Susceptibility of Selected European Pear Cultivars to Infection by *Stemphylium vesicarium* and Influence of Leaf and Fruit Age

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ABSTRACT

Montesinos, E., Moragrega, C., Llorente, I., and Vilardell, P. 1995. Susceptibility of selected European pear cultivars to infection by *Stemphylium vesicarium* and influence of leaf and fruit age. Plant Dis. 79:471-473.

Nonwounded fruit of 16 European pear cultivars (*Pyrus communis*) and potted Conference pear plants were inoculated with conidia of *Stemphylium vesicarium*. Cultivars Passe Crassane, Alexandrine, Conference, Doyenne du Comice, Duc de Bordeaux, Abate Fetel, and General Leclerc were highly susceptible. Cultivars Williams, Blanquilla, Beurre Hardy, Louis Bonne, Grand Champion, and Highland were slightly or not susceptible. The susceptibility of fruit decreased logarithmically from fruit set to harvest in very susceptible cultivars. Younger leaves developed 1.8–3 times more disease than older leaves.

Brown spot of pear (*Pyrus communis* L.), caused by *Stemphylium vesicarium* (Wallr.) E. Simmons, is an important disease in fruit-growing areas of Europe (2,8,10). Infection and necrosis occur on leaves, fruit, and, to a lesser extent, on twigs. Maximum levels of disease incidence generally occur just before harvest, and infected fruit are unmarketable.

Control of the disease is achieved by preventive sprays with dithiocarbamate fungicides applied at 7-day intervals and with dicarboximides applied at 15-day intervals (3,4,6,10). Commercial control of brown spot requires 15–25 fungicide applications from fruit set to preharvest.

There is little information available on the susceptibility of pear cultivars to *S. vesicarium* or on the variability of susceptibility through the vegetative period. The susceptibility of a few European pear cultivars to fungal isolates from France and Italy has been tested (2,7); however, quantitative information is not available on the susceptibility of other European cultivars or on changes in susceptibility of fruit and leaves with age.

The current study was undertaken to determine the susceptibility of 16 selected European pear cultivars to isolates of *S. vesicarium* from Spain and to evaluate the influence of fruit and leaf age on disease severity.

MATERIALS AND METHODS

Plant and fruit material. Self-rooted pear plants of cultivar Conference (CAV clone) obtained by micropropagation (Agromillora Catalana, S.A., Barcelona, Spain) were used for leaf infection experiments. Plants were 2–3 yr old, about 1 m high, and were grown in 20-cm-diameter plastic pots. Plants were maintained in a controlled environment chamber at 25 C under a 16 h light/8 h dark photoperiod at 150 $\mu E\ m^{-2}\ s^{-1}$ and were watered with a fertilizer solution (20-10-20 N-P-K).

Pear fruit were obtained from the cultivar collection of Mas Badia Agricultural Experiment Station (Girona, Spain). A set of 16 pear cultivars was evaluated (Fig. 1). These cultivars were among the most widely planted or of greatest commercial interest in Europe (1).

Inoculum production. A highly virulent isolate of *S. vesicarium*, EPS26, was used for inoculations. The isolate was obtained from a necrotic lesion on a Passe Crassane pear fruit collected from a commercial orchard near Girona. The fungus was grown in petri dishes on V8 agar prepared by adding 100 ml of V8 juice, 20 g of agar, and $CaCO_3$, to adjust the pH to 7, to 1 L of distilled water. Dishes were incubated at 20 C under a photoperiod of 12 h of fluorescent light at 150 $\mu E\ m^{-2}\ s^{-1}$.

Conidial suspensions were prepared from 8- to 10-day-old cultures by gently rubbing the agar surface with a sterile cotton swab wet with a diluted solution of Tween 20 in distilled water (one drop of Tween 20 per liter). The conidial suspension was shaken vigorously with a vortex mixer to dislodge conidia from mycelium, filtered through two layers of cheesecloth, and the resulting conidial suspension was kept at 4 C to prevent germination during manipulations. The conidial concentration was determined with a hemacytometer and adjusted by dilution to about 10^6 conidia per milliliter. Conidial germination was determined after 2 h of incubation at 20 C in the hemacytometer chamber.

Suspensions with less than 90% germination of conidia were discarded. Stock conidial suspensions sufficient to perform many experiments were prepared in advance and maintained in 20% glycerol at –80 C. Under these conditions, >90% conidial germination was retained for several months. Reconstitution of the conidial suspensions was carried out by defrosting the suspensions overnight at 4 C and centrifuging for 15 min at 5,000 *g*. The pellet that contained conidia was resuspended in a solution of Tween 20 and distilled water, and the suspension was kept overnight at 4 C before use.

Inoculations. Conidial suspensions of 5×10^5 conidia per milliliter were used for inoculations. Plants were inoculated by spraying the conidial suspension to runoff with a compressed-air atomizer operated at 2 kPa. Two spray passes were made across each plant—one to wet the adaxial leaf surface and another to wet the abaxial surface. Pear fruit were surface-disinfested by immersion for 30 s in a diluted solution of commercial NaOCl (5.25%) and rinsed three times in deionized water. Inoculations were performed by immersion of fruit for 1 min in the conidial suspension. Noninoculated checks were processed using a sterile Tween 20 and distilled water solution.

Experimental design and disease assessment. For cultivar susceptibility experiments, inoculated fruit were placed on a gridded container in a tray filled with sterile distilled water. The fruit peduncle was placed in contact with the water surface to avoid dehydration of fruit during incubation. Trays were placed within sealed transparent plastic bags and incubated for 15 days at 20 C under continuous darkness. Noninoculated fruit of each cultivar type were used as controls. The experiment was designed as a split-plot of 16 cultivars with six replicates of five fruit per cultivar and was conducted twice. The first trial was performed 2–3 mo before harvest, and the second trial was performed 7–10 days before harvest.

To study the effect of fruit age on susceptibility, fruit were inoculated at different stages of development from fruit set to harvest. Six replicates of five fruit were used for each fruit age. The experiment was performed with Passe Crassane during 1993 and repeated with Conference pear during 1994. Disease severity was recorded 10

Accepted for publication 30 January 1995.

days after inoculation by determining the number of lesions per fruit.

Inoculated pear plants were placed in moist chambers under darkness for 24 h. For the first trial, plants were incubated at 15, 20, and 25 C, and for the second trial, they were incubated at 10, 15, 20, 25, and 30 C. The moist chambers consisted of sealed tubular plastic bags containing each plant. The inner sides of the plastic bags were sprayed with water before use to provide 100% relative humidity during incubation. After the wetness period, moistened plants were dried gently at room temperature (22 C) by placing them in front of a fan for approximately 30 min.

For symptom development, plants were transferred to a controlled environment chamber at 20 ± 2 C, 70–80% relative humidity and 16 h light/8 h dark photoperiod at 150 μE m^{-2} s^{-1}. Disease severity was recorded for 10–15 young and 10–15 old leaves per plant 9 days after inoculation. Leaves were considered young when produced on the current-season wood; they were considered old when they were produced on wood that was more than 1 yr old. Three replicates of three plants each were used for each temperature. The experiment was conducted twice.

Statistical analysis. Preliminary analysis indicated that severity data were not normally distributed; therefore, a $\log_{10}$ transformation of severity data was necessary to fit the analysis of variance (ANOVA) assumptions. ANOVA was performed with the general linear models procedure and regression analysis with the REG procedure of SAS (9).

RESULTS

Cultivar susceptibility. The 16 cultivars varied in susceptibility to *S. vesicarium* in the first ($F = 347.9$, $P = 0.0001$) and second trials ($F = 42.2$, $P = 0.0001$). Susceptibility was divided arbitrarily into two categories: slightly or not susceptible (less than one lesion per fruit) and highly susceptible (more than one lesion per fruit). Cultivars Passe Crassane, Alexandrine, Conference, Doyenne du Comice, Duc de Bordeaux, Abate Fetel, and General Leclerc were highly susceptible in the two trials, and Williams, Blanquilla, Beurre Hardy, Louis Bonne, Grand Champion, and Highland were slightly or not susceptible (Fig. 1). Cultivars Winter Nellis, Rocha, and Kaiser were highly susceptible in the first trial but slightly susceptible in the second trial. The slightly or not susceptible cultivars did not differ significantly in susceptibility between both trials ($F = 1.3$, $P = 0.249$). However, for all highly susceptible cultivars, except General Leclerc, disease severity was significantly higher in the first trial than in the second trial ($F = 716.7$, $P = 0.0001$).

Fruit and leaf age effect. Susceptibility of fruit decreased with time after fruit set for cultivars Passe Crassane and Conference (Fig. 2). Comparisons of the data for both cultivars with a repeated measures analysis of variance using a first degree polynomial contrast for time showed no significant differences ($F = 0.07$, $P = 0.79$) and allowed pooling of the data. The following equation for combined data was derived: $\text{Log}_{10}(S + 1) = 2.126 - 0.0135t$ ($R^2 = 0.88$, $P = 0.002$), where S is the severity in lesions per fruit and t is the time in days after fruit set. Consequently, the susceptibility of fruit decreased logarithmically with physiological age.

Disease severity (lesions per leaf) decreased as leaf age increased for inoculations on potted plants of cultivar Conference (Fig. 3). Younger leaves developed 1.8–3 times more disease than older leaves at all temperatures studied ($P < 0.01$); the results were consistent for both trials. However, because the pear plants used in the second trial were younger than in the first trial, overall disease severities were higher in the second trial.

DISCUSSION

The 16 European pear cultivars tested vary widely in susceptibility of fruit to a highly virulent isolate of *S. vesicarium* from Spain. The cultivars Passe Crassane, Alexandrine, Conference, Doyenne du Comice, and Abate Fetel are the most susceptible and the most frequently planted, commercially accepted cultivars in the European Community (up to 52%) (1).

Blancard et al (2) studied the susceptibility of leaves in detached shoots of 12 pear cultivars to an isolate of *S. vesicarium* from France and observed that Alexandrine, Packam's Triumph, Conference, Beurre Hardy, and Abate Fetel were the most susceptible. These results partially agree with our results with pear fruit, but they found cultivars Passe Crassane and Doyenne du Comice less susceptible. Ponti and Cavanni (7) studied the susceptibility of fruit from six pear cultivars to conidia of an isolate of *S. vesicarium* from Italy and observed that Abate Fetel was highly susceptible; Doyenne du Comice, Passe Crassane, Conference, and Kaiser were moderately susceptible.

Except for minor differences, results with isolates from Italy, France, and Spain agree with observations in orchards in the main epidemic areas of Emilia-Romagna, Italy; Bouches-du-Rhone, France; and Catalunya, Spain (2,8,10), where the cultivars most affected by the disease are Abate

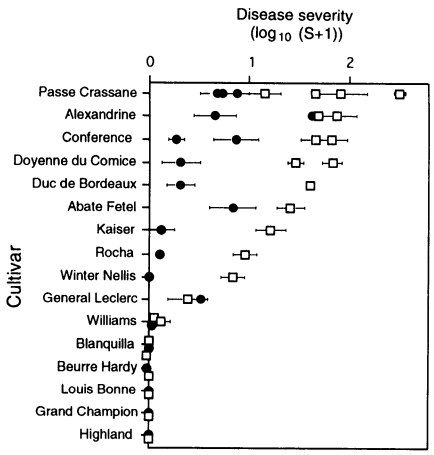

Fig. 1. Susceptibility of fruit of selected pear cultivars inoculated with *Stemphylium vesicarium* 2–3 mo before harvest (□ = trial 1) and at harvest (● = trial 2). Bars indicate confidence intervals for the mean ($P = 0.05$) of six replicates of five fruit per replicate. S is disease severity (lesions per fruit).

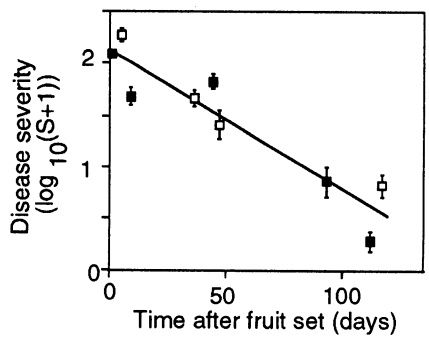

Fig. 2. Changes in susceptibility of pear cultivars Passe Crassane (□) and Conference (■) fruit to *Stemphylium vesicarium* at different stages of fruit development. Bars indicate confidence intervals for the mean ($P = 0.05$) of six replicates of five fruit per replicate. S is disease severity (lesions per fruit).

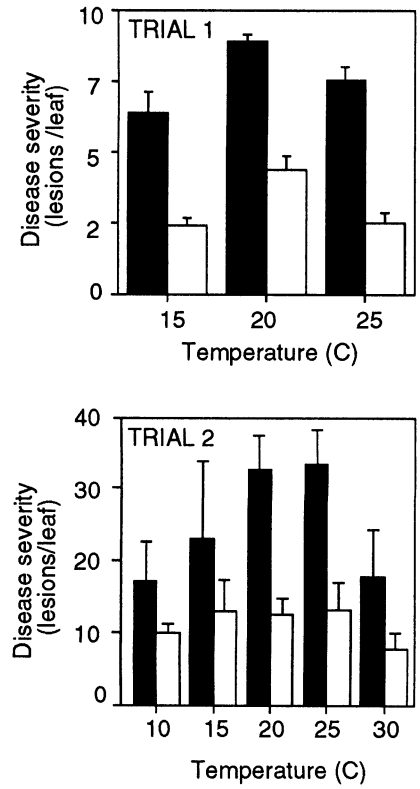

Fig. 3. Disease severity on young (solid bars) and old (open bars) leaves on pear plants of cultivar Conference inoculated with *Stemphylium vesicarium* at several temperatures. Bars indicate confidence intervals for the mean ($P = 0.05$) of three replicates of three plants per replicate.

Fetel, Alexandrine, and Passe Crassane, respectively.

Although a high dose of inoculum was used in our experiments (5×10^5 conidia per milliliter), on cultivars Williams, Blanquilla, Beurre Hardy, Louis Bonne, Grand Champion, and Highland there were no lesions or less than one lesion per fruit (mean value), and they can be considered resistant. The existence of such a significant number of resistant cultivars, some with valuable commercial interest, opens possibilities for a disease management program based on cultivar resistance.

The susceptibility of pear to *S. vesicarium* decreased with the physiological age of plant tissues. Fruit susceptibility decreased logarithmically with time as the fruit matured; fruit were 40 times more susceptible at fruit set compared to harvest for Passe Crassane and Conference pear. Young leaves were up to three times more susceptible than older ones. A decrease in susceptibility with increase in physiological age of leaves also has been reported for other fungal diseases of pear, such as scab, caused by *Venturia pyrina,* and Japanese pear black spot, caused by *Alternaria alternata* (5). Consequently, according to the phenology of pear trees, maximal leaf susceptibility encompasses the two vegetative periods, late spring-early summer and early autumn, when shoot growth occurs and young leaves develop. Also, the susceptibility of fruit is greater during the late spring-early summer period and least at the time of fruit maturation. This result is in contrast to field observations that disease progress is faster on fruit just before harvest (6). However, this may be a reflection of the combination of a high inoculum density and suitable weather conditions for infection during late summer-early autumn.

In conclusion, the wide range in susceptibility among cultivars and the significant effect of physiological age on fruit susceptibility are important factors in the epidemiology of brown spot of pear and should be taken into account in disease management programs.

ACKNOWLEDGMENTS

This research was supported by grants from the D.G.U-C.I.R.I.T of the Generalitat de Catalunya, Comisión Interministerial de Ciencia y Tecnologia, and Fundación La Caixa. We thank the Associació de Defensa Vegetal de Productors de Pera de Girona for field work facilities and A. Bonaterra, J. Cervantes, and J. Pereda for helpful assistance.

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