Fungal wars: the underlying molecular repertoires of combating mycelia

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Abstract

Non-self contact between fungi elicits strong morphological and biochemical reactions in the mycelia of interacting species. Although these reactions appear to be species- and interaction-specific, some responses such as pigmentation, increased secretion of phenol-oxidases, barrage formation and sealing of the mycelia front are common responses in most interactions. Hence, some species recruit similar molecular machineries in response to non-self. Increasing number of fully sequenced and annotated fungal genomes and advances in genome-wide and global proteome analytical tools now allow researchers to use techniques such as RNA sequencing, micro and macroarray analysis, 2-dimensional protein gel profiling, and differential display of mRNA to probe the underlying molecular mechanisms of combative mycelial interactions. This review provides an overview of the genes and proteins found to be differentially expressed in conflicting fungal mycelia by the use of 'omics' tools. Connections between observed gene and protein repertoires of competing mycelia and the attendant morphological and biochemical changes are presented.

Introduction

Fungi play a central role in the recycling of nutrients in nature (Hiscox and Boddy, 2017; van der Wal *et al*., 2013). Often, dead plant materials are simultaneously colonized by multiple species. As each species spreads in an attempt to gain control of colonized substrate, they come in contact with competing species. Depending on the arsenal and/or defense mechanisms available to each competitor, non-self mycelial contact is characterized by a range of biochemical and physiological reactions including pigmentation, formation of mycelial barrage to stave off opposing fungal mycelia; sealing of the mycelial front, and up-regulation of hydrophobic compounds (Rayner *et al*., 1994, 1995; Boddy, 2000; Peiris *et al*., 2008; Ujor *et al*., 2012a; Hiscox et al., 2015). These interactions are also characterized by the secretion of extracellular enzymes such as laccase, manganese peroxidase, lignin peroxidase, and chitinase (Boddy, 2000; Kubicek *et al*., 2001).

Antagonism between competing fungi may also occur at a distance, mediated by volatile organic compounds (VOCs) including alcohols, aldehydes, ketones, and aromatic compounds, which may possess antifungal properties (El Ariebi et al., 2016; Evans *et al*., 2008; Hynes *et al*., 2007; Humphris *et al*., 2002), as well as by non-volatile antifungal compounds (antibiosis) secreted by one or both interacting species (Waing et al., 2015). Broadly, interspecific mycelial interactions can result in replacement, where one species annexes the territory occupied by another, or deadlock where neither fungus gain the territory occupied by the other (Hiscox and Boddy, 2017; Boddy, 2000). Partial replacement is also reported where one species momentarily grows into the territory occupied by the other, but does not completely replace the opposition, and there is mutual replacement, which entails encroachment upon the opposing fungus' territory by each competing species (Boddy, 2000).

Extensive study of antagonistic mycelial interactions over the past four to five decades has led to the commercial use of aggressive species such as *Phlebiopsis gigantea* and *Trichoderma* species in the control of wood-rot and phytopathogenic fungi (Boddy, 2000; Kubicek *et al*., 2001; Adomas *et al*., 2006). More recently, studies of fungal interactions have revealed that *Fusarium verticillioides* has the potential to reduce the severity of corn smut caused by *Ustilago maydis* (Jonkers *et al*., 2012). However, the molecular mechanisms underlying characteristic responses during fungus-fungus interactions, vis-à-vis the genes and proteins that mediate these responses have only begun to emerge. Such knowledge is required to improve the robustness and persistence of biocontrol fungi in the field as well as to shed more light on the structure and dynamics of fungal communities, and how these interactions influence wood degradation and nutrient cycling (Boddy, 2000).

This is a rapidly evolving field of research due to the use of fungal-derived enzymes for accelerating lignocellulose hydrolysis to generate fermentable sugars in biofuel production, as well as other diverse applications relevant to bioremediation, food, paper, wine and textile industries (Hatakka, 2001; Hatvani *et al*., 2002; Binder and Raines, 2010). Early studies of combative interactions largely focused on the outcomes of interactions; namely, the patterns of pigmentation, enzyme activity profiling of the interaction interface, the metabolite repertoire and morphological changes associated with the combat zone. Recent advances in genomics and proteomics coupled with a marked increase in the availability of fully sequenced fungal genomes now make it possible for researchers to attempt to unravel the molecular mechanisms underlying the complex, biochemical and morphological reactions triggered by non-self contact between fungi. This has led to the identification of specific genes and proteins whose roles hitherto were poorly described or unknown in relation to non-self interactions between fungi. In this review, we summarize the

cellular aspects of interspecific mycelial interactions between fungi with an emphasis on the genes and proteins whose differential expressions have highlighted likely response mechanisms (**Table 1**) recruited by various species during combat, and the occurrence of some of these mechanisms across different species.

Mycelial stabilization and cell wall fortification

Different fungi growing on the same substrate may interact from a distance by exchanging chemical cues, thereby resulting in mutual or selective inhibition without contact (Heilmann-Clausen & Boddy, 2005). In contrast, some species only respond to, or exhibit signs of response to the opposition after direct mycelial contact. In a combative scenario, modifications to the structure and strength of the targeted hyphae are warranted to withstand an invasive opponent. To stave off an aggressive competitor, most species form a dense mat of aerial mycelia (a barrage; **Fig. 1A**) at the interaction interface (Rayner *et al*., 1994; Boddy, 2000). Interestingly, global mRNA profiling of non-self-interacting fungal mycelia showed increased expression of genes encoding hydrophobins. These are a group of small fungal proteins that have been implicated in hyphal aerial growth (Wösten *et al*., 1999; Torkkeli *et al*., 2002; Adomas *et al*., 2006). In fact, hydrophobins are involved in cell wall assembly, lowering surface tension, formation of amphipathic films, and assembly of surface layers in mycelia, which collectively enhance the formation of aerial hyphae (Wösten et al., 1999; Linder et al., 2002; Torkkeli et al., 2002; Adomas et al., 2006). In a similar study using cDNA macroarray, Adomas *et al*. (2006) reported varying expression levels for homologs of hydrophobins I, II, and III in *Phlebiopsis gigantea* and *Heterobasidion parviporum* at different stages of competitive interaction. Interestingly, hydrophobin I, which forms stable aggregates (Wösten *et al*., 1999) was strongly up-regulated within the confluence zone (barrage zone). Further, Jonkers *et al*. (2012) reported up-regulation of

hydrophobin-encoding genes in the mycelia of *Ustilago maydis* and *Fusarium verticillioides* interacting with each other. It is likely that hydrophobins orchestrate barrage formation as they are upregulated in barrage-forming mycelia; most plausibly to seal off mycelial front against the approaching competitor (**Fig. 1A**). In addition to sealing of mycelia front, more antagonistic species that metabolize competitor cell wall appear to recruit hydrophobins for attachment to host mycelia (Jonkers *et al*., 2012).

Hydrophobins are not the only biochemical players thought to participate in the sealing of the mycelial front and in barrage formation. Eyre *et al*. (2010) reported up-regulation of glycoside hydrolase, 1,3-beta glucan synthase, and α-1,2-mannosyltransferase genes in *Trametes versicolor*, while Ujor *et al*. (2012b) detected an increase in the amount of a glycosyltransferase in *Schizophyllum commune* during various interaction pairings. In addition to their roles in carbohydrate metabolism, glycoside hydrolase, 1,3-beta glucan synthase, and α-1,2-mannosyltransferase have been implicated in the strengthening and improvement of the plasticity of fungal cell wall, as well as in hyphal branching (Yuan *et al*., 2008; Latgé, 2007; Häusler *et al*., 1992). Similarly, glycosyltransferases are involved in the construction and polymerization of cell wall components in both prokaryotes and eukaryotes (Lim & Bowles, 2004; Hashimoto *et al*., 2009). Increases in the levels of these enzymes during combative interactions may indicate an attempt to fortify the mycelia following non-self contact. Such mycelial fortification would not only prevent the progress of the competitor (in the case of barrage formation), but also attempt to limit the passage of toxic metabolites often associated with combative mycelial interactions via the cell wall barrier.

Barrage formation and modification of cell wall plasticity serve as transient resistance when faced with an aggressive cell wall-lysing fungus such as a *Trichoderma* species (**Fig. 1**).

Once the cell wall barrier is breached (**Fig. 1 D & E**) it is critical to protect the cell membrane and cytosolic components. This entails recruitment of defence mechanisms that operate underneath the cell wall. Recent findings implicate hexagonal protein-1 (HEX-1) in such mechanism. HEX-1 is the major component of the Woronin body, a peroxisome-derived microbody that is used by fungi for septal plugging and sealing of central pores upon cellular injury (such as cell wall lysis) or during starvation (**Fig. 2**; Alymore et al., 1984; Soundarajan *et al*., 2004). Ujor *et al*. (2012b) reported significant increase in the amount of HEX-1 in *S. commune* antagonized by *T. viride* (**Fig. 1F**). Similarly, Kwon *et al*. (2009) reported increased expression of HEX-1 in *Fusarium graminearum* infected by the mycovirus *F. graminearum* virus-DK21 (FgV-DK21). Septal plugging and sealing of central pores is most likely an attempt to localize the effects of cellular injury to the affected hyphal compartments, thereby preventing the loss of cytoplasmic contents from unaffected compartments (Alymore *et al*., 1984). Therefore, upregulation of HEX-1 in antagonized *S. commune* and F. *graminearum* (in both cases by aggressive species capable of breaching the cell wall barrier) is indicative of an attempt by both fungi to limit cellular damage by increased biosynthesis of Woronin body.

Enhancement/reduction of primary or secondary metabolism in competing mycelia

One of the key responses that characterize non-self mycelial contact is reduction or abolition of mycelial extension post contact in one or both species, perhaps as a result of switch to secondary metabolic phase. The molecular signatures of different interacting species underscore this suggestion. However, this depends to a great extent on the arsenal available to a species relative to the threat presented by the competitor(s). Some species appear to expedite nutrient acquisition, thus, primary metabolic activities, while others scale-down such activities with concomitant decreases in the levels of primary metabolic proteins and genes that suggest a switch

to secondary metabolism. Below, we discuss different molecular signatures of interacting mycelia that suggest possible arrest or a slowing of mycelial growth and metabolic activities in some competing species, and upregulation of primary metabolic activities in other species during competitive interactions.

Indicators of reduced growth and increased secretion of secondary metabolites: mRNA profiling of *Physisporinus sanguinolentus* paired against *Heterobasidion annosum* revealed repression of homologs of a fimbrin protein-encoding gene, cystathionine gamma-lyase and a mitochondrial protein import component in the former (Iakovlev *et al*., 2004). Fimbrin protein is an active participant in cellular growth, as it contributes to the polarization of the actin cytoskeleton during mycelial growth (Iakovlev *et al*., 2004). On the other hand, cystathionine gamma-lyase is involved in cysteine biosynthesis (Yamagata *et al*., 1993), while mitochondrial protein import machinery is involved in protein biogenesis and energy generation (Dudek *et al*., 2013). Simultaneous down-regulation of these genes in *P. sanguinolentus* confronted by *H. annosum* is an indication of likely halt of primary metabolism and mycelial growth in *P. sanguinolentus*. Although *P. sanguinolentus* secretes toxic compounds that have been shown to inhibit germination of *H. annosum* spores (Svensson et al., 2001), Iakovlev et al. (2004) did not screen for production of such toxic compounds in this study. Additionally, results of the competition between both species (overgrowth, deadlock, mutual or partial overgrowth) were not reported. However, downregulation of primary metabolic genes in the supposedly more antagonistic *P. sanguinolentus* paired against the phytopathogen, *H. annosum*, suggests that the biochemical arsenal of *P. sanguinolentus* might not have recruited in this experimental set up. Environmental conditions influence combative interactions between fungi (Boddy, 2000), thus, the conditions under which the above study was conducted likely contributed to the observed results.

Jonkers *et al*. (2012) observed increases in the amounts of phenylalanine ammonia lyase (PAL) mRNA in the mycelia of *Ustilago maydis* antagonized by *F. verticillioides*, while Ujor *et al*. (2012b) showed significant increases in the protein levels of PAL in *S. commune* paired against *T. viride*. PAL is a stationary phase enzyme, which catalyzes deamination of L-phenylalanine into *cis*-cinamic acid, the entry point into the phenylpropanoid pathway; a major source of phenolic compounds in fungi and plants (Kim *et al*., 2001; Gómez-Vàsquez *et al*., 2004). Enhanced secretion of phenolic compounds is an established secondary metabolic trait, which is particularly recurrent during interspecific mycelial combat (Boddy, 2000; Griffith *et al*., 1994b; Humphries *et al*., 2002; Ujor *et al*., 2012a; Peiris *et al*., 2008). Upregulation of the secondary metabolism-associated PAL in less antagonistic *U. maydis* and *S. commune*, when paired against robust antagonists such as *F. verticillioides* and *T. viride* might be indicative of switch from primary to secondary metabolism, most plausibly to ensure judicious utilization of scarce metabolic resources when under attack. Specifically, upregulation of PAL could be attributed to an attempt by these species (*Ustilago maydis* and *S. commune*) to recruit their chemical arsenal against their competitors, albeit less potent given the outcome of both interactions. Also, induction of secondary metabolism in less combative species may be indicative of defeat. Often, outcompeted species undergo cessation of growth following superior nutrient sequestration by an aggressive competitor and competitor-induced mycelial damage. In fact, possible depletion of essential nutrients, accumulation of secondary metabolites and pH alterations by early colonizers of wood have been reported to influence the patterns/outcome of competition when substrates were precolonized by early colonizers before inoculation of late colonizers (Hiscox et al., 2016). Therefore, the cellular signature of outcompeted mycelia would be most likely characterized by reduced expression of primary metabolic genes and enhanced expression of their secondary

metabolic counterparts. In this case of *T. viride*, which degrades and metabolizes competitor mycelia (Kubicek et al., 2001), primary metabolism increases during competition (Ujor et al., 2012b). Therefore, it appears outcompeted species might downregulate primary metabolism and vice versa. Deadlock involving cessation of growth in both competing species could most likely result in downregulation of proteins involved in primary metabolism, particularly in the interaction interface, although this would likely depend on the timing of sample collection for DNA/mRNA/protein profiling. Possibly, early on during deadlock, one or both species may upregulate nutrient uptake machinery in an effort to colonize the substratum and out-compete the opposing fungus.

Down-regulation of glycolysis: In *S. commune* antagonized by *T. viride*, three different proteins (likely subunits of a polymeric protein) carrying the enolase conserved domain were significantly downregulated in addition to a triphosphate isomerase (TIM; Ujor, 2010; Ujor *et al*., 2012b). Enolase catalyses the penultimate step in glycolysis - conversion of 2-phospho-D-glycerate to phosphoenolpyruvate (Pandey *et al*., 2009) - whereas triphosphate isomerase (TIM), catalyses the reversible inter-conversion of dihydroxyacetone phosphate and D-glyceraldehyde-3-phopshate (Cui & Karplus, 2001; Walden et al., 2004). Down-regulation of these proteins indicates reduction in glycolytic activities. Generally, down-regulation of glycolysis is a means of regulating cellular metabolism (Shenton & Grant 2003; Grüning *et al*., 2014).

Under conditions of oxidative stress, down-regulation of glycolysis favours increased flux of glucose equivalents via the pentose phosphate pathway (PPP), which ultimately enhances NADPH generation (**Fig. 3**; Shenton & Grant 2003; Grüning et al., 2014), and both TIM and enolase were down-regulated in *S. cerevisiae* during exposure to the oxidative stressor, H_2O_2 (Shenton & Grant 2003). NADPH supplies the reducing power critical for physiological defence

against oxidative stress (Klatzien *et al*., 1994). Cancer cells and rapidly respiring or proliferating cells, including yeast cells enlist a similar mechanism (down-regulation of glycolysis and up-regulation of the PPP) in an attempt to tide over oxidative stress. Interestingly, indicators of oxidative stress such as lipid peroxidation and protein carbonylation increased in the mycelia of *S. commune* confronted by *T. viride* (Ujor et al., 2012a). Although the authors did not detect increased levels of PPP proteins under the conditions of study, using more sensitive silver-staining technique in a 2-dimensional protein profiling, Peiris (2009) observed significant increases in the levels of both glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in the mycelia of *Stereum hirsutum* confronted by both *Coprinus micaceus* and *Coprinus disseminates*. G6PDH and 6PGDH catalyse the first two steps of the PPP, both of which are key NADPH-generating reactions (Shenton & Grant 2003; **Fig 2**). Notably, where *S. hirsutum* overgrew *C. micaceus*, *S. hirsutum* was outcompeted by *C. disseminates* (Peiris, 2009). It appears therefore, that *S. hirsutum* upregulates similar mechanisms irrespective of the competitor or both competing species recruit similar mechanisms, possibly increased secretion of the same or similar chemical compounds during competition. Interestingly, Peiris et al. (2008) observed similar metabolic profiles in *S. hirsutum* irrespective of the competitor. The authors ascribed this to likely competition-induced stress or merely a defence mechanism.

TIM has been identified as a key regulatory enzyme in the glycolytic pathway whose down-regulation or inhibition confers considerable benefits under conditions of oxidative stress in *S. cerevisiae* and *Caenoharbditis elegans*, by stimulating the accumulation of PPP metabolites (Rasler *et al*., 2006, 2007; Grüning *et al*., 2014). It is worthy of note however, that low levels of TIM were retained in the *T. viride*-confronted mycelia of *S. commune* (Ujor *et al*., 2012b), indicating that glycolysis was not completely inhibited at the time of sample collection for

proteomic analysis. Therefore, concomitant reduction in the levels of enolase may serve to further truncate glycolysis. Furthermore, down-regulation of enolase results in the accumulation of phosphoglycerate (**Fig. 3**). In *Aspergillus nidulans*, exposure to osmotic stress led to down-regulation of enolase (Kim *et al*., 2007), most likely to accumulate phosphoglycerate for enhanced cell wall/membrane construction, in an effort to withstand osmotic stress. Similarly, during interaction with *T. viride,* osmolytes in the form of sugar alcohols increased significantly in the mycelia of *S. commune* (Ujor *et al*., 2012a).

Accumulation of sugar alcohols such as glycerol and arabitol is an established response to osmotic stress in fungi (Davis *et al*., 2000; Ramirez *et al*., 2004), and the mycelia of *S. commune* antagonized by *T. viride* exhibited signs of osmotic stress upon cell wall lysis, and consequently ruptured (**Fig. 1E&F**; Ujor *et al*., 2010). Hence, down-regulation of enolase in *S. commune* may furnish the cell with phosphoglycerate, which harnesses membrane biosynthesis for improved resistance of osmotic stress. This notion is supported by the fact that following the immediate stresses exerted on *S. commune* during mycoparasitic attack by *T. viride*, Chitin synthase gene was strongly up-regulated in *S. commune* 24 hours after contact, when compared to self-paired or unpaired mycelia, with concomitant up-regulation of a gene encoding an acyl carrier protein (Ujor, 2010). Chitin synthase is a key player in cell wall biogenesis, which supplies the cell with chitin, a major component of the fungal cell wall.

Up-regulation of nutrient uptake machinery: Whether fungi adopt down-regulation of primary metabolism or up-regulation of secondary metabolism depends on the mechanisms of attack and/or defense which they employ during combat, as well as the threat posed by the competitor. For instance, while glycolysis appeared to be down-regulated in *S. commune*, glycolytic enzymes were detected in higher amounts in *T. viride* when both fungi were paired, with significant increases in

the amounts of two glyceraldehyde-3-phosphate dehydrogenase (GAPDH) homologs (Ujor *et al*., 2012a). *T. viride* hydrolyzes the cell walls of its competitors and utilizes the released carbohydrates as substrates (Kubicek *et al*., 2001). This ultimately stimulates increased glycolytic flux. Similarly, some species such as *Phlebiopsis gigantea* replace their competitors by hyphal interference (Ikediugwu *et al*., 1970; Ikediugwu, 1976; Behrendt and Blanchette, 2001; Jonkers *et al*., 2012), and/or enhanced nutrient acquisition (Holdendrieder & Grieg 1998).

Increased nutrient uptake is accompanied by increased expression of nutrient processing enzymes. Using a macroarray in combination with virtual northern blotting, Adomas *et al*. (2006) observed considerable up-regulation of genes that putatively encode fructose biphosphate aldolase, GAPDH, endo-galacturonase, arginase, and glutamine synthetase, in the interaction interface between *P. gigantea* and *Heterobasidion parviporum*. This may reflect a direct attempt to more rapidly sequester nutrients from the interaction zone by one or both fungi. Interestingly, further analyses revealed that majority of the nutrient uptake genes originated from *P. gigantea*, which more often than not, out-competed *H. parviporum* (Adomas *et al*., 2006).

Using global protein profiling, Ujor *et al*. (2012a) reported an increase in the levels of an aspartyl protease, while Jonkers *et al*. (2012) observed up-regulation of different genes coding for an aspartyl protease, serine-like proteases, carboxylesterases, amidases and an endo-chitinase in separate fungal pairings, respectively. Conversely, aspartyl protease genes (25 transcripts) were both up- and down- regulated in *Pycnoporus coccineus* paired against *Botrytis cinerea* and *Coniophora puteana* (Arfi *et al*., 2013). However, majority of the transcripts were up-regulated. Proteases are among the complex web of enzymes employed by antagonistic, invasive fungal species for hydrolysis of competitor cell wall components (Dana *et al*., 2001; Schirmböck *et al*., 1994), which most likely accounts for their increases in the above-mentioned studies. Likewise,

genes whose protein products are involved in carbohydrate and nitrogen metabolism such as glycoside hydrolase, 1,3-beta glucan synthase, glutamate-cysteine ligase, α-1,2-mannosyltransferase, and phosphopyruvate dehydratase, were significantly up-regulated in *T. versicolor* during contact with *Stereum gausapatum* and *Bjekandera adusta*, which culminated in replacement by *Trametes versicolor* and deadlock, respectively (Eyre *et al*., 2010). Interestingly, these genes were not up-regulated when *T. versicolor* was confronted by *Hypholoma fasciculare*, in which the former was out-competed, possibly highlighting the relevance of nutrient acquisition during combat. It is likely though that compounds secreted into the immediate environment by *H. fasciculare* or other attack mechanisms employed by the latter impair the ability of *T. versicolor* to recruit its nutrient uptake machinery when both fungi are paired.

Implication of iron metabolism in mycelial combat: Genes involved in iron uptake namely, siderophore peptide synthetase (*fer3*), ferrichrome siderophore peptide synthetase (*sid2*), L-ornithine N5-oxygenase (*sid1*), dehydrogenase (*fer8*), and siderophore iron transporter (*fe7*) were significantly up-regulated in *Ustilago maydis* at day 2 of mycelial contact with *Fusarium verticillioides* (Jonkers *et al*., 2012). This may represent an attempt by *U. maydis* to sequester iron from the medium, which may serve to support its metabolism or starve *F. verticillioides* of iron, an essential nutrient in fungal metabolism (Johnson, 2008; Jo et al., 2009; Parente et al., 2011). Iron limitation has been reported to induce accumulation of heat shock proteins, due likely to cytoplasmic accumulation of unfolded proteins (Seo *et al*., 2008). Owing to its critical role in the coordination of redox chemistry, which influences multiple metabolic processes in both eukaryote and prokaryotes, microbes have evolved multiple mechanisms for iron uptake and storage, as well as for evading iron toxicity (Haas, 2014). Therefore, due to these salient functions of iron in fungal metabolism, and the iron-chelating properties of siderophores, siderophore-mediated iron

sequestration may be employed by microbes for defense or attack during antagonistic interactions, as well as for establishing mutual relations with other organisms (Haas, 2014). For instance, during antagonistic interactions, by scavenging iron from the environment, which are stored in organism-specific siderophores (xenosiderophores), one species may attempt to severely disrupt the metabolism, and hence growth of its competitor by limiting its access to iron. It is thought that some organisms neutralize this strategy during competitive interactions by evolving transporters capable of recognizing and importing opposition xenosiderophores into their cytoplasm from the immediate environment, thereby converting a growth-limiting to a growth-enhancing molecule (Haas, 2014).

Significant up-regulation of multiple siderophore-related genes in *U. maydis* confronted by *F. verticillioides* highlights likely importance of iron sequestration to the former during this interaction. The fact that siderophore biosynthesis is metabolically expensive, taking up L-arginine, which is also critical to protein biosynthesis, and L-ornithine a polyamine precursor, and the cofactors, NADPH and FAD (Frederick *et al*., 2011; Haas, 2014), upregulation of iron sequestering genes further underlines likely relevance of iron to *U. maydis* when antagonized by *F. verticillioides*. It is not clear though, whether this strategy was targeted at starving *F. verticillioides* of iron, or at supplying the cellular iron requirements of *U. maydis*. Investigation of the effects of iron on the outcome of *U. maydis* vs *F. verticillioides* competition, and the attendant cellular signature of both species under iron-limited and iron-replete conditions may prove instructive. Further, if extended to other fungal pairings, this may shed more light on yet to be identified salient roles that certain nutrients play during fungal interactions.

Overall, competition between fungi elicits the production of numerous enzymatic effectors (Score *et al*., 1997; Hiscox *et al*., 2010), and secondary metabolites (Boddy, 2000), and up-

regulation of repair machineries to fix damaged DNA, cell wall, and other cellular structures (Iakovlev *et al*., 2004; Ujor *et al*., 2012b; Arfi *et al*., 2013). Consequently, establishment of attack (even in the most fiercely invasive species) and defense mechanisms during competition is metabolically expensive, thus, accounting for the scale-down of primary metabolism to accommodate immediate physiological needs during combative interactions (Jonkers et al., 2012; Arfi *et al*., 2013). This is typified by the growth pattern of *P. coccineus* during interactions against 14 different species. Although *P. coccineus* out-competed the majority of its competitors in liquid cultures, this was accompanied by considerable decreases in biomass, and under-expression of primary metabolic genes, when compared to mono-cultures (Arfi *et al*., 2013). Clearly, the genes and proteins employed by a fungus, during competition, which determine its prevalent metabolic phase, depend on their attack or defense repertoires relative to those of their competitor(s). It appears fungi manage their metabolic economy in the face of opposition to ensure efficient utilization of resources during combat. This would be more pronounced in the wild where nutrient supply is far more limited, and even where nutrient is abundant, it is often in less readily utilizable forms, which are expensive to process pre-utilization.

Genes & proteins associated with detoxification and efflux of toxic metabolites during non-self interactions

Detoxification of toxic metabolites: A major feature of combative mycelial interaction is increased secretion of extracellular metabolites, particularly phenolic compounds, which are thought to contribute to the discoloration of mycelia in the confluence zone (Griffith et al., 1994a, b, & c; Rayner et al., 1994; Score et al., 1997; Boddy, 2000). Some of these metabolites can be toxic to one or both competing species, thereby eliciting the conscription of detoxifying mechanisms to contend with the ensuing toxicity (**Fig. 4**). Although their specific roles during

combative interactions remain to be well understood (Baldrian, 2004), laccases and peroxidases, whose activities have been repeatedly shown to increase significantly during combative interactions (Freitag & Morrell, 1991; White & Boddy, 1992; Griffith et al., 1994a, b, & c; Rayner et al., 1994; Score et al., 1997; Boddy, 2000; Iakovlev & Stenlid, 2000; Gregorio et al., 2006) are thought to play a key role in the detoxification of the confluence zone. This stems largely from their broad substrate range and particularly high activity against phenolic compounds.

Laccases oxidize phenolic compounds and aromatic amines, while class II peroxidases (heme peroxidases) including lignin peroxidase, and manganese peroxidase (MnP) catalyze hydrogen peroxide-mediated oxidation of aromatic and non-phenolic compounds, and lignin-derived phenolic compounds, respectively (Martinez et al., 2005; Bödeker et al., 2009; Hiscox et al., 2010). Surprisingly, to date, molecular profiling (transcriptomic or proteomic) of interacting fungi has yet to reveal significant differential expression of a laccase or a peroxidase (phenoloxidase). It is deserving of mention however, that Jonkers *et al*. (2012) and Ujor (2010) observed slight increases in the expression of a laccase gene in the interacting mycelia of *U. maydis* and *S. commune,* respectively.

Conversely, Arfi *et al*. (2013) noted that four laccase transcripts were expressed at high levels in both non-self-paired and self-paired mycelia of *P. coccineus*. Similarly, Hiscox *et al*. (2010) reported that despite increases in the activities of laccase and MnP in the confluence zones of the confronted mycelia of *T. versicolor*, transcriptional changes in the encoding genes were not dramatic. Consequently these authors (Hiscox et al., 2010; Arfi et al., 2013) suggested that changes in gene expression during mycelial competition may be rapid and transient, particularly for phenoloxidases. Therefore, they inferred that a narrow window during which changes in phenoloxidase gene expression occurs may have been missed in these studies. It is pertinent to

point out however, that although laccase transcripts were detected at high levels in both self-paired and non-self-paired mycelia of *P. coccineus* (Arfi *et al*., 2013), laccase activity increased significantly only in non-self-paired cultures, as with most interacting fungal species. It appears therefore, that posttranscriptional changes might be at play in regards to increases in laccase activity during antagonistic mycelial interactions. It has been suggested that laccase may be synthesized and stored in dormant form pre-contact, and released rapidly from intracellular stores upon contact, such that *de novo* synthesis (transcription) is not significant during non-self contact (Savoie *et al*., 1998; Hiscox *et al*., 2010). Also, it is likely that phenoloxidases, which are stable in the extracellular environment (Hiscox *et al*., 2010) are secreted into the culture medium pre-contact, and are activated by interaction-induced co-substrates or mediators absent in self-paired cultures. Such scenario would result in increased activity in mixed cultures relative to their self-paired counterparts, without significant differences in laccase gene expression.

Different studies have demonstrated increased laccases activity against recalcitrant aromatics and chlorophenols following the addition of co-substrates or mediators such as 2,2ʹ-azino-bis(3-ethylbenzthiazoline sulfonic acid) (ABTS), humic acid, 1-hydroxybenzotriazole, and 2,6-dimethoxyphenol (Roper *et al*., 1995; Keum and Li, 2004; Uhnáková *et al*., 2009). Similarly, Gregorio *et al*. (2006) showed that addition of filter-sterilized culture supernatant of *Marasmius pallescens* to liquid cultures of *Marasmiellus troyanus* elicited a rapid and strong increase in laccase activity, but not *vice versa* (addition of culture broth of *Marasmiellus troyanus* to cultures of *Marasmius pallescens*). Further, addition of autoclaved culture broth of *M. pallescens* to liquid cultures of *M. troyanus* induced a gradual and more prolonged increase in laccase activity. Clearly, the compounds secreted by one or both competitors have a considerable bearing on the level of ensuing laccase activity post-contact. Studies targeted at unravelling the complex interplay

between metabolites secreted during mycelial conflict and laccase activity may shed more light on the underlying mechanisms that govern the increases in phenoloxidase activity when non-self mycelia interact. Additionally, most fungi possess multiple copies of phenol-oxidases (Hiscox *et al*., 2010). As a result, depending on the molecular profiling tool employed (global or specific), up-regulation of a gene encoding a different, possibly more active isozyme would result in higher enzyme activity, but might not be captured during transcriptomic analysis (Hiscox *et al*., 2010).

In addition to phenoloxidases, other enzymes such as aldo/keto reductase, alcohol dehydrogenase, flavonol/cinnamoyl-CoA reductase, dioxygenase, short chain dehydrogenase/reductase (SDR), cytochrome P450 oxygenase, and glutathione S-transferase, which are involved in the detoxification of toxic compounds have been shown to accumulate in combating mycelia or in the interaction interface (**Table 2**). This underscores the significance of transforming the toxicants generated by one or both species during interspecific mycelial combat. Intracellular detoxification offers a secondary line of defence against toxic metabolites that evade the detoxifying enzymes that operate extracellularly (**Fig. 4**). For example, the expression levels of cytochrome P450 oxygenases increased in the competing mycelia of *P. gigantea, F. verticillioides,* and *U. maydis* (Adomas *et al*., 2006; Jonkers *et al*., 2012). Among other functions, cytochrome P450 oxygenases are involved in resistance to antifungal agents, and biotransformation of toxic compounds (van den Brink *et al*., 1998; O'Keeffe and Harder, 1991). Similarly, Eyre *et al*. (2010), Ujor *et al*. (2012a), and Arfi *et al*. (2013) reported increases in the expression levels of short chain dehydrogenase/reductases (SDR) in studies of different fungal pairings. SDRs are highly multifunctional intracellular enzymes that catalyse mainly redox reactions, utilising a broad spectrum of substrates including alcohols, sugars, steroids, and aromatics (Kallberg *et al*., 2002; Shafqat *et al*., 2006).

Efflux of toxic metabolites: Up-regulation of a transcriptional activator of an ATP-binding cassette (ABC) transporter gene was observed in paired mycelia of *S. commune* (Ujor *et al*., 2012a), whilst an ABC transporter was up-regulated in *P. gigantea* paired against *H. parviporum* (Adomas *et al*., 2006). Similarly, a gene coding for a multidrug transporter (similar to ABC transporters) was over-expressed in *U. maydis* during competition with *F. verticillioides* (Jonkers *et al*., 2012). Such transporters may serve to export any toxic metabolites that are not degraded by extracellular and intracellular enzyme defences (**Fig. 5**). Similar to antibiotic resistance in bacteria, it has been reported that the product of an ABC transporter gene in *Botrytis cinerea* plays a critical role in its resistance to a broad spectrum antibiotic-producing *Pseudomonas* spp. when both organisms were paired against each other (Schoonbeek *et al*., 2002). Conversely, *T. versicolor* under-expressed ABC transporters during combat with *S. gausapatum* (Eyre *et al*., 2010). This is a likely reflection of the reduced threat posed by *S. gausapatum* to *T. versicolor*, perhaps due to secretion of less potent or non-toxic metabolites by this fungus (*S. gausapatum*), which was replaced by *T. versicolor* on agar plates.

Genes and proteins that participate in the production and tolerance to reactive oxygen species (ROS)

Numerous studies have demonstrated the accumulation of ROS in the contact interface of interacting fungi (Tonberg and Olsson, 2002; Silar, 2005; Eyre et al., 2010). Both NADPH oxidase and a MAP kinase cascade have been implicated (at the level of gene regulation) in the generation of ROS during interspecific mycelial combat (Silar, 2005; Eyre *et al*., 2010). The exact role(s) of ROS during mycelial combat remain unclear, although signalling roles have suggested (Silar, 2005; Eyre et al., 2010). It is likely however, that they might constitute a part of the biochemical machinery employed by competing fungi for attack and defence purposes. For example, classII

peroxidases mentioned above as potential key players in the detoxification of the interaction interface require hydrogen peroxide for catalysis. Given their high reactivity, hence, toxicity to macromolecular structures, it is reasonable that ROS production would be accompanied by counter-active mechanisms targeted at mitigating ROS-mediated toxicity. Eyre *et al*. (2010), observed increases in the expression levels of catalase-encoding genes in *T. versicolor* confronted by *H. fasciculare*, *B. adusta* and *S. gausapatum*. However, these increases were not significant, indicating that *T. versicolor* might employ additional ROS-detoxifying mechanisms during combat. On the other hand, Iakovlev *et al*. (2004) reported up-regulation of a gene in *H. annosum* during combat with *Physisporinus sanguinolentus*, which bears significant semblance to *Coprinus cinereus recA*. The protein product of *recA*, RAD51 is a multifunctional enzyme that plays a key role in the repair of DNA damage and homologous recombination (Game, 1993; Stassen *et al*., 1997). RAD51 is strongly induced by γ-irradiation, a potent producer of DNA-damaging hydroxyl radicals (Stassen *et al*., 1997; Mikosch *et al*., 2001). Thus, the authors inferred that DNA-destructive free radicals were the likely trigger for *recA* up-regulation. Similarly, glutathione S-transferases were strongly up-regulated in *P. coccineus* when confronted by *Botrytis cinerea* (Arfi *et al*., 2013). Although glutathione S-transferases are involved in intracellular detoxification of toxic compounds (**Table 2**), they also participate in ROS detoxification (Veal *et al*., 2002; Thuillier *et al*., 2011). Whereas, a range of factors such as nutrient exhaustion (which disrupts cellular metabolism, hence, electron transport leading to ROS accumulation), active production of ROS as a signalling, defence, or attack response may account for the high levels of ROS associated with competing mycelia.

Upregulation of genes and proteins associated with protein and RNA stabilization

Phosphoglucomutase: It is likely that interacting fungi recruit trehalose for stress response. Trehalose is a non-reducing disaccharide consisting of two glucose units linked together by α, α-1,1-glycosidic bond, which is found in most forms of life including bacteria, yeast, fungi, and insects (Elbein *et al*., 2003). Trehalose functions as a stabilizer and protectant of proteins and membranes during various stress conditions (Elbein *et al*., 2003; Benaroudj *et al*., 2001; Davis *et al*., 2000), hence, it is thought that trehalose accumulation in stressed cells is in part a response to heat-, osmotic shock-, cold-, desiccation-, or dehydration-mediated perturbation of the cell wall (Levin, 2005). In fact, the cell wall integrity signaling pathway has been implicated in trehalose accumulation (Levin, 2005).

A phosphoglucomutase-encoding gene was up-regulated in *P. gigantea* paired against *H. parviporum* (Adomas *et al*., 2006), and in *T. versicolor* interacting with *B. adusta* (Eyre *et al*., 2010). Phosphoglucomutase primarily interconverts glucose-1-phosphate and glucose-6-phosphate, and the latter is the source of uridine diphosphate-glucose (UDP-glucose), the precursor of oligosaccharides and trehalose (Fu *et al*., 1995; Adomas *et al*., 2006). Interestingly, nutrient depletion during the growth of *Saccharomyces cerevisiae* (Fu *et al*., 1995), challenge of *H. annosum* with an antifungal agent, WS-5995 B from a potential biocontrol bacterium, *Streptomyces* sp. AcH 505 (Lehr, *et al*., 2009), and exposure of *S. cerevisiae* to high sugar concentration (40%, w/v; Erasmus *et al*., 2003), all elicited up-regulation of phosphoglucomutase. Further, glucose depletion in the growth medium of *S. cerevisiae* (Fu *et al*., 1995) has been reported to trigger trehalose accumulation owing to consequent shift of growth to the stationary phase (Benaroudj *et al*., 2001) with the attendant stresses. These findings therefore, suggest possible involvement of an underlying molecular cue that is perhaps shared by the interacting fungi mentioned above.

WS-5995 is a napthoquinone; a class of compounds which have been shown to disrupt electron flow resulting in the generation of reactive oxygen species (Lehr, *et al*., 2009). On the other hand, the other two treatments mentioned above (nutrient depletion and exposure to high sugar concentration), both drastically affect cell wall integrity. Akin to WS-5995, nutrient depletion can also result in increased generation of reactive oxygen species due to onset of secondary metabolism. Oxidative stress and cell wall damage can lead to the accumulation of trehalose, which is contingent on the availability of UDP-glucose furnished by the activity of phosphoglucomutase. Consequently, we speculate that up-regulation of phosphoglucomutase might be in connection with likely accumulation of trehalose in the confronted mycelia of *P. gigantea* and *T. versicolor*.

This assumption that up-regulation of phosphoglucomutase in interacting mycelia is associated with trehalose accumulation is supported by the work of Ujor *et al*. (2012b), who reported significant reduction in the levels of trehalose phosphorylase in *S. commune* confronted by *T. viride*. Trehalose phosphorylase is a key trehalose catabolic enzyme; hence, reduction of intracellular levels of trehalose phosphorylase during mycelial combat suggests an attempt to preserve intracellular trehalose levels. On the other hand, trehalose has been found to take part in fungal response to various abiotic stresses (van Laere, 1989). Ocón *et al*. (2007) reported increase in trehalose levels in the arbuscular mycorrhizal fungus *Glomus intaradices* with concomitant transient increase in the expression of a gene coding for trehalose-6-phophate phosphatase, a key enzyme in trehalose synthesis during prolonged or severe exposure to chemical or heat stresses. The chemistry of the interaction interface and its impact on the cell wall may mimic the effects of abiotic stresses, thereby causing increased trehalose accumulation in conflicting mycelia. In fact, Peiris *et al*. (2008) did detect trehalose and other disaccharides in samples from different

interacting species grown on agar, although control samples from self-paired cultures also harbored significant amounts of disaccharides, thereby impairing definitive identification and relative quantification. However, in a more recent study combining Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) with positive ion mode electrospray ionization, UPLC-MS/MS with negative ion mode electrospray ionization, and Gas Chromatography-Mass Spectroscopy (GC-MS), Luo et al. (2017) demonstrated increased mycelial accumulations of trehalose during combative interactions between *T. versicolor* and *Dichomitus squalens* and *T. versicolor* and *Pleurotus ostreatus*. Interestingly, Ocón et al. (2007) observed rapid catabolism of trehalose in *G. intaradices* upon cessation of stress. This is because, whereas trehalose stabilizes cell structures and allows proteins to maintain their native conformation during stress, high concentrations interfere with the refolding of proteins, hence, trehalose is rapidly degraded as soon as the stress terminates (Singer and Lindquist, 1998; Eroglu et al., 2000; Ocón et al., 2007). Consequently, depending on the dynamics and outcome of interaction, trehalose accumulation may be short-lived; hence, missed during metabolite profiling. This scenario fits well with the expression pattern observed for a gene coding for neutral trehalase, a key trehalose-degrading enzyme in *S. commune* confronted by *T. viride* (Ujor, 2010). Whereas the neutral trehalase gene was down-regulated early (24 hours) during contact with the biocontrol agent (*T. viride*), its expression increased significantly at 48 hours of contact, which would result in rapid degradation of trehalose. Therefore, specific time course investigation of expression patterns of trehalose metabolic genes and trehalose levels in competing fungi may help to unravel what roles trehalose might play during non-self mycelial combat.

Cyclophilins and heat shock proteins: Another emerging molecular signature of interspecific mycelial combat is up-regulation of cyclophilins (Adomas *et al*., 2006; Ujor *et al*., 2012a).

Cyclophilins are remarkably conserved proteins found in all forms of life, which underscores their relevance to cellular metabolism (Schonbrunner et al., 1991; Andreeva et al., 1999; Viaud et al., 2003; Wang & Heitman, 2005). Largely, they catalyze the otherwise slow *cis-trans* isomerisation of peptide bonds preceding proline residues, thereby accelerating protein folding; hence, they are referred to as peptidyl-prolyl *cis-tran*s isomerases (PPIases; Fisher and Schmid, 1990; Viaud et al., 2003; Wang & Heitman, 2005).

Some environmental stresses are 'proteotoxic', hence, they disrupt protein folding, which results in the accumulation of improperly folded nascent proteins; a lethal threat to cell survival (Andreeva *et al*., 1999). Similarly, mycelia in the barrage zone are subjected to a battery of stresses that are capable of disrupting protein folding. For example, insult to cell wall as with fungi paired against *Trichoderma* species triggers increases in the synthesis of proteins involved in the organization of the cell wall architecture (Krysan, 2009). Such specific increases in protein synthesis flux may engender up-regulation of cyclophilins to enhance protein folding, and prevent the delivery of aberrant proteins to the cell wall repair machinery. In a similar vein, *T. versicolor* was reported to over-express a gene encoding a GrpE chaperone domain-containing protein during mycelial conflict with *S. gausapatum* (Eyre *et al*., 2010), while genes that putatively encode heat shock proteins were up-regulated in the interacting mycelia of *U. maydis* (Jonkers *et al*., 2012). Analogous to cyclophilins, protein chaperones are known to maintain protein stability, enhance protein folding, assembly, and export (Schlesinger, 1990). Whereas heat shock proteins are constitutively expressed in most cells, they are particularly abundant under the conditions of physiological stress.

Despite the stresses stemming from mycelial combat, protein biosynthesis does not appear to cease during combat as ribosomal proteins were strongly up-regulated in the confronted mycelia

of *S. commune* (Ujor *et al*., 2012a), *T. versicolor* (Eyre *et al*., 2010), and *P. gigantea* (Adomas *et al*., 2006). *H. annosum* challenged with the antifungal agent, WS 599-B also exhibited significant increases in the expression of genes encoding ribosomal proteins (Lehr, *et al*., 2009). While mycelial combat may reduce the levels of specific proteins, overall flux through the protein biosynthesis machinery appears to increase, perhaps in an attempt to synthesize proteins critical for the containment of the competitor, and the attendant stresses of combat. In fact, contact with *Fusarium oxysporum* elicited increased protein synthesis in *Trichoderma harzianum* T-E5 (Zhang *et al*., 2014). Such increases in protein biosynthesis flux may account for the physiological need for cyclophilins and heat shock proteins. This notion is further supported by strong increase in the amount of a putative ubiquitin ligase and increased expression of a putative polyubiquitin gene in *S. commune* (Ujor *et al*., 2010; 2012a), and increased mRNA levels of multiple transcripts of an ubiquitin activating enzyme in *T. versicolor* (Eyre *et al*., 2010) during combat with different species. The ubiquitin pathway specifically recycles improperly folded proteins, which may accumulate significantly following increase in protein biosynthesis flux (Staszczak, 2008; Richie *et al*., 2009). Interestingly, up-regulation of ubiquitin activating enzyme in *T. versicolor* was observed in all interaction pairings studied by the authors - against *S. gausapatum*, *B. adusta* and *H. fasciculare* (Eyre *et al*., 2010).

Concluding remarks

In this review, we have summarized the genes and proteins differentially expressed in combating fungal mycelia and attempted to provide likely bases for their differential expression. Taken together, the strong morphological and biochemical reactions that ensue fungus-fungus contact are accompanied by equally robust shifts in the transcriptional and translational machinery of

interacting mycelia. Clearly, different species recruit different mechanisms for combat and this is influenced by the degree of threat posed by the competitor, and the metabolic response arsenal that each species possesses. Nevertheless, some salient molecular similarities exist between different interacting species. Namely, out-competed species tend to downregulate primary metabolism and vice versa. Also, interacting mycelia appear to recruit several lines of defence against toxic chemicals secreted into the confluence zone during combat and these include intracellular and extracellular enzymes involved in the detoxification of toxic metabolite secreted by one or both competing species. Similarly, some species appear to recruit efflux pumps for the ejection of toxic metabolites that perhaps elude the detoxifying enzymes. Despite multiple indications of switch from primary to secondary metabolism for most fungi during combat, especially the less aggressive species, protein biosynthesis does appear to increase, as evidenced by increased levels of ribosomal proteins or their encoding genes in different species. Increased biosynthesis of specific in-demand proteins during combat may account for this trend, which may in turn, increases protein biosynthesis flux and consequently, mechanisms targeted at streamlining protein biosynthesis such as cyclophilins and ubiquitin proteins. The chemistry of the confluence zone might include ROS employed by some combating species for signalling and attack or defence purposes. Increased ROS in the combat zone may likely accounts for the upregulation of mechanisms targeted at mitigating ROS-induced stresses. Furthermore, the molecular profiles of some of the thus far studied aggressive species suggest that they employ robust sequestration of nutrients and/or direct onslaught on competitor mycelia during combat. There is need to better understand the underlying mechanism(s) of increased laccase and peroxidase activities in the confluence zone relative to gene expression. Similarly, the specific roles played by nutrients such as iron, amino acids and different carbohydrate types remain to be elucidated. Knockout of some

salient genes implicated in the patterns and outcomes of interspecific mycelial interactions such as laccases, cyclophilins, ribosomal proteins, trehalose-associated proteins and toxicant-detoxifying enzymes in select fungal combatants would shed greater light on their roles in these interactions. Excitingly, more fully sequenced and annotated fungal genomes are now available on public databases, with several more nearing complete sequencing and annotation. This will certainly aid the use of 'omics' technologies to further probe the underlying cellular cues elicited by non-self fungus-fungus contact. As pointed out by Hiscox and Boddy (2017), future studies aimed at unravelling the underlying molecular mechanisms that govern the strong biochemical reactions that accompany interspecific mycelial interactions need to focus on ecologically relevant fungus-fungus pairings (involving multiple species, as often found in the field), with a view to better understanding the ecological as well as the biochemical aspects of these interactions. More so, the use of wood, soil, and leaf litters as substrates in the study of these interactions would better mimic interactions in the wild, as nutrient-rich laboratory media such as malt extract and potato dextrose agar, tom some degree, likely trigger different waves of molecular cues not replicated in the field.

Conflict of interest

The authors declare no conflict of interest.

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Table 1: Mechanisms of attack and defense recruited by fungal mycelia interacting with non-self

Table 2: Toxic metabolite-detoxifying enzymes whose activities, protein or mRNA levels have been reported to increase during interspecific mycelial combat

Figure legends

Figure 1: Representation of interspecific mycelial combat between *Trichoderma viride* and *Schizophyllum commune*: a) Barrage formation (arrow) by *S. commune* at the interaction interface at 24 hours of contact with *T. viride*; b) Self-paired *S. commune* with no barrage formation; c) Self-paired *T. viride*; d) Micrograph showing mycelia of *T. viride* coiling around *S. commune* mycelia on agar plate (arrows denote zones of dense coiling around *S. commune* mycelia); e) Micrograph of Nile Red-stained mycelia of *T. viride* and S. *commune* from the contact zone after 48 hours of contact, revealing loss of cell wall by *S. commune*; f) Complete rupture and disintegration of *S. commune* mycelia (following loss of cell wall), after 120 hours (5 days) of contact with *T. viride*.

Figure 2: Septal plugging in fungal mycelia following cellular injury (such as cell wall lysis). Woronin body is used by fungi for septal plugging and sealing of central pores upon cellular injury.

Figure 3: A simplified version of the glycolytic pathway depicting NADPH-producing reactions of the pentose phosphate pathway (PPP; depicted in blue). Enzymes depicted in red (TIM and enolase) were down-regulated in *S. commune* when confronted by *T. viride*, while G6PDH and 6PGDH in the PPP were up-regulated in *Stereum hirsutum* during interactions with *Coprinus*

micaceus and *Coprinus disseminatus*. Fructose-1,6-P: Fructose-1,6-biphosphate; G6PDH: Glucose-6-phosphate dehydrogenase; 6PGDH: 6-phosphoguconate dehydrogenase; TIM: triosephosphate isomerase; PEP - phosphoenol pyruvate (adapted with modifications from Shenton & g Grant, 2007).

Figure 4: Secretion of toxic metabolites is a recurring response in the mycelia of interacting fungi. Fungi with the capacity to enzymatically detoxify the toxic metabolites released into the surrounding medium/colonized material by the opposition fungus [extracellularly (A) or intracellularly (B) or both] tend to withstand the onslaught of opposition species, and some cases out-compete the opposition.

Figure 5: Some fungal species evade opposition biochemical attack by the use of efflux pumps to export toxic metabolites secreted by opposing fungal mycelia during combative interspecific mycelial interactions.