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Secondary metabolite toxins and nutrition of plant pathogenic fungi

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Fungal pathogens derive nutrition from the plants they invade. Some fungi can subvert plant defence responses such as programmed cell death to provide nutrition for their growth and colonisation. Secondary metabolite toxins produced by fungi often play a role in triggering these responses. Knowledge of the biosynthesis of these toxins, and the availability of fungal genome sequences and gene disruption techniques, allows the development of tools for experiments aimed at discovering the role of such toxins in triggering plant cell death and plant disease.

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Introduction

Fungi use three main nutritional strategies (biotrophy, necrotrophy and hemi-biotrophy) during the infection of plants. Although such classifications are useful, they are simplistic and arbitrary. Indeed some fungi are classified (by different researchers) as belonging to all of these three categories [1•]. Broadly accepted definitions are as follows: biotrophs rely on living plant tissue, whereas necrotrophs kill plant cells to derive nutrition. Hemi-biotrophs usually have an initial biotrophic phase, then become necrotrophic. Fungi such as *Leptosphaeria maculans*, the blackleg pathogen of canola, have alternating periods of biotrophy and necrotrophy [2]. For most fungi, the biotrophic lifestyle is associated with the development of complex feeding structures, such as haustoria and primary and secondary hyphae. In general, necrotrophs have broader host ranges than biotrophs and often enlist cell-wall-degrading enzymes and toxins, which can be small peptides or secondary metabolites. This review highlights recent findings on the interplay between fungi and their hosts, focussing on the role of secondary metabolite toxins in fungal nutrition and virulence.

Fungi can exploit the oxidative burst and programmed cell death for nutrition

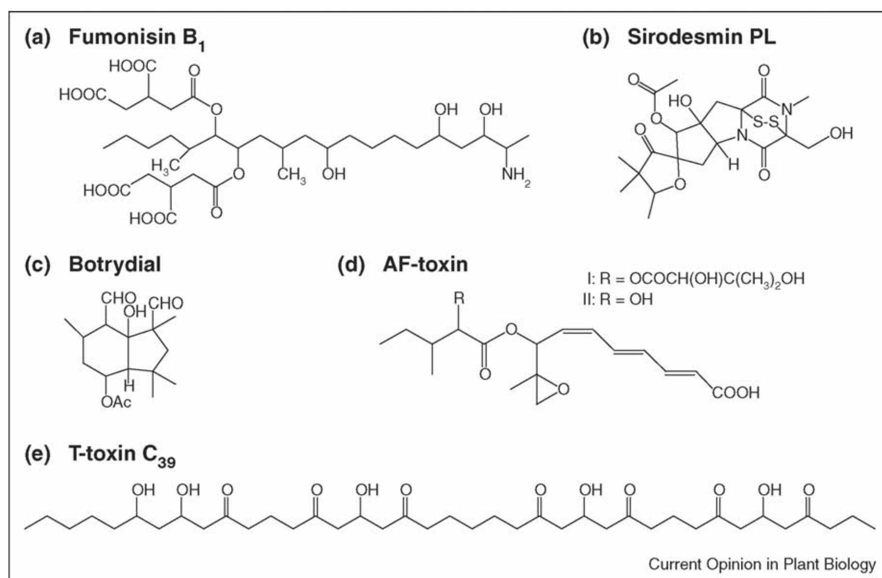
In many plant–pathogen interactions, defence responses include the hypersensitive response (HR), whereby an oxidative burst by the plant generates reactive oxygen species that are associated with the programmed cell death (PCD) of host cells. These responses arrest pathogen growth, particularly of that of biotrophs. It is becoming apparent that necrotrophic fungi can subvert this plant defence process to derive nutrition from the dead host tissue. *Botrytis cinerea* and *Sclerotinia sclerotiorum* have broad host ranges that include *Arabidopsis thaliana*. Several lines of evidence suggest that these fungi require PCD for successful invasion of their hosts. The degree of their infection is directly related to the amount of superoxide or hydrogen peroxide generated during the HR [3]. Furthermore an *A. thaliana* mutant (*dnd1*) that is defective in hypersensitive cell death is resistant to both fungi [4]. Govrin *et al.* [5••] have also shown that a small molecular weight elicitor that is derived from the apoplastic fluid of *Botrytis*-infected *A. thaliana* induces a HR on *A. thaliana* and other plants. This molecule promotes disease, but necrosis is suppressed in the *A. thaliana dnd1* mutant. The nature and origin (fungus or plant) of this elicitor is unknown, but it appears to be a pathogenicity factor that acts by eliciting a HR in the host.

Fungi that are not necrotrophic can exploit the oxidative burst for nutrition. *Cladosporium fulvum* is a hemi-biotroph that colonises the apoplastic space of tomato without eliciting a HR. During colonization, the fungus induces formation of γ-aminobutyric acid (GABA) whose role is to protect the plant from oxidative stress [6]. In return, the fungus appears to gain a nitrogen source, as suggested by the finding that fungal GABA aminotransferase is induced *in vitro* in response to the addition of GABA. Higher levels of this enzyme occur in compatible (susceptible host) compared to incompatible (resistant host) interactions. Utilisation of GABA by the fungus has not been investigated, but the fungus appears to be manipulating the stress responses of the host [7]. Whether this happens in other fungal–plant interactions is unknown.

Fungal toxins can induce PCD

In many fungal diseases, toxins are important virulence factors [8]. In contrast to necrotrophs, biotrophs are not reported to produce toxins, which is unsurprising given the need for such fungi to keep their host alive. Toxins can be host-specific or non-host specific. Some are small peptides but many are secondary metabolites,

Figure 1



Structures of secondary metabolite toxins from plant pathogenic fungi. **(a)** Fumonisin B1 from *Fusarium* species. **(b)** Sirodesmin PL from *Leptosphaeria maculans*. **(c)** Botrydial from *Botrytis cinerea*. **(d)** AF toxin I and II from *Alternaria alternata*. **(e)** T-toxin C $_{39}$ from *Cochliobolus heterostrophus*.

low-molecular-weight molecules with a diverse range of structures (Figure 1). These secondary metabolites are dispensable for the fungus but presumably confer some selective advantage. Many toxins elicit PCD: the sphingoid-derived molecule fumonison B_1 (Figure 1a) mediates this effect by depletion of extracellular ATP, which alters the abundance of particular intracellular proteins in the plant [9••]. This toxin is produced by *Fusarium* species that are pathogens of maize but does not appear to be a primary virulence factor. It is interesting that light is a requirement for this toxin to exert its effect and also for the induction of PCD during *Botrytis* infections [4]. This might reflect the plant's need for light to produce reactive oxygen during the oxidative burst.

Mutational strategies can determine the role of secondary metabolites in virulence

Knowledge of pathways for the biosynthesis of secondary metabolite toxins can lead to the development of tools to investigate the role of toxins in disease. Biosynthetic genes for secondary metabolites are usually clustered in the fungal genome. Toxin biosynthetic genes can often be deduced thanks to the recent availability of increasing amounts of sequence data, including complete genome sequences [10]. Diagnostic enzymes for secondary metabolite production include non-ribosomal peptide synthases or polyketide synthases, as well as those responsible for modifications of the core moiety (a peptide or polyketide). Such enzymes include methyl transferases, acetyl transferases, prenyl transferases, oxido-reductases and cytochrome P450 mono-oxygenases. The biosynthetic genes that encode these proteins are usually co-regulated, often by a transcriptional regulator in the cluster itself. Those genes flanking the cluster have different patterns of transcriptional regulation. If a gene in the predicted cluster is mutated and the resultant mutants are unable to produce the toxin, this will unequivocally prove that the cluster encodes the biosynthetic enzymes.

Such an approach has been used to identify the biosynthetic pathway for the epipolythiodioxopiperazine toxin sirodesmin PL (Figure 1b) in *L. maculans*. Sequencing of a cosmid clone of *L. maculans* that contained a non-ribosomal peptide synthase revealed the presence of linked genes with best matches to genes that are predicted to be involved in sirodesmin biosynthesis [11]. A sirodesmin-deficient mutant (which has a deletion in the peptide synthetase) makes lesions on cotyledons of canola that are of similar size to those made by the wildtype isolate. However, this mutant colonises stem tissue less effectively than the wildtype (C Elliott, D Gardiner, B Howlett, unpublished). These findings implicate sirodesmin as a virulence determinant in the late stages of canola infection, during necrosis of the stem, but not in the early stages when necrotic cotyledonary lesions form. Fungi need to be able to protect themselves from the toxins they produce. The ABC transporter SirA, a member of a multigene family responsible for efflux of small molecules, is present in the sirodesmin gene cluster. Disruption of this gene does not affect sirodesmin production, but it increases the sensitivity of *L. maculans* to

sirodesmin [12]. This indicates a difference between the mechanisms of primary toxin export and self-protection in this fungus.

The sesquiterpene-derived toxin botrydial (Figure 1c) from *B. cinerea* has been implicated in virulence, as it can be detected *in planta* and because addition of it facilitates fungal penetration and colonisation of plants [13]. Deletion of a gene in the biosynthetic pathway for botrydial in four different strains of *B. cinerea* led to botrydial-deficient mutants [14••]. Three of the four strains have similar virulence to that of wildtype isolates and are still able to infect beans and detached leaves and fruits of tomato, but the fourth mutant strain has reduced virulence on these hosts. Thus, botrydial appears to be a virulence factor only for some strains of *B. cinerea*. This fungus makes a range of toxins, and some of these might be involved in the virulence of other *B. cinerea* strains.

Mutations in biosynthetic pathways for secondary metabolites can also lead to altered products and consequent changes in the host range of a fungal pathogen. *Alternaria alternata* has seven variants (pathotypes) that produce different host-specific toxins and cause disease on different hosts. Three of these classes of toxin (ACT, AF and AK) are derived from a common structural moiety and are produced by a core set of three biosynthetic genes, which are located in multiple copies on a conditionally dispensable chromosome. Isolates that lack this dispensable chromosome are non-pathogenic on all hosts [15]. AF toxins I and II (Figure 1d) are produced by the strawberry pathotype; AF toxin I is toxic to strawberry and pear, whereas AF toxin II is toxic only to pear. In this strawberry pathotype, a gene with best match to an aldo-ketoreductase is located between two of the core biosynthetic genes. When this gene is disrupted, the resultant mutant produces only AF toxin II and can infect pear, but not strawberry. This phenotype is similar to that of isolates of the pear pathotype, which lacks the aldo-ketoreductase gene [16••]. Thus, mutation in a toxin biosynthetic gene limits the host range of a pathotype of this fungus.

In the examples discussed above, biosynthetic genes for secondary metabolites are at a single locus or are closely linked, which makes the identification of these genes relatively straightforward. However, for *Cochliobolus heterostrophus*, biosynthesis of the polyketide T toxin (Figure 1e) by some strains of race T involves genes at two loci on different chromosomes. One locus encodes two polyketide synthases, one of which is located in a repeat-rich region [17]. Mutation of these genes, as well as a decarboxylase gene at the other locus, leads to loss of toxin production and reduced virulence on maize [17,18]. The necessity for two polyketide synthases for biosynthesis of a single linear polyketide is intriguing, and suggests that two polyketides are produced, with one acting as starter for the mature toxin [17]. By contrast, polyketides in most other filamentous fungi are synthesised iteratively, whereby one unit is added at a time by a single polyketide synthase gene.

Secondary metabolite gene clusters and genes such as those encoding non-ribosomal peptide synthases or polyketide synthases seem to be present in greater numbers in pathogens compared to saprophytes such as *Neurospora crassa* [19]. Obligate biotrophic fungi for which expressed sequence tag (EST) and genome data are available (the powdery mildew fungus, *Blumeria graminis* [20,21] and the bean rust fungus *Uromyces faba*e [22]) do not appear to have such genes, nor are these fungi predicted to make secondary metabolites. However, the sequence data for such fungi are limited. The genome of the wheat stem rust fungus, *Puccinia graminis*, will be sequenced shortly, so a complete set of genes for a biotroph will be available. It will be interesting to see if secondary metabolite gene clusters are present in this genome.

Secondary metabolites and their biosynthetic genes can be identified by the use of mutants in a global transcriptional regulator

A novel way of detecting fungal secondary metabolites takes advantage of the global regulation of secondary metabolite production by the *LaeA* gene, which has been characterised in *Aspergillus* species [23]. Bok *et al.* [24••] examined microarrays of *Aspergillus nidulans* that were hybridised to RNA prepared from strains that overexpressed or underexpressed *LaeA*. A range of secondary metabolite gene clusters that are regulated by LaeA were identified. Deletion of a gene in one of these clusters resulted in a mutant that was unable to make the anti-tumour compound terrequinone, which had not previously been described in *A. nidulans*. Thus, a new biosynthetic pathway and resultant molecule was discovered. This approach has great potential for finding biosynthetic genes for toxins (and the toxins themselves) in fungi in which the *LaeA* homologue can be identified. Identification of such homologues is challenging as LaeA lacks conserved motifs other than an S-adenosyl methionine domain that is found in some methyl transferases. Nonetheless, strong matches have been found in the saprophyte *Neurospora crassa*, the human pathogen *Coccidioides immitis*, and two plant pathogens, *Magnaporthe grisea* and *Fusarium sporotrichioides* [23]. As yet there are no reports of the deletions or overexpression of this gene in these fungi.

Toxin production can be regulated by plant molecules

Toxin production can be regulated by signals or even substrates from the plant. For instance, *Aspergillus* species such as *A. flavus*, *A. parasiticus* and *A. nidulans* are proposed to derive acetyl CoA for the biosynthesis of toxins (i.e. sterigmatocystin and aflatoxin) from fatty acids in the

maize kernels that they colonised [25]. This role of seed fatty acids is suggested by several pieces of evidence. Added oleic acid induces the biogenesis of fungal peroxisomes, as well as catalase activity and beta oxidation. This correlates with the increased expression of biosynthetic genes for sterigmatocystin and aflatoxin in hyphae colonising the embryo and aleurone layer, where most seed lipids are stored. A recent example of a signal from a plant that regulates toxin production is the plant polysaccharide amylopectin, which induces fumonison B_1 production by *Fusarium verticillioides* during the colonisation of maize kernels [26]. The mechanism for this induction is unknown. There is a complex 'back and forth' interplay between plant and fungus in which the fungus metabolises a plant polysaccharide to produce molecules that trigger fumonison B_1 production. This leads to plant cell death, presumably by depletion of extracellular ATP as described above. Another example of a plant saccharide signal that influences toxin production is the discovery that AB toxin production by germinating spores of *Alternaria brassicicola* is induced by a 1.3 kDa oligosaccharide that is released by the leaves of *Brassica* plants [27]. Of course, there are many examples of plants that can inactivate fungal toxins; a recently discovered example are wheat varieties that can detoxify the mycotoxin deoxynivenol, an ability that co-localises with a quantitative trait locus (QTL) that confers resistance to the head scab fungus, *Fusarium graminaerum* [28].

Conclusions

Necrotrophic fungi do not just kill plant cells then live saprophytically; instead, they appear to be able to subvert host defences such as the HR to colonise the plant. Often, this effect is triggered by toxins. The availability of complete fungal genome sequences has allowed the biosynthetic pathways for secondary metabolite toxins to be identified, and the increasing amenability of fungi to gene disruption techniques allows toxin-deficient mutants to be produced. Thus, the role of such toxins in plant disease can be determined. For those toxins that are not primary virulence factors, it would be interesting to know the roles they play and the advantages (if any) that they confer on the fungus that produces them. Toxins have not been described from all necrotrophic fungi. Often purified toxins show toxic effects *in vitro* but cannot be detected *in planta*, so their role in disease is unknown. However, such molecules might be produced in small amounts and fungi might use toxins that are as yet undescribed. Recently developed analytical equipment, such as liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS), that achieves sensitive levels of detection provides opportunities to identify many more of these metabolites both *in vitro* and *in planta*.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Oliver RP, Ipcho SVS: ***Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens**. *Mol Plant Pathol* 2004, **5**:347-352.
Fungi have a range of lifestyles and infection mechanisms, and analysis of *Arabidopsis* pathogens provides insights into nutritional strategies and signalling pathways in the host.

2. Rouxel T, Balesdent MH: **The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters the genomic era**. *Mol Plant Pathol* 2005, **6**:225-241.

3. Govrin EM, Levine A: **The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea***. *Curr Biol* 2000, **10**:751-757.

4. Govrin EM, Levine A: **Infection of *Arabidopsis* with a necrotrophic pathogen, *Botrytis cinerea*, elicits various defense responses but does not induce systemic acquired resistance (SAR)**. *Plant Mol Biol* 2002, **48**:267-276.

5. •• Govrin EM, Rachmilevitch S, Tiwari BS, Solomon M, Levine A: **An elicitor from *Botrytis cinerea* induces the hypersensitive response in *Arabidopsis thaliana* and other plants and promotes the gray mold disease**. *Phytopathology* 2006, **96**:299-307.
Necrosis by an elicitor is suppressed by molecules that inhibit the oxidative burst and in *Arabidopsis* mutants that are compromised in the HR.

6. Solomon PS, Oliver RP: **Evidence that gamma-aminobutyric acid is a major nitrogen source during *Cladosporium fulvum* infection of tomato**. *Planta* 2002, **214**:414-420.

7. Oliver RP, Solomon PS: **Does the oxidative stress used by plants for defence provide a source of nutrients for pathogenic fungi?** *Trends Plant Sci* 2004, **9**:472-473.

8. Markham JE, Hille J: **Host-selective toxins as agents of cell death in plant–fungus interactions**. *Mol Plant Pathol* 2001, **2**:229-239.

9. •• Chivasa S, Ndimba BK, Simon WJ, Lindsey K, Slabas AR: **Extracellular ATP functions as an endogenous external metabolite regulating plant cell viability**. *Plant Cell* 2005, **17**:3019-3034.
The fungal toxin fumonison B1 depletes extracellular ATP to cause PCD in plants.

10. Keller NP, Turner G, Bennett JW: **Fungal secondary metabolism — from biochemistry to genomics**. *Nat Rev Microbiol* 2005, **3**:937-947.

11. Gardiner DM, Cozijnsen AJ, Wilson LM, Pedras MSC, Howlett BJ: **The sirodesmin biosynthetic gene cluster of the plant pathogenic fungus *Leptosphaeria maculans***. *Mol Microbiol* 2004, **53**:1307-1318.

12. Gardiner DM, Jarvis RS, Howlett BJ: **The ABC transporter gene in the sirodesmin biosynthetic gene cluster of *Leptosphaeria maculans* is not essential for sirodesmin production but facilitates self-protection**. *Fungal Genet Biol* 2005, **42**:257-263.

13. Deighton N, Muckenschnabel I, Colmenares AJ, Collado IG, Williamson B: **Botrydial is produced in plant tissues infected by *Botrytis cinerea***. *Phytochemistry* 2001, **57**:689-692.

14. •• Siewers V, Viaud M, Jimenez-Teja D, Collado IG, Gronover CS, Pradier JM, Tudzynski B, Tudzynski P: **Functional analysis of the cytochrome P450 monooxygenase gene *Bcbot1* of *Botrytis cinerea* indicates that botrydial is a strain-specific virulence factor**. *Mol Plant Microbe Interact* 2005, **18**:602-612.
Mutational analysis of a biosynthetic gene for botrydial shows that this toxin is a virulence factor for only some strains of *B. cinerea*.

15. Hatta R, Ito K, Hosaki Y, Tanaka T, Tanaka A, Yamamoto M, Akimitsu K, Tsuge T: **A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen *Alternaria alternata***. *Genetics* 2002, **161**:59-70.

16. Ito K, Tanaka T, Hatta R, Yamamoto M, Akimitsu K, Tsuge T:
•• **Dissection of the host range of the fungal plant pathogen *Alternaria alternata* by modification of secondary metabolism**. *Mol Microbiol* 2004, **52**:399-411.
The host range of a fungal pathogen can be restricted by modification of the structure of a secondary metabolite toxin.

17. Baker SE, Kroken S, Inderbitzin P, Asvarak T, Li BY, Shi L, Yoder OC, Turgeon BG: **Two polyketide synthase-encoding genes are required for biosynthesis of the polyketide virulence factor, T-toxin, by *Cochliobolus heterostrophus***. *Mol Plant Microbe Interact* 2006, **19**:139-149.

18. Rose MS, Yun SH, Asvarak T, Lu SW, Yoder OC, Turgeon BG: **A decarboxylase encoded at the *Cochliobolus heterostrophus* translocation-associated Tox1B locus is required for polyketide (T-toxin) biosynthesis and high virulence on T-cytoplasm maize**. *Mol Plant Microbe Interact* 2002, **15**:883-893.

19. Yoder OC, Turgeon BG: **Fungal genomics and pathogenicity**. *Curr Opin Plant Biol* 2001, **4**:315-321.

20. Thomas SW, Rasmussen SW, Glaring MA, Rouster JA, Christiansen SK, Oliver RP: **Gene identification in the obligate fungal pathogen *Blumeria graminis* by expressed sequence tag analysis**. *Fungal Genet Biol* 2001, **33**:195-211.

21. Soanes DM, Talbot NJ: **Comparative genomic analysis of phytopathogenic fungi using expressed sequence tag (EST) collections**. *Mol Plant Pathol* 2006, **7**:61-70.

22. Jakupovic M, Heintz M, Reichmann P, Mendgen K, Hahn M: **Microarray analysis of expressed sequence tags from haustoria of the rust fungus *Uromyces fabae***. *Fungal Genet Biol* 2006, **43**:8-19.

23. Bok JW, Balajee SA, Marr KA, Andes D, Nielsen KF, Frisvad JC, Keller NP: **LaeA, regulator of morphogenetic fungal virulence factors**. *Eukaryot Cell* 2005, **4**:1574-1582.

24. Bok JW, Hoffmeister D, Maggio-Hall LA, Murillo R, Glasner JD,
•• Keller NP: **Genomic mining for *Aspergillus* natural products**. *Chem Biol* 2006, **13**:31-37.
Examining transcription patterns of mutants that either overexpress or are deleted in a global transcriptional regulator of secondary metabolite biosynthesis allows the identification of novel metabolites, including toxins.

25. Maggio-Hall LA, Wilson RA, Keller NP: **Fundamental contribution of beta-oxidation to polyketide mycotoxin production *in planta***. *Mol Plant Microbe Interact* 2005, **18**:783-793.

26. Bluhm BH, Woloshuk CP: **Amylopectin induces fumonisin B-1 production by *Fusarium verticillioides* during colonization of maize kernels**. *Mol Plant Microbe Interact* 2005, **18**:1333-1339.

27. Oka K, Akamatsu H, Kodama M, Nakajima H, Kawada T, Otani H: **Host-specific AB-toxin production by germinating spores of *Alternaria brassicicola* is induced by a host-derived oligosaccharide**. *Physiol Mol Plant Pathol* 2005, **66**:12-19.

28. Lemmens M, Scholz U, Berthiller F, Dall'Asta C, Koutnik A, Schuhmacher R, Adam G, Buerstmayr H, Mesterhazy A, Krska R *et al.*: **The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat**. *Mol Plant Microbe Interact* 2005, **18**:1318-1324.