Potential alternative hosts for the pea powdery mildew pathogen *Erysiphe trifolii*

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Powdery mildew is an important disease of peas grown in both greenhouses and in the field. The latter is obviously important for commercial production, but greenhouses are often used to increase the number of generations per year in pea breeding programs. Even though *Erysiphe pisi* (often reported as *E. communis* or *E. polygoni* in earlier literature) is the most commonly documented pathogen species causing powdery mildew of peas, *E. baeumleri* (1) and *E. trifolii* (2) were recorded as powdery mildew pathogens on peas. It has been presumed that the putative breakdown of resistance in previously resistant pea cultivars observed in the US Pacific Northwest (US PNW) was actually due to the presence of more than one species of *Erysiphe* (2). Attanayake et al. (2) observed severe disease symptoms caused by *E. trifolii* on resistant pea cv. 'Lifter' grown in greenhouse conditions. Greenhouse-grown pea breeding materials often get infected with powdery mildew in the US PNW (K. McPhee, personal communication). However, the inoculum source, particularly for greenhouse-grown peas during the winter months, has not been determined. Since during winter months no pea crops are growing in fields in the PNW, inoculum would have to originate from pea debris of the previous growing season, volunteer pea plants or from powdery mildew-infected wild legume plants serving as alternative hosts. Many powdery mildew pathogens are known to have broad host ranges (3). *E. trifolii* has been reported on peas and lentils in the US PNW (2, 4) and on *Trifolium* (as the specific epithet indicates) and other genera of the Fabaceae such as *Acacia, Arachis, Lathyrus,* and *Melilotus* (5). Species of *Arachis, Dolichos, Lathyrus, Lens, Lupinus, Medicago, Melilotus, Phaseolus, Trifolium* and *Vicia* are known hosts for *E. pisi* (5). The above abbreviated host lists make it clear that *E. trifolii* and *E. pisi* have numerous hosts, including some hosts in common.

Powdery mildew-infected *Medicago lupulina, Melilotus* spp., *Lathyrus* spp. and *Vicia* spp. plants are abundant along road sides, recreational areas and commercial fields during the periods of July-November in the Palouse region of Idaho and Washington. We hypothesized that these weedy legumes can serve as alternative hosts for *E. trifolii.* Detailed studies on host range of *E. trifolii* in the US PNW are lacking, so we tested these common weedy legumes from the region as potential alternative hosts of *E. trifolii.*

Materials and Methods

Plant germplasm

To assess susceptibility to *E. trifolii,* we obtained germplasm (seeds) from the USDA-ARS National Plant Germplasm System for nine wild legume species: *Medicago lupulina* L. (PI189128), *Medicago scutellata* (L.) Mill. (PI161415), *Melilotus albus* Medik. (PI90186), *Melilotus officinalis* L. (PI539020), *Medicagopolymorpha* L. (PI186329), *Lathyrus latifolius* L. (PI358888), *Trifolium pratense* L. (PI631906), *Vicia amoena* Fisch. (PI428330), and *Vicia cracca* L. (PI371785), all from the North Central Plant Introduction Station at Ames, IA. *Viciafaba* L. seeds were obtained from Mountain Valley, Inc. in Salt Lake City, UT. To test cultivated legumes as potential alternative hosts, seeds of powdery mildew-susceptible *Glycine max* (L.) Merr. (cvs. L84-2237 - PI547870 and Harosoy- PI548573) seeds were obtained from the USDA Soybean Germplasm Collection in Urbana, IL and seeds of susceptible *Lens culinaris* Medik. cultivar 'Crimson' were obtained from the USDA Grain Legume Genetics and Physiology Research Unit, Washington State University (WSU), Pullman, WA. *Glycine max* cultivars, L84-2237 -PI547870 and Harosoy- PI548573 are susceptible to *E. diffusa* (6, 7).

This latter powdery mildew species was of interest, since a powdery mildew reported from lentil in Canada was identified as *E. diffusa* on the basis of morphology (8).

To maintain powdery mildew inoculum, seeds from four powdery mildew-susceptible pea cultivars; 'Dark Skin Perfection', 'Medora', 'Radley' and a previously registered powdery mildew resistant cv. 'Lifter' (9) were obtained from USDA-ARS Grain Legume Genetics and Physiology Research Unit, WSU, Pullman, WA.

Greenhouse inoculation

All the inoculations were carried out at the WSU Plant Growth Facility. Pea seeds of all varieties were grown in the greenhouse and allowed to get natural infections to serve as inoculum for all the experiments. Once pea plants became infected, the pathogen was identified by isolation of DNA from colonies of powdery mildew and sequencing the rDNA ITS 1 and ITS 2 regions as described by Attanayake et al. (4). Sequences were compared with those in GenBank. Powdery mildew conidia were collected from 3-4 plants of each pea cultivar and ITS sequencing was determined separately (by cultivar) in order to identify the pathogen species. Morphological characters of the pathogen were observed under a compound microscope and diagnostic characters of chasmothecia, if present, were documented.

Seeds of each of the above mentioned plant species were planted in 15cm pots. Plants were kept side by side in the same greenhouse with powdery mildew-infected pea plants. Twenty-four seeds of each plant accession were planted using six replicate pots with four seeds per pot. Three pots of each species served as controls and the rest of the plants were artificially inoculated. Inoculations were carried out when plants were 21 days old. Powdery mildew conidia were collected in a plastic weighing boat and carefully dusted onto the adaxial surfaces of the first six fully expanded younger leaves until white colored conidia were visible on the surfaces. Symptom development was observed every two days after inoculation until the flowering stage.

Results

All pea cultivars exhibited severe powdery mildew symptoms. Amplified PCR products were 650 bp in size. BLAST search results showed that the ITS sequences were identical to one another and 99% similar to *Erysiphe trifolii.* 97% similarity was exhibited to *E. pisi* deposited by Saenz and Taylor (10). Severe powdery mildew infections were produced on the pea cv. 'Lifter', a cultivar previously reported to be resistant to powdery mildew caused by *E. pisi.* Chasmothecia were observed on all pea cultivars and appendages were long, flexuous and dichotomously branched, typical of an authentic specimen of *E. trifolii* and isolates previously collected in the US PNW (2).

In the greenhouse inoculation studies, powdery mildew colonies were observed on all plants tested at 10¬14 days after inoculation, except in the case of *Glycine max* on which no colonies were observed. Those soybean genotypes were previously known to be susceptible to *E. diffusa* (6, 7). Due to the high disease pressure all control plants, except those of *G. max,* ultimately became infected within 15-18 days after the inoculation date.

Discussion

Results of cross inoculation studies conducted in the greenhouse showed that *Lathyrus latifolius, Medicago polymorpha, M. lupulina, M. scutellata, Melilotus albus, M. officinalis, Trifolium pretense, Vicia amoena, V. cracca,* and *V. faba* are potential alternative hosts for the pea powdery mildew pathogen, *E. trifolii,* and confirmed our previous reports that *Lens culinaris* and *Pisum sativum* are also hosts (2, 4). Our previous cross inoculation studies using a detached leaf bioassay showed that conidia of *E. trifolii* collected from *Melilotus albus* and *Lens culinaris* caused powdery mildew on *Pisum sativum* and conidia of *E. trifolii* collected from *Pisum sativum* caused powdery mildew on *Melilotus albus* and *Lens culinaris* (2, 4), which further supports the conclusions

of the current study. The lack of any symptom development of powdery mildew on the soybean cultivars reported to be susceptible to *E. diffusa* indicates that the powdery mildew species in this study was not *E. diffusa* and soybean is resistant to *E. trifolii.*

The findings of this study broaden the understanding of the wide host range of powdery mildew pathogens infecting peas. Findings are particularly pertinent to weed management, screening for powdery mildew resistance, provide information for consideration in developing strategies to select for resistance in pea breeding programs and to manage powdery mildew of pea in the field.

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