.000	-.1362	-.0718
		Phi 6 apps	Control	.50700*	.01197	.000	.4748	.5392
			Pi	.30900*	.01197	.000	.2768	.3412
			Phi 1 app	.10400*	.01197	.000	.0718	.1362
	dpi8	Control	Pi	.08400*	.00997	.000	.0572	.1108
			Phi 1 app	-.18700*	.00997	.000	-.2138	-.1602
			Phi 6 apps	-.20800*	.00997	.000	-.2348	-.1812
		Pi	Control	-.08400*	.00997	.000	-.1108	-.0572
			Phi 1 app	-.27100*	.00997	.000	-.2978	-.2442
			Phi 6 apps	-.29200*	.00997	.000	-.3188	-.2652
		Phi 1 app	Control	.18700*	.00997	.000	.1602	.2138
			Pi	.27100*	.00997	.000	.2442	.2978
			Phi 6 apps	-.02100	.00997	.170	-.0478	.0058
		Phi 6 apps	Control	.20800*	.00997	.000	.1812	.2348
			Pi	.29200*	.00997	.000	.2652	.3188
			Phi 1 app	.02100	.00997	.170	-.0058	.0478
	dpi10	Control	Pi	.04500*	.01089	.001	.0157	.0743
			Phi 1 app	-.26400*	.01089	.000	-.2933	-.2347
			Phi 6 apps	-.32900*	.01089	.000	-.3583	-.2997
		Pi	Control	-.04500*	.01089	.001	-.0743	-.0157
			Phi 1 app	-.30900*	.01089	.000	-.3383	-.2797
			Phi 6 apps	-.37400*	.01089	.000	-.4033	-.3447
		Phi 1 app	Control	.26400*	.01089	.000	.2347	.2933
			Pi	.30900*	.01089	.000	.2797	.3383
			Phi 6 apps	-.06500*	.01089	.000	-.0943	-.0357
		Phi 6 apps	Control	.32900*	.01089	.000	.2997	.3583
			Pi	.37400*	.01089	.000	.3447	.4033
			Phi 1 app	.06500*	.01089	.000	.0357	.0943

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.