SUMMARY

Strawberry powdery mildew, caused by *Podosphaera aphanis*, is one of the most serious diseases on strawberry worldwide. In contrast, raspberry powdery mildew is usually less severe. Both diseases are believed to be caused by the same fungal species (*P. aphanis*). However, this paper shows that the mildews on these two hosts are genetically distinct. Sequencing the ITS region of a number selected samples from the two fungi clearly indicates that these two fungi are genetically different.

Key words: rDNA ITS, species concept, powdery mildew.

The powdery mildew fungi (*Erysiphales*) are common obligate plant pathogens that are easily recognised visually. However, species identifications are often difficult or impossible (Glawe, 2008). Taxonomy and identification of the *Erysiphales* have traditionally been based on the morphology of the teleomorph and host range. Recently, emphasis has moved to the anamorphic morphology (Braun, 1987; Cook et al., 1997) and the use of ribosomal DNA internal transcribed spacer (rDNA ITS) sequences (Saenz and Taylor, 1999; Hirose et al., 2005; Cook et al., 2006; Kiss et al., 2006; Inuma et al., 2007; Chen et al., 2008; Jankovics et al., 2008; Kiss et al., 2008).

Powdery mildew, caused by *Podosphaera aphanis* (Wallr.) U. Braun et S. Takamatu (known earlier as *Sphaerotheca macularis* sensu auct. NZ), can infect leaves, leaf petioles, flower trusses, flowers and fruit, and is a serious disease of cultivated strawberries (*Fragaria x ananassa*) (Miller et al., 2003; Blanco et al., 2004; Amsalem et al., 2006; Willocquet et al., 2008). This pathogen is also believed to cause powdery mildew on raspberry (Ellis et al., 1991). Although fungal isolates from strawberry have been shown not to infect raspberry and vice versa (Ellis et al., 1991), it is now being claimed that recent severe epidemics of powdery mildew on strawberry in some parts of the UK arose from inoculum originating from raspberry plantations nearby. If confirmed, it will have considerable implications on disease management on both crops. Preliminary investigations were thus carried out at East Malling Research (UK) to compare powdery mildew on strawberry and raspberry. Scanning electron microscopic examinations found no apparent morphological differences between mildews from the two hosts but cross-inoculation failed to produce successful infections in polythene tunnel, glasshouse compartment or controlled environmental conditions (15-20°C at 70-95% relative humidity) (X.-M. Xu, unpublished information).

The failure of numerous cross-inoculation efforts led us to investigate whether strawberry and raspberry powdery mildews can be practically considered as two distinct species. Specifically, we selected a number of samples from each pathogen for sequencing their rDNA ITS region since recent studies suggested that ITS sequencing can be used to distinguish powdery mildews that are morphologically indistinguishable (Jankovics et al., 2008).

Individual leaves with sporulating mildew lesions were from strawberry plants in the UK, USA, China, Italy and Israel, whereas mildew raspberry samples were sampled from three sites in the UK only (Dundee, Cambridge and East Malling). A leaf disc (diameter of 0.5 cm) with a single lesion was cut in the field and placed immediately into a centrifuge vial containing 1-2 ml of 95% ethanol; the vials were then shaken manually for 10-20 seconds and kept under ambient conditions until DNA extraction. After at least 24 h, the disc was removed from each vial and the vial was then left open to dry. For Italian and Israeli samples of strawberry mildew, leaves with lesions were taken, dried under ambient conditions and shipped. Disks with a lesion were then cut out from these dried leaf samples and treated with ethanol, as above. A total 720 samples were obtained for strawberry mildew and 19 for raspberry mildew (11 from East Malling, 2 from Cambridge and 6 from Dundee).
To each dried sample vial, 600 µl cell lysis solution (5 mM TRIS, 10 mM EDTA, 0.5% SDS) and 5 µl of 20 mg/ml proteinase K were added. Tubes were incubated overnight at 55ºC and vortexed vigorously for 1 min. After samples were cooled to room temperature, 200 µl of ice-cold protein precipitation solution (3 M ammonium acetate) was added and tubes spun at 13,000 rpm for 10 min. The supernatant was decanted and mixed with 600 µl isopropanol, and precipitate spun down at 13,000 rpm for 10 min. The pellet was washed in 70% ethanol, followed by a final spin at 13,000 rpm (10 min). The pellet was air-dried and re-suspended in 50 µl water.

The universal primers EKITSF (CTTGGTCATTTAGGAAAGTAA) and Ek28R (ATATGCTTAAGTTCAGCGGG) were used to PCR across the whole ITS1 and ITS2 regions (Anderson and Cairney, 2004). Bands were cut from gels and cloned; both strands were sequenced, and the sequences matched to the GenBank database using the BLASTN program (http://www.ncbi.nlm.nih.gov/blast). A total of 28 samples were sequenced for their ITS region: 20 strawberry mildew samples (7 from the UK, 4 from Israel, 3 from Italy, 3 from USA, and 3 from China) and 8 raspberry mildew samples (2 from Cambridge, 3 from Dundee and 3 from East Malling). The sequences are deposited in GenBank as accession Nos GU942442 to GU942462 (Table 1).

Relationships among the ITS sequences were analysed using MEGA4 (Tamura et al., 2007). Four sequences from GenBank were included in the analysis: DQ139429 (P. pannosa isolate P-M from host Prunus laurocerasus), DQ139433 (P. pannosa isolate R-P from host Rosa sp.), AB026136 [P. aphanis var. aphanis from host F. grandiflora (Takamatsu et al., 2000)] and AF073355 [P. aphanis from host F. x ananassa = F. grandiflora (Cunnington et al., 2003)]. Preliminary analysis including two other P. aphanis sequences (AB000938 and AB026141 in Agrimonia) in GenBank revealed these sequences to be distantly related to P. aphanis isolated from strawberry and were therefore excluded from this study. Isolates with the same ITS sequence from the same region were excluded from phylogenetic analysis. Both parsimony and neighbour joining analyses were performed with insertions and deletions included; bootstrap was based on 500 replicates.

Because universal primers were used, a number of contaminating ascomycetes and basidiomycetes were present among the bands sequenced. However, all the samples produced a sequence of either 545 bp, 546 bp or 547 bp (when primers removed) which closely matched Sphaerotheca and Podosphaera in GenBank (sequences are available upon request). The topology produced based on 479 bp is the same for both distance and parsimony-based analyses and is shown in the neighbour joining tree in Fig. 1. The outgroup sequence is DQ139430 (P. pannosa from Rosa).

The samples from Eurasian (China, Japan, Italy, Israel and UK) strawberry and raspberry formed two distinct and well-supported clades, with bootstraps of 93% and 65%, respectively. However, the three strawberry-derived sequences from California and the GenBank sequence AF073355 remained unresolved outside these two well-supported clades. Nucleotide differences are greater between the three groups of samples (Eurasia strawberry, raspberry and California strawberry) than the within-group differences. As a group, the Eurasia group on average differed by 4.4 nucleotides with the raspberry group, and 3.3 nucleotides with the California group, whereas there are on average 2.4 nucleotide differences between raspberry and California groups.

As claimed in literature (Ellis et al., 1991), there was

<table>
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<th>Sample ID</th>
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no cross-infection between strawberry and raspberry mildew, which was confirmed by our recent unpublished results. Recent microsatellite-based studies indicated that there was no clear cut evidence for pathogen adaptation to particular hosts in strawberry mildew or particular geographic regions (Harvey and Xu, 2010). Furthermore, there is also the lack of strong race-specific interactions between strawberry cultivars and powdery mildew (Xu et al., 2008), unlike other mildews such as wheat (Wolfe and McDermott, 1994), cucurbits (del Pino et al., 2002), melon (McCright, 2006) and groundsel (Clark, 1997). This is consistent with the finding of the importance of the additive component in strawberry resistance to powdery mildew (Hsu et al., 1969; McNicol and Gooding, 1979; Simpson, 1987; Nelson et al., 1995; Davik and Honne, 2005).

In summary, powdery mildews on strawberry and raspberry are genetically distinct and might represent two cryptic species of the Erysiphales. The differences in the rDNA ITS region between Eurasian strawberry and raspberry mildew samples is about 4.4 bp, less than 1%. However, the average differences in nucleotides between P. pannosa sequences, and raspberry, Eurasia strawberry and California samples are only 4, 5 and 3 bp, respectively. Recent results from cross-inoculation, ITS sequencing and AFLP analyses have led to the conclusion that for morphologically indistinguishable powdery mildews 1-5 single nucleotide positions in their ITS region and different host ranges are to be considered as different taxa with distinct host ranges.

Fig. 1. Phylogenetic relationships among powdery mildews samples from strawberry and raspberry in different countries inferred using the Neighbour-Joining method (Saitou and Nei, 1987). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 479 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). Sample details are given in Table 1.
Raspberry and strawberry mildews are different

(Jankovics et al., 2008) for powdery mildew fungi belonging to the genus *Erysiphe*. There is also no clear evidence for cross-infection between the two pathogens, particularly given the fact that recent research suggested there is no strong evidence for race-specific interactions in strawberry. Therefore, we may consider raspberry mildew a separate species from *P. aphanis* on strawberry.

Further research is needed to understand why mildew samples from strawberry in California do not fit into the strawberry clade as defined in this study. This work showed that the ITS sequences of strawberry powdery mildew samples determined in the present and previous studies are more variable than those of other powdery mildew species infecting the same host plant species. The same is true for the ITS sequences of raspberry powdery mildew samples.

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REFERENCES


McCreight J.D., 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii*.