MOLECULAR PLANT PATHOLOGY (2004) **5**(4), 235–252 DOI: 10.1111/J.1364-3703.2004.00226.X

Pathogen profile

Gaeumannomyces graminis, the take-all fungus and its relatives

JACQUELINE FREEMAN AND ELAINE WARD*

Wheat Pathogenesis Programme, Plant–Pathogen Interactions Division, Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK

SUMMARY

Take-all, caused by the fungus *Gaeumannomyces graminis* var. *tritici*, is the most important root disease of wheat worldwide. Many years of intensive research, reflected by the large volume of literature on take-all, has led to a considerable degree of understanding of many aspects of the disease. However, effective and economic control of the disease remains difficult. The application of molecular techniques to study *G. graminis* and related fungi has resulted in some significant advances, particularly in the development of improved methods for identification and in elucidating the role of the enzyme avenacinase as a pathogenicity determinant in the closely related oat take-all fungus (*G. graminis* var. *avenae*). Some progress in identifying other factors that may be involved in determining host range and pathogenicity has been made, despite the difficulties of performing genetic analyses and the lack of a reliable transformation system.

TAKE-ALL DISEASE OF WHEAT AND ITS CONTROL

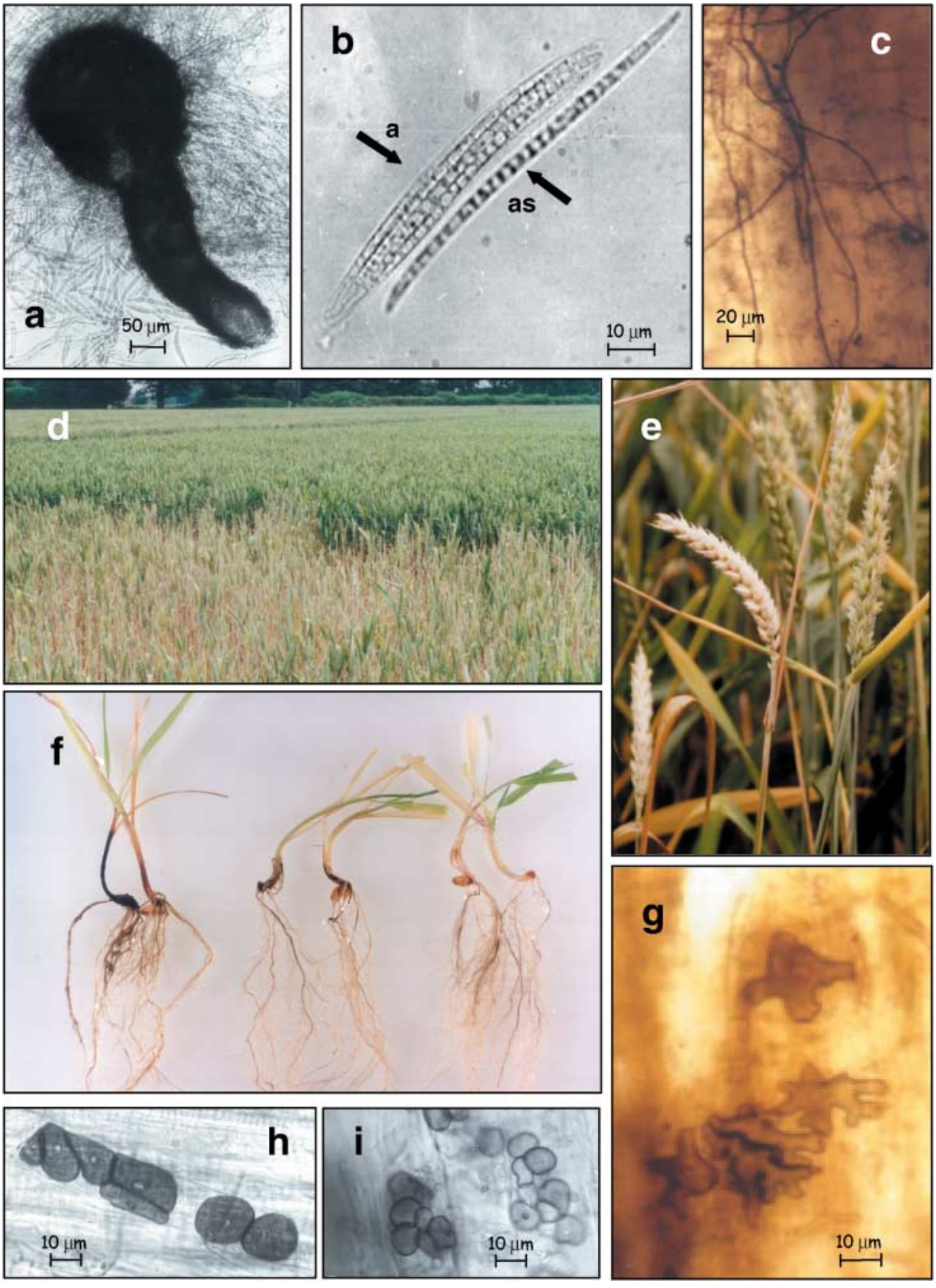
Take-all of wheat is caused by the fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) and is the most important root disease of wheat worldwide. The same pathogen (Ggt) also affects triticale, barley and rye, but to lesser extents. Yield losses, due to stunting and premature ripening resulting in shrivelled grain, can be large in severely affected crops. Substantial financial losses result from decreases in yield and quality. Using husbandry to minimize the financial impact of the disease imposes restrictions on the cropping systems and rotations that can be employed. Despite considerable progress resulting from decades of intensive research (summarized in Asher and Shipton, 1981; Cook, 2003; Hornby *et al.*, 1998) there is still much that we do not understand about the disease and the fungi that cause it. In the UK, most of Europe and parts of the USA, when consecutive susceptible cereal crops are grown, the disease often rises to a peak over 2–4 years after a break crop, then typically declines in severity (take-all decline). Although there have been reports of a decline in take-all associated with cultivation of continuous wheat in Australia, it may be different from that described above and appears to be less robust than in Europe and the USA.

During the intercrop period, Ggt survives saprophytically on the crop debris and is the main source of inoculum for the following crop. The quantity of viable inoculum remaining in the soil falls sharply in the absence of a susceptible crop and this is usually sufficient to reduce disease severity to an insignificant level in a first susceptible cereal following a 1-year break. Perithecia, which subsequently release ascospores (Fig. 1a,b), are sometimes produced on the cereal stem bases or stubble although this is believed to be of limited importance as a source of inoculum in the field. Ggt can also survive as a pathogen on other grasses and volunteer cereal plants.

On autumn-sown winter wheat primary infection of the seminal roots of seedlings occurs with the growth of dark runner hyphae on the root surface (Fig. 1c). Hyaline branches from these runner hyphae penetrate the root by invading the root cortex and then colonize and destroy the vascular tissue. In early spring the root lesions develop further followed by secondary infections resulting from root-to-root contact. In favourable years the disease may be severe by late spring when uneven crop growth can be observed. More typically in early summer, just before ear emergence, the flag leaves of infected plants may begin to roll as a result of drought-stress, and during summer patches of stunted plants with yellowed leaves (Fig. 1d) and whiteheads (Fig. 1e), a symptom of premature ripening, are observed. After harvest infected crop debris remains in the soil and if another susceptible crop is sown the cycle begins again.

The most important cultural practice used to control take-all is crop rotation. Other cultural practices that influence take-all severity include sowing date, tillage practices, the application of fertilizers and grass weed control. Cultivation of non-susceptible break crops, delaying sowing date and grass weed control reduce take-all severity as a result of decreased inoculum. Nitrogen-, phosphate- and manganese-deficiency can all increase susceptibility of a crop to take-all, but the relative importance of these

**Correspondence*: E-mail: elaine.ward@bbsrc.ac.uk


a
50 μm
b
a
as
10 μm
c
20 μm
d
e
f
g
10 μm
h
10 μm
i
10 μm

nutrients varies according to geographical location, with nitrogen and phosphate more important in the UK and manganese in the USA and Australia (reviewed in Hornby *et al*., 1998). Until recently these were the only effective methods available to farmers for controlling take-all. The best control is achieved by rotating susceptible cereal crops with non-susceptible break crops in which grass weeds and cereal volunteers are controlled effectively. Tillage has contributed to reducing take-all severity in the USA (Weller *et al*., 2002) but the effects of cultural practices involving reduced tillage, such as direct-drilling, are less consistent in the UK than in the USA (Hornby *et al*., 1998; Yarham, 1981).

Differences between wheat cultivars in their resistance to take-all are generally considered to be too small and too inconsistent to be useful (Cook *et al*., 1995; Hornby *et al*., 1998; Weller *et al*., 2002). There has been relatively little published work concerning resistance of wheat to Ggt during the last 20 years (Eastwood *et al*., 1994; Penrose, 1985, 1991, 1995; Penrose and Neate, 1994; Rengel *et al*., 1993, 1994). Rye has greater tolerance of take-all than does wheat and it was hoped that the wheat–rye hybrid triticale might retain this property. However, levels of susceptibility were found to be intermediate, and there is generally no advantage in changing to triticale, except in some situations where severe take-all is expected (Hornby *et al*., 1998). The grass species *Dasapyrum* (*Haynaldia*) and *Aegilops* also show resistance to take-all, but the prospects for introducing this into wheat seem poor (Linde-Laursen *et al*., 1973).

Some soils are known to be suppressive to take-all disease. The reasons for this are often uncertain but it may result from effects on the pathogen (growth rate and/or population structure), the host (host susceptibility) or the balance of antagonistic microflora in the soil (Bateman *et al*., 1997; Hornby, 1983; Rengel, 1997; Rovira and Wildermuth, 1981; Walker, 1975). General suppression increases with increasing microbial biomass in the soil (Weller *et al*., 2002). Various mechanisms for specific suppression exist (Hornby, 1983; Rovira and Wildermuth, 1981; Walker, 1975). The fastest developing durable mechanism of specific suppression of take-all is take-all decline (TAD), which was first demonstrated experimentally at Rothamsted in the 1960s (Hornby *et al*., 1998). Walker (1975) defined TAD as 'suppression developed within a few seasons in the presence of the severely diseased host'. TAD has been reported from many different geographical locations, but although the cause is probably mainly microbial, the microorganisms responsible may be different in different soils. Much research has been aimed at understanding the causes of TAD and at identifying the microorganisms responsible for suppression in TAD or other suppressive soils. Sometimes wheat is grown continuously to exploit take-all decline, but the yields obtained rarely match those of a first cereal after a break. In addition, TAD usually takes 4–6 years to become established over a whole field (Hornby *et al*., 1998; Weller *et al*., 2002) and even when TAD is established, take-all can still cause considerable losses if weather and soil conditions are conducive to disease.

A range of different microorganisms have been investigated as potential biological control agents for take-all, many isolated from TAD soils. Particular attention has been paid to fluorescent pseudomonads, which have been demonstrated to provide effective biological control of take-all in pot and field experiments (Chapon *et al*., 2002; de Souza *et al*., 2003; Duffy and Weller, 1995, 1996; Ryder *et al*., 1990; Weller *et al*., 2002). Production of antibiotics has been demonstrated to provide the mechanism by which many *Pseudomonas fluorescens* strains protect wheat roots aginst take-all. Phenazine-1-carboxylic acid (PCA) was the first such antibiotic to be identified but production of 2,4-diacetylphloroglucinol (DAPG) by fluorescent psuedomonads is now believed to be more important in TAD than production of PCA (see reviews by Cook, 2003; Weller *et al*., 2002). A number of other bacterial species and a few fungal species have also been investigated (Dewan and Sivasithamparam, 1988; Duffy *et al*., 1996; Kim *et al*., 1997; Ross *et al*., 2000; Rovira *et al*., 1992; Ryder and Rovira, 1993; Sivasithamparam, 1998). Some of the fungi that have been found to be effective are closely related to the take-all fungus, namely avirulent/hypovirulent isolates of *G. graminis* and *Phialophora* species (Andrade *et al*., 1994; Duffy and Weller, 1995, 1996; Mathre *et al*., 1998; Sivasithamparam, 1975; Wong *et al*., 1996; Zriba *et al*., 1999). The accumulated research on potential biological control agents for take-all of wheat demonstrates that screening *in vitro*, and even in pot experiments, does not necessarily give a reliable indication of effectiveness in the field (Cook *et al*., 1995; Elsherif and Grossmann, 1994). It is also evident that agents that give control in one geographical location do not necessarily have any beneficial effects elsewhere (e.g. Capper and Higgins, 1993; Hornby *et al*., 1993). Reports of successful biological control of take-all are mainly from the USA and Australia, with very few from Europe.

Take-all has long attracted the interest of agrochemical companies but until recently there were no fungicides that could

Fig. 1 Infection of wheat by *Gaeumannomyces graminis* and *Phialophora graminicola*. (a) *G. graminis* var. *tritici*: perithecia produced on wheat roots in a rotting test, surrounded by released asci; (b) *G. graminis* var. *tritici*: ascospores in a single ascus (arrowed, a), with one ascospore separated from the ascus (arrowed, as); (c) *G. graminis* var. *tritici*: dark runner hyphae on the surface of a wheat root; (d) a patch of stunted and yellowed wheat plants (foreground), typical of take-all, in the summer; (e) whiteheads of infected plants in a wheat crop with take-all, in late summer; (f) similarities in discoloration of wheat roots, and differences in stem-base blackening of wheat, caused by infection with *G. graminis* var. *tritici* (Ggt) (left), *G. graminis* var. *graminis* (Ggg) (centre) and *P. graminicola* (right), in a pathogenicity test; (g) *Phialophora* sp. (lobed-hyphopodia): lobed hyphopodia on the leaf sheath at the stem base of a wheat plant; (h) *Phialophora* sp. (lobed-hyphopodia): swollen cells with pores (known as growth cessation structures or vesicles) in a wheat root; (i) *P. graminicola*: swollen cells with pores (known as growth cessation structures or vesicles) in a wheat root. Images (a), (b), (d), (f), (h) and (i) were kindly provided by Richard Gutteridge (Rothamsted Research).

be used commercially (Bateman, 1989), and management of take-all relied on husbandry. Recently, however, two fungicides have become available as seed treatments for controlling the disease. Fluquinconazole (a trade name of the seed treatment formulation is Jockey®) is a triazole fungicide with a relatively broad spectrum of activity (Bateman *et al*., 2003; Löchel *et al*., 1998). Silthiofam (previously MON65500, trade name Latitude®), by contrast, is specific to Ggt (Beale *et al*., 1998; Schoeny and Lucas, 1999; Spink *et al*., 1998). Silthiofam has a novel mode of action, with the mitochondrial adenine nucleotide transporter identified as its probable target (Joseph-Horne *et al*., 2000). Host defence responses are also enhanced following silthiofam treatment (Huang *et al*., 2001b).

TAXONOMY AND PHYLOGENY OF *G. GRAMINIS* AND RELATED FUNGI (THE *GAEUMANNOMYCES–PHIALOPHORA* COMPLEX)

Gaeumannomyces is an ascomycete that was previously classified in the order *Diaporthales*. However, more recently, links with *Magnaporthe* species were noted and *Gaeumannomyces* has now been transferred to the family *Magnaporthaceae*, which is yet to be assigned to an order (Cannon, 1994). The genus *Gaeumannomyces* var. Arx and Olivier (emend. Walker) contains seven known species: *G. graminis*, *G. caricis*, *G. cylindrosporus*, *G. incrustans*, *G. medullaris*, *G. amomi* and *G. wongoonoo* (Bussaban *et al*., 2001a; Hornby *et al*., 1998; Wong, 2002). *G. graminis* and *G. cylindrosporus* infect cereals and grasses, *G. caricis* infects sedges, *G. incrustans* was isolated from turf-grass, *G. medullaris* from the rush *Juncus roemerianus* (Kohlmeyer *et al*., 1995; Landschoot and Jackson, 1989) and, recently, *G. amomi* from wild ginger (*Amomum siamense*; Bussaban *et al*., 2001b) and *G. wongoonoo* from buffalo grass (*Stenophram secundatum*; Wong, 2002).

The anamorph of *G. medullaris* is *Trichocladium medullare*, whereas the known anamorphs of the other *Gaeumannomyces* species are all species of *Phialophora* [the commonly accepted name, used here for convenience, but see reference to Gams (2000) below]. For example, the anamorph of *G. cylindrosporus* is *Phialophora graminicola*, and that of *G. graminis* var. *graminis* is *Phialophora* sp. (lobed-hyphopodia) (see below). *G. graminis* and related *Phialophora* species found on cereal and grass roots are known collectively as the *Gaeumannomyces–Phialophora* (G-P) complex, some of which cause superficially similar discoloration of wheat roots (Fig. 1f). The fungi of the G-P complex attack root systems, particularly of *Gramineae* and *Cyperaceae*, and most are necrotrophic parasites (Hornby *et al*., 1998). *Phialophora* is a highly polymorphic genus containing anamorphs of *Discomycetes*, *Pyrenomycetes* and *Loculoascomycetes*. Gams (2000) has therefore proposed a reclassification of *Phialophora* spp., which involves segregating the *Phialophora* anamorphs of *Gaeumannomyces* species into a new genus *Harpophora*, which also contains the related fungus *Cephalosporium maydis*.

Four varieties of *G. graminis*, which differ in host range, pathogenicity, ascospore size range or hyphopodial structure, have been identified (Table 1). *G. graminis* varieties *tritici* (Ggt), *avenae* (Gga) and *maydis* (Ggm) have simple hyphopodia, whereas *G. graminis* var. *graminis* (Ggg) has characteristically lobed hyphopodia (Fig. 1g), and its anamorph is therefore referred to as *Phialophora* sp. (lobed-hyphopodia). Ggt and Gga are pathogenic on several cereal crops, whereas Ggg is pathogenic on rice, but is weakly/non-pathogenic on some other cereals and Ggm

Table 1 Summary of the major morphological and pathogenicity characteristics of *Gaeumannomyces graminis* that are used to distinguish between varieties.

G. graminis variety	Abbreviation	Pathogenicity	Mean ascospore length (μm)	Morphology of hyphopodia	References
G. graminis var. *tritici*	Ggt	Causes take-all of wheat, triticale, barley and rye (in decreasing order of susceptibility)	70–105	Simple	Deacon (1981); Walker (1981); Hornby *et al*. (1998)
G. graminis var. *avenae*	Gga	Similar to Ggt but also causes take-all of oats and take-all patch of turf-grasses	100–130	Simple	Deacon (1981); Walker (1981); Hornby *et al*. (1998)
G. graminis var. *graminis*	Ggg	Causes dieback of Bermuda grass and sheath blight of rice	80–105	Lobed	Ou (1972); Walker (1981); McCarty and Lucas (1989); Elliott (1991); Datnoff (1993); Hornby *et al*. (1998)
G. graminis var. *maydis*	Ggm	Causes take-all of maize	55–85	Simple	Yao (1992); Hornby *et al*. (1998)

causes take-all of maize, but only slight infections on some other cereals. Although Ggt and Ggm can occur on some grass species, Gga and Ggg are pathogenic on turf-grasses and Bermuda grass, respectively. Ggt and Gga are highly invasive on wheat roots. Hyaline branches (infection hyphae) from the dark runner hyphae penetrate and invade the root cortex then penetrate the endodermis and invade the stele. The phloem is rapidly destroyed followed by slower colonization and blockage of the xylem with gum-like deposits (Deacon, 1981). While colonization of the internal root tissue within root lesions occurs, growth of the runner hyphae along the surface of wheat roots continues. Further root lesions develop at points of invasion by hyaline branches. Eventually growth of the pathogen may reach the stem base and, under suitable conditions, the culm (beneath the enclosing leaf sheaths) can become completely covered with darkly pigmented mycelium (Cook, 2003). Ggg and *P. graminicola* are less invasive. Ggg rarely penetrates the steles of seminal root axes of wheat but sometimes invades the steles of root laterals. Colonization of wheat roots by *P. graminicola* is almost always limited to the outer cortical cells, even in lateral roots (Deacon, 1981). Both *Phialophora* sp. (lobed-hyphopodia) (Ggg) and *P. graminicola* form growth cessation structures within the cortex of wheat roots (Fig. 1h,i). Ggt, Gga and Ggg all produce hyphopodia, from branching pigmented hyphae, on infected plant tissue (see above; Skou, 1981), but their role, if any, in the infection process is unclear. It is interesting that the related *Magnaporthe grisea* (a foliar pathogen that infects leaves via melanized appressoria) can also colonize wheat roots and, when it does so, it produces hyphopodia (Sesma and Osbourn, 2003).

Nuclear ribosomal DNA (rDNA) sequences have been used to determine phylogenetic relationships between fungi in the *Gaeumannomyces–Phialophora* complex. *G. incrustans*, *G. cylindrosporus*/*P. graminicola* and *G. graminis* formed distinct groups when internal transcribed spacer (ITS) rDNA sequences were analysed using distance-based (Ward and Bateman, 1999) and parsimony-based (Bryan *et al.*, 1995) methods of phylogenetic analysis. Within *G. graminis*, isolates of Ggt, Gga and Ggm formed distinct groups according to variety, whereas Ggg formed three distinct groups, with the distance-based method (Ward and Bateman, 1999). Using the parsimony-based method isolates of Ggt classified as R (rye-attacking) or N (non-rye-attacking) were grouped separately and Australian oat-attacking isolates, classified as Ggt on the basis of ascospore length (Yeates *et al.*, 1986), were grouped with Gga isolates (Bryan *et al.*, 1995). Phylogenetic analysis of ITS sequences has also confirmed that *P. graminicola* is the anamorph of *G. cylindrosporus* (Bryan *et al.*, 1995; Ward and Bateman, 1999), and that *P. zeicola* and *P. radicicola* Cain are likely to be the same fungus and the anamorph of Ggm (Ward and Bateman, 1999). A new *Phialophora* species (typified by isolate GP57), which appears to be the closest known relative to *G. graminis* and its *Phialophora* anamorphs, was recently recorded from wheat in the UK (Ward and Bateman, 1999). Ribosomal ITS sequence analysis (E. Ward, unpublished results) indicates that this fungus may be identical to a new *Phialophora* species identified in Germany (Ulrich *et al.*, 2000). The late wilt pathogen of maize, *Cephalosporium maydis*, was shown to be closely related to *G. graminis* but, contrary to earlier suggestions (Gams, 1971, cited in Walker, 1981), it is a distinct taxon (Ward and Bateman, 1999). Sequence analysis has also confirmed links between the *Gaeumannomyces–Phialophora* complex and *Magnaporthe* species (Bryan *et al.*, 1995; Bunting *et al.*, 1996; Ward and Bateman, 1999).

DETECTION AND IDENTIFICATION OF *G. GRAMINIS* VARIETIES BY DNA-BASED METHODS

Classification of *G. graminis* varieties on the basis of disease symptoms, host range and/or morphological characteristics in culture is difficult, time consuming and laborious. Various semiselective media have proved useful for identification of Ggt, particularly in the USA (Hornby *et al.*, 1998; Juhnke *et al.*, 1984). However, some of these are not suitable for use where avirulent *Phialophora* spp., which behave similarly to Ggt in culture, are present (Hornby *et al.*, 1998).

Various molecular methods have been used to detect and identify *G. graminis* at the species and variety level.

Restriction fragment length polymorphism (RFLP) analyses using labelled DNA probes

Several different DNA probes have been used for identification of isolates of *G. graminis* and related species. A mitochondrial DNA probe from Ggt (pMSU315) hybridized specifically to *Gaeumannomyces* and *Phialophora* species with RFLP patterns characteristic of particular species and varieties (Bateman *et al.*, 1992; Henson, 1989, 1992). Ggt, Gga, Ggg and *G. cylindrosporus*/*P. graminicola* were conclusively identified by RFLP analysis using a mitochondrial small-subunit rDNA probe (GggMR1/pEG34) from Ggg (Bateman *et al.*, 1997; Ward and Bateman, 1994; Ward and Gray, 1992). RFLP analysis using GggMR1 with *Eco*RI-digested DNA also identified subgroups of some species/varieties, but even greater discrimination among Ggt isolates is possible using a mitochondrial small-subunit rDNA probe from Ggt (pGgtMS7, J. Freeman, unpublished results). Attempts in our laboratory to develop a PCR assay based on this region have been unsuccessful. Various primers for this region have been exhaustively tested but reliable amplification from all isolates of Ggt has not been achieved (J. Freeman, unpublished results). A PCR product amplified from an isolate of Ggt (L3.4A) with MSU1 and MSU7 (Zhou and Stanosz, 2001) hybridizes to some genomic fragments homologous to pEG34 and pGgtMS7, but not to others. A partial DNA sequence

of this fragment is almost identical to sequences within introns 1 and 3 of the mtSSU gene of *Cryphonectria parasitica* (accession number AF029891). Optional introns are common in fungal mitochondrial genes (Rosewich and Kistler, 2000; Salvo *et al*., 1998) and it is possible that the variety of RFLP patterns identified by hybridization of pGgtMS7 to *Eco*RI-digested genomic DNA of Ggt (more than two bands greater than 1.8 kb usually hybridize), and the difficulties amplifying this gene from Ggt by PCR, may be due to the presence of optional introns.

RFLP analysis using a nuclear 26S rDNA probe grouped most *G. graminis* isolates according to variety and identified subgroups of Ggt (Tan *et al*., 1994). One RFLP band appeared to be associated with virulence on oats and bentgrass, but the correlation was not absolute. Isolates with the same or similar host preference and pathogenicity were often grouped together by RFLP patterns identified using labelled total DNA of Ggt and a wheat rDNA probe (Bryan *et al*., 1999; O'Dell *et al*., 1992).

Polymorphisms in length or restriction patterns of PCR products

PCR product size and/or restriction site polymorphisms in the ITS region and 18S rRNA gene of the nuclear rDNA can be used to identify *Geaumannomyces* species and varieties of *G. graminis* (Fouly *et al*., 1997; Ward and Akrofi, 1994). Size variation in nuclear 18S and 26S rRNA gene PCR products was found to be due to presence/absence and size variation of group I introns (Fouly and Wilkinson, 2000a; Tan and Wong, 1996). Size variation of 18S rRNA gene introns was used to differentiate between isolates of Ggt, Gga, Ggg, *G. incrustans*, *G. cylindrosporus* and *Ophioceras leptosporum* (misnamed *G. leptosporus* in Fouly *et al*., 1997). Isolates of Ggg had no introns in the 26S gene, whereas isolates of Ggt and Gga had two or three introns (Tan, 1997). PCR primers were designed that subdivided isolates of Ggt into four groups on the basis of presence/absence of the three introns, and, in combination with restriction digestion, could discriminate between isolates of Ggt and Gga (Tan, 1997). Presence/absence of these introns was used for rapid identification of *G. graminis* isolates causing take-all patch disease in Bermuda grass (Wong *et al*., 2000).

Random amplified polymorphic DNA (RAPD) analyses

Various research groups have used RAPD analysis to discriminate between *Gaeumannomyces* and *Phialophora* species, varieties and isolates (Augustin *et al*., 1999; Bryan *et al*., 1999; Fouly *et al*., 1996; Ulrich *et al*., 2000; Wetzel *et al*., 1996), and to identify subgroups within *G. graminis* varieties (Augustin *et al*., 1999; Bryan *et al*., 1999; Fouly *et al*., 1996). RAPD analysis also successfully discriminated *Gaeumannomyces* spp., and some close relatives, from other darkly pigmented fungi commonly found on turf-grass roots (Wetzel *et al*., 1996). Isolates of Ggg showed more genetic variability than isolates of Ggt and Gga, consistent with results obtained using other molecular methods. Although genetic groupings identified by RAPD analyses often correlate well with identification by other methods, there are often problems in obtaining reproducible results and it is difficult to know whether different results obtained by different laboratories are genuine or result from slight differences in procedure. For example, the OPA-02 band identified by Wetzel *et al*. (1996) as characteristic of *G. cylindrosporus*/*P. graminicola* was not scored in the isolate of this species analysed by Fouly *et al*. (1996).

Dendrograms produced from analysis of RAPD data grouped *G. graminis* isolates according to variety (Bryan *et al*., 1999). Isolates of Ggt classified as rye-adapted (R) or non-rye-adapted (N) formed distinct groups in dendrograms. There was good correlation between these RAPD data and RFLP data and with phylogenetic analysis of nuclear rDNA sequences (see above; Bryan *et al*., 1995).

Specific PCR assays

Several PCR primer-pairs for specific detection of particular *Gaeumannomyces* species and varieties of *G. graminis* have been developed. Most are based on nuclear rDNA sequences. Bryan *et al*. (1995) reported specific detection of Ggt and Gga using PCR primer pairs pGt1/pGt2 and pGa1/pGa2, respectively, but sequence data presented in the same report suggest that pGt1/pGt2 may amplify DNA from at least one isolate of Ggg (E. Ward and J. Freeman, unpublished results).

Reverse PCR primers GGT-RP and GGA-RP, with NS5 (White *et al*., 1990) as the forward primer, amplify DNA from Ggt and Gga isolates but not DNA from isolates of Ggg or other *Gaeumannomyces* species tested (Fouly and Wilkinson, 2000b). Amplification by NS5/GGT-RP can distinguish between isolates of Ggt and isolates of Gga on the basis of PCR product size. A nested PCR assay specific to *Gaeumannomyces*, *Phialophora* and *Magnaporthe* species (Henson, 1992; Schesser *et al*., 1991) was subsequently simplified to a single-step PCR assay by Ward (1995). Ggt and Gga could be separated from the other varieties and species tested on the basis of PCR product size but PCR products from isolates of Ggm, *P. radicicola* Cain, *P. zeicola* and *Cephalosporium maydis* were all within the range of PCR product size obtained with Ggg (Ward and Bateman, 1999).

PCR primers GG1 and GG2 were designed for diagnosis of take-all patch of turf-grasses (Goodwin *et al*., 1995). These PCR primers amplified DNA from all isolates of Gga and Ggg tested, but not from the isolates of Ggt, *P. graminicola* or some other turf-grass pathogens and saprophytes that were tested.

Specific PCR assays for identification of Ggt, Ggg and Gga, based on avenacinase and avenacinase-like gene sequences, have recently been developed (Rachdawong *et al*., 2002). Variety-specific forward PCR primers were used, singly and together, with a common

reverse PCR primer to give amplification products of different sizes from DNA of Ggt, Ggg and Gga isolates. However, some Ggt isolates gave PCR products of the size expected for Ggg isolates and the authors suggest further testing to confirm variety specificity.

Comparisons between different identification methods

A summary of the published molecular methods that can be used for identification of *G. graminis* is given in Table 2. Although many diverse methods are available, few of these can reliably identify all varieties of *G. graminis*, and there are many isolates that give atypical results. The difficulties of identification by traditional methods mean that many isolates that have been tested have not been identified to species or variety level, and this can lead to confusion in the literature. Generally, the numbers of isolates tested for each method have been small, or unrepresentative of the wide range of isolates that occur worldwide. There is only one report of molecular testing of the maize take-all fungus, *G. graminis* var. *maydis* (Ggm) and its putative anamorphs, *P. zeicola* and *P. radicicola* Cain, and this included only one isolate of each (Ward and Bateman, 1999). Several studies indicate that there is more variation in Ggg/*Phialophora* sp. (lobed-hyphopodia) than the other varieties, and more work is needed to determine the extent and significance of this variation. In addition, methods for identifying the fungi on turf-grasses, for example, may not be applicable on wheat or vice versa. Very little research on genetic diversity within populations of *G. graminis* has been reported. Harvey *et al.* (2001) demonstrated that similar cropping history was generally associated with close genetic identity of populations of *G. graminis*, whereas geographical proximity was not and concluded that populations may consist of series of host-adapted clonal lines. Unless isolated from the same field at the same time and therefore possibly clonally related, isolates of *G. graminis* are usually vegetatively incompatible with other isolates (Hornby *et al.*, 1998). In view of the difficulties in achieving anastomosis between isolates of *G. graminis* in the laboratory (Asher, 1981), and of producing hybrid perithecia (see below), it seems unlikely that sexual hybridization is important in field populations of *G. graminis*.

Very little work has been done to detect *G. graminis* directly in soil samples or in infected roots by molecular methods. Most of the work has involved isolating and culturing of the fungus. There are no methods available for reliably isolating *G. graminis* directly from soil, and therefore most isolates are obtained from field-grown plants or by growing susceptible plants in the soil as baits. Isolates are usually made from surface-sterilized lesioned root pieces (Hornby *et al.*, 1998), but this could bias sampling towards more virulent isolates. Choosing an appropriate strategy for sampling fields for the presence of the take-all fungus, and determining its population structure, can be difficult, particularly when the disease is patchy. It is desirable that as much of the area is sampled as is practicable and several different procedures have been used to achieve this (Hornby *et al.*, 1998).

GENETICS AND TRANSFORMATION OF *G. GRAMINIS*

The sexual stage in the life cycle of *G. graminis* involves the production of flask-shaped perithecia. Each of the many asci within the perithecia usually contains eight ascospores, resulting from one meiotic and one mitotic division. Because *G. graminis* is homothallic (self-fertile), attempts to perform genetic crosses often result in relatively small proportions of hybrid perithecia compared with the numbers that result from selfing of each parental strain (Asher, 1981; Blanch *et al.*, 1981). In intra-isolate crosses, using mutants or antibiotic-resistant transformants, the percentage of hybrid perithecia was generally around 10–25% but sometimes higher frequencies were obtained (Hornby *et al.*, 1998; Musker, 1994; Pilgeram and Henson, 1992). Also, numerous vegetative incompatibility groups exist within *G. graminis* (Asher, 1981; Jamil and Buck, 1991; Jamil *et al.*, 1984) and this places restrictions on the isolate combinations that can be sucessfully paired. Attempts to make forced heterokaryons by pairing isolates on selective medium produced stable heterokaryons only when mutants of the same isolate were used. Other pairings between different isolates of the same variety or between isolates of Ggt and Gga were unsuccessful (Hornby *et al.*, 1998; Musker, 1994). Further complications arise because the ascospores of *G. graminis* are loosely coiled together within the ascus (Fig. 1b) and it is not possible to distinguish the first (meiotic) and second (mitotic) generation products (i.e. the tetrad is unordered). This makes genetic analysis more difficult and involves the analysis of random meiotic products (Asher, 1981).

Understanding the genetics of *G. graminis* has also been hampered by the lack of available mutants. Mutations would be useful, for example in facilitating gene mapping and in providing novel phenotypic markers for use in genetic crosses and population studies. The generation of *G. graminis* mutants has been hindered by problems in producing sufficient numbers of germinable propagules from uninucleate material; uninucleate conidia are difficult to produce and/or germinate, and ascospores and the vegetative haploid hyphae are multinucleate. Various treatments, such as irradiation with UV or exposure to the chemical mutagens nitrosoguanidine and 4-nitroquinolene oxide, have been used to mutate *G. graminis*. The most successful approach has been to treat protoplasts with mutagenic agents, although unpredictable regeneration frequencies can be a problem. Despite the difficulties, several mutants have been isolated, including those with resistance to the fungicides benomyl and carboxin, altered morphology or pigmentation and a variety of auxotrophic mutations (Bowyer *et al.*, 1992; Frederick *et al.*, 1999).

Another method for generating mutants is by stable transformation. All published reports of transformation of *G. graminis*

Table 2 Summary of features of the various DNA-based methods for the analysis of isolates of *G. graminis* and related species.

Target	Method	References	Diagnostic features	Comments
Nuclear rDNA—ITS region	DNA sequencing	Ward and Bateman (1999) Bryan *et al.* (1995)	Conclusive identification possible.	Useful for phylogenetic analysis. Widely used—many sequences available in databases. Time consuming—not practical for large-scale studies.
	RFLP analysis and size variation of PCR products	Ward and Akrofi (1994) Fouly *et al.* (1997)	Identifies isolates to variety.	More than one enzyme required to identify all isolates to variety. Some isolates give RFLPs typical of other varieties.
	PCR with specific primers pGt1 and pGt2 (Ggt) or pGa1 and pGa2 (Gga)	Bryan *et al.* (1995)	Specific identification of Ggt and Gga, respectively.	pGt1 and pGt2 may possibly amplify DNA from some isolates of Ggg.
	PCR with specific primers GG1 and GG2	Goodwin *et al.* (1995)	PCR specific for Ggg and Gga.	Used to identify *G. graminis* isolates pathogenic to turf-grasses (Ggg and Gga) in the US. May not amplify DNA of UK isolates.
Nuclear rDNA—18S rDNA	Size variation of NS5 and NS6 PCR products	Fouly *et al.* (1997) Fouly and Wilkinson (2000a)	Isolates of Ggt and Gga have characteristic PCR product size.	Based on very few isolates. Size of PCR products of Ggg isolates varies. Conclusive identification to species/variety not possible.
	PCR with GGT-RP or GGA-RP and NS5	Fouly and Wilkinson (2000b)	PCR specific for Ggt and Gga.	PCR with GGT-RP and NS5 identifies isolates as Ggt or Gga on the basis of PCR product size. PCR with GGA-RP and NS5 can be used to identify isolates pathogenic to cereals (Ggt and Gga).
	RFLP analysis with 18S rDNA probe	Tan *et al.* (1994)	Size of hybridizing fragment of different varieties varies.	Does not identify isolates to species/variety.
Nuclear rDNA—26S rDNA	RFLP analysis with 26S rDNA probe	Tan *et al.* (1994)	Can be used to discriminate cereal pathogens (Ggt and Gga) from Ggg. Identifies subgroups of Ggt isolates.	Does not discriminate between isolates of Ggt and Gga.
	RFLP analysis and size variation of PCR products	Tan *et al.* (1994) Tan and Wong (1996) Tan (1997) Wong *et al.* (2000)	Identifies isolates of *G. graminis* to variety.	Size variation of PCR products discriminates cereal pathogens (Ggt and Gga) from Ggg. RFLP analysis of PCR products discriminates between Ggt and Gga.
Other repeated DNA sequences	RFLPs of repeated DNA sequences (total Ggt DNA and wheat rDNA probes)	O'Dell *et al.* (1992) Bryan *et al.* (1999)	Can be used to indicate host preference of *G. graminis* isolates.	For a few isolates RFLP results did not correlate with host range.
	Quantification of Ggt and RFLP analysis using probe pG158	Herdina *et al.* (1996) Herdina *et al.* (1997) Herdina and Roget (2000)	Probe is relatively specific for Ggt.	Weak hybridization to Gga, even at high stringency.
Mitochondrial DNA	RFLP analysis with pMSU315	Henson (1989) Henson (1992) Bateman *et al.* (1992) Ward and Bateman (1999)	Can be used to discriminate cereal pathogens (Ggt and Gga) from Ggg.	Does not discriminate between isolates of Ggt and Gga. Some isolates give atypical RFLP patterns. Does not discriminate between G-P complex isolates from maize.
	RFLP analysis with pEG34	Ward and Gray (1992) Ward and Bateman (1994) Bateman *et al.* (1997) Ward and Bateman (1999)	Identifies isolates of G-P complex to species and variety and identifies subgroups of *G. graminis* varieties.	Cannot distinguish Ggm (and *P. zeicola*, *P. radicicola* Cain) from some isolates of Ggt.

Table 2 *continued.*

Target	Method	References	Diagnostic features	Comments
	Nested PCR with KS1F and KS2R followed by KS4F and KS5R	Henson (1989) Schesser *et al.* (1991) Henson (1992) Elliott *et al.* (1993) Henson (1993)	PCR specific to G-P complex and *Magnaporthe* spp.	Does not identify isolates to species/variety.
	PCR with KS1F and KS2R (including modifications by Ward, 1995)	Ward (1995)	Specific PCR for *G. graminis*. Discriminates cereal pathogens (Ggt and Gga) from Ggg.	Does not identify isolates of Ggt and Gga to variety.
Random	RAPD analysis	Fouly *et al.* (1996) Wetzel *et al.* (1996) Bryan *et al.* (1999) Augustin *et al.* (1999) Ulrich *et al.* (2000)	Can identify isolates to species and variety and identify subgroups within varieties.	Generally analysis with more than one RAPD primer is required. Results can be inconsistent.
Avenacinase and avenacinase-like genes	PCR analysis with variety-specific (Ggt, Gga and Ggg) forward primers and a common reverse primer (AV3)	Rachdawong *et al.* (2002)	Variety-specific PCR.	All primers can be included in a single PCR reaction. Based on a single-copy gene, therefore may not be as sensitive as methods using rDNA or mitochondrial targets. Some Ggt isolates gave results typical of Ggg isolates.
Laccase genes	DNA sequencing	Litvintseva and Henson (2002a, 2002b)	Potential alternative to nuclear rDNA sequencing for phylogenetic analyses.	Time consuming therefore not practical for large-scale studies.

have used PEG-$CaCl_2$-mediated protoplast transformation. Benomyl- and phleomycin-resistant transformants of Ggg and Ggt have been obtained using this method, but the transformation efficiency is very low (1–5 transformants/μg DNA per 10^7 protoplasts) (Bowyer *et al.*, 1995; Henson *et al.*, 1988; Pilgeram and Henson, 1990), and varies greatly from experiment to experiment (P. Bowyer, personal communication). The stability of the integrated DNA varies. Transgenes in the majority of transformants are mitotically stable, but their stability through meiosis varies with copy number and with different genes (Pilgeram and Henson, 1990, 1992; Pilgeram *et al.*, 1993). For example, benomyl- and phleomycin-resistant transformants of Ggt and Ggg were mitotically stable irrespective of transgene copy number. In most cases, *G. graminis* transformants with a single copy of the transgene were phenotypically and genotypically stable through meiosis, but, of six transformants with multiple transgene copies, four were both phenotypically and genotypically unstable, and stability did not correlate with either benomyl or phleomycin resistance (Pilgeram and Henson, 1992).

Various mutants have been isolated from *G. graminis* following transformation and the random insertion of plasmids conferring benomyl- and phleomycin-resistance. These include a nicotinic acid auxotroph of Ggt, a pigment mutant and several Ggg mutants with abnormal/no hyphopodia (Epstein *et al.*, 1994; Pilgeram and Henson, 1992). Targeted gene disruption is a much more effective and precise method of generating mutants provided the requisite gene has been cloned, but we have found only one successful report of its use in *G. graminis*, namely to produce avenacinase mutants of Gga (Bowyer *et al.*, 1995).

The poor transformation frequencies that are obtained using *G. graminis* has caused great difficulties understanding gene function in Ggt via gene disruption experiments. As for most fungal species, the technique at present is inadequate for identification of DNA clones from genomic libraries by complementation of mutants. A novel method, involving recombination between linear genomic DNA and a fungal replicating vector (pHELP1), and complementation of *Aspergillus nidulans* mutants, was used to clone the *argB* and *pyrG* genes of Gga (Bowyer *et al.*, 1994). This method relies on a suitable mutant strain being available and on the abilty of the target species gene to complement the mutant gene in *A. nidulans*. Only two of seven mutants were complemented and the genes responsible for complementation were rescued into *Escerichia coli* for further analyses.

FACTORS IMPLICATED IN PATHOGENICITY AND HOST RANGE DETERMINATION

Avenacinase

Isolates of the wheat take-all fungus (Ggt) are unable to infect oats, whereas Gga can. Oat roots produce the saponin avenacin,

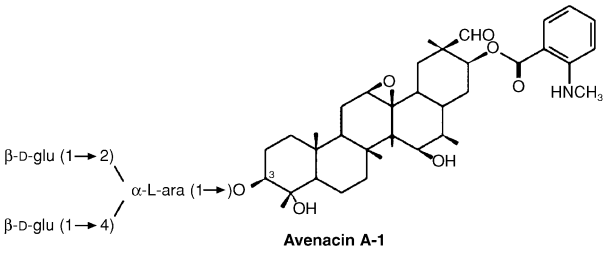


Fig. 2 Structure of the oat saponin avenacin A-1. Avanacin is produced in the roots of oat plants, but not in wheat or barley roots, and is located in a circle around the periphery of healthy oat roots. The oat-attacking variety of *Gaeumannomyces graminis* (*G. graminis* var. *avanae* (Gga)) produces the saponin-detoxifying enzyme, avenacinase, that detoxifies avanacin by removing the 1→2- and 1→4-linked terminal D-glucose (glu) sugar molecules.

a glycosylated triterpenoid secondary metabolite (Fig. 2), which is a constitutively present antimicrobial agent (phytoanticipin) that can inhibit a wide range of fungi (Osbourn, 1996a,b). Some fungi, including Gga, can detoxify avenacin using the enzyme avenacinase (Fig. 2), whereas others such as Ggt cannot. A series of studies have demonstrated that avenacinase is required for infection of oats by Gga, and that it is involved in host-range determination. The Gga avenacinase gene was cloned using anti-avenacinase antisera to screen cDNA expression libraries of Gga, and this was then used to generate mutants using targeted gene disruption (Bowyer *et al*., 1995). The avenacinase-minus mutant was no longer pathogenic on oats but was still able to infect wheat. Ggt and some other members of the *Gaeumannomyces–Phialophora* complex also contain DNA sequences homologous to the avenacinase gene even though they are unable to inactivate avenacin effectively (Osbourn, 1996a; Osbourn *et al*., 1994a; Rachdawong *et al*., 2002). The role of these avenacinase-like genes is uncertain but it has been suggested that they might be involved in producing enzymes that detoxify other saponins in hosts other than oats (e.g. grasses). When a wide range of *Avena* species were screened, *Avena longiglumis* was the only one found not to produce avenacin, and this was also the only species that was susceptible to Ggt (Osbourn *et al*., 1994b). Further evidence for the role of avenacin as a resistance determinant was obtained by studies of saponin deficient (*sad*) mutants of the diploid oat species *Avena strigosa*, which were isolated using the UV fluorescence properties of avenacin as a preliminary screen (Papadopoulou *et al*., 1999). These mutants were compromised in their resistance to Ggt as well as *Fusarium culmorum* and *F. avenaceum*, but not the leaf-infecting fungi *Stagonospora nodorum* and *S. avenae*. The gene encoding the enzyme that catalyses the first committed step in avenacin biosynthesis (β-amyrin synthase, AsbAS1) has recently been cloned and shown to be a novel oxidosqualene cyclase (Haralampidis *et al*., 2001). It was demonstrated that *Sad1* mutants of *A. strigosa* are mutated in the gene encoding this enzyme. The *AsbAS1* gene is highly conserved in oat, but is absent from other cereals such as wheat. Manipulation of these pathways, by genetic engineering or other means, might eventually provide a means of generating resistance to Ggt and other pathogens in wheat, although this could be compromised if there were an increase in the prevalence of Gga, or Ggt isolates that can infect oats (Bryan *et al*., 1999; Hornby *et al*., 1998).

Melanin

Various studies have investigated the synthesis and function of melanin in *G. graminis* varieties. Melanin is a dark polymeric pigment, found in many phytopathogenic fungi, which has a role in the pathogenicity of some fungal species, whereas in other species it protects against environmental stresses such as extremes of temperature and pH, ionizing radiation, oxidative stress, desiccation, metals, plant defence mechanisms and the lytic action of microorganisms in soil (Bell and Wheeler, 1986; Butler and Day, 1998; Butler *et al*., 2001; Henson *et al*., 1999; Langfelder *et al*., 2003).

There are several different pathways for fungal melanin biosynthesis, but the best characterized and most commonly utilized pathway is the dihydroxynaphthalene (DHN)–melanin pathway. Studies with melanin inhibitors have shown that this is also the pathway used by *G. graminis* (Elliott, 1995; Kelly, 1997). Melanin can, however, be produced from L-dihydroxyphenylalanine (L-DOPA), when added to synthetic media, by *G. graminis* and this is the basis of a diagnostic selective medium for *G. graminis* (Juhnke *et al*., 1984). Extracellular laccases secreted by the fungus are able to convert the L-DOPA to melanin, but there is no evidence that the L-DOPA pathway for melanin synthesis occurs under normal growth conditions.

In certain phytopathogenic fungi, such as *Magnaporthe grisea*, which use melanized appressoria as their sole means of host surface penetration, melanin is essential for pathogenicity (Henson *et al*., 1999; Howard and Valent, 1996). In *M. grisea,* it has been demonstrated that melanin limits cell-wall permeability, allowing osmolyte accumulation and the build up of enormous turgor pressures in the appressoria (Howard *et al*., 1991; Money, 1995). This process creates the forces required for the penetration peg to break the host cell cuticle and epidermal cell wall. However, it has been proposed that this may not be necessary to overcome the less substantial barriers to infection in the roots; *M. grisea* mutants unable to synthesize DHN–melanin (*alb1* and *buf1*) were unable to infect wheat leaves but were still able to infect wheat roots (Dufresne and Osbourn, 2001; Osbourn, 2001). *G. graminis* produces melanized appressorium-like structures called hyphopodia, but these develop from the end of hyphae rather than conidial germ tubes, and their role, whether as a platform for host infection or otherwise, is unclear.

The role of melanin in *G. graminis* has been investigated in several studies using mutants and inhibitors of melanin synthesis. In Ggg, a mutant (*thr*) was generated that was unable to synthesize melanin and another (*moe*) that was constitutively melanized (Frederick *et al*., 1999). The non-melanized mutant was found to be unaltered in its pathogenicity on rice compared with the wild-type, but the constitutively melanized mutant was actually less pathogenic than the wild-type on rice. The explanation for this phenotype may be that increased amounts of melanin in the hyphopodia limit their ability to secrete the various extracellular lytic enzymes that are required for host tissue degradation and subsequent invasion. Heavily melanized variants of Ggt have also been reported to be non-pathogenic on wheat (Goins *et al*., 2002). The wild-type and constitutively melanized Ggg mutant were found to be more hydrophobic, and more resistant to lytic enzymes, benomyl, restrictive temperature and UV light than the non-melanized mutant (Frederick *et al*., 1999). Measurements of hyphopodial turgor were performed on these Ggg strains (Money *et al*., 1998) and, although it was demonstrated that melanin synthesis was associated with changes in hyphopodial turgor, permeability and cell-wall rigidity, the pressures generated were much lower than in *M. grisea*.

In Ggt, there is some evidence that melanin is required for pathogenicity because an albino mutant was demonstrated to be non-pathogenic and the DHN melanin synthesis inhibitors tricyclazole and PP389 reduced the pathogenicity of Ggt isolates (Kelly, 1997; Kelly *et al*., 1997). Earlier work by Elliott (1995) had indicated that inhibitors of DHN–melanin synthesis did not alter pathogenicity, but this study used lower concentrations of the inhibitors. It seems likely that any requirement for melanin in pathogenicity of Ggt may not be for host penetration, but may instead be needed for the production of runner hyphae on roots (Dufresne and Osbourn, 2001; Henson *et al*., 1999).

The role of melanin in *G. graminis* therefore remains unclear, particularly in relation to pathogenesis, and may be different in different members of the *Gaeumannomyces–Phialophora* complex. However, it seems likely that melanin plays a role in protection against environmental stresses, a property that may be particularly relevant in a soil-borne pathogen such as *G. graminis* that survives saprophytically in the field between susceptible host crops.

Laccases

G. graminis isolates secrete various laccases that accumulate in their cell walls and extracellularly. This is the basis of a diagnostic semiselective medium for *G. graminis* (Juhnke *et al*., 1984), which converts L-DOPA in the medium to melanin. The final polymerization reaction in the DHN–melanin pathway, the oxidation of 1,8-DHN into melanin, is also thought to be mediated by a laccase; extracellular laccase purified from Ggt has been shown to convert 1,8-DHN into polymers (Edens *et al*., 1999). A monoclonal antibody has been produced for which the target antigen appears to be a Ggt laccase gene and this has been used in immunofluorescence microscopy studies and in diagnostic assays for *G. graminis* (Thornton *et al*., 1997). The immunofluorescence studies showed that the antigen was associated with both the melanized runner hyphae and the hyaline mycelia.

Laccases are widely distributed enzymes and individual fungi can produce several different laccase enzymes, each of which may have a variety of roles. This is likely to complicate the determination of their individual functions, and mutants with multiple disruptions may be necessary. One of the main roles of laccases is in melanin synthesis. They may also be involved in degradation of lignin, metabolizing phytotoxic substances and oxidation of humic acids and manganese ions (Litvintseva and Henson, 2002a). The Ggt extracellular laccase will decolorize the lignin-like dye poly B-411, indicating that it may have a role in lignin depolymerization in the plant host (Edens *et al*., 1999).

Three genes encoding laccase enzymes have recently been cloned from Ggt by screening genomic libraries using a laccase gene probe from *Neurospora crassa*. Their relative expression patterns were studied using RT-PCR (Litvintseva and Henson, 2002a). *LAC1* was transcribed constitutively, but *LAC2* was copper inducible and corresponded to the enzyme purified by Edens *et al*. (1999). All three genes were transcribed *in planta*, but transcription of *LAC3* was found only *in planta* or in the presence of wheat homogenate.

A full-length *LAC1* genomic clone was isolated from Ggg using the Ggt *LAC1* gene as a probe, and the *LAC2* genomic clone was isolated using PCR with primers based on the Ggt sequence (Litvintseva and Henson, 2002b). The *LAC1* and *LAC2* sequences were highly conserved between different Ggg and Ggt isolates, but the expression patterns were different. In Ggg, a high level of *LAC1* transcription was seen only in the presence of the plant, and copper had no effect on the transcription of *LAC2*. There was some evidence for the presence of a *LAC3* gene in Ggg but, using RT-PCR, no transcription was detected under any of the conditions used. Expression of the *LAC1* and *LAC2* genes was also monitored in mutants with altered melanin synthesis. The unmelanized (*thr*) mutant showed similar levels of expression of the two genes to the wild-type, but the over-melanized mutant showed higher levels of *LAC1* expression and lower levels of *LAC2* expression than the wild-type.

Cell-wall-degrading enzymes

Fungal plant pathogens produce a range of enzymes capable of degrading the components of plant cell walls, and these enzymes may play a role in nutrition and cell penetration (Annis and Goodwin, 1997; Cooper, 1983; Cooper *et al*., 1988). Various biochemical studies have demonstrated that Ggt can produce cellulases, xylanases and pectinases in infected wheat roots and/or *in vitro*,

but there are discrepancies between some of these reports as to the particular enzymes that are produced by Ggt, and in the correlations between their activities and pathogenicity (reviewed by Hornby *et al.*, 1998; Sivasithamparam and Parker, 1981). Also, in other fungal pathogens, studies with mutants that are defective in individual enzymes have usually failed to show that these enzymes are needed for pathogenicity or virulence. This is probably because most fungi produce multiple forms of many of these enzymes and mutation of a single enzyme does not therefore affect the ability of the fungus to degrade the cell-wall polymer (Annis and Goodwin, 1997). It may therefore prove difficult to demonstrate any links between cell-wall-degrading enzymes and pathogenicity in Ggt even if mutants defective in these enzymes could be isolated.

Cell-wall-degrading enzymes of *G. graminis* have also been studied indirectly by monitoring their effects on wheat roots (Huang *et al.*, 2001a; Kang *et al.*, 2000). Various cell-wall components were studied in uninoculated and Ggt-infected wheat roots using electron microscopy with enzyme-gold and immunogold labelling techniques (Kang *et al.*, 2000). The labelling densities of cellulose, xylan and pectin in the cell walls of infected roots were significantly reduced compared with healthy wheat roots, indicating that Ggt produces enzymes that degrade these cell-wall components. Comparisons were also made between root tissues infected by Ggt or *P. graminicola* (Huang *et al.*, 2001a). The results indicated that *P. graminicola* caused only slight modification of the cortical cell walls compared with Ggt, suggesting that *P. graminicola* produces less cell-wall-degrading enzymes than Ggt during infection of wheat roots.

Enzymes involved in fatty acid metabolism

A number of recent studies of *G. graminis* have focused on enzymes involved in the production of oxylipins, biologically active compounds generated by oxidative metabolism of polyunsaturated fatty acids. Two fatty acid dioxygenase enzymes were recently purified and characterized from *G. graminis* and the resulting peptide sequence information used to clone the genes. These were a lipoxygenase gene that contains manganese at its active site (Hörnsten *et al.*, 2002b; Su and Oliw, 1998), and a linoleate diol synthase (Hörnsten *et al.*, 1999; Su and Oliw, 1996). The roles of these enzymes remain to be elucidated but, in other organisms, oxylipins are thought to have important biological roles in signalling, the degradation of cellular membranes, reproduction and development (Feussner *et al.*, 2001; Herman, 1998; Hörnsten *et al.*, 1999, 2002a; Howe and Schilmiller, 2002). The *G. graminis* linoleate diol synthase gene was recently shown to be similar to a gene thought to be involved in spore formation in *Ustilago maydis* (Huber *et al.*, 2002). There is currently much research activity in this area and it is likely that there will be interesting developments in the near future.

Cyclic hydroxamic acids

The first identification of antifungal activity of a hydroxamic acid from a cereal was nearly 50 years ago (Virtanen and Hietala, 1955). Since then, evidence has suggested roles for hydroxamic acids in defence against fungi, bacteria and insects, in the detoxification of herbicides and in allelopathic effects (Niemeyer, 1988). Cyclic hydroxamic acids are found almost exclusively in the *Gramineae* (Frey *et al.*, 1997). They have a 4-hydroxy-1,4-benzoxazin-3-one structure, and are found constitutively in wheat, rye, triticale, maize and sorghum, but are not present in barley, rice or oats (Morrissey and Osbourn, 1999; Niemeyer, 1988). *In planta*, hydroxamic acids are sequestered as inactive glucosides, but are hydrolysed after infection or tissue damage to aglucones, e.g. 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which decompose to BOA and MBOA, respectively (Friebe *et al.*, 1998; Morrissey and Osbourn, 1999). Friebe *et al.* (1998) studied the ability of *G. graminis* varieties to degrade and tolerate MBOA and BOA. The results did not reveal any clear correlations with pathogenicity. However, other work has demonstrated correlations between susceptibility of cereals to take-all and hydroxamic acid production (Wilkes *et al.*, 1999). Wheat, rye and triticale, which differ in their susceptibility to Ggt, all contain DIMBOA, whereas only rye and some cultivars of triticale contain DIBOA in addition to DIMBOA. Root extracts from wheat did not inhibit the growth of Ggt, but those of rye and triticale did, and DIBOA and BOA were more potent growth inhibitors of Ggt than DIMBOA and MBOA. The effects of hydroxamic acids on the growth of *G. graminis* are fungistatic rather than fungicidal (Frey *et al.*, 1997; Wilkes *et al.*, 1999). Wilkes *et al.* (1999) estimated the concentration of DIBOA *in planta* to be in the range 0.3–2 mM. DIBOA severely inhibited the growth of Ggt at 0.5–1.0 mM, and thus growth inhibition can occur at concentrations estimated to occur *in planta* (Wilkes *et al.*, 1999). In cereals, hydroxamic acids are found in all plant parts, but are generally most abundant in the vascular tissues and in young seedlings (Morrissey and Osbourn, 1999; Niemeyer, 1988). In wheat, rye and triticale roots, amounts of hydroxamic acids are greater in the growing root tip and the root stele than behind the root meristem or in the cortex (Niemeyer, 1988; Wilkes *et al.*, 1999), consistent with a role in defence against Ggt infection. A gene cluster encoding the enzymes of the DIBOA biosynthetic pathway has been identified in maize (Frey *et al.*, 1997). Transfer of these genes to wheat may offer an alternative approach to the development of wheat lines less susceptible to take-all.

A PMK1-related MAP kinase

Magnaporthe grisea, although closely related to *G. graminis*, usually infects the leaves of cereals by means of appressoria formed by conidial germ tubes. This pathogen has recently been

demonstrated to cause lesions on wheat and barley roots, but infection of roots does not require the formation of appressoria (Dufresne and Osbourn, 2001). A number of *M. grisea* mutants were tested for their ability to infect wheat and barley leaves and roots (Dufresne and Osbourn, 2001). Some of the mutants (*pmk1*) were deficient in MAP (mitogen activated protein) kinase activity. PMK1-related MAP kinases have been shown to be required for virulence of a number of fungi, including the root-infecting fungus *Fusarium oxysporum* f.sp. *lycopersici* (Di Pietro *et al.*, 2001; Xu, 2000). The *M. grisea pmk1* (MAP kinase deficient) mutants were unable to infect leaves of rice and barley and were also unable to cause lesions on wheat and barley roots. A *G. graminis* homologue of *PMK1* (*GMK1*) was cloned, using a probe made using degenerate PCR primers designed for amplification of FUS3-related MAP kinases, and expressed in *M. grisea pmk1* mutants. All defects of the *pmk1* mutants were complemented by the *G. graminis* gene, and transformants were able to infect leaves and roots, but whether *GMK1 has* a role in the pathogenicity of *G. graminis* is still unclear. Until more efficient transformation of *G. graminis* and other related root-infecting fungi is possible, the complementation of *M. grisea* mutants offers some prospects for studying genes that may be involved in the pathogenicity of *G. graminis* and that have previously been identified in other fungal species.

VIRUSES AND EXTRACHROMOSOMAL NUCLEIC ACIDS

Viruses and double-stranded RNAs (dsRNAs) are widespread in *G. graminis* but, to date, it is not clear what effects they have on their hosts. In several fungi, dsRNA has been shown to be associated with hypovirulence, notably *Cryphonectria parasitica*, where a cause and effect relationship has been proven (Dawe and Nuss, 2001). In some fungi (e.g. *Saccharomyces cerevisiae* and *Ustilago maydis*) dsRNAs have been shown to be associated with toxin production, and in others (e.g. *Helminthosporium victoriae* and *Ophiostoma novo-ulmi*) they may cause disease of the fungus (Buck, 1998). Viruses present in *G. graminis* mycelium were the subject of much research in the 1970s and early 1980s when it was thought that they might be associated with hypovirulence and/or take-all decline (reviewed by Buck, 1986, 1997; Rawlinson and Buck, 1981).

The viruses do not have an extracellular phase and are probably transmitted by vegetative (somatic hyphal) fusion or during mating of isolates. They are sometimes, but not always, eliminated during ascospore formation. *G. graminis* viruses are isometric and have dsRNA genomes. There is much variation in the properties of these viruses and they have been classified into five groups depending on the physical properties of the particle, the capsid polypeptide serology, and the numbers and sizes of the dsRNAs they contain (Buck, 1986). Similar viruses have been observed in different vegetative compatibility groups of Ggt, the other varieties of *G. graminis* and *P. graminicola* (Jamil and Buck, 1991; Jamil *et al.*, 1984; Liang and Chen, 1990; Liang *et al.*, 1989; McGinty *et al.*, 1981). The distribution of *G. graminis* viruses is complex; Ggt isolates from a single field can have different combinations of several different viruses. This has created difficulties in understanding the relationships among virus, fungus and pathogenicity. The work done to date suggests that viruses in general do not modify pathogenicity, but specific viral RNAs (or combinations of RNAs) may have a role in this. In *Rhizoctonia solani*, there was also much controversy for many years concerning the effect of dsRNAs on virulence. More recently, however, an explanation for some of the conflicting findings emerged, when distinct dsRNAs were found to be associated with enhanced or diminished virulence (Jian *et al.*, 1997). Further characterization of dsRNAs using molecular techniques could help clarify their role(s), if any, in *G. graminis*. There appears to have been no research in this area since the early 1990s.

Linear dsDNA plasmids also appear to be common in *G. graminis* (Henson and Ton That, 1995; Honeyman and Currier, 1986). These range from around 4 kb to 11 kb and are found in the mitochondria. A wide range of *G. graminis* strains from diverse geographical locations contain these elements but some strains are plasmid-free. Most *G. graminis* strains analysed had one or two plasmids and the most observed in any one strain was four. The dsDNAs show varying degrees of homology to one another. In other fungi, plasmids have been shown to encode toxins or virulence factors or to be involved in senescence but, to date, it has not been possible to ascribe a function to any of the *G. graminis* plasmids (Henson and Ton That, 1995).

CONCLUDING REMARKS

Considerable progress has been made as a result of many years of intensive research on take-all, but there is still much that we do not understand about the disease and the fungi that cause it. Take-all control is hampered by the economics of cereal growing and the need for consecutive cropping, and presents a major challenge in plant pathology. Experimental studies on take-all and effective disease control are slow to perform because of the unpredictability of the disease and its occurrence in patches (epidemics can progress at different rates in different parts of the same field). As for many diseases caused by soil-borne pathogens, the impacts of environmental factors (biological and physical) on disease severity are difficult to measure and predict, and, especially for take-all, can be very different in different parts of the world. Control measures that are effective in the USA, for example, may not be effective in Europe or Australia (Hornby *et al.*, 1998). Much innovative research on potential biological control agents has not led to any widely available, effective commercial products (reviewed in Cook, 2003). However, the recently available fungicides silthiofam and fluquinconazole, applied as

seed treatments, offer some promise for controlling the disease, but their effective and economic use depends on accurate targeting of those crops that will benefit from treatment (Bateman *et al.*, 2003). Also, their long-term future could be compromised if resistance/insensitivity or other factors reduce their effectiveness.

Identification of pathogenic and non-pathogenic *Gaeumannomyces* and *Phialophora* species has improved with the advent of molecular diagnostic methods although, again, techniques developed in one part of the world may not be universally applicable. There have also been significant advances in elucidating the role of avenacinase in the pathogenicity of the oat take-all fungus. However, progress in understanding other mechanisms determining pathogenicity, and differences between *G. graminis* varieties, has been hampered by the difficulties of performing genetic analyses with the fungi and the lack of an efficient, stable transformation system. Future advances may be helped by the availability of genome sequence data from other fungal plant pathogens, which could allow the isolation of interesting related genes from *G. graminis*, and then testing for function in other fungi such as the related *Magnaporthe grisea* (Osbourn, 2001). Wheat germplasm that is naturally resistant to take-all does not exist. A transgenic plant approach may offer research opportunities. For example, transfer of the genes encoding enzymes involved in biosynthesis of avenacin or cyclic hydroxamic acids to wheat would offer the ability to test whether these compounds could play a role in making wheat lines less susceptible to take-all.

Achieving effective and complete control of take-all is unlikely to be achieved by a single approach, because of the number of interacting factors involved in disease incidence and severity. The use of cultural control measures is likely to remain an important component of any future disease management strategy.

ACKNOWLEDGEMENTS

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. J.F. was funded by Defra, HGCA and Aventis through the Sustainable Arable LINK project (LK0907). We thank Richard Gutteridge for providing photographs and Geoff Bateman, John Jenkyn, Richard Gutteridge and Kim Hammond-Kosack for helpful comments on the manuscript.

REFERENCES

Andrade, O.A., Mathre, D.E. and Sands, D.C. (1994) Natural suppression of take-all disease of wheat in Montana soils. *Plant Soil*, **164**, 9–18.

Annis, S.L. and Goodwin, P.H. (1997) Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. *Eur. J. Plant Pathol*, **103**, 1–14.

Asher, M.J.C. (1981) Pathogenic variation. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 199–218.

Asher, M.J.C. and Shipton, P.J. (1981) *Biology and Control of Take-All*. London: Academic Press.

Augustin, C., Ulrich, K., Ward, E. and Werner, A. (1999) RAPD-based inter- and intravarietal classification of fungi of the *Gaeumannomyces–Phialophora* complex. *J. Phytopathol*, **147**, 109–117.

Bateman, G.L. (1989) Progress in research on the control of take-all in cereals with fungicides—a review. *Crop Prot.*, **8**, 75–81.

Bateman, G.L., Freeman, J., Gutteridge, R.J., Jenkyn, J.F. and Ward, E. (2003) *Strategies for Fungicidal Control of Take-All*. HGCA project report 309. London: Home-Grown Cereals Authority.

Bateman, G.L., Ward, E. and Antoniw, J.F. (1992) Identification of *Gaeumannomyces graminis* var. *tritici* and *G. graminis* var. *avenae* using a DNA probe and non-molecular methods. *Mycol. Res.* **96**, 737–742.

Bateman, G.L., Ward, E., Hornby, D. and Gutteridge, R.J. (1997) Comparisons of isolates of the take-all fungus, *Gaeumannomyces graminis* var. *tritici*, from different cereal sequences using DNA probes and non-molecular methods. *Soil Biol. Biochem.* **29**, 1225–1232.

Beale, R.E., Phillion, D.P., Headrick, J.M., O'Reilly, P. and Cox, J. (1998) MON65500: a unique fungicide for the control of take-all in wheat. In *Proceedings of the 1986 British Crop Protection Conference—Pests and Diseases*. Farnham, UK: British Crop Protection Council, pp. 343–350.

Bell, A.A. and Wheeler, M.H. (1986) Biosynthesis and functions of fungal melanins. *Annu. Rev. Phytopathol.* **24**, 411–451.

Blanch, P.A., Asher, M.J.C. and Burnett, J.H. (1981) Inheritance of pathogenicity and cultural characters in *Gaeumannomyces graminis* var. *tritici*. *Trans. Br. Mycol. Soc.* **77**, 391–399.

Bowyer, P., Clarke, B.R., Lunness, P., Daniels, M.J. and Osbourn, A.E. (1995) Host-range of a plant-pathogenic fungus determined by a saponin detoxifying enzyme. *Science*, **267**, 371–374.

Bowyer, P., Musker, R., Osbourn, A.E., Clarke, B., Caten, C. and Daniels, M.J. (1992) Production of mutants of *Gaeumannomyces graminis* var. *tritici* and var. *avenae* by 4-nitroquinolene-oxide treatment of protoplasts. *Fungal Genet. Newsletter*, **39**, 13–14.

Bowyer, P., Osbourn, A.E. and Daniels, M.J. (1994) An instant gene bank method for heterologous gene cloning—complementation of 2 *Aspergillus nidulans* mutants with *Gaeumannomyces graminis* DNA. *Mol. Gen. Genet.* **242**, 448–454.

Bryan, G.T., Daniels, M.J. and Osbourn, A.E. (1995) Comparison of fungi within the *Gaeumannomyces–Phialophora* complex by analysis of ribosomal DNA sequences. *Appl. Environ. Microbiol.* **61**, 681–689.

Bryan, G.T., Labourdette, E., Melton, R.E., Nicholson, P., Daniels, M.J. and Osbourn, A.E. (1999) DNA polymorphism and host range in the take-all fungus, *Gaeumannomyces graminis*. *Mycol. Res.* **103**, 319–327.

Buck, K.W. (1986) Viruses of the wheat take-all fungus *Gaeumannomyces graminis* var. *tritici*. In *Fungal Viruses* (Buck, K.W., ed.). Boca Raton, FL: CRC Press, pp. 221–236.

Buck, K.W. (1997) Viruses of plant pathogenic fungi. In *Genetics and Plant Pathogenesis* (Day, P.R. and Jellis, G.J., eds). Oxford, UK: Blackwell Scientific Publications, pp. 111–126.

Buck, K.W. (1998) Molecular variability of viruses of fungi. In *Molecular Variability of Fungal Pathogens* (Bridge, P.D., Couteaudier, Y. and Clarkson, J.M., eds). Wallingford, UK: CAB International, pp. 53–72.

Bunting, T.E., Plumley, K.A., Clarke, B.B. and Hillman, B.I. (1996) Identification of *Magnaporthe poae* by PCR and examination of its relationship to other fungi by analysis of their nuclear rDNA ITS-1 regions. *Phytopathology*, **86**, 398–404.

Bussaban, B., Lumyong, S., Lumyong, P., Hyde, K.D. and McKenzie, E.H.C. (2001a) Two new species of endophytes (ascomycetes) from *Zingiberaceae* sporulating in culture. *Nova Hedwigia*, **73**, 487–493.

Bussaban, B., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and Hyde, K.D. (2001b) Endophytic fungi from *Amomum siamense*. *Can. J. Microbiol.* **47**, 943–948.

Butler, M.J. and Day, A.W. (1998) Fungal melanins: a review. *Can. J. Microbiol.* **44**, 1115–1136.

Butler, M.J., Day, A.W., Henson, J.M. and Money, N.P. (2001) Pathogenic properties of fungal melanins. *Mycologia*, **93**, 1–8.

Cannon, P.F. (1994) The newly recognized family *Magnaporthaceae* and its interrelationships. *Systema Ascomycetum*, **13**, 25–42.

Capper, A.L. and Higgins, K.P. (1993) Application of *Pseudomonas fluorescens* isolates to wheat as potential biological control agents against take-all. *Plant Pathol.* **42**, 560–567.

Chapon, A., Guillerm, A.Y., Delalande, L., Lebreton, L. and Sarniguet, A. (2002) Dominant colonisation of wheat roots by *Pseudomonas fluorescens* Pf29A and selection of the indigenous microflora in the presence of the take-all fungus. *Eur. J. Plant Pathol.* **108**, 449–459.

Cook, R.J. (2003) Take-all of wheat. *Physiol. Mol. Plant Pathol.* **62**, 73–86.

Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G. and Kim, D. (1995) Molecular mechanisms of defense by rhizobacteria against root disease. *Proc. Natl Acad. Sci. USA*, **92**, 4197–4201.

Cooper, R.M. (1983) The mechanisms and significance of enzymic degradation of host cell walls by parasites. In *Biochemical Plant Pathology* (Callow, J.A., ed.). Chichester, UK: Wiley, pp. 101–137.

Cooper, R.M., Longman, D., Campbell, A., Henry, M. and Less, P.E. (1988) Enzymic adaptation of cereal pathogens to the monocotyledonous primary wall. *Physiol. Mol. Plant Pathol*, **32**, 33–47.

Datnoff, L.E. (1993) Black sheath rot caused by *Gaeumannomyces graminis* var. *graminis* on rice in Florida. *Plant Dis.* **77**, 210.

Dawe, A.L. and Nuss, D.L. (2001) Hypoviruses and chestnut blight: exploiting viruses to understand and modulate fungal pathogenesis. *Annu. Rev. Genet.* **35**, 1–29.

Deacon, J.W. (1981) Ecological relationships with other fungi: competitors and hyperparasites. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 75–101.

Dewan, M.M. and Sivasithamparam, K. (1988) Idebtity and frequency of occurence of *Trichoderma* spp. in roots of wheat and rye-grass in Western Australia and their effects on root rot caused by *Gaeumannomyces graminis* var. *tritici*. *Plant Soil*, **109**, 93–101.

Di Pietro, A., García-Maceira, F.I., Méglecz, E. and Roncero, M.I.G. (2001) A MAP kinase of the vascular wilt fungus *Fusarium oxysporum* is essential for root penetration and pathogenesis. *Mol. Microbiol.* **39**, 1140–1152.

Duffy, B.K., Simon, A. and Weller, D.M. (1996) Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. *Phytopathology*, **86**, 188–194.

Duffy, B.K. and Weller, D.M. (1995) Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. *Plant Dis.* **79**, 907–911.

Duffy, B.K. and Weller, D.M. (1996) Biological control of take-all of wheat in the Pacific Northwest of the USA using hypovirulent *Gaeumannomyces graminis* var. *tritici* and fluorescent pseudomonads. *J. Phytopathol.* **144**, 585–590.

Dufresne, M. and Osbourn, A.E. (2001) Definition of tissue-specific and general requirements for plant infection in a phytopathogenic fungus. *Mol. Plant–Microbe Interact.* **14**, 300–307.

Eastwood, R.F., Kollmorgen, J.F., Hannah, M. and Williams, W.M. (1994) Reaction of somaclonal variants of wheat to the take-all fungus (*Gaeumannomyces graminis* var. *tritici*). *Plant Pathol.* **43**, 644–650.

Edens, W.A., Goins, T.Q., Dooley, D. and Henson, J.M. (1999) Purification and characterization of a secreted laccase of *Gaeumannomyces graminis* var. *tritici*. *Appl. Environ. Microbiol.* **65**, 3071–3074.

Elliott, M.L. (1991) Determination of an etiological agent of bermudagrass decline. *Phytopathology*, **81**, 1380–1384.

Elliott, M.L. (1995) Effect of melanin biosynthesis inhibiting compounds on *Gaeumannomyces species*. *Mycologia*, **87**, 370–374.

Elliott, M.L., Des Jardin, E.A. and Henson, J.M. (1993) Use of a polymerase chain reaction assay to aid in identification of *Gaeumannomyces graminis* from different grass hosts. *Phytopathology*, **83**, 414–418.

Elsherif, M. and Grossmann, F. (1994) Comparative investigations on the antagonistic activity of fluorescent pseudomonads against *Gaeumannomyces graminis* var. *tritici in vitro* and *in vivo*. *Microbiol. Res.* **149**, 371–377.

Epstein, L., Kaur, S., Goins, T., Kwon, Y.H. and Henson, J.M. (1994) Production of hyphopodia by wild-type and 3 transformants of *Gaeumannomyces graminis* var. *graminis*. *Mycologia*, **86**, 72–81.

Feussner, I., Kuhn, H. and Wasternack, C. (2001) Lipoxygenase-dependent degradation of storage lipids. *Trends Plant Sci.* **6**, 268–273.

Fouly, H.M. and Wilkinson, H.T. (2000a) A group I intron in the nuclear small subunit ribosomal DNA of *Gaeumannomyces graminis*. *Curr. Microbiol.* **40**, 291–296.

Fouly, H.M. and Wilkinson, H.T. (2000b) Detection of *Gaeumannomyces graminis* varieties using polymerase chain reaction with variety-specific primers. *Plant Dis.* **84**, 947–951.

Fouly, H.M., Wilkinson, H.T. and Chen, W.D. (1997) Restriction analysis of internal transcribed spacers and the small subunit gene of ribosomal DNA among four *Gaeumannomyces* species. *Mycologia*, **89**, 590–597.

Fouly, H.M., Wilkinson, H.T. and Domier, L.L. (1996) Use of random amplified polymorphic DNA (RAPD) for identification of *Gaeumannomyces* species. *Soil Biol. Biochem.* **28**, 703–710.

Frederick, B.A., Caesar-Tonthat, T.C., Wheeler, M.H., Sheehan, K.B., Edens, W.A. and Henson, J.M. (1999) Isolation and characterisation of *Gaeumannomyces graminis* var. *graminis* melanin mutants. *Mycol. Res.* **103**, 99–110.

Frey, M., Chomet, P., Glawischnig, E., Stettner, C., Grun, S., Winklmair, A., Eisenreich, W., Bacher, A., Meeley, R.B., Briggs, S.P., Simcox, K. and Gierl, A. (1997) Analysis of a chemical plant defense mechanism in grasses. *Science*, **277**, 696–699.

Friebe, A., Vilich, V., Hennig, L., Kluge, M. and Sicker, D. (1998) Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. *tritici*, *G. graminis* var. *graminis*, *G. graminis* var. *avenae*, and *Fusarium culmorum*. *Appl. Environ. Microbiol.* **64**, 2386–2391.

Gams, W. (2000) *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. *Stud. Mycol.* **45**, 187–199.

Goins, T.Q., Edens, W.A. and Henson, J.M. (2002) Heavily-melanized variants of the sexual *Gaeumannomyces graminis* var. *tritici* are non-pathogenic and indistinguishable from the asexual, *Phialophora* state. *Mycol. Res.* **106**, 1179–1186.

Goodwin, P.H., Hsiang, T., Xue, B.G. and Liu, H.W. (1995) Differentiation of *Gaeumannomyces graminis* from other turf-grass fungi by amplification with primers from ribosomal internal transcribed spacers. *Plant Pathol.* **44**, 384–391.

Haralampidis, K., Bryan, G., Qi, X., Papadopoulou, K., Bakht, S., Melton, R. and Osbourn, A. (2001) A new class of oxidosqualene cyclases

directs synthesis of antimicrobial phytoprotectants in monocots. *Proc. Natl Acad. Sci. USA*, **98**, 13431–13436.

Harvey, P.R., Langridge, P. and Marshall, D.R. (2001) Genetic drift and host-mediated selection cause genetic differentiation among *Gaeumannomyces graminis* populations infecting cereals in southern Australia. *Mycol. Res.* **105**, 927–935.

Henson, J.M. (1989) DNA probe for identification of the take-all fungus, *Gaeumannomyces graminis*. *Appl. Environ. Microbiol.* **55**, 284–288.

Henson, J.M. (1992) DNA hybridization and polymerase chain reaction (PCR) tests for identification of *Gaeumannomyces*, *Phialophora* and *Magnaporthe* isolates. *Mycol. Res.* **96**, 629–636.

Henson, J.M., Blake, N.K. and Pilgeram, A.L. (1988) Transformation of *Gaeumannomyces graminis* to benomyl resistance. *Curr. Genet.* **14**, 113–117.

Henson, J.M., Butler, M.J. and Day, A.W. (1999) The dark side of the mycelium: melanins of phytopathogenic fungi. *Annu. Rev. Phytopathol.* **37**, 447–471.

Henson, J.M. and Ton That, T.C.C. (1995) Mitochondrial plasmids of the *Gaeumannomyces–Phialophora* complex and their detection by primed *in situ* fluorescence labeling. *Exp. Mycol.* **19**, 263–274.

Herdina and Roget, D.K. (2000) Prediction of take-all disease risk in field soils using a rapid and quantitative DNA soil assay. *Plant Soil*, **227**, 87–98.

Herdina, Harvey, P.H. and Ophel-Keller, K. (1996) Quantification of *Gaeumannomyces graminis* var. *tritici* in infected roots and soil using slot-blot hybridization. *Mycol. Res.* **100**, 962–970.

Herdina, Yang, H.A. and Ophel-Keller, K. (1997) Correlation of take-all disease severity and inoculum level of *Gaeumannomyces graminis* var. *tritici* using a slot-blot hybridization assay. *Mycol. Res.* **101**, 1311–1317.

Herman, R.P. (1998) Oxylipin production and action in fungi and related organisms. In *Eicosanoids and Related Compounds in Plants and Animals* (Rowley, A.F., Kuhn, H. and Schewe, T., eds). London: Portland Press, pp. 115–130.

Honeyman, A.L. and Currier, T.C. (1986) Isolation and characterization of linear DNA elements from the mitochondria of *Gaeumannomyces graminis*. *Appl. Environ. Microbiol.* **52**, 924–929.

Hornby, D. (1983) Suppressive soils. *Annu. Rev. Phytopathol.* **21**, 65–85.

Hornby, D., Bateman, G.L., Gutteridge, R.J., Lucas, P., Osbourn, A.E., Ward, E. and Yarham, D.J. (1998) *Take all Disease of Cereals: a Regional Perspective*. Wallingford, UK: CABI International.

Hornby, D., Bateman, G.L., Payne, R.W., Brown, M.E., Hendon, D.R. and Campbell, R. (1993) Field-tests of bacteria and soil-applied fungicides as control agents for take-all of winter wheat. *Ann. Appl. Biol.* **122**, 253–270.

Hörnsten, L., Su, C. and Oliw, E. (2002a) Linoleate diol synthase and PGH synthase—A new gene family of fatty acid heme dioxygenases? *Adv. Exp. Med. Biol.* **507**, 557–561.

Hörnsten, L., Su, C., Osbourn, A.E., Garosi, P., Hellman, U., Wernstedt, C. and Oliw, E.H. (1999) Cloning of linoleate diol synthase reveals homology with prostaglandin H synthases. *J. Biol. Chem.* **274**, 28219–28224.

Hörnsten, L., Su, C., Osbourn, A.E., Hellman, U. and Oliw, E.H. (2002b) Cloning of the manganese lipoxygenase gene reveals homology with the lipoxygenase gene family. *Eur. J. Biochem.* **269**, 2690–2697.

Howard, R.J., Ferrari, M.A., Roach, D.H. and Money, N.P. (1991) Penetration of hard substrates by a fungus employing enormous turgor pressure. *Proc. Natl Acad. Sci. USA*, **88**, 11281–11284.

Howard, R.J. and Valent, B. (1996) Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annu. Rev. Microbiol.* **50**, 491–512.

Howe, G.A. and Schilmiller, A.L. (2002) Oxylipin metabolism in response to stress. *Curr. Opin. Plant Biol.* **5**, 230–236.

Huang, L.L., Kang, Z. and Buchenauer, H. (2001a) Comparison of infection of wheat roots by *Phialophora graminicola* and *Gaeumannomyces* graminis var. *tritici* by ultrastructural and cytochemical studies. *Z. Pflanzenk. Pflanzens.* **108**, 593–607.

Huang, L.L., Kang, Z., Heppner, C. and Buchenauer, H. (2001b) Ultrastructural and immunocytochemical studies on effects of the fungicide Mon65500 (Latitude R.) on colonization of roots of wheat seedlings by *Gaeumannomyces graminis* var. *tritici*. *Z. Pflanzenk. Pflanzens.* **108**, 188–203.

Huber, S., Lottspeich, F. and Kamper, J. (2002) A gene that encodes a product with similarity to dioxygenases is highly expressed in teliospores of *Ustilago maydis*. *Mol. Genet. Genomics*, **267**, 757–771.

Jamil, N. and Buck, K.W. (1991) Effect of vegetative incompatibility on double-stranded-RNA and mycovirus transmission in *Gaeumannomyces graminis* var. *tritici*. *Pak. J. Bot.* **23**, 160–164.

Jamil, N., Buck, K.W. and Carlile, M.J. (1984) Sequence relationship between virus double stranded RNA from isolates of *Gaeumannomyces graminis* in different vegetative incompatibility groups. *J. Gen. Virol.* **65**, 1741–1747.

Jian, J., Lakshman, D.K. and Tavantzis, S.M. (1997) Association of distinct double-stranded RNAs with enhanced or diminished virulence in *Rhizoctonia solani* infecting potato. *Mol. Plant–Microbe Interact.* **10**, 1002–1009.

Joseph-Horne, T., Heppner, C., Headrick, J. and Hollomon, D.W. (2000) Identification and characterization of the mode of action of MON 65500: a novel inhibitor of ATP export from mitochondria of the wheat 'take-all' fungus, *Gaeumannomyces graminis* var. *tritici*. *Pest. Biochem. Physiol.* **67**, 168–186.

Juhnke, M.E., Mathre, D.E. and Sands, D.C. (1984) A selective medium for *Gaeumannomyces graminis* var. *tritici*. *Plant Dis.* **68**, 233–236.

Kang, Z., Huang, L. and Buchenauer, H. (2000) Cytochemistry of cell wall component alterations in wheat roots infected by *Gaeumannomyces graminis* var. *tritici*. *Z. Pflanzenk. Pflanzens.* **107**, 337–351.

Kelly, C. (1997) Genetics of pigmentation and pathogenicity in *G. graminis*. PhD thesis, University of Birmingham.

Kelly, C., Osbourn, A.E. and Caten, C.E. (1997) The genetics of *Gaeumannomyces graminis* with particular reference to pigment production and pathogenicity. *Fungal Genet. Newsletter*, **44A**, 108.

Kim, D.S., Cook, R.J. and Weller, D.M. (1997) *Bacillus* sp. L324–92 for biological control of three root diseases of wheat grown with reduced tillage. *Phytopathology*, **87**, 551–558.

Kohlmeyer, J., Volkmannkohlmeyer, B. and Eriksson, O.E. (1995) Fungi on *Juncus roemerianus*. 4. New marine ascomycetes. *Mycologia*, **87**, 532–542.

Landschoot, P.J. and Jackson, N. (1989) *Gaeumannomyces incrustans* sp. nov., a root-infecting hyphopodiate fungus from grass roots in the United-States. *Mycol. Res.* **93**, 55–58.

Langfelder, K., Streibel, M., Jahn, B., Haase, G. and Brakhage, A.A. (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genet. Biol.* **38**, 143–158.

Liang, P.-Y. and Chen, K.-Y. (1990) Homology between *Gaeumannomyces graminis* virus and other mycoviruses by dot blot hybridization. *Chin. J. Virol.* **6**, 245–249.

Liang, P.-Y., Chou, S.-M. and Chen, K.-Y. (1989) Properties of double stranded RNA viruses from different strains of *Gaeumannomyces graminis* in different vegetative compatible groups. *Virologica Sinica*, **4**, 68–75.

Linde-Laursen, I., Jensen, H.P. and Jørgensen, J.H. (1973) Resistance of *Triticale*, *Aegilops* and *Haynaldia* species to the take-all fungus, *Gaeumannomyces graminis*. *Z. Pflanzenzüchtung*, **70**, 200–213.

Litvintseva, A.P. and Henson, J.M. (2002a) Cloning, characterization, and transcription of three laccase genes from *Gaeumannomyces graminis* var. *tritici*, the take-all fungus. *Appl. Environ. Microbiol.* **68**, 1305–1311.

Litvintseva, A.P. and Henson, J.M. (2002b) Characterization of two laccase genes of *Gaeumannomyces graminis* var. *graminis* and their differential transcription in melanin mutants and wild type. *Mycol. Res.* **106**, 808–814.

Löchel, A.M., Wenz, M., Russell, P.E., Buschhaus, H., Evans, P.H., Cross, S., Puhl, T. and Bardsley, E. (1998) Root protection using fluquinconazole: a new approach to controlling cereal take-all. *Proceedings of the 1998 British Crop Protection Conference—Pests and Diseases*, pp. 89–96.

Mathre, D.E., Johnston, R.H. and Grey, W.E. (1998) Biological control of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* under field conditions using a *Phialophora* sp. *Biocontrol. Sci. Technol.* **8**, 449–457.

McCarty, L.B. and Lucas, L.T. (1989) *Gaeumannomyces graminis* associated with spring dead spot of bermudagrass in the Southeastern United States. *Plant Dis.* **73**, 659–661.

McGinty, R.M., Buck, K.W. and Rawlinson, C.J. (1981) Virus particles and double-stranded RNA in isolates of *Phialophora* with lobed hyphopodia, *Phialophora graminicola* and *Gaeumannomyces graminis* var. *graminis*. *Phytopathol. Z.* **102**, 153–162.

Money, N.P. (1995) Turgor pressure and the mechanics of fungal penetration. *Can. J. Bot.* **73**, S96–S102.

Money, N.P., Caesar-TonThat, T.C., Frederick, B. and Henson, J.M. (1998) Melanin synthesis is associated with changes in hyphopodial turgor, permeability, and wall rigidity in *Gaeumannomyces graminis* var. *graminis*. *Fungal Genet. Biol.* **24**, 240–251.

Morrissey, J.P. and Osbourn, A.E. (1999) Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol. Mol. Biol. Rev.* **63**, 708–724.

Musker, R. (1994) Genetics and population studies of *Gaeumannomyces graminis*. PhD thesis, University of Birmingham.

Niemeyer, H.M. (1988) Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defense chemicals in the *Gramineae*. *Phytochemistry*, **27**, 3349–3358.

O'Dell, M., Flavell, R.B. and Hollins, T.W. (1992) The classification of isolates of *Gaeumannomyces graminis* from wheat, rye and oats using restriction-fragment-length-polymorphisms in families of repeated DNA sequences. *Plant Pathol.* **41**, 554–562.

Osbourn, A.E. (1996a) Saponins and plant defence—A soap story. *Trends Plant Sci.* **1**, 4–9.

Osbourn, A.E. (1996b) Preformed antimicrobial compounds and plant defense against fungal attack. *Plant Cell*, **8**, 1821–1831.

Osbourn, A.E. (2001) Plant mechanisms that give defence against soil-borne diseases. *Aust. Plant Pathol.* **30**, 99–102.

Osbourn, A.E., Bowyer, P., Bryan, G., Lunness, P., Clarke, B. and Daniels, M. (1994a) Detoxification of plant saponins by fungi. In *Advances in Molecular Genetics of Plant–Microbe Interactions* (Daniels, M.J., Downie, J.A. and Osbourn, A.E., eds). Dordrecht: Kluwer, pp. 215–221.

Osbourn, A.E., Clarke, B.R., Lunness, P., Scott, P.R. and Daniels, M.J. (1994b) An oat species lacking avenacin is susceptible to infection by *Gaeumannomyces graminis* var. *tritici*. *Physiol. Mol Plant Pathol*, **45**, 457–467.

Ou, S.H. (1972) *Rice Diseases*. Kew, UK: Commonwealth Mycological Institute.

Papadopoulou, K., Melton, R.E., Leggett, M., Daniels, M.J. and Osbourn, A.E. (1999) Compromised disease resistance in saponin-deficient plants. *Proc. Natl Acad. Sci. USA*, **96**, 12923–12928.

Penrose, L. (1985) Evidence for resistance in wheat cultivars grown in sand culture to the take-all pathogen, *Gaeumannomyces graminis* var. *tritici*. *Ann. Appl. Biol.* **107**, 105–108.

Penrose, L.D.J. (1991) Disease in wheat genotypes naturally infected with *Gaeumannomyces graminis* var. *tritici*. *Ann. Appl. Biol.* **118**, 513–526.

Penrose, L.D.J. (1995) Two wheat genotypes differ in root disease due to *Gaeumannomyces graminis* without interaction with site. *Soil Biol. Biochem.* **27**, 133–138.

Penrose, L.D.J. and Neate, S.M. (1994) Resistance to *Gaeumannomyces graminis* in wheat genotypes grown in field environments and sand culture. *Soil Biol. Biochem.* **26**, 719–726.

Pilgeram, A.L., Goins, T. and Henson, J.M. (1993) The fate of integrated DNA in *Gaeumannomyces graminis* transformants. *FEMS Microbiol. Lett.* **113**, 309–314.

Pilgeram, A.L. and Henson, J.M. (1990) Transformation and cotransformation of *Gaeumannomyces graminis* to phleomycin resistance. *Phytopathology*, **80**, 1124–1129.

Pilgeram, A.L. and Henson, J.M. (1992) Sexual crosses of the homothallic fungus *Gaeuannomyces graminis* var. *tritici* based on use of an auxotroph obtained by transformation. *Exp. Mycol.* **16**, 35–43.

Rachdawong, S., Cramer, C.L., Grabau, E.A., Stromberg, V.K., Lacy, G.H. and Stromberg, E.L. (2002) *Gaeumannomyces graminis* vars. *avenae*, *graminis*, and *tritici* identified using PCR amplification of avenacinase-like genes. *Plant Dis.* **86**, 652–660.

Rawlinson, C.J. and Buck, K.W. (1981) Viruses in *Gaeumannomyces graminis* and *Phialophora*. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 151–172.

Rengel, Z. (1997) Decreased capacity of take-all fungus to oxidize manganous ions is associated with take-all decline. *J. Plant Nutr.* **20**, 455–460.

Rengel, Z., Graham, R.D. and Pedler, J.F. (1993) Manganese nutrition and accumulation of phenolics and lignin as related to differential resistance of wheat genotypes to the take-all fungus. *Plant Soil*, **151**, 255–263.

Rengel, Z., Graham, R.D. and Pedler, J.F. (1994) Time-course of biosynthesis of phenolics and lignin in roots of wheat genotypes differing in manganese efficiency and resistance to take-all fungus. *Ann. Bot.* **74**, 471–477.

Rosewich, U.L. and Kistler, H.C. (2000) Role of horizontal gene transfer in the evolution of fungi. *Annu. Rev. Phytopathol.* **38**, 325–363.

Ross, I.L., Alami, Y., Harvey, P.R., Achouak, W. and Ryder, M.H. (2000) Genetic diversity and biological control activity of novel species of closely related pseudomonads isolated from wheat field soils in South Australia. *Appl. Environ. Microbiol.* **66**, 1609–1616.

Rovira, A., Ryder, M. and Harris, A. (1992) Biological control of root diseases with psuedomonads. In *Biological Control. of Plant Diseases-Progress and Challenges for the Future* (Tjamos, E.S., Papavizas, G.C. and Cook, R.J., eds). New York: Plenum Press, pp. 175–184.

Rovira, A.D. and Wildermuth, G.B. (1981) The nature and mechanisms of suppression. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 385–415.

Ryder, M.H., Brisbane, P.G. and Rovira, A.D. (1990) Mechanisms in the biological control of take-all of wheat by rhizosphere bacteria. In *Biological Control of Soil-Borne Plant Pathogens* (Hornby, D.H., ed.). Wallingford: CAB International, pp. 123–130.

Ryder, M.H. and Rovira, A.D. (1993) Biological control of take-all of glasshouse-grown wheat using strains of *Pseudomonas corrugata* isolated from wheat field soil. *Soil Biol. Biochem.* **25**, 311–320.

Salvo, J.L., Rodeghier, B., Rubin, A. and Troischt, T. (1998) Optional introns in mitochondrial DNA of *Podospora anserina* are the primary source of observed size polymorphisms. *Fungal Genet. Biol.* **23**, 162–168.

Schesser, K., Luder, A. and Henson, J.M. (1991) Use of polymerase chain reaction to detect the take-all fungus, *Gaeumannomyces graminis*, in infected wheat plants. *Appl. Environ. Microbiol.* **57**, 553–556.

Schoeny, A. and Lucas, P. (1999) Modeling of take-all epidemics to evaluate the efficacy of a new seed-treatment fungicide on wheat. *Phytopathology*, **89**, 954–961.

Sesma, A. and Osbourn, A.E. (2003) Root colonization by *Magnaporthe grisea*: novel developmental processes associated to a foliar pathogen. In *Abstracts of the 11th International Congress on Plant–Microbe Interactions, July 18th–26th 2003, St. Petersburg, Russia*, p. 221.

Sivasithamparam, K. (1975) *Phialophora* and *Phialophora*-like fungi occuring in the root region of wheat. *Aust. J. Bot.* **23**, 193–212.

Sivasithamparam, K. (1998) Root cortex—The final frontier for the biocontrol of root-rot with fungal antagonists: a case study on a sterile red fungus. *Annu. Rev. Phytopathol.* **36**, 439–452.

Sivasithamparam, K. and Parker, C.A. (1981) Physiology and nutrition in culture. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 125–150.

Skou, J.P. (1981) Morphology and cytology of the infection process. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 175–197.

de Souza, J.T., Weller, D.M. and Raaijmakers, J.M. (2003) Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. *Phytopathology*, **93**, 54–63.

Spink, J.H., Wade, A.P., Paveley, N.D., Griffin, J.M., Scott, R.K. and Foulkes, M.J. (1998) The effects of a novel seed treatment, MON65500, on take-all severity and crop growth in winter wheat. In *Proceedings of the 1998 British Crop Protection Conference—Pests and Diseases*, pp. 913–920.

Su, C. and Oliw, E.H. (1996) Purification and characterization of linoleate 8-dioxygenase from the fungus *Gaeumannomyces graminis* as a novel hemoprotein. *J. Biol. Chem.* **271**, 14112–14118.

Su, C. and Oliw, E.H. (1998) Manganese lipoxygenase—Purification and characterization. *J. Biol. Chem.* **273**, 13072–13079.

Tan, M.K. (1997) Origin and inheritance of group I introns in 26S rRNA genes of *Gaeumannomyces graminis*. *J. Mol. Evol.* **44**, 637–645.

Tan, M.K. and Wong, P.T.W. (1996) Group I introns in 26S rRNA genes of *Gaeumannomyces graminis* as possible indicators of host specificity of *G. graminis* varieties. *Mycol. Res.* **100**, 337–342.

Tan, M.K., Wong, P.T.W. and Holley, M.P. (1994) Characterization of nuclear ribosomal DNA (rDNA) in *Gaeumannomyces graminis* and correlation of rDNA variation with *G. graminis* varieties. *Mycol. Res.* **98**, 553–561.

Thornton, C.R., Dewey, F.M. and Gilligan, C.A. (1997) Production and characterization of a monoclonal antibody raised against surface antigens from mycelium of *Gaeumannomyces graminis* var. *tritici*: evidence for an extracellular polyphenol oxidase. *Phytopathology*, **87**, 123–131.

Ulrich, K., Augustin, C. and Werner, A. (2000) Identification and characterization of a new group of root-colonizing fungi within the *Gaeumannomyces–Phialophora* complex. *New Phytol.* **145**, 127–135.

Virtanen, A.I. and Hietala, P.K. (1955) 2 (3)-Benzoxazolinone, an anti-*Fusarium* factor in rye seedlings. *Acta Chemica Scand.* **9**, 1543–1544.

Walker, J.L. (1975) Take-all diseases of *Gramineae*: a review of recent work. *Rev. Plant Pathol.* **54**, 113–144.

Walker, J. (1981) Taxonomy of take-all fungi and related genera and species. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 15–74.

Ward, E. (1995) Improved polymerase chain reaction (PCR) detection of *Gaeumannomyces graminis* including a safeguard against false negatives. *Eur. J. Plant Pathol.* **101**, 561–566.

Ward, E. and Akrofi, A.Y. (1994) Identification of fungi in the *Gaeumannomyces–Phialophora* complex by RFLPs of PCR-amplified ribosomal DNAs. *Mycol. Res.* **98**, 219–224.

Ward, E. and Bateman, G.L. (1994) Identification of fungi in the *Gaeumannomyces–Phialophora* complex associated with take-all of cereals and grasses using DNA probes. In *Modern Detection Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification* (Schots, A., Dewey, F.M. and Oliver, R., eds). Wallingford, UK: CAB International, pp. 127–134.

Ward, E. and Bateman, G.L. (1999) Comparison of *Gaeumannomyces*- and *Phialophora*-like fungal pathogens from maize and other plants using DNA methods. *New Phytol.* **141**, 323–331.

Ward, E. and Gray, R. (1992) Generation of a ribosomal probe by PCR and its use in identification of fungi within the *Gaeumannomyces–Phialophora* complex. *Plant Pathol.* **41**, 730–736.

Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M. and Thomashow, L.S. (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* **40**, 309–348.

Wetzel, H.C., Dernoeden, P.H. and Millner, P.D. (1996) Identification of darkly pigmented fungi associated with turfgrass roots by mycelial characteristics and RAPD-PCR. *Plant Dis.* **80**, 359–364.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols. A Guide to Methods and Applications* (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., eds). San Diego, CA: Academic Press, pp. 315–322.

Wilkes, M.A., Marshall, D.R. and Copeland, L. (1999) Hydroxamic acids in cereal roots inhibit the growth of take-all. *Soil Biol. Biochem.* **31**, 1831–1836.

Wong, P.T.W. (2002) *Gaeumannomyces wongoonoo* sp. nov., the cause of a patch disease of buffalo grass (St Augustine grass). *Mycol. Res.* **106**, 857–862.

Wong, P.T.W., Mead, J.A. and Holley, M.P. (1996) Enhanced field control of wheat take-all using cold tolerant isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. (lobed hyphopodia). *Plant Pathol.* **45**, 285–293.

Wong, P.T.W., Tan, M.K. and Beehag, G.W. (2000) Confirmation of take-all patch disease in Tifdwarf hybrid couch grass (bermudagrass) by morphological and DNA methods. *Austr. Plant Pathol.* **29**, 19–23.

Xu, J.-R. (2000) MAP kinases in fungal pathogens. *Fungal Genet. Biol.* **31**, 137–152.

Yao, J.M., Wang, Y.C. and Zhu, Y.G. (1992) A new variety of the pathogen of maize take-all. *Acta Mycol. Sinica*, **11**, 99–104.

Yarham, D.J. (1981) Practical aspects of epidemiology and control. In *Biology and Control of Take-All* (Asher, M.J.C. and Shipton, P.J., eds). London: Academic Press, pp. 353–384.

Yeates, J.S., Fang, C.S. and Parker, C.A. (1986) Distribution and importance of oat-attacking isolates of *Gaeumannomyces graminis* var. *tritici* in Western Australia. *Trans. Br. Mycol. Soc.* **86**, 145–152.

Zhou, S.G. and Stanosz, G.R. (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria and* associated anamorphic fungi. *Mycol. Res.* **105**, 1033–1044.

Zriba, N., Sherwood, J.E. and Mathre, D.E. (1999) Characterization and effectiveness of *Phialophora* spp. isolated from a Montana take-all suppressive soil in controlling take-all disease of wheat. *Can J. Plant Pathol.* **21**, 110–118.