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Pear decline resistance in progenies of *Pyrus* taxa used as rootstocks

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Abstract Progenies of 39 open-pollinated genotypes belonging to 26 *Pyrus* taxa were examined for pear decline resistance and pomological traits when used as rootstocks. Following graft inoculation and observation over 18 years, considerable differences in pear decline resistance between and within the progenies were observed. Not affected or little affected and moderately to severely affected trees were observed in all progenies. However, great quantitative differences among them were observed. In the progenies of about one third of the pollinated trees most of the individuals showed a high level of resistance to grafted trees. Significantly different from this group was another third of the progenies that mostly showed high susceptibility in grafted trees. Between these two groups there were progenies that statistically neither differed from the resistant nor from the susceptible group. These progenies were defined as moderately resistant. Significant differences in resistance were also observed between progenies of genotypes of the same species that originated from different locations. These data indicate segregation of the resistance trait and show that seedling progenies are unsuitable as rootstocks in commercial pear growing. Instead, careful selection of suitable genotypes for propagation is required. Great differences between and within the progenies examined were also observed in vigour and yield efficiency.

Keywords *Candidatus* Phytoplasma pyri · Fruit trees · Phytoplasma

Abbreviation

DAPI	4-6-diamidino-2-phenylindole

Introduction

Pear decline (PD) is one of the most important disorders of the cultivated European or French pear *Pyrus communis*. The disease is widespread in Europe and North America and is caused by the wall-less bacterium *Candidatus* Phytoplasma pyri that is closely related to phytoplasmas infecting other deciduous fruit trees (Seemüller and Schneider 2004). *Ca.* P. pyri causes a range of non-specific symptoms such as foliar reddening, small and pale green leaves, leaf curl, premature leaf drop, undersized fruits, growth suppression and decline. *Cacopsylla pyricola* (pear psylla) and *C. pyri* have been identified as vectors of

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the pathogen (Carraro et al. 1998; Jensen et al. 1964). Because these psyllids are difficult to control by insecticide application, spread of the disease cannot satisfactorily be prevented. Also, other measures such as roguing of diseased trees to remove infection sources are not sufficiently effective. The most promising approach to control PD is the use of resistant plants. As phytoplasmas depend on intact phloem sieve tubes, the pathogen declines during winter in the aerial parts in which the phloem degenerates. In contrast, phytoplasmas survive in the roots where functional sieve tubes are present all year. From the roots the stem may be recolonised in the following spring (Schaper and Seemüller 1982; Seemüller et al. 1984). This fluctuation in stem colonisation has led to the presumption that PD can be controlled by the use of resistant rootstocks, as shown for apple proliferation (Seemüller et al. 1992, 2008).

First observations on the different response of pear rootstocks to PD infection stem from the outbreak of the disease at Pacific coast fruit growing areas of North America in the fifties and sixties of the last century. During this epiphytotic, trees on the oriental rootstocks *P. pyrifolia* (syn.: *P. serotina*) and *P. ussuriensis* suffered severely from disease and declined in large numbers. In contrast, trees on seedlings of some *P. communis* cultivars and *P. betulifolia* were much less affected (Blodgett et al. 1962; Westwood et al. 1971). Resistance was also observed in seedling progenies of crosses of *P. communis* cvs. Old Home x Farmingdale. Several genotypes of such seedlings were developed as rootstocks (OH x F rootstocks) and were described to be PD-resistant. In contrast, *P. communis* seedling grown from imported French seeds (‘French Seedling’) showed susceptibility to grafted trees (Westwood et al. 1976; Westwood and Lombard 1982). Similarly, in Germany, trees on *P. communis* cv. Kirchensaller seedlings proved considerably more susceptible than trees on clonal quince (*Cydonia oblonga*) rootstocks (Seemüller et al. 1986).

In Europe, pear is mainly grown on clonal stocks of quince selections and *P. communis* clonal or seedling rootstocks (Wertheim 2002). Trees on *P. communis* stocks are often severely affected by PD. Due to the unsatisfactory PD resistance of at least some of the *P. communis* seedling rootstocks used in Europe, and the unsuitability of quince rootstocks under certain soil conditions, in areas with severe winter frost (Westwood and Lombard 1982), or when psyllid infestation is high (Giunchedi et al. 1994), work was initiated to identify PD-resistant genotypes by screening seedling progenies of many *Pyrus* taxa. The results obtained are presented in this paper. A preliminary report on this screening has been published elsewhere (Seemüller et al. 1998).

Materials and methods

Seeds from 26 open-pollinated *Pyrus* taxa consisting of 39 genotypes were obtained from the botanical gardens of Berlin, Darmstadt, Dortmund, Göttingen, Mainz and München (Germany), Oregon State University at Corvallis, University of North Carolina at Chapel Hill, and Appalachian Fruit Research Station at Kearneysville (USA), Belmonte Arboretum, Wageningen (The Netherlands), Fruit Tree Research Station, Jatabe, Ibaraki (Japan), Institute for Horticulture, Moscow (Russia), INRA, Beaucouzé-Angers (France), Horticulture International, East Malling (UK), or were collected at the Dossenheim Institute (Table 1). Seeds were planted in the greenhouse, and seedlings were grown in pots for one or two years until inoculation.

From 1982 up until 1987, the potted plants were inoculated by top- or side-grafting in August or the beginning of September. Scion wood was taken from naturally infected, virus-free pear trees (cvs Bartlett or Alexander Lucas) showing red-leaf symptoms. From each donor tree, several randomly selected shoots were examined for phytoplasma infection using the DAPI (4-6-diamidino-2-phenylindole) fluorescence method (Seemüller 1976). Trees were used as donor trees only when all examined scions tested phytoplasma-positive. From each progeny inoculated, eight to 15 plants were grafted with scions from healthy trees of the same cultivar as controls. The grafted plants were grown for another 1 or 2 years in pots and were then transplanted into the field at standard spacing. All inoculated plants not developing symptoms within three years were re-inoculated in the field. The plants were kept under appropriate insecticide coverage against natural infections by psyllids.

Following inoculation, the plants were observed over at least 18 years. In late summer or early fall, foliar symptoms, fruit size, vigour, and decline were

Table 1 Pear decline resistance, vigour and yield potential of trees on *Pyrus* taxa progenies

Seed parent	Origin[a]	Diseased trees (number)	Cumulative disease index/tree[b]	Not or slightly affected trees[c] (%)	Mortality (%)	Trunk girth (cm)	Cumulative yield value[d]
P. communis	Moscow	33	4.6 a[e]	88	3	29 ab	14.4 ab
P. calleryana cv. Bradford	Chapel Hill	33	6.9 a	61	0	31 ab	11.7 a
P. betulifolia	Ibaraki	38	7.2 a	73	12	35 b	15.0 ab
P. pyraster	München	26	8.5 a	54	8	30 ab	15.1 b
P. amygdaliformis	Göttingen	24	8.8 a	62	17	33 b	13.4 a
P. x *michauxii*	Dortmund	37	8.8 a	52	14	33 b	15.3 b
P. calleryana var. *tomentella*	Berlin	26	8.9 a	58	8	31 ab	16.1 b
P. cuneata	Bristol	43	9.1 a	60	14	34 b	12.6 a
P. fauriei	Ibaraki	60	9.1 a	60	10	29 ab	15.1 b
P. syriaca	Berlin	39	9.1 a	62	18	31 ab	13.4 a
P. cossonii	Darmstadt	32	9.2 a	56	16	32 b	15.1 b
P. nivalis	Berlin	24	9.6 a	58	17	35 b	11.3 a
P. elaeagrifolia	Mainz	25	9.9 a	52	22	31 ab	14.7 ab
P. ussuriensis	Dossenheim	62	11.1 ab	51	14	31 ab	13.6 ab
P. betulifolia	Corvallis	34	11.3 ab	38	21	36 b	14.0 ab
P. amygdaliformis	Wageningen	27	11.4 ab	48	15	31 ab	14.4 ab
P. bretschneideri	Berlin	28	11.4 ab	43	25	33 b	13.9 ab
P. elaeagrifolia	Dortmund	28	11.5 ab	50	25	34 b	16.3 b
P. nivalis	Dortmund	8	11.6 ab	50	25	33 b	11.5 a
P. gharbiana	Bristol	18	11.9 ab	39	17	35 b	11.5 a
P. hondoensis	Bristol	18	11.9 ab	39	17	29 ab	11.5 a
P. nivalis	Darmstadt	30	11.9 ab	33	33	29 ab	14.7 ab
P. ussuriensis	Mainz	7	12.3 ab	42	14	29 ab	15.8 b
P. serrulata	Berlin	34	12.4 ab	8	29	29 ab	11.5 a
P. calleryana	Dortmund	27	13.1 ab	44	22	34 b	15.3 b
P. elaeagrifolia	Berlin	26	13.2 ab	31	15	31 ab	13.6 ab
P. pyraster	Dortmund	9	13.6 b	33	33	31 ab	13.4 a
P. longipes	East Malling	48	13.7 b	27	27	32 b	15.5 b
P. pyrifolia	Ibaraki	27	14.1 b	41	44	29 ab	13.7 ab
P. caucasica	Dortmund	15	14.2 b	27	20	30 ab	11.6 a
P. pyrifolia	Dossenheim	24	14.3 b	38	42	33 b	12.8 a
P. betulifolia	Dortmund	44	14.4 b	16	25	28 ab	15.6 b
P. communis cv. Feudière	Beaucouzé	29	14.5 b	31	28	34 b	13.1 a
P. communis	Dortmund	33	15.1 b	36	24	25 ab	16.6 b
P. calleryana cv. Bradford	Kearneysville	31	15.4 b	19	45	28 ab	16.4 b
P. calleryana var. *gracilifolia*	Berlin	57	15.9 b	16	40	28 ab	15.9 b
P. kunariana	Wageningen	12	16.6 b	8	67	28 ab	14.2 ab
P. x *lecontei*	Dortmund	28	17.9 b	11	43	19 a	17.2 b
P. ussuriensis	Dortmund	31	20.1 b	16	48	19 a	15.0 ab

[a] See "Materials and methods" for details.

[b] Annual disease ratings accumulated over 18 years.

[c] Trees with cumulative disease index ≤10.0.

[d] Annual yield ratings accumulated over 7 years.

[e] Values with the same letter are not significantly different from each other at $\alpha=0.05$.

evaluated annually using a rating system from 0 (no symptoms) to 3 (severe symptoms). Symptom rating categories were: slight reddening or mild yellowing = 0.5; severe reddening or yellowing and premature leaf drop = 1; reduced vigour and smaller fruits than normal = 2; severe fruit size reduction and severe stunting = 3. Slow decline was rated as 10, and quick decline occurring within two years of inoculation as 20. Only the highest values per tree and year were considered. At the end of the observation period, the annual rating values of all inoculated trees of each progeny were accumulated and divided by the number of trees to obtain the cumulative disease index (CDI). Yield of surviving trees was estimated in the final 7 years of observation using a rating system from 0 (no yield) to 3 (high yield). The cumulative values were calculated as described for symptoms. Trunk girth of surviving trees was measured 40 cm above ground at the end of the observation period. For statistical analysis the differences between means were tested for significance using the simulation-based adjusted *P*-values for pairwise comparisons (SAS) at a significance level of α=0.05.

Results

Symptomatology

The inoculated trees responded very differently upon infection. The reactions ranged from rapid decline following inoculation to no symptoms during the entire observation period. Diseased trees showed all kinds of PD symptoms, including foliar reddening in late summer and fall, leaf roll, leaf curl, pale green leaves, poor growth, undersized fruits, slow decline, and quick decline. Quick decline occurred most often in the first two years following inoculation and then decreased steadily. Towards the end of the observation period, quick decline no longer appeared whereas there were still slowly-declining trees. A total of 283 out of 1,175 trees (24%) died. Some of the severely affected trees that survived the observation period regularly showed symptoms and were significantly reduced in size. Other trees developed symptoms only temporarily for one to three years and then recovered. Recovery was either permanent or the symptoms reappeared after a several-year period. Most of the permanently or temporarily recovered trees performed normally or nearly normally as far as terminal growth, fruit size, and yield are concerned. Only 2% of the trees never showed symptoms. Differences in symptomatology between cvs Bartlett and Alexander Lucas were not observed.

Most of the non-inoculated control trees became infected during the observation period so that a conclusive comparison with inoculated trees was not possible. The controls will thus not be considered further.

Resistance in *Pyrus* taxa progenies

The *Pyrus* progenies examined differed significantly in PD resistance. There was also a great variability within the progenies tested (Table 1). Not affected, little, moderately and severely affected trees were observed in all of the progenies. However, there were great quantitative differences in the occurrence of these resistance categories. In the progenies of about one third of the genotypes tested the majority of the individuals showed a substantial level of resistance to grafted trees as expressed by low CDI values (<10), >50% of not affected or slightly affected trees, and mostly low mortality rates. Most resistant were trees on the *P. communis* progeny from Moscow. Significantly different from the first group was another third of the progenies that rendered most grafted trees susceptible as evidenced by CDI values >13.5, a mostly low percentage of little or not affected trees and mostly high mortality rates. Most susceptible were trees on *P. kunariana*, *P. lecontii*, and *P. ussuriensis* (Dortmund) progenies.

Between these two groups there were progenies that were not statistically different from the resistant or the susceptible group. These progenies were defined as moderately resistant.

Considerable differences in susceptibility to PD were also observed between progenies of obviously different genotypes of the same taxon obtained from different institutions (Table 1). In all such cases the progenies were phenotypically similar and thus appeared as typical representatives of the taxon. Striking examples of such differences in resistance were *P. communis* and *P. betulifolia*, of which the different progenies were either among the most resistant, moderately resistant, or very susceptible. Similar differences were observed in *P. calleryana* cv. Bradford, *P. pyraster* and other taxa. Even in *P.*

ussuriensis, reported to be very susceptible, there were two progenies that were moderately resistant while one accession was the most susceptible.

Vigour and productivity

The vigour of the trees on the progenies examined also differed significantly (Table 1). As in PD resistance three groups could be distinguished consisting of trees with average trunk girths of 19 cm, 25 to 30 cm and 31 to 36 cm. There were also significant differences between different progenies of the same taxon such as in *P. betulifolia*, *P. ussuriensis*, *P. calleryana* cv. Bradford and *P. pyraster*. There was no strong correlation in vigour of trees on resistant and susceptible progenies, probably because the most susceptible trees declined and could not therefore be included in the trunk girth determination at the end of the observation period. However, severely affected trees were considerably reduced in size.

The progenies also differed significantly in the yield efficiency they showed in the scion cultivar (Table 1). As in resistance and vigour, three groups could be distinguished that ranged in values from 11.3 to 13.4, 13.6 to 15.0, and 15.1 to 17.2, respectively. High productivity was shown by trees on most *P. calleryana*-related progenies, whereas the yield of the *P. nivalis* progenies was below the average. The *P. communis* and *P. betulifolia* progenies were slightly above the average. The differences between sources of the same taxon obtained from different institutions were small except for *P. calleryana* cv. Bradford where the susceptible progeny showed much higher values than the resistant one. This might be due to the fact that fruit set of affected trees was often higher than of non-symptomatic trees. On average, however, the estimated yield of the more resistant group of progenies did not differ markedly from the group of the more susceptible ones.

Discussion

A great variability in PD resistance was observed in the progenies examined that derived from open-pollinated seeds of many *Pyrus* taxa. This variability was observed between taxa, between genotypes of the same taxon, and within the progenies. In all progenies there were individuals that were not affected, or little, moderately, or severely affected. However, the number of plants with different susceptibility varied considerably between the progenies so that groups with different resistance levels could be distinguished. This segregation of resistance corresponds to data obtained by Westwood (1976) in analysing progenies of experimental crosses of *Pyrus* genotypes of different susceptibility under natural infection conditions. In his work, crosses of resistant parents, for instance of *P. betulifolia* genotypes, resulted in a high proportion of resistant offspring and few plants that were susceptible. In contrast, offspring of susceptible parents such as *P. pyrifolia* genotypes were predominantly susceptible while resistant seedlings were rare.

Following the epiphytotic at the Pacific coast pear growing areas of North America, extensive screening of *Pyrus* taxa for PD resistance was performed under natural infection conditions. The material examined included clonal rootstocks from *P. communis* cultivars and quince as well as open-pollinated progenies of several *Pyrus* species including *P. communis*. According to the results obtained the progenies were grouped into several resistance classes. For instance, progenies of *P. betulifolia*, *P. calleryana*, *P. elaeagrifolia*, *P. nivalis*, *P. syriaca*, and of some *P. communis* cultivars were classified as very resistant or resistant. In contrast progenies of *P. amygdaliformis*, *P. caucasica*, *P. fauriei*, *P. pyrifolia*, *P. ussuriensis*, and 'Imported French Seedling' were rated as susceptible or very susceptible (Blodgett et al. 1962; Westwood and Lombard 1977, 1982, 1983). However, our results show that resistance cannot be assigned to a certain taxon because it is a segregating trait. In taxa from which several progenies were examined, great differences in resistance were observed. There were, for example, highly susceptible progenies of the 'resistant' species *P. betulifolia* and *P. calleryana* and a quite resistant progeny of the 'susceptible' species *P. amygdaliformis*. Results similar to those of the American researchers were obtained with progenies from *P. communis* genotypes that showed a wide range of resistance. Also the high susceptibility of progenies of *P. pyrifolia* and the *P. pyrifolia* hybrid *P.* x *lecontei* has been confirmed. The overall resistance ranking of the material examined, is for most progenies, similar to that of the preliminary evaluation published previously (Seemüller et al. 1998). However, some of them such as *P. nivalis* (Berlin), *P. bretschneideri*, *P. communis* (Dortmund), and *P.*

kunariana changed their position considerably after a longer observation period, due to either slow decline or recovery events.

Pyrus communis seedling and clonal rootstocks are predominant in North America and are also important in European pear growing. Within this species genotypes were selected and used as clonal stocks and/or seed parents that show a fairly high level of PD resistance to grafted trees. These genotypes include scion cvs Bartlett, Winter Nelis, Old Home, and selections of crosses of Old Home x Farmingdale (OH x F clones) (Westwood and Lombard 1982). Also, own-rooted Conference trees are reported to be quite tolerant to infection (Giunchedi et al. 1995). In our work, the most resistant progeny derived from a Muscovite *P. communis* seed parent. In this progeny the values for resistance (CDI), vigour (trunk girth), and yield efficiency (cumulative yield) ranged from 0 to 14, 23 to 35 cm, and 9 to 20, respectively. Other progenies showed a similar variability. This indicates a high potential for selecting PD-resistant rootstocks with suitable pomological properties. Promising genotypes were observed not only in the most resistant but also in nearly all progenies examined. From the variability within progenies it can be concluded that unselected seedlings should not be used as rootstocks. This is even true when the seed parent is resistant but the pollen parent is unknown. Rather, suitable genotypes should be carefully selected and then vegetatively propagated for use as rootstocks.

Little is known about the mechanisms involved in PD resistance. As previously reported, the percentage of trees in which phytoplasmas could be detected in the rootstock by DAPI fluorescence microscopy ranged from 14 to 100% eight to ten years after inoculation (Seemüller et al. 1998). This indicates that all or nearly all trees were successfully inoculated and that in a proportion of the trees infection was either eliminated or the phytoplasma titer was reduced to a level not detectable by fluorescence microscopy. Some of the trees were examined using both fluorescence microscopy and a more sensitive PCR assay. In this comparison 62% of the trees tested negative using the DAPI test but only 27% following PCR analysis (Seemüller et al. 1998). These data show that in some of the trees the infection was probably eliminated or was reduced to a very low level.

Quantitative-PCR studies on apple proliferation-diseased apple trees revealed that the titer of the causal agent *Ca.* P. mali is in standard rootstocks based on *Malus* x *domestica* 100 to 5,000 times higher than in experimental apomictic rootstocks based on *Malus sieboldii* and *M. sargentii* (Bisognin et al. 2008). Of these hosts, *M. sargentii*-derived stocks are very susceptible, the standard stocks moderately susceptible, and the *M. sieboldii*-based stocks resistant. Thus, there is no direct correlation between phytoplasma titer and resistance. Although no quantitative phytoplasma determination was carried out in our work on pear, the same seems to be true for the pear progenies examined as no correlation between resistance and pathogen titer could be identified (Seemüller et al. 1998). However, on apple it could be shown that only a minority of the trees on resistant *M. sieboldii*-derived stocks were colonised in the top, in contrast to trees on *M. domestica* stocks and some selections with *M. sargentii* parentage where most or all trees were infected in the scion. Also, the phytoplasma titer in the top-infected scion of trees on resistant rootstocks was much lower than in top-infected trees on *M. domestica* roots (Bisognin et al. 2008). The low infection rate and the low titer in the stem of trees on resistant apomictic stocks seems to result from low phytoplasma concentrations in the roots. The low starting concentration in and poor host suitability of apomictic rootstocks may have a negative effect on the spread of the pathogen from the roots into the scion during recolonisation of the stem in spring. Thus, the low titer in the roots is likely to contribute to the resistance of *M. sieboldii*-derived stocks.

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