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Evaluation of the USDA National Clonal *Pyrus* Germplasm Collection for Resistance to *Podosphaera leucotricha*

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***Abstract.* Powdery mildew (PM) occurs worldwide and is prevalent on susceptible cultivars wherever pears are grown, causing economic losses due to russeted fruit and an increased need for fungicides. A core subset of the *Pyrus* germplasm collection at the USDA National Clonal Germplasm Repository in Corvallis, Ore., was evaluated for resistance to *Podosphaera leucotricha*, the causal agent of PM, using greenhouse and field inoculations of potted trees. The core collection consists of about 200 cultivars and species selections, representing most of the genetic diversity of pears and includes 31 Asian cultivars (ASN), 122 European cultivars (EUR), 9 EUR × ASN hybrids and 46 pear species selections. Three trees of each core accession were grafted on seedling rootstocks. In 2001–02, trees were artificially inoculated in a greenhouse, grown under conditions conducive for PM, and evaluated for symptoms. The same trees were subsequently evaluated for PM symptoms from natural field infections during 2003 and 2004. In the greenhouse, 95% of EUR and 38% of ASN were infected with PM. Average PM incidence (percent of leaves infected) in the greenhouse (8% for ASN and 30% for EUR) was much higher than incidence in the field (2% for ASN and 5% for EUR) during 2003. Symptoms were also more severe in the greenhouse, with 46% of ASN and 83% of EUR with PM symptoms having a mean PM incidence of >10%. In the field, 42% and 22% of EUR and 23% and 13% of ASN were infected with *P. leucotricha* in 2003 and 2004, respectively. Field infection was very low during both years, with percentage leaves infected in ASN and species selections significantly different from EUR. In the field, 6% of ASN with PM symptoms had a mean PM incidence >10% during both years, while 15% and 2% of EUR accessions with PM symptoms had a mean PM incidence >10% in 2003 and 2004 respectively. These results should be very useful to pear breeding programs to develop improved PM resistant cultivars in the future, by using accessions with consistent low PM ratings.**

Powdery mildew (PM), caused by *Podosphaera leucotricha* (Ell. and Ev.) E.S. Salmon, is an important foliar disease in apple and pear nurseries as well as newly planted orchards throughout the world (Hickey and Yoder, 1990). The fungus is genetically identical on apple and pear (Scholberg et al., 2003) and reduces growth and photosynthetic efficiency of actively-growing terminal shoots, resulting in stunted plants. Infected apple trees may serve as primary inoculum and infect nearby pear orchards, where *P. leucotricha* could produce russeted areas on the surface of fruit. These unsightly patches expand as the fruit enlarge and cause serious losses to growers (Hickey and Yoder, 1990; Spotts, 1984; Woodward, 1927). PM is of commercial concern mainly on fruit of pear cultivars ‘Beurre d’Anjou’, ‘Louise Bonne’, and ‘Doyenne du Comice’ (Hickey and Yoder, 1990). The first PM outbreak on pears was reported by Fisher (1922) for central Washington.

Most of the winter pears in the U.S. are grown in Oregon and Washington. Pear growers in these regions suffer serious losses due to *P. leucotricha,* as ‘Beurre d’Anjou’ is the most important winter pear cultivar in the U.S. and also susceptible to PM infection. Fungicide sprays are currently the primary method for PM control and are applied once at pink stage and again during petal fall (Castagnoli et al., 2003). Fungicides registered in the United States of America for pear PM control belong to the strobilurin and demethylation inhibitor (DMI) families. Resistance to both strobilurin and DMI fungicides has been reported in *Podosphaera* spp. (Ishii et al., 2001; McGrath, 2001; McGrath and Shishkoff, 2003). Thus, it is a concern that these fungicides may become less effective for control of *P. leucotricha* in the Pacific Northwest. The use of chemicals for disease control is expensive, and some consumers prefer fruit that is produced with the minimum amount of pesticides and fungicides (Labuschagné, 2002). Dormant-season pruning of visibly infected shoots may contribute to PM control, but fungicides still need to be applied. In pear orchards >200 m from susceptible apple cultivars, no fungicide applications are needed to control fruit russet (Spotts, 1984).

The use of resistant cultivars is a desirable method for PM prevention on pears. Pear fruit breeding programs today aim to combine durable resistance to the economically important diseases, which include pear scab (*Venturia pirina* Aderh.) and pear PM (Hjeltnes, 1988; Kemp and van Dieren, 2000). Apple breeders have been screening for resistance to *P. leucotricha* for several years (Labuschagné, 2002; Rosenberger et al., 1996; Yoder et al., 1994). Screening of pear cultivars for resistance to PM has been done in the Netherlands (Kemp and van Dieren, 2000) and Norway (Hjeltnes, 1988). Apart from breeding for disease resistance, these fruit also need to be of high quality, store well and have good shelf life.

During this study, *Pyrus* germplasm was evaluated for PM resistance in order to identify the most suitable accessions for commercial planting or as parents for breeding. The relationship between greenhouse and field infection was determined to aid in future predictions of field susceptibility using greenhouse evaluation data. A short summary of parts of this study has been published (Serdani et al., 2005).

Materials and Methods

The *Pyrus* germplasm collection at the USDA National Clonal Germplasm Repository (NCGR) in Corvallis consists of about 1700 accessions, representing 26 major *Pyrus* taxa and their hybrids (Hummer, 1993). Due to the large number of plants in the collection, a core subset of about 200 cultivars was selected to represent a broad genetic cross section of pear diversity. The core subset includes representatives of each *Pyrus* species, each geographic area where pears are grown or occur in the wild, and cultivars with important characteristics. It also includes commercially important cultivars such as ‘Bartlett’ (‘Williams’ Bon Chretien’), ‘Beurre Bosc’, ‘Beurre d’Anjou’, and ‘Doyenne du Comice’.

Each pear clone was identified as belonging to one of four groups: rootstocks and edible European cultivar (EUR = *Pyrus communis*), edible Asian cultivar (ASN = *P. pyrifolia, P. bretschneideri or P. ussuriensis*), edible hybrid cultivar (Asian × European), or *Pyrus* species selection. These groups were carefully considered based on *Pyrus* taxonomy and on distinct phenotypic differences in fruit characteristics and leaf serration between EUR and ASN clones. The natural range of EUR does not overlap that of ASN so there are no natural hybrids in the wild between EUR and ASN. The small number of edible hybrid

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Table 1. Incidence of foliar infection by crop group in *Pyrus* germplasm inoculated with *Podosphaera leucotricha* conidia in the greenhouse and exposed to *P. leucotricha* conidia in the field.

Crop	Leaves infected (%)			
	Greenhouse 2002[z]		Field 2003 and 2004[y,x]	
group	Mean	Range	Mean	Range
Asian cultivars	8 a	0–66.1	1.5 a	0–20.0
European cultivars	30 b	0–100.0	3.5 b	0–48.4
Hybrid cultivars	23 a	0–66.5	2.0 ab	0–16.2
Species selections	8 a	0–81.0	1.7 a	0–50.0

[z]Numbers followed by the same letter within columns are not significantly different at $P < 0.05$ according to an analysis of variance; means were compared through the Tukey-Kramer procedure.
[y]Mean for 2 years.
[x]Numbers followed by the same letter within columns are not significantly different according to the Wilcoxon signed-rank test; means were compared through the Bonferroni correction.

cultivars included in our study represents selections and introductions from breeding programs made during the past century. The fourth group, which includes all species other than the three above is an artificial group, created specifically to exclude all other species from the three cultivar groups. The pear core collection includes 31 Asian cultivars, 122 European cultivars, 9 hybrid (European × Asian) cultivars, and 46 species selections. Asian cultivars included 19 from China, 11 from Japan and one from India.

Greenhouse inoculations. Budwood from the 200 core accessions was grafted onto 'Bartlett' seedling rootstock in spring 2000. Three trees were grafted for each accession and planted in 15-cm pots. During 2000 and Spring 2001, the potted trees were used for greenhouse pear scab resistance studies (Postman et al., 2005). Trees were pruned in late Summer 2001 and placed randomly in a greenhouse at the Mid-Columbia Agricultural Research and Extension Center (MCAREC) in Hood River, Ore. In June 2002, plants were cut back to three buds and allowed to grow for three weeks. At the time of inoculation, plants were about 30 cm tall with a single shoot carrying 4 to 17 leaves. *P. leucotricha* conidia (a voucher specimen was deposited with the Oregon State University Herbarium, Corvallis—OSC# 123964) were produced in vivo on naturally infected 'Newtown' apple shoot terminals from orchards at MCAREC. Pear trees were inoculated daily from Monday to Friday, over 5 weeks, using infected apple shoots that were cut weekly. Inoculations were done by shaking *P. leucotricha* infected apple shoots above potted pear plants. Dispersal of conidia was aided by greenhouse fans. The greenhouse floor was wetted twice a day throughout the duration of the study to maintain high humidity. The aim was to inoculate potted pear plants with a massive dose of *P. leucotricha* conidia, much higher than found in the field, to ensure that no susceptible leaves escaped inoculation. Trees were evaluated for foliar PM symptoms five weeks after inoculation according to the percent leaves per plant with PM symptoms. Temperature in the greenhouse ranged from 7 to 29 °C and relative humidity was maintained between 60% to 100%.

Field inoculations. In April 2003, the potted plants from the core collection were placed in a completely randomized block in an apple orchard at MCAREC near unsprayed PM-infected apple trees. Plants were about 30 to 60 cm tall with one to several shoots carrying a total of 3 to 81 leaves per plant. Young, potted trees were left in the field for the duration of the field trial and evaluated for foliar symptoms in June 2003 and June 2004. Trees were evaluated according to the percentage of leaves per plant showing PM symptoms.

Statistical analysis. Data were evaluated to investigate whether there were any statistically significant differences between the four groups. Greenhouse data were analyzed using an ANOVA with SPlus software (Insightful Corporation, Seattle, Wash.). Means for greenhouse data were compared through the Tukey-Kramer procedure. Field data were analyzed using the nonparametric Wilcoxon signed-rank test (WSRT) with SPlus software (Hollander and Wolfe, 1973). WSRT is robust against any distribution assumption testing for significant differences among groups and was applied due to the many zeros in the field data. The Bonferroni correction was applied to compare means for field data (Table 1). SPlus software was used to calculate mean PM incidence referred to in Table 3. A chi square test was used to determine dependence of field PM on greenhouse PM (Table 4).

Results

Results for both greenhouse and field data are provided in Table 1. Pear accessions in the EUR group were significantly more susceptible to PM than the other three crop groups in the greenhouse (Table 1). In the field, EUR had significantly more PM than ASN and species selections, of which the latter two groups also had the least mildew in the greenhouse (Table 1). Disease incidence for all four crop groups was consistently higher in the greenhouse than in the field. The three most susceptible cultivars in the greenhouse were all EUR and included 'Doyenne du Comice Crimson Gem

Table 2. Pear accessions without powdery mildew symptoms in both the greenhouse (2002) and field (2003 and 2004).

Crop group	Accession[z]	PI no.[y]
Asian (China)	Ba Li Xiang [Ba Li Hsiang]	541985
Asian (China)	Hang Pa Li	315064
Asian (China)	Hansen Siberian Pear	542004
Asian (China)	Harbin (*P. ussuriensis*)	542019
Asian (China)	Kikusui	228014
Asian (China)	Laioyang 50	144035
Asian (China)	Manchuria	143978
Asian (Japan)	Meigetsu	97348
Asian (China)	Nan Guo Li [Nangon Li]	541965
Asian (India)	Naspati	250449
Asian (China)	*P. pyrifolia* No. 2	541900
Asian (China)	Pai Li	541998
Asian (China)	Ping Guo Li [Pingo Li]	267863
Asian (China)	Seuri Li	541904
Asian (China)	Tang Li	542024
Asian (China)	Xiangshui Li [Hsiang Sui-Li]	542022
Asian (China)	Ya Li	506362
European	Arganche	264694
European	B-12 rootstock	437067
European	Flemish Beauty	541189
European	Luscious	541322
European	Maxine	541231
European	Mustafabey	324134
Hybrid	Krylov	127715
Hybrid	Monterrey	541903
Hybrid	NY 10353	541809
Hybrid	Tioma	134606
Species selection	*P. betulifolia*	540942
Species selection	*P. calleryana*	541021
Species selection	*P. calleryana* 'Autumn Blaze'	541075
Species selection	*P. calleryana* 'Bradford'	617646
Species selection	*P. calleryana* v. graciliflora	313928
Species selection	*P. cordata*	541591
Species selection	*P. cossonii*	541592
Species selection	*P. dimorphophylla*	541601
Species selection	*P. dimorphophylla* (Inunashi)	318871
Species selection	*P. hondoensis* (Aonashi)	318874
Species selection	*P. salicifolia* hybrid–blister mite resistant	617559
Species selection	*P. syriaca* No. 1	541975

[z]Accession refers to a unique clonal genotype
[y]PI number refers to the United States Plant Inventory number and serves as a unique identifier for an accession in a particular Genetic Research Scheme.

Table 3. Powdery mildew (PM) ratings by crop group of *Pyrus* germplasm accessions inoculated with *Podosphaera leucotricha* conidia in the greenhouse and field.

Crop group	No. in 2002–03[z]	Greenhouse 2002 HPM[y] (%)	Greenhouse 2002 LPM[x] (%)	Field 2003 HPM[y] (%)	Field 2003 LPM[x] (%)	No. in 2004[z]	Field 2004 HPM[y] (%)	Field 2004 LPM[x] (%)
Asian cultivars	29	3.4	72.4	0.0	93.3	31	0.0	96.8
European cultivars	118	23.7	26.3	0.0	87.3	122	0.0	100.0
Hybrid cultivars	9	22.2	55.6	0.0	87.5	9	0.0	100.0
Species selection	46	6.5	71.7	0.0	92.5	45	0.0	97.8

[z]No. = total number of accessions for that year.
[y]HPM = high powdery mildew: percentage of accessions within that crop group with mean PM incidence ≥50%.
[x]LPM = low powdery mildew: percentage of accessions within that crop group with mean PM incidence <10%.

Table 4. Relationship between greenhouse and field powdery mildew (PM) evaluation from the core collection inoculated with *Podosphaera leucotricha* conidia.

Greenhouse disease incidence group (%)[z]	Accessions with field PM symptoms (%)[y, x]
0–25	25.6
26–74	53.6
75–100	100.0

[z]Incidence group containing all accessions and repetitions with a mean PM incidence (%) in given range.
[y]Percentage accessions (including all repetitions) with PM symptoms in the field in 2003.
[x]Values are significantly different at $P \geq 0.05$ according to chi-square tests.

#2', 'Gebhard Red d'Anjou' and 'Untoase de Geoagiu', with 100%, 100%, and 93% disease incidence respectively. The three most susceptible cultivars in the field during 2003 included one species selection and two EUR. They are *P. pashia*, 'Kalebasa Plocka' and 'Cascade', with 50%, 48%, and 45% disease incidence respectively. In 2004, the three most susceptible cultivars in the field all belonged to different groups, with 13%, 11%, and 8% for *P. communis* [Plant Inventory (PI) number 881.001] (species selection), 'Hung Li' (ASN) and 'Bartlett' (EUR) respectively. 'Cascade' was the only accession to rank among the five most susceptible cultivars both in the greenhouse in 2002 and in the field during 2003. Several cultivars were free of PM symptoms after both greenhouse and field inoculations in 2003–04 (Table 2). Included in this category is 5% of EUR, 55% of ASN, 44% of hybrids, and 26% of species selections. Differences in PM resistance were observed among Asian accessions. In the field, 87% of Chinese and 77% of Japanese accessions was PM resistant during both years. In the greenhouse, accessions from China were much more resistant than those from Japan, with 74% and 18% PM resistance, respectively. The only Indian accession evaluated, 'Naspati', was PM resistant during all trials. PI numbers are included in Table 2 as standard identifiers.

Accessions in the four crop groups were categorized as having a high PM incidence (mean rating ≥50%) or a low PM incidence (mean rating <10%), based on the mean percentage of leaves within an accession showing PM symptoms (Table 3). In the greenhouse, EUR and hybrid cultivars had the highest percentage (23.7% and 22.2%) of accessions in the high PM group. In the field, none of the crop groups had high PM ratings, and between 87% to 100% accessions of each crop group had a low PM rating for the two years. Number of accessions differs between 2003 and 2004 as some plants died and others were re-grafted, replacing some of the dead accessions (Table 3).

Results of the relationship between greenhouse and field data (Table 4), analyzed by chi-square tests, indicate that any accession that had a 75% to 100% PM incidence in the greenhouse developed PM symptoms when exposed to natural infection by *P. leucotricha* in the field. When this figure dropped to between 0% to 25% PM incidence in the greenhouse, only 25% of plants in the field developed PM symptoms. Accessions with field PM symptoms were significantly different at $P \geq 0.05$ according to chi-square tests.

Discussion

Greenhouse infection was more severe than field infection, which is similar to results from apple cultivar evaluations for resistance to PM in South Africa (Labuschagné, 2002). This may be attributed to more succulent growth and higher inoculum dose in the greenhouse, and conditions being more favorable (higher humidity) for PM development in the greenhouse when compared to the field. According to Labuschagné (2002), greenhouse conditions may favor infection to such an extent that host resistance could be obscured. Other contributing factors include plant age, soil type and amount of wind to which trees are exposed (Hjeltnes, 1988). Previous studies show that infection rate decreases with increasing age, which is why PM is usually not of major significance to foliage of mature trees (Spotts, 1984; Hjeltnes, 1988). Infection is also higher at windy sites with sandy soils than at calmer sites with heavy loam (Hjeltnes, 1988). In the present study, evaluations of potted plants in the field were done one and two years after greenhouse evaluation and thus greenhouse plants were younger than when they were rated in the field. Results from a previous study indicate a 43% increase in pear seedling resistance to PM for each year increase in plant age (Hjeltnes, 1988). In addition, plants were placed under apple trees where they were protected from the wind, with soils being a loam rather than sandy. These factors may have contributed to lower infection in the field than in the greenhouse. Hjeltnes (1988) also points out the importance of time of observation as the development of the disease progresses rapidly throughout the growing season. Evaluations done too early in the season may result in the false conclusion that field resistance is higher than greenhouse resistance. In the present study, however, evaluations were not done too early.

Observation time also needs to be considered in disease resistance studies. Labuschagné (2002) stated the importance of repeated tests in the field and greenhouse in order to identify disease resistance obtained through breeding programs. Brown (1959) showed that 2 years is sufficient to evaluate for large differences (resistant vs. susceptible) in PM resistance among young apple trees. Kemp and van Dieren (2000) suggest that an observation period of at least 7 to 10 years is required for pears to clearly distinguish between resistant and moderately resistant genotypes for PM. The present study supports Brown's (1959) conclusion that 2 years would be sufficient to evaluate for large differences in PM.

Results from the present study indicate a positive relationship between disease incidence in the greenhouse and that in the field. An accession exhibiting 75% to 100% disease incidence following greenhouse inoculation indicates that this accession also will be highly susceptible in the field. This information may be useful when doing future PM resistance evaluations in the greenhouse to predict disease incidence in the field.

Genotypes with consistent low PM ratings should be evaluated at other sites and over more seasons. Results will be combined with existing resistance information for other pear pathogens, including Fabraea leaf spot (Bell, 1990; Bell and van der Zwet, 1988; Lombard and Westwood, 1987), Mycosphaerella leaf spot (Bell, 1990) and pear scab (Postman et al., 2005). This information may be useful to pear breeders in selecting parents for combining PM resistance with resistance to other pathogens in future commercial cultivars. This study is not a prediction for susceptibility to fruit russet, although higher inoculum may increase chances for infection of cultivars susceptible to fruit russet. 'Bartlett' pears are known to be resistant to fruit russet (Hickey and Yoder, 1990), although the leaves were shown to be susceptible to PM infection both in the field and greenhouse. Results from this study have been loaded onto the USDA Germplasm Resources Information Network (GRIN) database as observation data and can be retrieved by following the evaluation link at http://www.ars-grin.gov/cor/pyrus/pyrinfo.html. This will allow breeders to select and develop pear cultivars with multiple pest and disease resistance and may translate into reduced use of fungicides to control pear PM.

Powdery mildew symptoms are generally much lighter on pear than on apple (Hickey and Yoder, 1990), and factors involved in resistance also may differ between these genera. Both polygenic resistance and single gene immunity to PM has been reported from apple (Janick et al., 1996), while studies indicate that few genes are involved in pear resistance to PM (Hjeltnes, 1988). Different PM resistance sources (*Pl* genes) have been identified in apple germplasm but are still under testing. *Pl2* is a major gene of resistance to apple powdery mildew originating from *Malus zumi* (Caffier and Laurens, 2005). Previous studies show disease symptoms of plants carrying the *Pl2* resistance gene to be consistently associated with a specific phenotype, consisting of few mildewed colonies without necrosis or chlorosis, both in the field and greenhouse (Caffier and Laurens, 2005; Knight, 1968). Even though chlorosis and necrosis were not associated with mildewed leaves in the present study, it is not known whether the *Pl* genes also are responsible for resistance to *P. leucotricha* on pear. The use of molecular markers linked to PM resistance will improve and speed up some selection procedures. Until useful molecular markers are developed for pear PM, breeding programs will continue to rely on visual evaluation of pear accessions for PM resistance.

Recently, Urbanietz and Duneman (2005) reported that different physiological races of *P. leucotricha* exist in Europe, displaying a high level of diversity in terms of virulence. It is possible that different races also may occur in the United States and that the resistance testing herein may involve several different races of *P. leucotricha*. Additional research is necessary to determine this.

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