

FIRE BLIGHT RESISTANCE IN VARIOUS LOQUAT, APPLE AND PEAR CULTIVARS AND SELECTIONS IN GREECE

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SUMMARY

Fire blight resistance of various pear, apple and, for the first time, loquat cultivars and selections were evaluated in Greece in 1997-1999. Shoots of accessions from Greece, Italy, Spain, France, and Great Britain were inoculated with a mixture of five Greek isolates of *Erwinia amylovora*, and the size of the lesions was recorded after six weeks. Based on their reactions, the cultivars were grouped into five classes according to the frequency of successful infections and their severity expressed as weighted mean of lesion length as percentage of total length of blighted shoots. All loquat cultivars were very susceptible, whereas apple and pear cultivars showed various levels of susceptibility. Variations were also observed in host reactions in different years.

Key words: *Erwinia amylovora*, *Pomoideae*, breeding, screening for resistance.

INTRODUCTION

Fire blight is a serious disease of *Pomoideae* caused by *Erwinia amylovora* (Burrill) Winslow *et al.*, reported from 40 countries (Bonn and van der Zwet, 2001). In Greece it was first identified in 1984 and, since then, it has spread all over the country (Psallidas, 1990).

Until now chemicals have not been able to completely control the disease (Psallidas and Tsiantos, 2000), and genetic disease resistance is recognized as an important feature of Integrated Pest Management (IPM). Developing varieties with good pomological traits and high levels of resistance to fire blight is a difficult task, as resistance seems always to be partial, involving a complex of polygenes (Lespinasse, 1990). Usually, resistance to fire blight has been evaluated by observing trees growing under nursery or natural conditions in orchards.

However, the disease can be affected by several factors, such as the rootstock (Keil and van der Zwet,

1975), tree age (Shaw, 1934), orchard topography, soil type (Fisher *et al.*, 1959), inoculum level (Beer, 1978), and climatic conditions (Aldwinckle and Beer, 1978). Artificial inoculation of trees of the same age in the orchard can avoid the effect of some of the above factors. Van der Zwet and Keil (1979) published results of ratings of pear and apple cultivars for fire blight susceptibility based on the literature. They also reported overall degrees of fire blight resistance for different *Pyrus* species.

In this study, we report the results of a three-years trial in which shoots were inoculated on field-grown pears (*Pyrus communis*), apples (*Malus domestica*) and loquats (*Eriobotrya japonica*). Some of the pear and apple cultivars tested are important Greek varieties, others were from France, Spain, Italy and Great Britain.

MATERIALS AND METHODS

Plant material. An experimental field was established near Volos (Central Greece) in 1992 and 1993. There were 35 pear cultivars and selections (on quince rootstock), 14 apples (on MM106 rootstock), three of which were cider cultivars, and four loquats (on quince rootstock). The cultivars tested, selections and their parents, as well as their origin are shown in Table 1. Ten trees of each cultivar were inoculated in 1997, 1998 and 1999.

Bacterial strains and inoculum. The bacterial strains used were isolated in Greece and maintained at the Plant Protection Institute of Volos. In preliminary unpublished experiments we tested if there was any virulence difference between strains, and the effect of different inoculum concentrations on three of the most important pear cultivars in Greece (Krystalli, Kondoula and Santa Maria). The inoculum consisted of a mixture of six Greek isolates. In the cultivar Krystalli different inoculum concentrations were tested (10^4 , 10^6 , 10^8 and 10^9 cfu ml⁻¹).

For each treatment, 20 to 30 actively growing shoots were inoculated as described below. Even though previous results had not shown any differential virulence (perhaps because of the limited number of cultivars and

Table 1. List of cultivars and selections under trial.

| Cultivar or selection | Parents of selections | Origin |
|-----------------------|--------------------------------|--------|
| LOQUATS | | |
| Amadeous (blue) | | Spain |
| Algerie (black) | | Spain |
| Golden Nagget (red) | | Spain |
| Gordones (white) | | Spain |
| APPLES | | |
| Baujade | | France |
| Belford | | France |
| Coloradona (cider) | | Spain |
| Firiki | | Greece |
| Florina | | France |
| Golden Delicious | | Greece |
| Jersey Mac | | France |
| Jonagold (cider) | | Spain |
| Mutsu | | France |
| Pilafa | | Greece |
| Raxaedo (cider) | | Spain |
| Regina (cider) | | Spain |
| Smoothe | | France |
| Starking | | France |
| PEARS | | |
| Blanquilla (Quince) | | Spain |
| Coscia | | Greece |
| Harrow Delight | | France |
| Harvest Queen | | France |
| Kondoula | | Greece |
| Krystali | | Greece |
| B.A. Lucas | | France |
| Magness | | France |
| Moonglow | | France |
| Peral Castell | | Spain |
| Peral Maggalon | | Spain |
| Pierre Corneille | | France |
| P83/13 | Williams x Moonglow | Greece |
| P155/3 | Comice x 19B29 | Greece |
| P384/40 | Conference x 13B83 | Greece |
| Santa Maria | | Greece |
| Starking | | France |
| Toska | | Italy |
| U.S. 309 | | France |
| Voutirata Korinthou | | Greece |
| Williams | | Greece |
| Yali (Asian pear) | | France |
| 80-36-71 | Coscia x Bella di Giugno | Italy |
| 80-51-72 | Coscia x Dr. Guyot | Italy |
| 80-57-83 | Conference x Dr. Guyot | Italy |
| 80-66-82 | Conference x Doyenne du Comice | Italy |
| 84-3348-40 | Doyenne du Comice x Morgen | Italy |
| 80-107-72 | Coscia x Dr. Guyot | Italy |
| 80-75-72 | Coscia x Dr. Guyot | Italy |
| 80-34-67 | Coscia x Bella di Giugno | Italy |
| 80-25-83 | Conference x Dr. Guyot | Italy |
| 80-09-82 | Conference x Doyenne du Comice | Italy |
| 1/6 | Santa Maria x Kondoula | Greece |
| 1/9 | Santa Maria x Kondoula | Greece |
| 1/7 | Santa Maria x Kondoula | Greece |

bacterial isolates used), it was decided to use as inoculum a mixture of six Greek strains, five of which (IPFV 708, 750, 850, 870 and 877) did not differ significantly in virulence, whereas the sixth isolate (IPFV 740) was less virulent. Using a mixture of strains has been recom-

mended by Norelli *et al.* (1986).

Inoculum was in distilled water from 24-h-old cultures grown on nutrient yeast agar at 26°C. Its concentration was adjusted turbometrically and verified by dilution plating at $4\text{-}6\cdot 10^8$ cfu ml⁻¹. The inoculum was prepared just before use and was kept in portable refrigerators.

Inoculation and pathogenicity determinations. The most useful index of fire blight susceptibility is the extent of lesion development on the infected shoot. Quamme *et al.* (1976) provided evidence that artificial inoculation is a valid procedure to evaluate fire blight susceptibility of pears. Thus, artificial inoculation was made on 20 to 30 vigorously growing shoots of ten trees of each cultivar.

Pears were inoculated first, followed in order by apples and loquats. Shoots were inoculated using a 26-gauge hypodermic needle, which was inserted just above the youngest unfolded leaf. Enough inoculum was introduced to fill the wound and leave visible drops at both ends of the wound. Lesion length was recorded as percentage of the total shoot length, after 6-7 weeks. When lesions extended into the previous season's growth, shoots were considered as 100% infected.

In spite of this heavy inoculation, in agreement with published procedures (Otto *et al.*, 1970; van der Zwet *et al.*, 1984; Thibault *et al.*, 1987; Lecomte, 1994) there was extensive variability in the results that made ranking of individual accessions difficult. Thus, reactions of the cultivars were classified according to Thibault *et al.* (1987, 1989), taking into account both the frequency (F), expressed as percentage of successful infections, and the severity (S) of infections, expressed as the weighted mean of the percentage of lesion length for a blighted shoot. The index of varietal susceptibility (I.V.S. = F x S) was also calculated. The cultivars were then grouped into five classes of susceptibility according to the above parameters. The I.V.S. and the ranking of each cultivar in different years are summarized in Tables 2 and 3.

RESULTS AND DISCUSSION

All accessions tested showed differing degrees of susceptibility to *E. amylovora*, perhaps because the infection took place by introducing a massive dose of bacterial cells directly into the shoots.

In loquats, the most and least severe infections were recorded in 1998 and 1999, respectively. As shown in Table 2, the four Spanish cultivars were highly susceptible, their average I.V.S. ranging from 44.6 for cv Golden Nagget to 57.1 for cv Algerie, so that they were all included in class D. By contrast, unidentified Greek varieties showed a high level of resistance. Since 1984, when

Table 2. Determination of shoot susceptibility to *Erwinia amylovora* of apple, pear and loquat varieties in 1997, 1998 and 1999 (class A lowest susceptibility, class E highest susceptibility).

| Cultivar or selection | 1997 | | 1998 | | 1999 | |
|-----------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|
| | Index of susceptibility (0-100) | Class of susceptibility (A-E) | Index of susceptibility (0-100) | Class of susceptibility (A-E) | Index of susceptibility (0-100) | Class of susceptibility (A-E) |
| LOQUATS | | | | | | |
| Amadeous | 43.8 | D | 71.6 | E | 41.5 | E |
| Algerie | 58.3 | D | 76.9 | E | 44.9 | D |
| Golden Nagget | 67.9 | E | 65.5 | E | 31.7 | C |
| Gordones | 47.5 | D | 58.8 | D | 39.6 | D |
| APPLES | | | | | | |
| Baujade | 6.0 | A | 13.5 | C | 12.5 | C |
| Belford | 60.7 | E | 27.4 | C | 66.0 | E |
| Coloradona | 44.4 | D | 60.1 | E | 84.3 | E |
| Firiki | 44.2 | D | 32.7 | C | 66.8 | E |
| Florina | 2.2 | A | 8.8 | A | 9.7 | B |
| Golden Delicious | 21.7 | C | 27.2 | C | 38.8 | C |
| Jersey Mac | 20.0 | C | 23.9 | C | 33.9 | C |
| Jonagold (cider) | 11.7 | B | 15.4 | C | 32.4 | C |
| Mutsu | 8.7 | A | 29.5 | C | 37.5 | C |
| Pilafa | 14.6 | B | 10.1 | B | 9.2 | A |
| Raxaedo (cider) | 40.9 | D | 51.0 | D | 84.7 | E |
| Regina (cider) | 52.3 | D | 56.5 | D | 73.5 | E |
| Smoothie | 11.3 | B | 19.2 | C | 36.3 | C |
| Starking | 9.7 | A | 7.6 | B | 15.9 | B |
| PEARS | | | | | | |
| Blanquilla | 15.5 | C | 25.2 | C | 41.4 | D |
| Coscia | 13.9 | B | 11.5 | C | 22.3 | C |
| Harrow Delight | 1.0 | A | 5.8 | A | 17.7 | B |
| Harvest Queen | 5.7 | A | 8.0 | A | 45.3 | D |
| Kondoula | 48.6 | D | 78.8 | E | 83.5 | E |
| Krystali | 23.8 | C | 32.3 | C | 50.6 | D |
| B.A. Lucas | 17.1 | B | 16.4 | C | 41.3 | E |
| Magness | 3.6 | A | 5.0 | A | 11.8 | B |
| Moonglow | 8.3 | A | 4.3 | A | 10.8 | B |
| Peral Castell | 11.3 | B | 7.6 | B | 20.1 | D |
| Peral Maggalon | 4.8 | A | 6.6 | A | 2.7 | A |
| Pierre Corneille | 32.3 | C | 9.2 | B | 35.0 | C |
| P83/13 | 62.4 | E | 17.2 | C | 65.8 | E |
| P155/3 | 49.8 | D | 22.7 | C | 40.0 | D |
| P384/40 | 18.9 | C | 15.3 | C | 16.4 | C |
| Santa Maria | 32.5 | C | 55.2 | D | 69.2 | E |
| Starking | 13.0 | B | 9.0 | A | 16.0 | B |
| Toska | - | | 6.0 | A | 19.9 | C |
| U.S. 309 | 9.6 | A | 14.3 | C | 29.3 | C |
| Voutirata Korinthou | 12.8 | C | 13.0 | C | 31.5 | C |
| Williams | 36.4 | C | 60.4 | E | 90.8 | E |
| Yali (Asian pear) | 23.9 | C | 34.1 | C | 36.8 | C |
| 80-36-71 | 32.4 | C | 32.4 | C | 36.6 | C |
| 80-51-72 | 7.3 | A | 7.7 | B | 4.8 | A |
| 80-57-83 | 26.3 | C | 29.8 | C | 52.2 | D |
| 80-66-82 | 24.5 | C | 33.2 | C | 57.7 | D |
| 84-3348-40 | 12.3 | C | 2.5 | A | 30.7 | C |
| 80-107-72 | 22.1 | C | 12.7 | B | 18.2 | C |
| 80-75-72 | 28.8 | C | 17.6 | B | 33.6 | C |
| 80-34-67 | 20.6 | C | 32.2 | C | 43.3 | D |
| 80-25-83 | 21.9 | C | 19.5 | C | 45.8 | D |
| 80-09-82 | 10.6 | B | 6.2 | A | 29.3 | C |
| 1/6 | 24.0 | C | 47.4 | D | 54.0 | D |
| 1/9 | 30.2 | C | 27.5 | C | 47.4 | D |
| 1/7 | 24.5 | C | 33.2 | C | 57.7 | D |

Table 3. Overall results of the experiments for determining shoot susceptibility to *E. amylovora* in the three years of trial (1997 to 1999).

| Cultivar or selection | Frequency (0-100) | Severity (0-100) | Index of susceptibility (0-100) | Class of susceptibility (A-E) |
|-----------------------|-------------------|------------------|---------------------------------|-------------------------------|
| LOQUATS | | | | |
| Amadeous | 90 | 57 | 51.2 | D |
| Algerie | 98 | 58 | 57.1 | D |
| Golden Nagget | 98 | 52 | 51.3 | D |
| Gordones | 92 | 50 | 46.0 | D |
| APPLES | | | | |
| Baujade | 41 | 25 | 10.4 | B |
| Belford | 85 | 66 | 56.1 | D |
| Coloradona | 89 | 78 | 69.2 | E |
| Firiki | 88 | 58 | 51.0 | D |
| Florina | 43 | 17 | 7.4 | A |
| Golden Delicious | 81 | 38 | 30.7 | C |
| Jersey Mac | 60 | 46 | 27.3 | C |
| Jonagold | 70 | 31 | 21.4 | C |
| Mutsu | 72 | 39 | 28.0 | C |
| Pilafa | 55 | 20 | 10.8 | B |
| Raxeado | 83 | 77 | 63.8 | E |
| Regina | 87 | 72 | 62.7 | E |
| Smoothe | 63 | 40 | 25.2 | C |
| Starking | 55 | 22 | 12.0 | B |
| PEARS | | | | |
| Blanquilla | 94 | 32 | 30.0 | C |
| Coscia | 53 | 32 | 17.0 | B |
| Harrow Delight | 48 | 21 | 9.9 | A |
| Harvest Queen | 65 | 37 | 23.7 | C |
| Kondoula | 95 | 76 | 72.7 | E |
| Krystali | 93 | 41 | 38.1 | C |
| B.A. Lucas | 76 | 35 | 26.6 | C |
| Magness | 41 | 18 | 7.6 | A |
| Moonglow | 52 | 16 | 8.4 | A |
| Peral Castell | 74 | 26 | 19.4 | C |
| Peral Magallon | 48 | 9 | 4.4 | A |
| Pierre Corbeille | 73 | 38 | 28.0 | C |
| P83/13 | 79 | 69 | 54.6 | D |
| P155/3 | 76 | 55 | 41.7 | D |
| P384/40 | 83 | 20 | 16.9 | C |
| Santa Maria | 99 | 55 | 54.7 | D |
| Starking | 60 | 23 | 13.5 | B |
| Toska | 95 | 17 | 16.2 | B |
| U.S. 309 | 73 | 24 | 17.8 | C |
| Voutirata Korinthou | 89 | 22 | 19.5 | C |
| Williams | 88 | 66 | 58.2 | D |
| Yali | 73 | 44 | 31.9 | C |
| 803671 | 78 | 43 | 33.8 | C |
| 805172 | 48 | 13 | 6.3 | A |
| 805783 | 88 | 35 | 30.9 | C |
| 806682 | 88 | 29 | 25.9 | C |
| 84334840 | 59 | 30 | 17.5 | B |
| 8010772 | 63 | 28 | 17.9 | B |
| 807572 | 83 | 32 | 26.8 | C |
| 803467 | 82 | 40 | 32.7 | C |
| 802583 | 83 | 38 | 31.9 | C |
| 800982 | 74 | 30 | 22.0 | C |
| 1/6 | 91 | 47 | 43.1 | D |
| 1/9 | 97 | 38 | 36.8 | C |
| 1/7 | 100 | 41 | 40.5 | D |

fire blight first appeared in Greece, natural shoot infections have been seen only once in two trees growing in a very heavily infected pear orchard, and their shoots were in close contact with those of pears. Flower infections were never observed, maybe because loquats bloom in winter, a time when the disease progresses very little or not at all. To our knowledge, this is the first report on resistance of loquat to fire blight.

Among pears, the local cv Kondoula proved to be the most susceptible of all (class E), its I.V.S. ranging from 48.6 to 83.5 (mean I.V.S. 72.67). Greek selections 1/6, 1/7, P155/3, P83/13 and cvs Santa Maria and Williams were also highly susceptible (class D), whereas cvs Magness, Moonglow, and Harrow Delight, the Italian selection 805172 and the Spanish cv Peral Magallon were the least susceptible and were assigned to class A. The Italian selections 84334840, 8010772, and the cvs Tosca and Coscia showed low susceptibility (class B). All the other selections were moderately susceptible (class C).

Spanish cider apples Raxeado, Colarodona and Regina were the most susceptible and subject to bloom infection. All were assigned to class E. The Greek apple cv Firiki, which produces a small but very sweet fruit was highly susceptible (class D) and also showed natural bloom infections. The cv Florina was the least susceptible (class A). Its I.V.S. was very low throughout the three-year experiment as it varied from 2.2 to 9.7 (average 7.4). The cvs Baujade, Pilafa and Starking showed low susceptibility (class B). All other accessions were in class C (moderately susceptible).

Even though we tried to operate under the same conditions of inoculation in the three years of the trial, susceptibility of the host tested varied in according to the year (Table 3). For example, the pear cv Harvest Queen had an I.V.S. of 5.7 in 1997 (class A), whereas in 1999 its I.V.S. was 45.3 (class D). For loquats most severe infections were observed in 1997 while for apples and pears infection was most severe in 1999. Variations in the phenological stage of the trees and climatic conditions occurred in the years of the trial, thus affecting the progress of artificial infections. This is in line with reports by Thibault *et al.* (1987) and Lecomte (1993) and stresses the need to test trees for as many years as possible.

It will be noted that two cultivars or selections with the same I.V.S. can be allocated to different classes because they differ in disease severity and frequency. Thus the pear selection ISF-FO 8010772 with I.V.S. 17.9 was placed in class B, while the cultivar US 309 with a smaller I.V.S. (17.8) was placed in class C (Table 2). The same happened with the selection ISF-FO 84334840 and the cv Coscia.

Most susceptible, at least under the conditions of the experiment, were the loquats. The pears cultivars, as in other European countries, proved to be more susceptible than apples (Zeller, 1990).

Some of our results are in agreement with those of others. For cvs Golden Delicious and Coscia there is agreement with the results of Lezec *et al.* (1997), for cv Starking with those of Maroofi and Mostafavi (1996), and for cv Florina with those of Aldwinckle *et al.* (1999).

However, some of our results diverged from those in the literature, as in the case of cvs Mutsu and Smoothe, which we found to be moderately susceptible while they were classified as lightly susceptible by Lezec and Paulin (1984). These contradictory results can be explained by the different climatic conditions during the experiments. Indeed, in 1997 both the above cultivars showed susceptibility also in our experiments (Table 3).

Accessions should also be tested for blossom susceptibility because in certain cultivars there is no correlation between blossom and shoot susceptibility (Fischer and Richter, 1993). In our trial only a few cultivars showed natural blossom susceptibility as for instance, the three apple cider cultivars. Apple cv Firiki and the pear cv Coscia are also known to be very susceptible to blossom infection, with low susceptibility to shoot infection.

Our field trial took place in a southern European climate and the results seem to have overestimated susceptibility, even though for most cultivars, evaluation of susceptibility based on the outcome of artificial inoculations agreed with that observed under natural orchard conditions. They should therefore be considered with caution because all tested plants were young and were bearing vigorous, actively growing and succulent shoots that received a very high inoculum. For example, the pear cv Williams proved to be very susceptible in our artificial inoculations, but under natural conditions it does not seem to be susceptible. Likewise, Duron *et al.* (1987), reported that cv Evereste which is resistant in the field, behaved as susceptible.

In conclusion, our data cannot be compared directly with susceptibility under natural infection conditions since natural infections depend on many uncontrolled factors. Our data can be regarded as the potential of a cultivar to be infected by *E. amylovora* under natural conditions favorable for the disease.

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