Evaluation of a Cider Apple Germplasm Collection of Local Cultivars from Spain for Resistance to Fire Blight (*Erwinia amylovora*) Using a Combination of Inoculation Assays on Leaves and Shoots

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Abstract. Fire blight, caused by the bacterium *Erwinia amylovora*, is among the three most important diseases of apple. A major effective method for its integrated management is the reduction of the susceptibility of the host. Cider apple production in Spain is based on local apple cultivars with minimum crop management and phytosanitary control. After the entry of fire blight in Spain, the selection and planting of cultivars with low susceptibility to this disease has thus become of paramount importance. In consequence, and as part of a wider characterization effort, we undertook the evaluation of an apple germplasm collection of local apple cultivars from Spain for susceptibility to fire blight. Because of the quarantine status of *E. amylovora* in Europe, we evaluated the use of a detached leaf inoculation assay in combination with a traditional shoot inoculation assay to reduce the amount of plant material to evaluate and to minimize pathogen manipulation. Comparison of the susceptibility values for 78 apple cultivars indicated a low but significant correlation ($r = 0.56$; $\alpha = 0.01$) between the leaf and shoot inoculation methods. Although the detached leaf assay was not reliable for the direct selection of cultivars with low susceptibility, it was useful to optimize resources and limit the potential dispersal of the pathogen by allowing the exclusion of medium and highly susceptible cultivars from further evaluation. Shoot inoculation of 103 apple cultivars allowed the identification of 48 cultivars with high levels of resistance to fire blight, which could serve as starting material both for apple production and for breeding programs.

Fire blight, caused by the bacterium *Erwinia amylovora* (Burr.) Winsl. et al., is one of the most important diseases of apple and many other economically important genera in Rosaceae, not only for the economical losses that it generates, but also because it is very difficult to manage (Johnson and Stockwell, 1998; van der Zwet and Beer, 1995). Disease control relies on preventive methods and in the implementation of an integrated management strategy that includes reduction of inoculum, limitation of the establishment of the pathogen on the host, and, as a very effective management tactic, the reduction of the susceptibility of the host (Norelli et al., 2003). Indeed, the danger of fire blight in the eastern United States and many other parts of the world has dramatically increased in the last years as a result of the increase in high-density production systems that use highly susceptible, dwarfing rootstocks such as M.9 and M.26 in combination with equally susceptible scion cultivars (Norelli et al., 2003). A priority of modern fire blight management strategies is incorporating the use of host resistance, one of the most effective control methods, which will become more important in the future as the options for chemical control of apple pests become more limited and because of the trend to reduce pesticide use.

Fire blight was first detected in Spain in 1995 in an apple cider orchard and later in different locations in center and northern Spain (Donat et al., 2007). Effective eradication and containment protocols were immediately implemented after the first detection report, and Spain is still considered a protected fire blight country in the European Union. Taking into account the rapid progression of the disease in other Mediterranean countries (van der Zwet and Beer, 1995), it is necessary to implement appropriate management strategies to minimize the potential losses if the pathogen becomes widespread in Spain. In particular, natural (nonsparkling) cider apple production in northern Spain is potentially at a great risk because of the widespread use of self-rooted local cultivars whose susceptibility to fire blight, and to other important diseases of apple such as apple scab [*Venturia inaequalis* (Cooke) G. Wint.], is largely unknown. In different places in northern Spain, apples for natural cider production mostly come from extensive apple orchards established over natural prairies that are also exploited (Lizar, 1996). Apple cultivation follows traditional methods, with minimum crop management, and is based on the use of local cultivars, which often are mixed in the orchard. The agricultural potential and response to pathogens of many of these local cultivars have not been explored, and selection of cultivars for planting does not consider a long-term integrated pest management strategy. Therefore, it is imperative to characterize the response to different diseases of these local cultivars to maintain the cider production industry with minimal losses and minimal use of phytosanitary products.

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A collection of more than 250 cider and table apple cultivars originating from north-east Spain has been established and maintained by the Instituto Técnico de Gestión Agrícola (ITGA; Navarre) and is being characterized (Itoiz and Royo, 2003; Lizar, 1996; Lizar et al., 2008). This germplasm collection could be exploited in the future as a source for resistance against fire blight and other diseases, either in rootstock or scion breeding programs, or as choice cultivars for cider production. We have thus undertaken the evaluation of this collection for resistance to diseases starting with the evaluation of their susceptibility to fire blight. Because of the quarantine status of *E. amylovora* in Spain, manipulation of the bacterium and inoculation of plant material had to be done under a very strict security protocol to avoid dispersion of the pathogen. We therefore tested the use of a leaf inoculation protocol to reduce the amount of plant material to evaluate, and the results of this assay were compared with the susceptibility ratings estimated using an inoculation assay with young shoots.

Materials and Methods

Bacterial strains

E. amylovora strains (Table 1) were generally propagated using King's medium B (King et al., 1954) at 28 °C and were stored in this medium with 20% glycerol at -80 °C for long-term conservation.

Plant inoculation

Inoculation of detached leaves. Young leaves, 2 to 4 cm in lamina length, were collected during April and May from ≈15-year-old trees located in an ITGA experimental orchard at Sartaguda (Navarre, Spain). Leaves were maintained refrigerated under high humidity until inoculation, which was carried out within 4 h from collection. Twenty-one leaves (seven leaves from each of three trees) were inoculated per cultivar; each set of seven leaves was considered a repetition because variation was larger between trees than among leaves from the same tree (data not shown). Inoculation was done essentially as described by Donovan (1991) with some modifications as follows. Leaves were surface-disinfected by immersion for 30 seconds in 1% household bleach and rinsed thoroughly three times, 1 min each, with sterile distilled water. Then, leaves were deposited over moist sterile filter paper in plastic boxes with a lid and 10 µL of a suspension of strain UPN500 (10⁸ cfu·mL⁻¹) was deposited on a fresh incision made in the midrib with a scalpel. Bacterial suspensions used for inoculation were obtained from cultures grown overnight at 28 °C on King's medium B, which always were started from an original set of vials preserved at -80 °C in 20% glycerol. Cells were scraped from the agar surface and suspended in sterile distilled water adjusting the suspension to 10⁸ cfu·mL⁻¹ by dilution using a previously prepared viable-absorbance relationship. After

Table 1. *Erwinia amylovora* strains used in this work.

Strain ^a	Host, place, and year of isolation	Reference
CFBP1430	<i>Crataegus oxyacantha</i> , France, 1972	(Laurent et al., 1989)
Ea273	<i>Malus ×domestica</i> , USA, 1971	(Bocsanczy et al., 2008)
EPS101	<i>Pyrus communis</i> , Lérida, Spain, 1999	(Cabrefiga and Montesinos, 2005)
IVIA1614-2	<i>Pyracantha</i> sp., Segovia, Spain, 1996	(Cabrefiga and Montesinos, 2005)
NCPPB311	<i>P. communis</i> , Canada, 1952	(Rico et al., 2004)
NCPPB3159	<i>M. sylvestris</i> , The Netherlands, 1980	(Donat et al., 2007)
NCPPB3548	<i>Eriobotrya japonica</i> , Turkey, 1987	(Donat et al., 2007)
NCPPB595	<i>P. communis</i> , UK, 1958	(Donat et al., 2007)
NCPPB683	<i>P. communis</i> , UK, 1959	(Donat et al., 2007)
OMP-BO1185	<i>Malus</i> sp., Bologna, Italy	(Donat et al., 2007)
SV4576	<i>P. communis</i> , Lérida, Spain, 1999	(Cabrefiga and Montesinos, 2005)
UPN500	<i>P. communis</i> , Guipúzcoa, Spain, 1998	(Rico et al., 2004)
UPN529	<i>Pyracantha</i> sp., Navarre, Spain, 1997	(Rico et al., 2004)
UPN546	<i>Cydonia</i> sp., Navarre, Spain, 1998	(Rico et al., 2004)

^aCFBP = Collection Française de Bactéries Phytopathogènes, Angers, France; EPS = Universidad de Gerona, Gerona, Spain; IVIA = Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, UK; OMP-BO = Osservatorio delle Malattie delle Pianta, Bologna, Italy; SV = Servei de Protecció dels Vegetals, Barcelona, Spain; UPN = Universidad Pública de Navarra, Pamplona, Navarre, Spain.

5 d in a growth chamber at 20 °C with a 16-h light (20000 lx) photoperiod, severity was estimated using a procedure modified from that of Duron et al. (1987). Briefly, progression of necrosis was evaluated using an arbitrary scale: 0 = no necrosis; 1 = necrosis limited to the inoculation point; 2 = necrosis advancing into the midrib; 3 = necrosis reaching the lateral veins; and 4 = necrosis over the whole leaf, reaching the end of the lateral veins (Fig. 1). These values were used to compute a necrosis severity index (NSI) using the formula: $NSI = [(0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)] / 4 \times N \times 100$, where n_n is the number of leaves on each category in the scale to evaluate the progression of necrosis and N is the total number of leaves inoculated per repetition. For comparison with the inoculation assay on shoots, the NSI values for the detached leaves assay were transformed to an integer index value because this improved the correlation between the detached leaves and the shoot assays. The index transforms NSI percentage intervals into a 0- to 9-point scale as follows: 0 = 0%; 1 = 1% to 3%; 2 = 4% to 6%; 3 = 7% to 12%; 4 = 13% to 25%; 5 = 26% to 50%; 6 = 51% to 75%; 7 = 76% to 88%; 8 = 89% to 99%; and 9 = 100%. The NSI values of the different cultivars evaluated by both the detached leaf and the shoot assays were compared using the linear regression and Pearson's correlation method.

Shoot inoculation. Inoculations were carried out essentially as described by Norelli et al. (1988) by inoculating young shoots by cutting the youngest leaf with scissors dipped in a pathogen suspension; this method performs better than others and results in optimal conditions in high disease severity levels (Ruz et al., 2008). To evaluate the susceptibility to *E. amylovora*, cultivars were grafted on M.9 rootstock toward the end of August and kept in 2-L pots in the field; ≈3 weeks before inoculation, toward the beginning of April, they were moved to a greenhouse and then transferred to a growth chamber at 20 °C, 70% relative humidity, and a 16/8 h light/dark photoperiod (20,000 lx) for 1 week

Fig. 1. Arbitrary scale used to evaluate progression of necrosis in inoculated detached apple leaves.

before inoculation. Five to eight plants were assayed per cultivar, and one to three actively growing, 20 to 30 cm long shoots per plant were inoculated by cutting the youngest leaf with a scissor dipped into a 10⁸ cfu·mL⁻¹ suspension of strain UPN500 prepared as indicated for the inoculation of detached leaves. As a result of the short length of the shoots, progression of the disease was estimated using an average NSI for each cultivar (Duron et al., 1987), which was recorded after incubating the plants for 12 d in a growth chamber. Before a one-way analysis of variance and a Student-Newman-Keuls test to determine differences in means ($P = 0.05$), the NSI values were transformed to $\arcsin [(\sqrt{NSI}) \times 0.01] \times 57.2958$; this last figure was used in the transformation to convert radians into degrees. According to their NSI, cultivars were classified into three arbitrary susceptibility groups (Le Lézec et al., 1997): low susceptible (0% to 40%), moderately susceptible (41% to 60%), and highly susceptible (61% to 100%).

Additionally, the virulence of diverse *E. amylovora* strains was evaluated on shoots of 1-year-old self-rooted apple plants of cv. Gezamiña, maintained in 5-L pots, which were inoculated and rated as described previously.

Results

Virulence of *E. amylovora* strains. We were compelled to use a local isolate of the pathogen to test plant susceptibility and, in

consequence, we evaluated the virulence on apple of a collection of 14 strains of *E. amylovora* (Table 1), including six strains isolated in Spain. NSI values indicated that the strains showed significantly different degrees of virulence to apple (Table 2). Strain UPN500, isolated in Spain, was highly virulent to pear fruits (Cabrefiga and Montesinos, 2005) and apple (Table 2) and was therefore chosen for further plant testing.

Susceptibility of cultivars in the detached leaves assay. Although the collection comprised more than 250 cultivars, we could evaluate only 233 cultivars using the detached leaves assay. In the more susceptible cultivars, symptoms appeared 2 to 3 d after inoculation and necrosis rapidly progressed through the midrib, the lateral veins, and the lamina, eventually covering the complete leaf by Day 5; bacterial exudates oozed out of some of these leaves. The mean NSI values for more than one-third of the cultivars were in the ranges of 21% to 40% and 41% to 60%, and only 6% of the cultivars scored in the extreme intervals (Table 3). We observed the variability typically associated with this type of assay with a SE of the mean higher than 8.4 for 15.9% of the NSI values, although it was lower than 5.5 for 34.7% of the NSI values.

Susceptibility of cultivars in inoculated shoots. As a result of operational limitations, mostly of space for inoculation under containment conditions, we only evaluated 103 local cultivars by shoot inoculation, which included the 40 cultivars with the lowest NSI in the detached leaves assay (up to 34%) plus 63 cultivars that were of interest for the local cider growers because of their pomological characteristics (B. Lizar, personal communication). In general, symptoms appeared 3 to 4 d later, and NSI values were recorded 12 d after inoculation. The 103 cultivars showed differential susceptibility to *E. amylovora*, displaying NSI values between 5% and 98% (Table 4) that allowed their classification into three arbitrary susceptibility groups. Forty-

Table 3. Distribution of local apple cultivars according to their susceptibility to *Erwinia amylovora* in a detached leaf assay and in a young shoot assay.

Susceptibility group	NSI ²	Number of cultivars	
		Detached leaf assay	Shoot assay
Low susceptibility	0% to 20%	8	17
	21% to 40%	89	31
Moderate susceptibility	41% to 60%	71	27
High susceptibility	61% to 80%	59	18
	81% to 100%	6	10
Total		233	103

²Intervals of necrosis severity index (NSI) values

Table 4. Classification of 103 local apple cultivars according to their susceptibility to *Erwinia amylovora* UPN500 in a shoot inoculation assay.

Susceptibility groups and cultivars	Interval of NSI $\pm$ SD ²	Significance level ³
Low susceptibility		
1.1.24; 1.1.53; 2.1.21; 2.1.6; 5.1.13; 3.1.101	5 $\pm$ 1.8–8 $\pm$ 2.1	a
3.1.93; 1.1.20	9 $\pm$ 2.8	ab
3.1.109; 3.1.102; 5.1.23; 1.1.12; 3.1.75	10 $\pm$ 2.9–11 $\pm$ 2.2	abc
Miguela de Ademuz; 5.1.71; 4.1.18; 3.1.50	14 $\pm$ 2.2–18 $\pm$ 6.4	bcde
3.1.78; 3.1.90; 2.1.1; 1.1.14	21 $\pm$ 2.4	cdefg
3.1.100; 2.1.14	22 $\pm$ 3.3	defghi
Manyaga; 3.1.80; Morro de Liebre	24 $\pm$ 3.5–25 $\pm$ 0.0	defghij
3.1.71; 3.1.87; 3.1.70; Orache; Reineta Asturias; 3.1.28; Pero Cervera	27 $\pm$ 1.6–30 $\pm$ 3.3	efghijkl
3.1.3; 1.1.9	31 $\pm$ 2.4–32 $\pm$ 3.2	fghijklm
5.1.66; 3.1.84; 1.1.13	33 $\pm$ 3.8–35 $\pm$ 5.7	fghijklmn
2.1.19; 3.1.14; 3.1.40; 5.1.17; 5.1.14; 5.1.58; 2.1.25; 3.1.59; 2.1.26; 3.1.94	36 $\pm$ 3.7–40 $\pm$ 1.9	fghijklmno
Moderate susceptibility		
5.1.16; 1.1.21	41 $\pm$ 3.1	ghijklmnop
3.1.15	43 $\pm$ 2.5	ghijklmnopq
1.1.38; 5.1.50	45 $\pm$ 4.0	hijklmnopqr
3.1.63	47 $\pm$ 2.0	ijklmnopqrs
3.1.56; 3.1.85; 3.1.96; 3.1.86; 3.1.79; 5.1.1	47 $\pm$ 2.0–50 $\pm$ 2.4	ijklmnopqrst
5.1.73; 5.1.12; 3.1.68	51 $\pm$ 8.1	ijklmnopqrstu
2.1.30; 5.1.69; 3.1.47	53 $\pm$ 3.7	jklmnopqrstuv
5.1.77; 3.1.55; 3.1.33	54 $\pm$ 3.7	klmnopqrstuv
3.1.42	55 $\pm$ 4.0	lmnopqrstuv
3.1.105; 5.1.63; Reineta Regil	56 $\pm$ 3.1–56 $\pm$ 4.8	mnopqrstuv
3.1.72	59 $\pm$ 3.9	nopqrstuv
5.1.76	60 $\pm$ 5.3	opqrstuv
High susceptibility		
5.1.43; 5.1.53; 4.1.7; 3.1.51	61 $\pm$ 3.7	pqrstuv
1.1.10; 3.1.82; 1.1.11; 3.1.43	65 $\pm$ 4.2–66 $\pm$ 3.6	qrstuv
5.1.18; 5.1.75	68 $\pm$ 5.7–69 $\pm$ 5.3	rstuv
Granny Smith ⁴ ; 3.1.8; 1.1.32; 5.1.65; 5.1.10; Txalaka; Pero Sangüesa; 5.1.72; 5.1.70	73 $\pm$ 4.3–79 $\pm$ 6.1	stuv
3.1.32; 5.1.27; 3.1.92; 3.1.61; 4.1.21; 3.1.67	83 $\pm$ 5.9–86 $\pm$ 6.1	tuv
3.1.30; F8 Pasillo; Pero Pardo	89 $\pm$ 4.4–94 $\pm$ 3.5	uv
5.1.19	98 $\pm$ 1.6	v

²Mean necrosis severity index (from the inoculation of five to eight plants per cultivar and one to three actively growing, 20- to 30-cm long shoots per plant) $\pm$ SD.

³Same letters indicate no significant differences according to one-way analysis of variance and Student-Newmans-Keuls test ($\alpha = 0.05$).

⁴Cultivar used as highly susceptible control.

eight cultivars were included in the low susceptibility group, whereas 27 were considered moderately susceptible and 28 cultivars as highly susceptible. ‘Granny Smith’ was used as a positive control and, as expected (van der Zwet and Beer, 1995), was included in the highly susceptible group (Table 4). Variability was low, as expected for this kind of assay, and the analysis of variance showed significant differences of susceptibility to *E. amylovora* among the cultivars (Table 4).

A total of 78 cultivars were evaluated by both the detached leaf and the shoot assays

and the resulting regression line ($y = 0.0171x + 3.6738$) had a low but significant correlation coefficient of $r = 0.56$, with $\alpha = 0.01$ (Fig. 2).

Discussion

The adequate use of plant resistance is one of the more efficient, cheap, and environmentally sound control methods for fire blight; however, the majority of the most successful apple cultivars and rootstocks are highly susceptible to this disease, which has prompted the deployment of diverse strategies to incorporate the use of host resistance into fire

Table 2. Virulence of different *Erwinia amylovora* strains on apple cv. Gezamiña.

Strain	NSI $\pm$ SD ²	Classification ³
CFBP1430	87.8 $\pm$ 1.6	A
NCPBP3159	87.8 $\pm$ 1.6	A
UPN546	85.7 $\pm$ 2.5	A
EPS101	84.1 $\pm$ 3.0	A
UPN500	84.1 $\pm$ 3.7	A
NCPBP3548	81.3 $\pm$ 2.5	A
SV4576	80.7 $\pm$ 2.8	A
NCPBP683	80.5 $\pm$ 2.2	A
UPN529	79.2 $\pm$ 1.6	A
Ea273	76.0 $\pm$ 1.9	AB
IVIA1614-2	70.1 $\pm$ 2.0	B
OMP-BO1185	57.8 $\pm$ 3.0	C
NCPBP595	53.9 $\pm$ 2.2	C
NCPBP311	28.1 $\pm$ 1.5	D

²Mean necrosis severity index (from eight inoculated plants) $\pm$ SD.

³The virulence level of strains classified in the same category are not significantly different according to one-way analysis of variance and Student-Newmans-Keuls test ($\alpha = 0.05$).

Fig. 2. Correlation between susceptibility scores observed in a detached leaf assay and in a shoot inoculation method. Leaves and shoots were inoculated with *E. amylovora* UPN500, and symptoms were scored 5 or 12 d after inoculation, respectively; necrosis severity index (NSI) scores for the detached leaf assay were transformed to an integer severity scale. The regression line ($y = 0.0171x + 3.6738$) had a Pearson correlation coefficient of $r = 0.56$ ($\alpha = 0.01$). The horizontal line marks an arbitrary score limit (equivalent to NSI = 40) for the selection of cultivars with low susceptibility to fire blight in the detached leaf assay; the vertical line marks the upper score limit for the low susceptibility group in the shoot assay.

blight management (Norelli et al., 2003). In particular, cider production in Spain could be severely hampered in the future because the lack of appropriate cultivars to facilitate management of the principal apple diseases, including fire blight. We report the first results into the evaluation of a local apple germplasm collection for resistance to diseases, which resulted in the evaluation of 103 local apple cultivars for susceptibility to *E. amylovora* by artificial inoculation of actively growing shoots. The assayed cultivars were classified in three susceptibility groups (Table 4) that were compatible with previous classification schemes (Le Lézec et al., 1997; Paulin and Primault, 1993; Thibault and Le Lézec, 1990; Thomas and Jones, 1992; van der Zwet and Beer, 1995). Forty-eight of the cultivars were classified in the low susceptibility group and showed significant differences in their NSI scores as compared with the 28 cultivars of the highly susceptible group. Also, 17 of the cultivars had very low NSI scores equal or less than 20, making them promising candidates for further testing and breeding because cultivars included in this category, as well as in the highly susceptible category, tend to show a similar susceptibility behavior under different climatic conditions (Le Lézec et al., 1997). Furthermore, the evaluation of different agronomic parameters for a group of cultivars in an independent study (Lizar et al., 2008) indicated that cultivars 1.1.9, 3.1.71, and 3.1.90, which were classified in the low susceptibility group (Table 4), were highly adequate for cider production.

Fourteen strains of *E. amylovora* showed different degrees of virulence to apple cv. Gezamiña (Table 2), which in general correlated with their virulence on pear immature

fruit (Cabrefiga and Montesinos, 2005); a relevant exception was strain UPN546 that showed a very low virulence on pear fruit but was included in the group of highly virulent strains to apple. For evaluation we used strain UPN500, which was highly virulent to both pear fruit (Cabrefiga and Montesinos, 2005) and apple (Table 2) and was as virulent as other *E. amylovora* strains previously used for the evaluation of susceptibility to fire blight such as CFBP1430 or Ea273 (Cabrefiga and Montesinos, 2005; Laurent et al., 1989). Nevertheless, some apple cultivars are differentially susceptible to specific strains of *E. amylovora* (Norelli et al., 2003), indicating the need to continue the evaluation of the cultivars with low susceptibility identified in this work using other bacterial strains, including those known to have exhibited differential aggressiveness. The high number of local cultivars showing low susceptibility to the disease is probably attributable, at least in part, to the fact that 40 of the 103 evaluated cultivars were selected among the less susceptible in the detached leaf assay; additionally, it is still possible that the germplasm collection is enriched in cultivars that have an increased resistance to diseases.

Because of the limitations for the manipulation of *E. amylovora* in Spain, and to speed up the identification of cultivars with low susceptibility, we applied a rapid susceptibility assay using detached leaves to narrow down the number of cultivars to be assayed using actively growing shoots, which is a more precise and reproducible method (Chevreau et al., 1998). The assay with detached leaves has the important advantages that it requires very little space for the inoculations, limiting the possibilities for pathogen escape, that a large number of cultivars can be assayed in a short period of time, and that there is a large supply of inexpensive material (i.e., leaves) to carry out the analyses. Previously, this type of analysis was successfully applied to evaluate the susceptibility of pear cultivars to *Pseudomonas syringae* (Moragrega et al., 2003), although it was reported to have limited reproducibility for the analysis of apple susceptibility to *E. amylovora* (Donovan, 1991; Donovan et al., 1994). In our hands, the detached leaf assay also showed large variability and low resolution, because scores for the leaf response to the pathogen were concentrated in less than half of the complete evaluation scale (Fig. 2), whereas scores in the shoot assay were distributed practically along the whole scale (Table 4; Fig. 2). On evaluation of the susceptibility of 78 apple cultivars by the detached leaf method and by inoculation of actively growing shoots, we found a low, but significant, correlation ($r = 0.56$; $\alpha = 0.01$) between the disease progression indices estimated for each cultivar with both methods (Fig. 2). However, the cultivars with low susceptibility to fire blight selected in the detached leaf assay (i.e., those having a score lower than 5, which is equivalent to an NSI of 40%) were distributed in the three suscepti-

bility groups in the shoot assay; conversely, the large majority of cultivars showing a score higher than 5 in the detached leaf assay were later classified in the moderate or high susceptibility groups in the shoot assay (Fig. 2). Therefore, our results supports previous observations that the detached leaf assay is not reliable for the direct selection of cultivars with low susceptibility to fire blight (Donovan, 1991; Donovan et al., 1994), although it was useful to identify some cultivars with a medium or high susceptibility to the pathogen. In our case, the use of the detached leaf assay was highly beneficial because the exclusion of these cultivars from shoot assays contributed to the optimization of resources and to limit the potential dispersal of the pathogen; these advantages would also be especially relevant in those situations in which it is necessary to evaluate a large number of cultivars such as during breeding for resistance.

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