Review

Formae speciales of cereal powdery mildew: close or distant relatives?VERONIQUE TROCH^{1,2,*}, KRIS AUDENAERT^{1,2}, REBECCA A. WYAND³, GEERT HAESAERT^{1,2}, MONICA HÖFTE² AND JAMES K. M. BROWN³¹Associated Faculty of Applied Bioscience Engineering, University College Ghent (Ghent University Association), Valentin Vaerwyckweg 1, BE-9000 Ghent, Belgium²Department of Crop Protection, Laboratory of Phytopathology, Ghent University, Coupure links 653, BE-9000 Ghent, Belgium³John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK**SUMMARY**

Powdery mildew is an important disease of cereals, affecting both grain yield and end-use quality. The causal agent of powdery mildew on cereals, *Blumeria graminis*, has been classified into eight *formae speciales* (ff.spp.), infecting crops and wild grasses. Advances in research on host specificity and resistance, and on pathogen phylogeny and origins, have brought aspects of the subspecific classification system of *B. graminis* into ff.spp. into question, because it is based on adaptation to certain hosts rather than strict host specialization. Cereals therefore cannot be considered as typical non-hosts to non-adapted ff.spp. We introduce the term 'non-adapted resistance' of cereals to inappropriate ff.spp. of *B. graminis*, which involves both pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). There is no clear distinction between the mechanisms of resistance to adapted and non-adapted ff.spp. Molecular evolutionary data suggest that the taxonomic grouping of *B. graminis* into different ff.spp. is not consistent with the phylogeny of the fungus. Imprecise estimates of mutation rates and the lack of genetic variation in introduced populations may explain the uncertainty with regard to divergence times, in the Miocene or Holocene epochs, of ff.spp. of *B. graminis* which infect cereal crop species. We propose that most evidence favours divergence in the Holocene, during the course of early agriculture. We also propose that the *forma specialis* concept should be retained for *B. graminis* pathogenic on cultivated cereals to include clades of the fungus which are strongly specialized to these hosts, i.e. ff.spp. *hordei*, *secalis* and *tritici*, as well as *avenae* from cultivated *A. sativa*, and that the *forma specialis* concept should no longer be applied to *B. graminis* from most wild grasses.

Keywords: *Blumeria graminis*, divergence time, *forma specialis*, host specificity, molecular phylogeny, non-adapted resistance, powdery mildew.

INTRODUCTION

Many species of plant-pathogenic fungi are classified below the species level into special forms (*formae speciales*, ff.spp.). This taxonomic grouping describes a situation in which each *forma specialis* (f.sp.) is classified as: (i) adapted to a specific host species; and (ii) shows no or minimal morphological difference from its closest relatives at the species level (Schulze-Lefert and Panstruga, 2011). Although the ff.spp. of a fungus often, although not always, relate to the specialization to various crop species, their evolutionary origins are largely obscure, as are the mechanisms by which they have become specialized to different hosts.

We review the biology and evolution of ff.spp. in *Blumeria graminis* (DC.) Speer, the causal agent of powdery mildew on cereal crop species and grasses (subfamily Pooideae). Powdery mildew is a widespread disease of many monocotyledonous and dicotyledonous plants, caused by Ascomycetes of the order *Erysiphales* (Braun *et al.*, 2002). Mildew pathogens are obligate biotrophic fungi, depending entirely on living plant cells for survival and reproduction. *Blumeria graminis* is a major problem for cereal production as it can reduce both quality and yield (Conner *et al.*, 2003; Everts *et al.*, 2001), leading to large economic losses either in reduced output or increased costs of production. Eight ff.spp. have been described for *B. graminis*, based on their host specialization, each having a capacity for infection limited to a single host genus (Marchal, 1902; Oku *et al.*, 1985). These hosts are wild grasses from the genera *Dactylis*, *Agropyron*, *Poa* and *Bromus* and four genera which include cereal crops: *Triticum* (wheat), *Hordeum* (barley), *Secale* (rye) and *Avena* (oats). The corresponding ff.spp. are named *dactylidis*, *agropyri*, *poae*, *bromi*, *tritici*, *hordei*, *secalis* and *avenae*, respectively (Fig. 1). *Agropyron* is now split into several genera, e.g. *A. repens* (couch grass) is now *Elymus repens* (Dewey, 1983), but the mildew pathogen is still called f.sp. *agropyri*. This subspecific taxonomy of *B. graminis* has been brought into question by several studies which have revealed that individual isolates of *B. graminis* have host ranges extending to plants from more than one genus and even in different tribes. Moreover, *B. graminis* is a common pathogen of many grasses

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Fig. 1 Schematic view of *Blumeria graminis* formae speciales (Marchal, 1902; Oku *et al.*, 1985). ^aThe wheat–rye hybrid triticale is a host for *B. graminis* related to *f.sp. tritici* (Troch *et al.*, 2012; Walker *et al.*, 2011). ^bOther grass genera, such as *Lolium*, are also infected by powdery mildew (Carver *et al.*, 1990; Eshed and Wahl, 1975; Hardison, 1945; Inuma *et al.*, 2007), but Marchal (1902) and Oku *et al.* (1985) did not name the formae speciales of *B. graminis* that infect them.

which are not hosts of named ff.spp., such as *Lolium perenne* or ryegrass (Carver *et al.*, 1990).

We describe the state of current knowledge about the f.sp. concept in *B. graminis*. We discuss the host specificity of the ff.spp. and recent advances in the molecular and cellular basis of adaptation of the fungus to different hosts. We then address the genetic diversity of *B. graminis* and the phylogenetic relationships among the ff.spp. Finally, we review the co-evolutionary history of the ff.spp. with their hosts, including their emergence and centre of origin, as well as possible dates of divergence of the ff.spp. The combination of current knowledge about pathogen evolution, host adaptation and genetic diversity indicates that, although groups of isolates within *B. graminis* can be recognized as being adapted to specific host species or genera, particularly the four main small-grain cereal crops, the concept of the f.sp. as a taxonomic group within the *B. graminis* species may no longer be valid.

HOST SPECIFICITY

The classification of *B. graminis* into eight ff.spp. based on host specialization (Marchal, 1902; Oku *et al.*, 1985) oversimplifies a complex situation (Brown *et al.*, 2002). Several studies have shown that specialization to the host genus may not be as absolute as the nomenclature suggests. Hardison (1944, 1945) showed that *B. graminis* ff.spp. can infect a wider host range than the genus from which each isolate was obtained, and a study of wheat powdery mildew in northern China found that 286 wild plant accessions belonging to 40 species in seven genera were susceptible to infection by *B. graminis* f.sp. *tritici* (Sheng *et al.*, 1993,

Table 1 General adaptation of *Blumeria graminis* formae speciales (ff.spp.) to cultivated cereal hosts*.

Source hosts (origin of isolates)	Inoculated hosts†				
	Oat	Barley	Wheat	Rye	Triticale
<i>B. graminis</i> f.sp. <i>avenae</i>	+++	–	–	–	–
<i>B. graminis</i> f.sp. <i>hordei</i>	–	+++	–	–	–
<i>B. graminis</i> f.sp. <i>tritici</i>	±	±	+++	–	+
<i>B. graminis</i> f.sp. <i>secalis</i>	–	–	±	+++	+
<i>B. graminis</i> f.sp. 'triticale'	–	–	++	±	+++

*Based on Eshed and Wahl (1970), Troch *et al.* (2012), Walker *et al.* (2011) and Wyand and Brown (2003).

†+, infection; –, no infection; more + corresponds to a higher aggressiveness.

1995). Eshed and Wahl (1970) demonstrated that *B. graminis* ff.spp. *hordei*, *tritici* and *avenae*, isolated from barley, wheat and oat crops in Israel, were compatible with wild grasses belonging to different genera in several tribes. Significantly, none of the ff.spp. *hordei* and *avenae* isolates induced infection on their non-adapted cereal hosts *Avena* or *Triticum*, and *Hordeum* or *Triticum*, respectively, although f.sp. *tritici* showed a compatible interaction with *Avena* and *Hordeum* plants. Wyand and Brown (2003) found that isolates of ff.spp. *hordei*, *tritici*, *secalis* and *avenae* sampled in the UK only infected species from which they had been sampled. Eshed and Wahl (1970) reported that isolates from Israel had a wider host range than those from elsewhere in the world, reflecting the broader genetic diversity of host plants in West Asia.

In research on the recent emergence of powdery mildew on triticale, cross-infection tests were conducted with isolates sampled from triticale, wheat and rye. Isolates from triticale were also pathogenic on wheat, supporting the hypothesis that triticale mildew emerged through a host range expansion of *B. graminis* f.sp. *tritici* (Troch *et al.*, 2012; Walker *et al.*, 2011). Moreover, isolates from wheat and rye were occasionally able to infect triticale (Linde-Laursen, 1977; Troch *et al.*, 2012; Walker *et al.*, 2011), underscoring the close relationship of this intergeneric hybrid with its parents wheat and rye.

In a study of the host specificity of *B. graminis* isolates sampled from wild grasses, such isolates were capable of causing infection on many other wild grass species, but were non-pathogenic on cultivated barley, wheat and oats (Eshed and Wahl, 1975). This contrasts sharply with the ability of isolates sampled from cereal crops in West Asia to infect wild grasses (Eshed and Wahl, 1970).

Altogether, these results indicate that the classification of *B. graminis* from cereals into ff.spp. is based on adaptation to certain hosts, summarized in Table 1, rather than on strict host specialization as described by Marchal (1902). They also suggest the possibility that wild grasses may play a dual role in epidemics of powdery mildew on cereals. First, wild grasses may act as temporary hosts, allowing the persistence of cereal powdery mildew between seasons. Chasmothecia (the sexual structures formerly known as cleistothecia) preserved on the debris of wild

grasses may enable the oversummering of *B. graminis* ff.spp. *hordei*, *tritici* and *avenae* (Eshed and Wahl, 1975). Second, wild grasses may act as common hosts for different ff.spp. of cereal powdery mildew, providing opportunities for hybridization between them.

Sexual hybridization between *B. graminis* ff.spp. has been demonstrated experimentally (Hiura, 1965). In particular, crosses between ff.spp. *tritici*, *secalis* and *agropyri* produced fertile chasmothecia, crosses between f.sp. *hordei* and ff.spp. *tritici*, *secalis* or *agropyri* produced sterile chasmothecia, whereas no chasmothecia were produced when f.sp. *avenae* was crossed with *tritici* or *hordei* (Hiura, 1978). In principle, hybridization may increase the host range and hence disease potential through recombination of avirulence (*AVR*) genes from the parental ff.spp. However, several studies have indicated that hybrids between ff.spp. have lower pathogenicity than the parental isolates on either parent's host plant (Oku *et al.*, 1986; Tosa, 1989a, b). An hypothesis for the emergence of powdery mildew on the wheat-rye hybrid triticale was that it was a hybrid of ff.spp. *tritici* and *secalis*. Combined pathology and molecular phylogenetic studies rejected this hypothesis, however, and revealed that this 'new' powdery mildew emerged through a host range expansion of *B. graminis* f.sp. *tritici* (Troch *et al.*, 2012; Walker *et al.*, 2011). It is possible that a few modifications in the effector repertoire of f.sp. *tritici* were sufficient to adapt to triticale, which is closely related to wheat. A similar breakdown of resistance was found for *Magnaporthe oryzae* f.sp. *oryzae*, which probably emerged from a host range expansion from a *Setaria* millet, possibly because the loss of a strain-specific effector gene allowed the fungus to colonize rice (Couch and Kohn, 2002; Couch *et al.*, 2005).

HOST RESISTANCE

The infection process of *B. graminis* and host resistance have been reviewed by Eichmann and Hückelhoven (2008), Green *et al.* (2002), Hückelhoven and Panstruga (2011) and Zhang *et al.* (2005), among others. Here, we focus on 'non-adapted' interactions of cereals with *B. graminis* ff.spp. Non-host resistance is usually defined as durable resistance of all known genotypes of a plant species to all known genotypes of a pathogen species (reviewed by Lipka *et al.*, 2008; Mysore and Ryu, 2004; Niks and Marcel, 2009; Nürnberger and Lipka, 2005; Schulze-Lefert and Panstruga, 2011; Schweizer, 2007). As these studies showed that the subspecific classification of *B. graminis* into ff.spp. is based on adaptation to certain hosts, rather than strict host specialization, cereals cannot be considered as typical non-hosts to inappropriate ff.spp.; hence, we prefer to use the term 'non-adapted resistance'.

B. graminis and the multilayered plant immune system

In the zigzag model of plant defence, plants recognize the presence of pathogens at various levels (Dodds and Rathjen, 2010;

Jones and Dangl, 2006). Initially, the recognition of conserved pathogen-associated molecular patterns (PAMPs) leads to PAMP-triggered immunity (PTI). In powdery mildew, early defences, which may be a form of PTI, are expressed in both susceptible and *R*-gene-resistant cultivars, and are associated with the induction of defence-related genes (Boyd *et al.*, 1996) and the formation of papillae (Zeyen *et al.*, 2002), involving the generation of NO (Prats *et al.*, 2005), H₂O₂ (Thordal-Christensen *et al.*, 1997), phenolics (Carver *et al.*, 1994) and the deposition of callose below the attempted site of penetration (Zeyen *et al.*, 2002). A papilla may either stop penetration or, if penetration succeeds, become a 'collar' for the neck of the haustorium (Zeyen *et al.*, 2002).

In a second layer of defence, the host recognizes specific effector (*AVR*) proteins. In several pathogens, these proteins suppress PTI and facilitate infection, leading to effector-triggered immunity (ETI) (Dodds and Rathjen, 2010; Jones and Dangl, 2006). In diseases caused by biotrophs, ETI involves a hypersensitive response (HR), including cell death, preventing the establishment of a biotrophic interaction (Schulze-Lefert and Panstruga, 2003). Most *R* genes, including those in cereals for mildew resistance, act against a subset of pathogen races. Two *AVR* genes with effector activity have been isolated from *B. graminis* (Nowara *et al.*, 2010; Ridout *et al.*, 2006), but the fungus has many homologues of these genes (Sacristan *et al.*, 2009). This family and other candidate effectors evolve rapidly and their diversity may contribute to host range and parasite speciation (Spanu *et al.*, 2010).

In addition, in barley, recessive loss-of-function *mlo* alleles mediate durable, broad-spectrum resistance towards otherwise virulent *B. graminis* f.sp. *hordei* (Jørgensen, 1994). This resistance is associated with papilla formation and H₂O₂ accumulation and stops the fungus at the pre-penetration stage (Hückelhoven *et al.*, 1999). Wild-type *Mlo* genes have been identified as negative regulators of PTI, required for susceptibility (Buschges *et al.*, 1997; Consonni *et al.*, 2006). Homologues of *Mlo* in wheat have a similar function to the barley gene (Elliott *et al.*, 2002; Konishi *et al.*, 2010). Several lines of evidence point to a functional link between *mlo*-mediated resistance in barley and non-host resistance (Humphry *et al.*, 2006; Schweizer, 2007; Trujillo *et al.*, 2004; Zellerhoff *et al.*, 2010).

Interactions of cereals with non-adapted ff.spp.

Inoculation of non-adapted *B. graminis* ff.spp. on different cereals has been employed to discover which resistance mechanisms form the basis of non-adapted resistance. Much of the research has investigated f.sp. *tritici* on barley.

Tosa *et al.* (1990) observed the incidence of papillae and cell wall penetration to infer compatibility between ff.spp. and non-adapted hosts. On the basis of the low incidence of papillae, ff.spp. *tritici*, *secalis* and *agropyri* were found to be more compatible with any accession of wheat, rye or wheatgrass than with barley.

However, *hordei* induced a low incidence of papillae on each of the four hosts. These four ff.spp. had low compatibility with oat, although *hordei* was the least incompatible, as indicated by the lower incidence of papillae. These responses suggest involvement of a form of PTI in non-adapted resistance.

Tosa *et al.* (1990) found that ff.spp. *agropyri* and *secalis* induced similar cellular responses in wheat leaves as the appropriate f.sp. *tritici*. The resistance of wheat to these ff.spp. was attributable to HR, a response conditioned by gene-for-gene interactions in cultivar specificity. Moreover, Tosa and Tada (1990) and Matsumura and Tosa (1995), who crossed isolates of these ff.spp. on common hosts and analysed the segregation of virulent and avirulent progeny, concluded that ff.spp. *agropyri* and *secalis* carry AVR genes corresponding to wheat genes for resistance to f.sp. *tritici*.

Non-adapted resistance of cereals to *B. graminis* ff.spp. is associated with penetration resistance caused by papilla formation, in some cases supplemented by single-cell HR. *Blumeria graminis* f.sp. *tritici* develops more slowly than f.sp. *hordei*, and this difference in timing is reflected in the expression of barley genes involved in defence (Boyd *et al.*, 1996). Olesen *et al.* (2003) found that, when barley, wheat or oat leaf epidermal cells were attacked by non-adapted ff.spp., penetration attempts failed in association with papilla deposition by epidermal cells. Attacked cells died or, if visible haustoria were formed, the plant cell died soon afterwards. In addition, when epidermal cells were first attacked by the adapted f.sp., as inducer, and later by the non-adapted ff.spp., as challenger, there was a dramatic suppression of defence responses within epidermal cells containing the inducer haustorium. This allowed most challenger attacks to form a haustorium which functioned to support colony development of the non-adapted f.sp. This implies that non-adapted isolates are capable of establishing a fully compatible infection if PTI and ETI are suppressed through previous infection by an adapted isolate of the appropriate f.sp.

Non-adapted resistance of barley to f.sp. *tritici* is associated with penetration resistance by H₂O₂ accumulation in papilla and HR of attacked cells (Hückelhoven *et al.*, 2001). Enhanced expression of barley BAX inhibitor-1 suppresses penetration resistance to the non-adapted f.sp. *tritici* (Eichmann *et al.*, 2004). This links barley non-adapted resistance to the induction of cell death because BAX inhibitor-1 has a role in modulating cell wall-associated defence and establishing full compatibility of f.sp. *hordei* with barley (Eichmann *et al.*, 2010).

In a microscopic analysis of barley defences against *B. graminis* f.sp. *tritici*, non-adapted resistance was associated with several mechanisms of cellular defence expressed at distinct stages of fungal development (Trujillo *et al.*, 2004). Non-adapted resistance of barley to f.sp. *tritici* is established not only by penetration resistance caused by papillae and early HR, but also by post-penetration resistance, leading to late hypersensitive cell death or

restricted fungal development. Using transient-induced gene silencing (TIGS), a role of the t-SNARE protein HvSNAP34 in race-nonspecific resistance of barley, mediated by *mlo*, was observed, with a proposed function in papilla formation (Douchkov *et al.*, 2005). ETI, however, was demonstrated to be independent of HvSNAP34 function and associated with HR instead of papilla formation. Miklis *et al.* (2007) showed a critical role for actin cytoskeleton function in *mlo*-mediated and non-adapted resistance of barley, which was highly effective against adapted and non-adapted powdery mildew species, respectively. These data show that barley defence against the non-adapted f.sp. *tritici* is determined by mechanisms similar to those involved in race-specific and race-nonspecific resistance to the adapted f.sp. *hordei*.

Aghnouch and Niks (2010) developed barley lines with substantial susceptibility to *B. graminis* f.sp. *tritici* at the seedling stage. Analysis of the infection of these lines by non-adapted ff.spp. showed that: (i) this resistance is quantitative, as indicated by differences in haustorium formation and colony development among accessions; (ii) genes for basal resistance have effects specific to pathogen developmental stages; and (iii) these genes are also specific to different *B. graminis* ff.spp. Zellerhoff *et al.* (2010) described the transcriptional responses of a near-isogenic pair of barley lines differing in the allelic status of the *Mlo* gene to three pairs of host/non-host pathogens causing powdery mildew, rust and blast on cereals. Results suggested that non-adapted and *mlo*-mediated defences of barley are functionally related. Moreover, non-host resistance to different fungi is associated with more robust regulation of complex, but largely non-overlapping, sets of pathogen-responsive genes involved in similar metabolic or signalling pathways.

Altogether, these studies indicate that the resistance of cereals to non-adapted ff.spp. of *B. graminis* involves both PTI and ETI, and that there is no clear distinction between mechanisms of resistance to adapted and non-adapted ff.spp. Schweizer (2007) concluded that non-adapted resistance in barley depends on non-compromised, PAMP-triggered basal defence, which implies that fungal effectors are largely ineffective against non-adapted hosts. Niks and Marcel (2009) argued that non-adapted resistance and basal resistance to adapted pathogens may rest on the same or similar mechanisms, and that effective suppression of PTI requires specific effectors. This second argument underscores the relevance of studies which found that *B. graminis* ff.spp. *agropyri* and *secalis* carry AVR genes corresponding to wheat genes for resistance to the adapted f.sp. *tritici* (Matsumura and Tosa, 1995; Tosa and Tada, 1990; Tosa *et al.*, 1990). Schulze-Lefert and Panstruga (2011) proposed that both PTI and ETI contribute to non-host resistance such that, with increasing phylogenetic divergence time between two plant species, the relative effectiveness of PTI increases, whereas the relative contribution of ETI decreases. This hypothesis is supported by the studies discussed above because

the resistance of cereals to infection by closely related ff.spp., such as wheat to ff.spp. *agropyri* or *secalis*, is based largely on HR, so that the interactions are compatible if AVR activity is lost, whereas infection by more distantly related ff.spp., such as f.sp. *tritici* on barley, is stopped by papilla formation. More diverse cytological and molecular studies, based on interactions other than *B. graminis* f.sp. *tritici* on barley, should be carried out to understand non-adapted resistance in more depth.

PHYLOGENY

Several studies have tried to elucidate the phylogenetic relationships among the ff.spp. of *B. graminis*. Nevertheless, the subspecific phylogeny of *B. graminis* is not yet resolved, particularly the relationship of ff.spp. from crops to isolates from wild grasses. Phylogenetic studies of rDNA internal transcribed spacer (ITS), β -tubulin, chitin synthase 1 and 28S rDNA (Inuma *et al.*, 2007; Wyand, 2001; Wyand and Brown, 2003) and of AVR-effector gene homologues (Sacristan *et al.*, 2009), analysis of the incidence of cell wall penetration (Tosa *et al.*, 1990) and data on reproductive isolation (Hiura, 1978) have all concluded that ff.spp. *tritici*, *secalis* and *agropyri* are more closely related to each other than to *hordei* or *avenae* (Fig. 2). Tosa *et al.* (1990) suggested that *hordei* is closer than the '*tritici* clade' to both the ancestral form of *B. graminis* and *avenae* (Fig. 2A). The latter proposal was supported by a phylogeny of β -tubulin genes (Wyand and Brown, 2003). This partially reflects host classification, because *Triticum*, *Secale* and *Agropyron* are more closely related to each other than to *Hordeum* within the tribe Triticeae. *Avena*, however, is in a different supertribe of Poaceae, the Poodae rather than Triticodae.

Fig. 2 Existing subspecific phylogenies of *Blumeria graminis*. (A) Diagram summarizing the conclusions of Tosa *et al.* (1990) on the relationships between *formae speciales* of *B. graminis* based on cytological evidence. The close relationship of ff.spp. *tritici*, *secalis* and *agropyri* is supported by molecular phylogenetic evidence (Inuma *et al.*, 2007; Wyand, 2001; Wyand and Brown, 2003). (B) Summary of the maximum parsimony phylogeny of Inuma *et al.* (2007), estimated from sequences of four genomic regions. Host genera of isolates are indicated. Note that isolates from wild *Avena* are not closely related to those from crops. (C) Maximum parsimony phylogeny of *B. graminis* isolates, estimated from rDNA internal transcribed spacer (ITS) sequences and rooted with reference to *Podospaera pannosa* and *Cystotheca lanestris* (Wyand, 2001). Numbers of isolates sampled from each host species are in parentheses; all isolates from the same host species had identical sequences (GenBank accession numbers: *B. graminis*, AJ313137–AJ313148; *C. lanestris*, AF011289 and AB000933; *P. pannosa*, AF011322). Bootstrap frequencies are shown for relevant clades. Note that isolates from *Avena sativa* lie within the clade containing isolates from other crops. Host classification in (B) and (C). All except *Diarrhena*: subfamily Pooideae. Black type, supertribe Triticodae; Roman, tribe Triticeae; italics, tribe Bromeae. White-on-black, supertribe Poodae; Roman, tribe Aveneae; italics, tribe Poeae. *Diarrhena*: subfamily Bambusoideae, supertribe Oryzodae, tribe Diarrheneae.

Contrasting conclusions have been published about the relationship of *B. graminis* on crops and wild grasses. A multilocus phylogenetic analysis revealed nine clades, generally reflecting the phylogeny of host genera (Inuma *et al.*, 2007; Fig. 2B). In most cases, isolates from each host genus were monophyletic, but f.sp. *bromi* and isolates from *Lolium* were polyphyletic. Isolates from *Avena* were not closely related to those from crops, ff.spp. *tritici*, *secalis* and *hordei*; one of these isolates was from wild *A. fatua* and one from an unknown *Avena* species. An rDNA-ITS phylogeny

placed an isolate from cultivated *A. sativa* in Switzerland in the same clade as the two other *Avena* isolates (fig. 2A in Inuma *et al.*, 2007). By contrast, another ITS phylogeny placed f.sp. *avenae* from *A. sativa* in the UK within a clade of isolates from crops, including wheat, barley, rye, *L. perenne* and the wild grass *E. repens*; ff.spp. *bromi* and *poae* were polyphyletic (Wyand, 2001; Fig. 2C). In another study, f.sp. *avenae* from the UK and Finland had identical ITS and β -tubulin sequences and were related to *hordei*, *secalis* and *tritici* (Wyand and Brown, 2003). Neither of the subspecific phylogenies of *B. graminis* (Inuma *et al.*, 2007; Wyand, 2001) reflected the classification of host genera or even tribes (Fig. 2). These results imply that isolates of *B. graminis* from cereal crops fall into a single clade representing a small fraction of the phylogenetic diversity of the species as a whole, that ff.spp. of *B. graminis* from most wild grasses are invalid because they are polyphyletic, and that f.sp. *agropyri* may be an exception to this situation as a group within the clade of *B. graminis* from crops.

To clarify these relationships, a phylogenetic analysis of 24 isolates belonging to the eight recognized *B. graminis* ff.spp. was conducted using β -tubulin gene sequences retrieved from GenBank (Inuma *et al.*, 2007; Troch *et al.*, 2012; Wyand and Brown, 2003). The question of particular interest is the relationship of ff.spp. on crops to isolates from wild grasses. Although only one gene was analysed, it contained 379 characters, 98 of which were parsimony informative. The 50% majority rule consensus tree (Fig. 3) underscores the close relatedness of powdery mildew on wheat, rye, triticale and *Agropyron*, all belonging to one clade. A second clade containing f.sp. *hordei* was closely related to this 'Triticum clade'. An isolate from *A. sativa* (cultivated oats) formed a sister group of the *hordei* clade, whereas isolates from other *Avena* spp. (wild oats) clustered in a separate clade. This implies that the classification of isolates from all *Avena* spp. into f.sp. *avenae* is invalid. It would be desirable to sample *B. graminis* isolates from more *Avena* species, cultivated and wild, to clarify the phylogeny of isolates classified in f.sp. *avenae*. Isolates from *Bromus* were placed in three clades, whereas isolates from *Poa* and *Dactylis* each belonged to one clade, as in Inuma *et al.* (2007).

Altogether, these molecular evolutionary data suggest that the taxonomic classification of *Blumeria graminis* into ff.spp. is not consistent with the phylogeny of the fungus. In the cladistic approach to biological classification, only monophyletic groups are recognized (Hörandl, 2006), and the nomenclature of ff.spp. is not governed by the International Code of Nomenclature for algae, fungi and plants (McNeil *et al.*, 2012). More comprehensive sequencing of genes with greater phylogenetic signal of isolates of all ff.spp., and from wild grasses, is required to clarify the subspecific phylogenetic relationships within *B. graminis*. This will lead to a better understanding of the origin and epidemiology of *B. graminis*, with implications for disease control.

Fig. 3 Phylogeny of *Blumeria graminis* formae speciales inferred by Bayesian analysis from the β -tubulin gene. The 50% majority rule consensus tree is shown. Maximum likelihood analysis recovered the same topology. Numbers above the branches are posterior probabilities. Branch lengths are proportional to the number of character changes. Labels on the phylogeny are hosts from which isolates were sampled and GenBank accession numbers. Coloured areas indicate genera from which isolates were obtained: red, *Dactylis*; dark blue, *Avena*; green, *Bromus*; yellow, *Triticum*; light blue, *Secale*; white, *Triticale*; orange, *Agropyron*; purple, *Hordeum*; grey, *Poa*.

ORIGIN OF THE FF.SPP.

Co-evolutionary history

Genetic and archaeological evidence indicates that the transformation of cereals from wild forms into domesticated crops originated in the Fertile Crescent at 10 000–12 000 BP (Balter, 2007; Feuillet *et al.*, 2008; Salamini *et al.*, 2002). Despite the lack of fossil records of most fungal plant pathogens, their evolutionary history can be reconstructed using tools based on population genetics, coalescence methods and phylogeography (Grünwald and Goss, 2011; Stukenbrock and McDonald, 2008). *Blumeria graminis* is an obligate biotroph and, as such, the evolution of the pathogen is influenced by the dispersal of its various host species. Several studies have addressed the co-evolutionary history of *B. graminis* and its hosts. Two main categories of event may explain the highly specialized associations between *Blumeria graminis* ff.spp. and their hosts: (i) co-speciation, in which two ff.spp. have separated in response to the speciation of the host, with which the fungus remains closely associated; and (ii) separation through host shifts or jumps, where the f.sp. switches from

the ancestral host to a new species, closely or distantly related, respectively. Uncertainties in the subspecific classification of *B. graminis* focus on the time of divergence of ff.spp. and the specificity of adaptation to host genera.

Wyand and Brown (2003) sequenced the rDNA ITS region and the β -tubulin gene of ff.spp. *tritici*, *secalis*, *hordei* and *avenae*, resulting in dendrograms with different topologies. As no topology was congruent with the phylogeny of host genera, they rejected the hypothesis that the ff.spp. co-evolved with their host. Instead, they suggested that the divergence between ff.spp. of *B. graminis* might reflect rapid evolution in agriculture, possibly beginning in the early Holocene epoch.

Matsuda and Takamatsu (2003) sequenced the 28S rDNA gene of ff.spp. *hordei* and *tritici*. They estimated that these ff.spp. diverged approximately 11 million years ago, in sharp contrast with the proposal of Wyand and Brown (2003). This divergence time was calculated in three steps: (i) based on the molecular clock of the *rbcl* (ribulose-bisphosphate carboxylase) gene in the sunflower alliance of plant families (Bremer and Gustafsson, 1997), they calculated the divergence time of the tribe Cardueae of the Asteraceae; (ii) from this divergence time, the nucleotide substitution rates of the D1 and D2 regions of 28S rDNA of *Golovinomyces* species (Erysiphaceae: Golovinomycetaceae), a parasite that co-speciates with host tribes in the Asteraceae, were estimated; and (iii) from this molecular clock, the divergence times of different nodes of the Erysiphales were calculated. As the hosts wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) diverged about 11.6 million years ago from their last common ancestor (Chalupska *et al.*, 2008), this divergence time indicates co-evolution of the ff.spp. with their hosts. This type of co-evolution is co-speciation, where host and pathogen diverge simultaneously, in contrast with host tracking, where the pathogen is likely to be younger than the host (Stukenbrock and McDonald, 2008).

Inuma *et al.* (2007) conducted a phylogenetic analysis of *B. graminis* based on the sequences of four DNA regions (ITS, 28S rDNA, chitin synthase 1 and β -tubulin). The topology of the patho-

gen phylogeny was shown not to be congruent with that of their host plants (Fig. 2), and the hypothesis of co-speciation between ff.spp. and their hosts was therefore not supported. Instead, the authors suggested that *B. graminis* expanded its host range together with host taxa, and that host jumping to phylogenetically distinct hosts occurred several times during the evolution of the fungus. In addition, they estimated the split between the *Hordeum* and *Triticum* clade to be 4.6 million years ago, applying Takamatsu and Matsuda's (2004) mutation rate of rDNA ITS. Again, this mutation rate was calculated from the molecular clock of *rbcl* in the sunflower alliance (Bremer and Gustafsson, 1997).

Oberhaensli *et al.* (2011) sequenced 273.3 kb of *B. graminis* f.sp. *tritici* BAC sequences and compared them with orthologous regions in the *B. graminis* f.sp. *hordei* genome. Protein-coding regions were collinear and well conserved, but the intergenic regions showed very low conservation, mostly because of different integration patterns of transposable elements. They estimated a divergence time between *tritici* and *hordei* of 10 million years ago, based on a total of 13 kb of conserved intergenic sequence, including orthologous transposable elements and non-coding sequences, using the same substitution rate as that of long terminal repeat (LTR)-retrotransposons in rice nuclear genomes (Ma and Bennetzen, 2004). This divergence time, approximately two million years after the hosts wheat and barley, suggested co-evolution of the ff.spp. with their hosts, possibly after a short phase of host expansion when the same ancestral pathogen species could grow on both hosts.

Divergence time of *B. graminis* ff.spp.

The wide range of estimated times of divergence between the different ff.spp. (Table 2) reflects two major challenges in the accurate dating of divergence times in plant pathogens (Grünwald and Goss, 2011). First, dating events in years is dependent on the knowledge of fairly accurate estimates of mutation rates per year or per generation, and also of the generation time, but these are not well understood for many plant pathogens. Inuma *et al.*

Table 2 Overview of divergence time estimates between *Blumeria graminis* formae speciales (ff.spp.) *hordei* and *tritici*, including the DNA sequences on which they were based and the calculation method.

Estimated divergence time	DNA sequences	Calculation method	Reference
14 000 years ago	rDNA ITS, <i>tub2</i>	Suggestion, no calculation	Wyand and Brown (2003)
11 million years ago	28S rDNA	Based on the molecular clock of the <i>rbcl</i> gene for the sunflower alliance (Bremer and Gustafsson, 1997)	Matsuda and Takamatsu (2003)
4.6 million years ago	rDNA ITS	Based on the molecular clock of the <i>rbcl</i> gene for the sunflower alliance (Bremer and Gustafsson, 1997)	Inuma <i>et al.</i> (2007)
10 million years ago	Conserved intergenic sequences including orthologous transposable elements	Based on the substitution rate of LTR-retrotransposons in rice nuclear genomes (Ma and Bennetzen, 2004)	Oberhaensli <i>et al.</i> (2011)

ITS, internal transcribed spacer; LTR, long terminal repeat; *rbcl*, ribulose-bisphosphate carboxylase gene.

(2007), Oberhaensli *et al.* (2011) and Takamatsu and Matsuda (2004) all calculated divergence times on the basis of substitution rates in plant DNA. A major objection to the use of these rates for powdery mildew fungi is that, most unusually for a eukaryote, all reproductive cells of almost all of these species are directly exposed to sunlight. Although their haustoria are embedded within the leaf (but, even then, only within epidermal cells, which lack chlorophyll), the mycelium from which conidiophores and thence conidia are formed, as well as sexual structures, are entirely superficial on the leaf surface. All nuclei which undergo mitosis or meiosis are therefore directly exposed to mutagens, including ultraviolet light, cosmic rays, ionizing radiation and chemicals, to an extent perhaps unmatched by any other group of eukaryotes. It is possible, therefore, that the rate of mutation in these fungi might be unusually high; this could be tested, in principle, by high-throughput sequencing of mildew colonies exposed to daylight in natural conditions. If this hypothesis is correct, the use of plant mutation rates to calibrate phylogenies of powdery mildew fungi, including ff.spp. of *B. graminis*, will overestimate divergence times considerably.

Moreover, mutation rates can vary greatly in different parts of the genome. The *rbcl* gene used to calibrate rates of DNA sequence evolution (Bremer and Gustafsson, 1997) encodes an important part of the metabolic mechanism of plants and thus may be expected to evolve slowly. By contrast, the intergenic sequences studied by Oberhaensli *et al.* (2011) are non-coding and are therefore expected to evolve much more rapidly than sequences of metabolic genes.

The generation time of *B. graminis* is not unusual for a fungus and is unlikely to have greatly affected its rate of molecular evolution. *Blumeria graminis* undergoes one sexual and several asexual generations each year (Koltin and Kenneth, 1970).

The second challenge is that plant pathogens may lack genetic variation in introduced populations, which may be the only known populations (Grünwald and Goss, 2011). With little polymorphism, population genetic parameters can be difficult to estimate. Indeed, accurate reconstruction of the timing and order of speciation depends critically on obtaining suitable characters for phylogenetic analyses (Brito and Edwards, 2009). This was not the case in the studies mentioned above, which all used sequences with imprecise phylogenetic signals, such as the ITS region, to estimate a divergence time for *B. graminis* f.sp. *hordei* and *tritici*. When sufficiently polymorphic population data for these ff.spp. become available, the accuracy of the estimated divergence times could be improved.

For all of these reasons, it is possible that the divergence times of *B. graminis* ff.spp. in the Miocene epoch (Inuma *et al.*, 2007; Matsuda and Takamatsu, 2003; Oberhaensli *et al.*, 2011) may have been considerably overestimated, and may not date back millions of years to when their hosts diverged. The existing evidence does not conclusively disprove the contention of Wyand and

Brown (2003) that the ff.spp. of *B. graminis*, which attack cultivated cereal species, may have diverged early in the pre-history of agriculture in the Holocene. More data are required to reach a firm conclusion one way or the other. The genome of f.sp. *hordei* has been sequenced and annotated (Spanu *et al.*, 2010), and the complete f.sp. *tritici* genome will soon be available (Parlange *et al.*, 2011). These data may go some way to resolving the question of the divergence of ff.spp. of *B. graminis*.

Centre of origin

If a parasite evolves by host tracking, it is expected that the centre of origin of the pathogen will correspond to that of the host (Stukenbrock and McDonald, 2008). It was further suggested that the centre of diversity of *B. graminis* may coincide with the centre of origin of the hosts in West Asia (Wyand and Brown, 2003). Phylogeographical analysis of β -tubulin and translation elongation factor 1- α of *B. graminis* revealed that Israeli isolates formed diverse haplotypes, mostly near the centre of the evolutionary network, consistent with the hypothesis that this region contains the ancestral population and the centre of diversity of *B. graminis* (Troch *et al.*, 2012). Parks *et al.* (2009) investigated the global evolutionary history of *B. graminis* f.sp. *tritici* by comparing 12 single-nucleotide polymorphisms in the USA, UK and Israeli populations. Although they concluded that USA isolates are direct descendants of the 'Old World' populations from the UK and Israel, it was not possible to test whether the centre of diversity corresponded to the centre of origin of the pathogen. It may be possible to infer the evolutionary history of *B. graminis* by coalescence analysis of isolates from wild grasses and from cultivated cereals collected both in the Middle East, the proposed centre of origin, and elsewhere. The possibility that host shifts and jumps may have confused the evolutionary relationship between *B. graminis* and its host species must be considered in any analysis.

Recent emergence of *B. graminis* on triticale

Triticale ($\times$ *Triticosecale* Wittmack) is an intergeneric hybrid between wheat (*Triticum* ssp.) and rye (*Secale cereale*). In contrast with wheat and rye, this artificial cereal is of very recent origin and was commercialized at the end of the 1960s (Ammar *et al.*, 2004; Oettler, 2005). During the last decade, triticale has gained considerable importance in Europe, as its production area has nearly doubled since 2000, to almost 3.3 Mha in 2010 (FAOSTAT). With the expansion of the growing area, however, powdery mildew has emerged and become a significant disease of triticale. Combined pathology and phylogenetic research demonstrated that this pathogen emerged through host range expansion of *B. graminis* f.sp. *tritici* from wheat (Troch *et al.*, 2012; Walker *et al.*, 2011). This implies that *B. graminis* has evolved the

capacity to colonize a new host, triticale, which is closely related to its long-standing host, wheat. Multilocus phylogeographical analysis revealed that this expansion occurred recently and at several times at different locations in Europe (Troch *et al.*, 2012). Other recent examples of host range expansions of pathogens to triticale include the spread of rice blast disease to wheat and triticale in Brazil (Mehta, 1998) and of scald (*Rhynchosporium secalis*) from rye to triticale (Zaffarano *et al.*, 2008). This host range expansion may eventually result in host shift speciation (Troch *et al.*, 2012), so that the population specialized to triticale will become incapable of infecting wheat, with the cessation of gene flow between the populations infecting the two crop species (Giraud *et al.*, 2010). A case of disease emergence consistent with ecological speciation by host shifts in prehistoric times was detected by molecular evolutionary analysis of the wheat fungal pathogen *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*; Stukenbrock *et al.*, 2007).

CONCLUSIONS

Current knowledge about the f.sp. concept in *B. graminis* points to the need for research on the divergence times of the ff.spp., host specificity, host resistance and phylogeny. Imprecise estimates of mutation rates may account for much of the uncertainty about the divergence times of the subspecific taxa of *B. graminis*.

If the Miocene divergence hypothesis of Inuma *et al.* (2007), Matsuda and Takamatsu (2003) and Oberhaensli *et al.* (2011) is correct, in contrast with the Holocene divergence hypothesis (Wyand and Brown, 2003), an explanation is required for the existence of four related clades of *B. graminis* which are highly specialized to the four main cereal crops which originated in the Fertile Crescent (rye, barley, wheat and oats), recognized as ff.spp. *secalis*, *hordei*, *tritici* and *avenae*. This contrasts with a lack of similar specialization of *B. graminis* on wild grasses (Eshed and Wahl, 1975; Inuma *et al.*, 2007; Wyand, 2001). Several observations are consistent with the Holocene hypothesis, but are harder to interpret with the Miocene hypothesis.

1. There is one clade of *B. graminis* pathogenic on cereal crop species. In the Holocene hypothesis, this could have originated from a regional population of the fungus in an area in which early agriculture was practised.
2. *Blumeria graminis* ff.spp. from cereals are highly specialized to their host species, but this is not the case for isolates from wild grasses, or even for isolates sampled from wild plants but classified into one of the ff.spp. pathogenic on crops. This specialization could have evolved during the course of agriculture, whereas, if it evolved during the previous 10 Myr, an explanation is required for why it evolved in clades that infected the grass species which, at a much later time, humans cultivated for food, but not for those that infected other species.

3. There are at least two clades of f.sp. *avenae*, one pathogenic only on cultivated oats and another on wild oats, even though *A. sativa* is cross-fertile with *A. fatua* and is sometimes classified as a subspecies of it. The 'cultivated' f.sp. *avenae* could have evolved from a local population at an early agricultural site, as in the Holocene hypothesis, whereas the Miocene hypothesis requires the evolution of two clades, one a weakly specialized clade pathogenic on wild *A. fatua* and the other a much more strongly specialized clade pathogenic on genotypes of *A. fatua*, which became cultivated by humans as *A. sativa*, and was the sister group of clades pathogenic on cultivated (but not wild) grasses in the Triticeae.
4. The ff.spp. *bromi* and *poae* are polyphyletic, implying that the classification of *B. graminis* into ff.spp. is not consistent with the phylogeny of the fungus, and that these ff.spp. are therefore not valid taxa.
5. Similar defences operate against isolates of adapted and non-adapted ff.spp., with little divergence in PTI mechanisms and effective ETI against non-adapted ff.spp. It is reasonable to suggest that this is consistent with fairly recent divergence of the ff.spp.
6. Finally, against this, the Holocene hypothesis is not consistent with the calculated divergence times of ff.spp. pathogenic on crop species, although these time points in the Miocene were calculated from rates of variation of functional genes in plants, rather than non-functional sequences in a fungus, which is exposed to high levels of mutagenic radiation throughout its life cycle.

To conclude, we propose that the f.sp. concept should be retained for *B. graminis* pathogenic on cultivated cereals, including clades which are strongly specialized to these hosts, namely ff.spp. *hordei*, *secalis* and *tritici*, as well as *avenae* from cultivated *A. sativa*; f.sp. *agropyri* may be valid as a distinct subclade related to ff.spp. *tritici* and *secalis*. We propose that the f.sp. concept should no longer be applied to *B. graminis* from other wild grasses, including wild *Avena* spp.; instead, the host species of origin can be mentioned when required when referring to the source of an isolate.

Recent advances in DNA analysis, including whole genome sequencing of *B. graminis* f.sp. *hordei* and *tritici* and other isolates, coalescence methods and cell biology of pathogenicity and host resistance, promise to advance our understanding of the evolution of host–pathogen specialization in cereal powdery mildew. This will hopefully resolve some of the uncertainties in the subspecific taxonomy of *B. graminis*.

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REFERENCES

- Aghnoum, R. and Niks, R.E. (2010) Specificity and levels of non-host resistance to nonadapted *Blumeria graminis* forms in barley. *New Phytol.* **185**, 275–284.
- Ammar, K., Mergoum, M. and Rajaram, S. (2004) The history and evolution of triticale. In: *Triticale Improvement and Production* (Mergoum, M. and Gomez-Macpherson, H., eds), pp. 1–9. Rome: FAO. FAO Plant Production and Protection Paper 179.
- Balter, M. (2007) Seeking agriculture's ancient roots. *Science*, **316**, 1830–1835.
- Boyd, L.A., Smith, P.H., Foster, E.M. and Brown, J.K.M. (1996) Defense gene induction in barley in response to the development of powdery mildew fungi. In: *Proceedings of the 9th European and Mediterranean Cereal Rusts and Powdery Mildews Conference*, 2–6 September 1996 (Kema, G.H.J., Niks, R.E. and Daamen, R.A., eds), pp. 40–43. Lunteren: COST.
- Braun, U., Cook, R.T.A., Inman, A.J. and Shin, H.D. (2002) The taxonomy of the powdery mildew fungi. In: *The Powdery Mildews* (Bélanger, R.R., Bushnell, W.R., Dik, A.J. and Carver, T.L.W., eds), pp. 13–55. St. Paul, MN: APS Press.
- Bremer, K. and Gustafsson, M.H.G. (1997) East Gondwana ancestry of the sunflower alliance of families. *Proc. Natl. Acad. Sci. USA*, **94**, 9188–9190.
- Brito, P.H. and Edwards, S.V. (2009) Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica*, **135**, 439–455.
- Brown, J.K.M., Hovmöller, M.S., Wyand, R.A. and Yu, D. (2002) Oases in the desert: dispersal and host specialization of biotrophic fungal pathogens of plants. In: *Dispersal Ecology* (Bullock, J.M., Kenward, R.E. and Hails, R.S., eds), pp. 395–409. Oxford: Blackwell Science.
- Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., van Daelen, R., van der Lee, T., Diergaarde, P., Groenendijk, J., Topsch, S., Vos, P., Salamini, F. and Schulze-Lefert, P. (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. *Cell*, **88**, 695–705.
- Carver, T.L.W., Thomas, B.J., Ingerson-Morris, S.M. and Roderick, H.W. (1990) The role of the abaxial leaf surface waxes of *Lolium* spp. in resistance to *Erysiphe graminis*. *Plant Pathol.* **39**, 573–583.
- Carver, T.L.W., Zeyen, R.J., Robbins, M.P., Vance, C.P. and Boyles, D.A. (1994) Suppression of host cinnamyl alcohol-dehydrogenase and phenylalanine ammonia-lyase increases oat epidermal-cell susceptibility to powdery mildew penetration. *Physiol. Mol. Plant Pathol.* **44**, 243–259.
- Chalupska, D., Lee, H.Y., Faris, J.D., Evrard, A., Chalhoub, B., Haselkorn, R. and Gornicki, P. (2008) *Acc* homeoeloci and the evolution of wheat genomes. *Proc. Natl. Acad. Sci. USA*, **105**, 9691–9696.
- Conner, R.L., Kuzyk, A.D. and Su, H. (2003) Impact of powdery mildew on the yield of soft white spring wheat cultivars. *Can. J. Plant Sci.* **83**, 725–728.
- Consonni, C., Humphry, M.E., Hartmann, H.A., Livaja, M., Durner, J., Westphal, L., Vogel, J., Lipka, V., Kemmerling, B., Schulze-Lefert, P., Somerville, S.C. and Panstruga, R. (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat. Genet.* **38**, 716–720.
- Couch, B.C. and Kohn, L.M. (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, **94**, 683–693.
- Couch, B.C., Fudal, I., Lebrun, M.-H., Tharreau, D., Valent, B., van Kim, P., Nottéghem, J.-L. and Kohn, L.M. (2005) Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics*, **170**, 613–630.
- Dewey, D.R. (1983) Historical and current taxonomic perspectives of *Agropyron*, *Elymus*, and related genera. *Crop Sci.* **23**, 637–642.
- Dodds, P.N. and Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* **11**, 539–548.
- Douchkov, D., Nowara, D., Zierold, U. and Schweizer, P. (2005) A high-throughput gene-silencing system for the functional assessment of defense-related genes in barley epidermal cells. *Mol. Plant–Microbe Interact.* **18**, 755–761.
- Eichmann, R. and Hückelhoven, R. (2008) Accommodation of powdery mildew fungi in intact plant cells. *J. Plant Physiol.* **165**, 5–18.
- Eichmann, R., Schultheiss, H., Kogel, K.H. and Hückelhoven, R. (2004) The barley apoptosis suppressor homologue bax inhibitor-1 compromises non-host penetration resistance of barley to the inappropriate pathogen *Blumeria graminis* f.sp. *tritici*. *Mol. Plant–Microbe Interact.* **17**, 484–490.
- Eichmann, R., Bischof, M., Weis, C., Shaw, J., Lacomme, C., Schweizer, P., Douchkov, D., Hensel, G., Kumlehn, J. and Hückelhoven, R. (2010) BAX INHIBITOR-1 is required for full susceptibility of barley to powdery mildew. *Mol. Plant–Microbe Interact.* **23**, 1217–1227.
- Elliott, C., Zhou, F.S., Spielmeier, W., Panstruga, R. and Schulze-Lefert, P. (2002) Functional conservation of wheat and rice *Mlo* orthologs in defense modulation to the powdery mildew fungus. *Mol. Plant–Microbe Interact.* **15**, 1069–1077.
- Eshed, N. and Wahl, I. (1970) Host ranges and interrelations of *Erysiphe graminis hordei*, *Erysiphe graminis tritici*, and *Erysiphe graminis avenae*. *Phytopathology*, **60**, 628–634.
- Eshed, N. and Wahl, I. (1975) Role of wild grasses in epidemics of powdery mildew on small grains in Israel. *Phytopathology*, **65**, 57–63.
- Everts, K., Leath, S. and Finney, P.L. (2001) Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. *Plant Dis.* **85**, 423–429.
- Feuillet, C., Langridge, P. and Waugh, R. (2008) Cereal breeding takes a walk on the wild side. *Trends Genet.* **24**, 24–32.
- Giraud, T., Gladioux, P. and Gavrillets, S. (2010) Linking the emergence of fungal plant diseases with ecological speciation. *Trends Ecol. Evol.* **25**, 387–395.
- Green, J.R., Carver, T.L.W. and Gurr, S.J. (2002) The formation and function of infection and feeding structures. In: *The Powdery Mildews* (Bélanger, R.R., Bushnell, W.R., Dik, A.J. and Carver, T.L.W., eds), pp. 66–82. St. Paul, MN: APS Press.
- Grünwald, N.J. and Goss, E.M. (2011) Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. *Annu. Rev. Phytopathol.* **49**, 249–267.
- Hardison, J.R. (1944) Specialization of pathogenicity in *Erysiphe graminis* on wild and cultivated grasses. *Phytopathology*, **34**, 1–20.
- Hardison, J.R. (1945) Specialization in *Erysiphe graminis* for pathogenicity on wild and cultivated grasses outside the tribe Hordeae. *Phytopathology*, **35**, 394–405.
- Hiura, U. (1965) Sexual compatibility between form species of *Erysiphe graminis* DC. and variability of the ascospore derived from the inter-form-specific hybridization. *Nogaku Kenkyu*, **51**, 67–73.
- Hiura, U. (1978) Genetic basis of *formae speciales* in *Erysiphe graminis* DC. In: *The Powdery Mildews* (Spencer, D.M., ed.), pp. 101–128. New York: Academic Press.
- Hörandl, E. (2006) Paraphyletic versus monophyletic taxa—evolutionary versus cladistics classifications. *Taxon*, **55**, 564–570.
- Hückelhoven, R. and Panstruga, R. (2011) Cell biology of the plant–powdery mildew interaction. *Curr. Opin. Plant Biol.* **14**, 738–746.
- Hückelhoven, R., Fodor, J., Preis, C. and Kogel, K.H. (1999) Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. *Plant Physiol.* **119**, 1251–1260.
- Hückelhoven, R., Dechert, C. and Kogel, K.H. (2001) Non-host resistance of barley is associated with a hydrogen peroxide burst at sites of attempted penetration by wheat powdery mildew fungus. *Mol. Plant Pathol.* **2**, 199–205.
- Humphry, M., Consonni, C. and Panstruga, R. (2006) *mlo*-based powdery mildew immunity: silver bullet or simply non-host resistance? *Mol. Plant Pathol.* **7**, 605–610.
- Inuma, T., Khodaparast, S.A. and Takamatsu, S. (2007) Multilocus phylogenetic analysis within *Blumeria graminis*, a powdery mildew fungus of cereals. *Mol. Phylogenet. Evol.* **44**, 741–751.
- Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.
- Jørgensen, J.H. (1994) Genetics of powdery mildew resistance in barley. *Crit. Rev. Plant Sci.* **13**, 97–119.
- Koltin, Y. and Kenneth, R. (1970) The role of the sexual stage in the overwintering of *Erysiphe graminis* DC. f.sp. *hordei* Marchal under semi-arid conditions. *Ann. Appl. Biol.* **65**, 263–268.
- Konishi, S., Sasakuma, T. and Sasanuma, T. (2010) Identification of novel *Mlo* family members in wheat and their genetic characterization. *Genes Genet. Syst.* **85**, 167–175.
- Linde-Laursen, I. (1977) Reaction of triticale, wheat and rye to the powdery mildew fungi, *Erysiphe graminis* f.sp. *tritici* and *E. graminis* f.sp. *secalis*. *Z. Pflanzenzüchtung*, **79**, 110–121.
- Lipka, U., Fuchs, R. and Lipka, V. (2008) Arabidopsis non-host resistance to powdery mildews. *Curr. Opin. Plant Biol.* **11**, 404–411.
- Ma, J. and Bennetzen, J. (2004) Rapid recent growth and divergence of rice nuclear genomes. *Proc. Natl. Acad. Sci. USA*, **101**, 12 404–12 410.
- Marchal, E. (1902) De la spécialisation du parasitisme chez l'*Erysiphe graminis*. *C. R. Acad. Sci. Paris*, **135**, 210–212.
- Matsuda, S. and Takamatsu, S. (2003) Evolution of host–parasite relationships of *Golovinomyces* (Ascomycete: Erysiphaceae) inferred from nuclear rDNA sequences. *Mol. Phylogenet. Evol.* **27**, 314–327.
- Matsumura, K. and Tosa, Y. (1995) The rye mildew fungus carries avirulence genes corresponding to wheat genes for resistance to races of the wheat mildew fungus. *Phytopathology*, **85**, 753–756.

- McNeil, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'Homme, W.F., Van Reine, G., Smith, F., Wiersema, J.H. and Turland, N.J. (eds) (2012) International code of botanical nomenclature (Melbourne code). Adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. *Regnum Vegetabile*, 154. Ruggell, Liechtenstein: Gantner.
- Mehta, Y.R.A. (1998) Variação patogênica entre isolados de *Magnaporthe grisea* atacando triticale e trigo no estado do Paraná. *Summa. Phytopathol.* **24**, 119–125.
- Miklis, M., Consonni, C., Bhat, R.A., Lipka, V., Schulze-Lefert, P. and Panstruga, R. (2007) Barley MLO modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. *Plant Physiol.* **144**, 1132–1143.
- Mysore, K.S. and Ryu, C.M. (2004) Non-host resistance: how much do we know? *Trends Plant Sci.* **9**, 97–104.
- Niks, R.E. and Marcel, T.C. (2009) Non-host and basal resistance: how to explain specificity? *New Phytol.* **182**, 817–828.
- Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kumlehn, J. and Schweizer, P. (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. *Plant Cell*, **22**, 3130–3141.
- Nürnberg, T. and Lipka, V. (2005) Non-host resistance in plants: new insights into an old phenomenon. *Mol. Plant Pathol.* **6**, 335–345.
- Oberhaensli, S., Parlange, F., Buchmann, J.P., Jenny, F.H., Abbott, J.C., Burgis, T.A., Spanu, P.D., Keller, B. and Wicker, T. (2011) Comparative sequence analysis of wheat and barley powdery mildew fungi reveals gene collinearity, dates divergence and indicates host-pathogen co-evolution. *Fungal Genet. Biol.* **48**, 327–334.
- Oettler, G. (2005) The fortune of a botanical curiosity—Triticale: past, present and future. *J. Agric. Sci.* **143**, 329–346.
- Oku, T., Yamashita, S., Doi, Y. and Nishihara, N. (1985) Host range and *forma specialis* of cocksfoot powdery mildew fungus (*Erysiphe graminis* DC) found in Japan. *Ann. Phytopathol. Soc. Jpn.* **51**, 613–615.
- Oku, T., Yamashita, S., Doi, Y. and Hiura, U. (1986) Genetic analysis of resistance of wheat cultivars to races and some *formae speciales* of *Erysiphe graminis* DC. *Ann. Phytopathol. Soc. Jpn.* **52**, 700–708.
- Olesen, K.L., Carver, T.L.W. and Lyngkjær, M.F. (2003) Fungal suppression of resistance against inappropriate *Blumeria graminis formae speciales* in barley, oat and wheat. *Physiol. Mol. Plant Pathol.* **62**, 37–50.
- Parks, R., Carbone, I., Murphy, J.P. and Cowger, C. (2009) Population genetic analysis of an eastern U.S. wheat powdery mildew population reveals geographic subdivision and recent common ancestry with U.K. and Israeli populations. *Phytopathology*, **99**, 840–849.
- Parlange, F., Oberhaensli, S., Breen, J., Platzter, M., Taudien, S., Šimková, H., Wicker, T., Doležal, J. and Keller, B. (2011) A major invasion of transposable elements accounts for the large size of the *Blumeria graminis* f.sp. *tritici* genome. *Funct. Integr. Genomics*, **11**, 671–677.
- Prats, E., Mur, L.A.J., Sanderson, R. and Carver, T.L.W. (2005) Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis* f.sp. *hordei*. *Mol. Plant Pathol.* **6**, 65–78.
- Ridout, C.J., Skamnioti, P., Porritt, O., Sacristan, S., Jones, J.D.G. and Brown, J.K.M. (2006) Multiple avirulence paralogs in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance. *Plant Cell*, **18**, 2402–2414.
- Sacristan, S., Vigouroux, M., Pedersen, C., Skamnioti, P., Thordal-Christensen, H., Micali, C., Brown, J.K.M. and Ridout, C.J. (2009) Coevolution between a family of parasite virulence effectors and a class of LINE-1 retrotransposons. *PLoS ONE*, **4**, e7463.
- Salamini, F., Ozkan, H., Brandolini, A., Schafer-Pregl, R. and Martin, W. (2002) Genetics and geography of wild cereal domestication in the Near east. *Nat. Rev. Genet.* **3**, 429–441.
- Schulze-Lefert, P. and Panstruga, R. (2003) Establishment of biotrophy by parasitic fungi and reprogramming of host cells for disease resistance. *Annu. Rev. Phytopathol.* **41**, 641–667.
- Schulze-Lefert, P. and Panstruga, R. (2011) A molecular evolutionary concept connecting non-host resistance, pathogen host range, and pathogen speciation. *Trends Plant Sci.* **16**, 117–125.
- Schweizer, P. (2007) Non-host resistance of plants to powdery mildew—new opportunities to unravel the mystery. *Physiol. Mol. Plant Pathol.* **70**, 3–7.
- Sheng, B., Zhou, Y., Duan, X. and Xiang, Q. (1993) The host range of wheat powdery mildew (*Erysiphe graminis* f.sp. *tritici*) in Shanxi province. *Acta Phytophyl. Sin.* **20**, 105–112.
- Sheng, B., Duan, X., Xiang, Q. and Zhou, Y. (1995) The study on the host range of wheat powdery mildew in the northern 11 provinces (regions and cities). *Scientia Agric. Sin.* **28**, 52–57.
- Spanu, P.D., Abbott, J.C., Amselem, J., Burgis, T.A., Soanes, D.M., Stüber, K., Ver Loren van Themaat, E., Brown, J.K.M., Butcher, S.A., Gurr, S.J., Lebrun, M.-H., Ridout, C.J., Schulze-Lefert, P., Talbot, N.J., Ahmadinejad, N., Ametz, C., Barton, G.R., Benjdia, M., Bidzinski, P., Bindschedler, L.V., Both, M., Brewer, M.T., Cadle-Davidson, L., Cadle-Davidson, M.M., Collemare, J., Cramer, R., Francisco, L.-R., Frenkel, O., Godfrey, D., Harriman, J., Hoede, C., King, B.C., Klages, S., Kleemann, J., Knoll, D., Koti, P.S., Kreplak, J., Lu, X., Maekawa, T., Mahanil, S., Milgroom, M.G., Montana, G., Noir, S., O'Connell, R.J., Oberhaensli, S., Parlange, F., Pedersen, C., Quesneville, H., Reinhardt, R., Rott, M., Sacristán, S., Schmidt, S.M., Schön, M., Skamnioti, P., Sommer, H., Stephens, A., Takahara, H., Thordal-Christensen, H., Vigouroux, M., Weßling, R., Wicker, T. and Panstruga, R. (2010) Genome expansion and gene loss in powdery mildew fungi reveal functional tradeoffs in extreme parasitism. *Science*, **330**, 1543–1546.
- Stukenbrock, E.H. and McDonald, B.A. (2008) The origins of plant pathogens in agro-ecosystems. *Annu. Rev. Phytopathol.* **46**, 75–100.
- Stukenbrock, E.H., Banke, S., Javan-Nikkhah, M. and McDonald, B.A. (2007) Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. *Mol. Biol. Evol.* **24**, 398–411.
- Takamatsu, S. and Matsuda, S. (2004) Estimation of molecular clocks for ITS and 28S rDNA in Erysiphales. *Mycoscience*, **45**, 340–344.
- Thordal-Christensen, H., Zhang, Z.G., Wei, Y.D. and Collinge, D.B. (1997) Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. *Plant J.* **11**, 1187–1194.
- Tosa, Y. (1989a) Genetic analysis of the avirulence of wheatgrass powdery mildew fungus on common wheat. *Genome*, **32**, 913–917.
- Tosa, Y. (1989b) Evidence on wheat for gene-for-gene relationship between *formae speciales* of *Erysiphe graminis* and genera of gramineous plants. *Genome*, **32**, 918–924.
- Tosa, Y. and Tada, S. (1990) Operation of resistance genes in wheat to *Erysiphe graminis* f.sp. *tritici* against *E. graminis* f.sp. *agropyri*. *Genome*, **33**, 231–234.
- Tosa, Y., Akiyama, T. and Ogura, H. (1990) Cytological aspects of interactions between *formae speciales* of *Erysiphe graminis* and genera of gramineous plants, and their evolutionary implications. *Can. J. Bot.* **68**, 1249–1253.
- Troch, V., Audenaert, K., Bekaert, B., Höfte, M. and Haesaert, G. (2012) Phylogeography and virulence structure of the powdery mildew population on its 'new' host triticale. *BMC Evol. Biol.* **12**, 76.
- Trujillo, M., Troeger, M., Niks, R.E., Kogel, K.-H. and Hückelhoven, R. (2004) Mechanistic and genetic overlap of barley host and non-host resistance to *Blumeria graminis*. *Mol. Plant Pathol.* **5**, 389–396.
- Walker, A.S., Bouguennec, A., Confais, J., Morgant, G. and Leroux, P. (2011) Evidence of host-range expansion from new powdery mildew (*Blumeria graminis*) infections of triticale (*Triticosecale*) in France. *Plant Pathol.* **60**, 207–220.
- Wyand, R.A. (2001) *Molecular evolution of Blumeria graminis*. PhD Thesis, University of East Anglia.
- Wyand, R.A. and Brown, J.K.M. (2003) Genetic and forma specialis diversity in *Blumeria graminis* of cereals and its implications for host–pathogen co-evolution. *Mol. Plant Pathol.* **4**, 187–198.
- Zaffarano, P.L., McDonald, B.A. and Linde, C.C. (2008) Rapid speciation following recent host shifts in the plant pathogenic fungus *Rhynchosporium*. *Evolution*, **62**, 1418–1436.
- Zellerhoff, N., Himmelbach, A., Dong, W., Bieri, S., Schaffrath, U. and Schweizer, P. (2010) Non-host resistance of barley to different fungal pathogens is associated with largely distinct quantitative transcriptional responses. *Plant Physiol.* **152**, 2053–2066.
- Zeyen, R.J., Carver, T.L.W. and Lyngkjær, M.F. (2002) Epidermal cell papillae. In: *The Powdery Mildews* (Bélanger, R.R., Bushnell, W.R., Dik, A.J. and Carver, T.L.W., eds), pp. 107–124. St. Paul, MN: APS Press.
- Zhang, Z., Henderson, C., Perfect, E., Carver, T.L.W., Thomas, B.J., Skamnioti, P. and Gurr, S.J. (2005) Of genes and genomes, needles and haystacks: *Blumeria graminis* and functionality. *Mol. Plant Pathol.* **6**, 561–575.