Resistance

The Effects of Virulence of Erwinia amylovora on the Evaluation of Fire Blight Resistance in Malus

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Abstract


Inoculation of greenhouse-grown apple (Malus) seedlings derived from controlled crosses with a mixture of five strains of Erwinia amylovora resulted in a larger percentage of the seedling population being evaluated as susceptible than when seedlings were inoculated with a single strain. Inoculation of field-grown progenies with five individual strains of E. amylovora and a mixture of strains indicated that the strain used for inoculation significantly affected the evaluation of resistance to E. amylovora. In general, the aggressiveness (sensu Vanderplank, 1968) of the strains most strongly affected the evaluation of resistance. The differential virulence of the strains to specific cultivars had less effect on the evaluation of resistance. Mixtures of strains masked the differential response of specific cultivar by strain interactions. Methods for selecting cultivars resistant to E. amylovora in an apple breeding program are discussed.

The apple (Malus) breeding program at the New York State Agricultural Experiment Station (NYSAES) in Geneva strives to develop apple scions and rootstocks that have pomological merit and possess high levels of resistance to several diseases (1,5,6,10), including fire blight, caused by Erwinia amylovora (Burr.) Winslow et al. Differential host by pathogen interactions exist among some sources of resistance to E. amylovora used in apple breeding programs and strains of E. amylovora (13).

In the breeding program at the NYSAES, resistance to E. amylovora is evaluated in two stages. In the greenhouse, seedling progeny derived from controlled crosses are inoculated with E. amylovora during the first 4-7 mo of growth to eliminate susceptible seedlings before planting in the field. Transplanted seedlings are later evaluated for pomological characteristics. Selections with good pomological traits are further screened for resistance to E. amylovora. Before 1980, seedlings were inoculated with a single strain of E. amylovora, Ea 273 (7); however, after the identification of differentially virulent strains of E. amylovora (12), a mixture of several strains has been used. A different segregation

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pattern among seedlings for resistance to *E. amylovora* has resulted from using a mixture of strains in the inoculum than when only strain Ea 273 was used (11).

The purpose of this study was to determine the effect of the virulence of *E. amylovora* strains on the evaluation of resistance of apple seedlings. Two aspects of virulence were considered, aggressiveness (sensu Vanderplank, 1968) and differential virulence of the strain to specific host genotypes. In addition, the effect of mixing strains in the inoculum on the evaluation of resistance was tested at the greenhouse and the field stages of the selection process.

**MATERIALS AND METHODS**

**Bacterial strains and inoculum.** The bacterial strains used are maintained in the Cornell University Collection of Phytopathogenic Bacteria (CCPPB) (Table 1). Strains Ea 224, Ea 273, and Ea 344 are pathogenic to most apple cultivars but cause little or no disease on the cultivars Quinzi, Ottawa 523, Novole, and *Malus* × *robusta* No. 5 (*Robusta* 5) (2, 12, 13, J. L. Norelli, unpublished data). Strain Ea 266 is differentially virulent to Quinci, Ottawa 523, Novole, and *Robusta* 5 (12, 13). Strain Ea 344 is more aggressive than strain Ea 273, and strains Ea 224 and Ea 266 are less aggressive than strain Ea 273 (12). Preliminary studies have indicated that Ea 404 is highly aggressive and virulent to Novole (J. L. Norelli, unpublished data).

Broth cultures used for inoculation were initiated from single colonies of freshly revived lyophilized cultures grown on nutrient broth yeast-extract-gluconic agar. Inoculum consisted of 18-h-old cultures grown in Kado 523 broth (9) at 28 C. Equal concentrations of each inocula were achieved by adjusting the turbidity of the suspension of each inoculum to an optical density of 1.0 at 620 nm. At this optical density there were no significant differences among the resulting concentrations of each strain, and the overall mean concentration was 3.17 × 10^7 colony-forming units per milliliter (95% confidence interval: 3.02–3.30 × 10^7).

**Plant material.** The crosses made and their progeny code number are listed in Table 2. Seedlings derived from these crosses were grown in the greenhouse for 6–12 mo and then transplanted to a field in rows 1 m spacing. Seedlings were grown in this orchard to maturity to allow for pollenological evaluation. Progenies 82313, 82315, 82316, 82319, 82220, 82231, 83105, 83121, 83134, and 83135 were used to test the effect of mixing strains in the inoculum on the evaluation of resistance in the greenhouse. Seedlings were grown in the greenhouse as single-shoot plants, which provided for a single inoculation. The 82-progeny series was tested in 1983 greenhouse evaluations and the 83-progeny series in 1984 greenhouse evaluations. Progenies 79507, 79511, 79537, 80319, 80324, and 80338 were used to determine the correlation between greenhouse and field evaluations of resistance to *E. amylovora* and to test the effect of inoculation with different strains of *E. amylovora* on the evaluation of resistance in the field. Each field-grown tree was inoculated with each of the five strains of *E. amylovora* and with a mixture of the five strains (one shoot each).

Grafted trees of different scion cultivars on M.7 rootstock had been planted in an orchard in a completely randomized design and were 8 and 9 yr old when inoculated. The cultivars Delicious, Idared, McIntosh, and Quinzi were inoculated with the strains of *E. amylovora* listed in Table 3. Ten shoots of each cultivar were inoculated with each of the strains and with the mixture of strains. The 10 shoots of a particular combination were distributed among 8–10 trees.

**Inoculation and disease measurement.** The evaluation of resistance of seedlings and cultivars was based on the severity of fire blight symptoms. Vigorously growing vegetative shoots at least 15 cm in length were selected for inoculation on greenhouse, orchard, and field plants. A 0.46-mm-diameter (26-gauge) hypodermic needle was inserted through the stem just above the youngest unfolded leaf. Sufficient inoculum was introduced to fill the wound until drops were observed at both ends of the wound. Lengths of the fire blight lesion and of the current season’s shoot growth were recorded in centimeters after all lesions had ceased to extend, as determined by formation of a determinate margin between necrotic and healthy tissue. Generally, a determinate margin formed 6 wk after inoculation; however, the exact number of weeks varied with each group of inoculations. The proportion of the current season’s shoot length that became necrotic was calculated by dividing the length of the fire blight lesion by the length of the current season’s shoot. Lesions that extended into previous season’s growth were recorded as 1.00.

To determine the effect of bacterial inoculum on the evaluation of resistance to *E. amylovora*, disease responses were categorized into three classes based on the proportion of the shoot length that became necrotic as follows: resistant, 0.0–0.15; intermediate, 0.16–0.84; and susceptible, 0.85–1.00. The numbers of seedlings in each class were compared using a chi-square analysis.

**Correlation between greenhouse and field evaluations of fire blight resistance.** In 1980, progenies of crosses made in 1979 were evaluated for resistance in the greenhouse by inoculation with strain Ea 273. The following year, progenies of crosses made in 1980 were evaluated in the greenhouse by inoculation with a mixture of *E. amylovora* strains Ea 224, Ea 266, Ea 273, Ea 344, and Ea 404. The proportion of the shoot length that became necrotic on individual seedlings from greenhouse inoculation was compared with the mean proportion of the shoot length that became necrotic when the same plants were inoculated in the field with the five individual strains of *E. amylovora* and the mixture of strains. For progenies 79507, 79511, and 79537, 1980 greenhouse evaluations were compared with 1985 field evaluations; for progenies 80319, 80324, and 80338, 1981 greenhouse evaluations were compared with 1985 field evaluations.

**RESULTS**

**Effect of mixing strains in the inoculum on the evaluation of resistance of greenhouse-grown seedlings.** Greenhouse-grown seedlings from controlled crosses were randomly separated in two groups and inoculated with either strain Ea 273 or the mixture of five strains in both the 1983 and 1984 greenhouse evaluations. Inoculation with the mixture had a significant effect on the evaluation of resistance (Table 4). In general, fewer seedlings inoculated with the mixture of *E. amylovora* strains were evaluated as resistant than when inoculated with the single strain, Ea 273. However, the mixed inoculum did not have a significant effect on the progeny of all crosses (Table 4). In 1983 greenhouse tests, a significant change in the observed distribution of the numbers of seedlings in resistance classes occurred with progenies 82313, 82320, and when all seedlings were considered together.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>CCPPB no.</th>
<th>Origin</th>
<th>Location</th>
<th>Isolator</th>
<th>Isolator’s original designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ea 224</td>
<td>0036</td>
<td>apple (Rome Beauty)</td>
<td>New York</td>
<td>S. V. Beer</td>
<td>1</td>
</tr>
<tr>
<td>Ea 266</td>
<td>0071</td>
<td>apple (R.I. Greening)</td>
<td>Ontario</td>
<td>W. G. Bonn</td>
<td>E4001A</td>
</tr>
<tr>
<td>Ea 273</td>
<td>0273</td>
<td>apple (R.I. Greening)</td>
<td>New York</td>
<td>S. V. Beer</td>
<td>1379-9</td>
</tr>
<tr>
<td>Ea 344</td>
<td>0386</td>
<td>pear (Passe Crassane)</td>
<td>France</td>
<td>J. P. Paulin</td>
<td>1</td>
</tr>
<tr>
<td>Ea 404</td>
<td>2191</td>
<td>apple (M.9 interstem)</td>
<td>New York</td>
<td>J. L. Norelli</td>
<td>1</td>
</tr>
</tbody>
</table>

*Strain designation in the Cornell University Collection of Phytopathogenic Bacteria.*
Similarly, in 1984 greenhouse tests, a significant change in the observed distribution of the numbers of seedlings in resistance classes occurred with progeny 83121 and when all seedlings were considered together. Chi-square comparisons of the distribution of seedlings in the three resistance classes inoculated with the single strain and the mixture of strains yielded chi-square values of 15.12, 3.87, 5.06, 1.53, and 11.62 for progeny 83121, 83103, 83134, 83135, and all seedlings, respectively (critical X² values: X² 0.05 [df = 2] = 5.99; X² 0.05 [df = 2] = 9.21). 

Evaluation of resistance of field-grown progeny with individual strains and a mixture of strains. After 4-5 yr of growth in the field, several shoots of each of the trees from each of six progenies were inoculated with five individual strains of \textit{E. amylovora} and with the mixture of the five strains. Analysis of variance on the severity of fire blight symptoms resulting from 1985 field inoculation indicated that there was a significant effect of the \textit{E. amylovora} strain used for inoculation ($F = 19.94$ [df = 5,1937]; critical $F$ value: $F = 0.05$ [df = 5,1937] = 3.03) and a significant effect of the cross ($F = 56.72$ [df = 5,1937]), but that there was no significant strain by cross interaction ($F = 1.28$ [df = 25,1937]; critical $F$ value: $F = 0.01$ [df = 25,1937] = 1.52). The overall mean proportion of the shoot lengths that became necrotic when progenies were inoculated with \textit{E. amylovora} strains Ea 224, Ea 266, Ea 273, Ea 344, Ea 404, and the mixture of five was 0.74, 0.66, 0.66, 0.50, 0.77, and 0.71, respectively.

Inoculation with different strains of \textit{E. amylovora} had a significant effect on the evaluation of resistance in field-grown progenies (Fig. 1). Inoculation of 4-yr-old seedlings of progeny 80319 with the more aggressive strain Ea 224 and Ea 404 resulted in a higher percentage of seedlings being evaluated as susceptible, whereas inoculation with the least aggressive strain, Ea 344, resulted in a higher percentage of seedlings being evaluated as resistant. The differential virulence of \textit{E. amylovora} strain Ea 266 had little effect on evaluation of resistance when compared with the nontoxically virulent strain Ea 273 (Fig. 1). In 1985, field inoculation with strain Ea 273 and Ea 266 resulted in the same overall mean proportions of the shoot lengths necrotic, 0.66. In 1985, strain dependent changes in the segregation of resistance were observed with progenies 79507, 79511, and 80319. Chi-square values of 27.38, 35.53, 11.96, 39.02, 15.55, and 13.00 were obtained for progenies 79507, 79511, 79537, 80319, 80324, and 80338, respectively (critical $X$² values: $X$² 0.05 [df = 10] = 18.31; $X$² 0.05 [df = 10] = 23.21). In general, the greater aggressiveness of strain Ea 244 and Ea 224 and the lesser aggressiveness of strain Ea 344 were the major contributors to the chi-square value. In general, the number of trees evaluated resistant, intermediate, or susceptible was close to that expected when these were inoculated with the mixture of five \textit{E. amylovora} strains.

Correlation between greenhouse and field evaluations of fire blight resistance. Data obtained from greenhouse evaluation of 1979 progenies inoculated with strain Ea 273 were not significantly correlated with those obtained from 1985 field inoculations with five individual strains and the mixture of strains ($r = 0.170$, df = 102). However, there was a highly significant correlation between the mixture of progeny 80319 inoculated with the single strains and those obtained from 1985 field inoculations ($r = 0.344$, df = 83). The difference between the correlation coefficients was tested using a z value transformation (16) to compare the two methods of greenhouse evaluation. Greenhouse evaluation with the mixture of strains was a more accurate measure ($P = 0.10$) of field susceptibility than evaluation with a single strain.

Effect of mixing strains in the inoculum on the evaluation of resistance of differentially resistant genotypes. Orchard trees of four apple cultivars were inoculated with five individual strains of \textit{E. amylovora} and the mixture of the five strains (Table 3). The differential susceptibility of Quinte to strain Ea 266 was masked by the use of a mixture of strains in the inoculum. The mean proportion of the shoot length necrotic of Quinte was much less when inoculated with the mixture of strains (0.22) than when inoculated with Ea 266 or a one-fifth dilution of Ea 266 (0.69 and 0.78, respectively). The mixture of five \textit{E. amylovora} strains had the same concentration of strain Ea 266 as the one-fifth dilution. In contrast, on Delicious and McIntosh the low virulence of Ea 266 (0.21 and 0.29) and Ea 344 (0.24 and 0.36, respectively) did not greatly reduce the virulence of the other strains in mixed inocula (0.89 and 0.72, respectively).

**DISCUSSION**

A major hindrance to progress in an apple breeding program is the limited number of seedlings that can be grown and evaluated in the field. In the program at the NYSAES, attempts are made to eliminate as many disease-susceptible seedlings as possible during the first months of growth in the greenhouse and before planting in the field. Seedlings are inoculated with \textit{Venturia inaequalis} (Cke.) Wint. and \textit{Gymnosporangium juniperi-virginianae} Schw. to screen for resistance to apple scab and cedar apple rust before inoculation with \textit{E. amylovora}. Cessation of vigorous growth of some seedlings before inoculation with \textit{E. amylovora} results in reduced susceptibility. Furthermore, seedlings in the greenhouse have single

**Table 3. Severity of fire blight on apple cultivars inoculated with individual strains and a mixture of strains of \textit{Erwinia amylovora} in orchard tests and summary of analysis of variance**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Strain</th>
<th>Delicious</th>
<th>Idared</th>
<th>McIntosh</th>
<th>Quinte</th>
<th>Strain mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ea 273</td>
<td>0.52</td>
<td>0.64</td>
<td>0.70</td>
<td>0.05</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>1/5 Ea 273</td>
<td>0.52</td>
<td>0.43</td>
<td>0.66</td>
<td>0.09</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Ea 266</td>
<td>0.21</td>
<td>0.39</td>
<td>0.29</td>
<td>0.69</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>1/5 Ea 266</td>
<td>0.23</td>
<td>0.62</td>
<td>0.31</td>
<td>0.78</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Ea 224</td>
<td>0.75</td>
<td>0.91</td>
<td>0.98</td>
<td>0.53</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Ea 344</td>
<td>0.24</td>
<td>0.97</td>
<td>0.36</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Ea 404</td>
<td>0.79</td>
<td>0.88</td>
<td>0.96</td>
<td>0.53</td>
<td>0.79</td>
</tr>
<tr>
<td>Mixture</td>
<td>0.89</td>
<td>0.72</td>
<td>0.72</td>
<td>0.22</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Cultivar mean</td>
<td>0.50</td>
<td>0.86</td>
<td>0.66</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analysis of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains</td>
<td>7</td>
<td>1.031</td>
<td>7.08*</td>
</tr>
<tr>
<td>Cultivars</td>
<td>3</td>
<td>2.304</td>
<td></td>
</tr>
<tr>
<td>Strains × cultivars</td>
<td>21</td>
<td>0.506</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>280</td>
<td>0.071</td>
<td></td>
</tr>
</tbody>
</table>

*Severity is expressed as the proportion of current season's shoot length that became necrotic (mean of 10 replicates).

* A mixture of five \textit{E. amylovora} strains: Ea 224, Ea 266, Ea 273, Ea 344, and Ea 404.

* One-fifth dilution of indicated strain.

*Mean calculated from severity resulting from inoculation with five individual strains only.

*Critical $F$ values: $F = 0.09$ (df = 21, 280) = 2.44.
TABLE 4. Effect of *Erwinia amylovora* inoculum on the evaluation of fire blight resistance of greenhouse-grown seedlings into resistant (R), intermediate (I), and susceptible (S) classes

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Progeny code</th>
<th>All seedlings ^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82313</td>
<td>82315</td>
</tr>
<tr>
<td>Ea 273</td>
<td>R-I-S</td>
<td>R-I-S</td>
</tr>
<tr>
<td></td>
<td>n = 119</td>
<td>n = 43</td>
</tr>
<tr>
<td>Mixture ^a</td>
<td>17-55-28 ^c</td>
<td>16-63-21</td>
</tr>
<tr>
<td></td>
<td>n = 130</td>
<td>n = 49</td>
</tr>
<tr>
<td></td>
<td>4-39-57</td>
<td>20-55-25</td>
</tr>
<tr>
<td></td>
<td>25.07</td>
<td>0.57</td>
</tr>
</tbody>
</table>

^a Resistant class (R): x = 0.00 - 0.15; intermediate class (I): x = 0.16 - 0.84; susceptible class (S): x = 0.85 - 1.00; x = proportion of shoot length that became necrotic.

^b Includes the four progeny listed plus progeny 82319 and 82321 (Table 2).

^c The percentage of seedlings in resistance classes R, I, and S (see footnote a).

^d A mixture of five *E. amylovora* strains: Ea 224, Ea 266, Ea 273, Ea 344, and Ea 404.

^e Critical X^2^ values: X^2^ (df = 2) = 5.99, X^2^ (df = 2) = 9.21.

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![Graphs showing proportion of shoot length infected](image)

**Fig. 1.** Effect of different strains of *Erwinia amylovora* on the evaluation of resistance to *E. amylovora* in 4-yr-old field-grown progeny 80319. Mixture includes one-fifth of the original concentration of five *E. amylovora* strains: Ea 224, Ea 266, Ea 273, Ea 344, and Ea 404.

shoots and, therefore, multiple or replicated inoculations are not possible. Because of these limitations, initial greenhouse inoculation of seedlings with *E. amylovora* does not reliably evaluate resistance but serves only to identify many susceptible seedlings that can be discarded before planting in the field.

In this study, the disease response of seedlings was categorized into three classes to analyze the effect of strain on the observed segregation of resistance using a chi-square statistic. This test analyzed the effect within the progeny derived from a single cross. An analysis of variance was used to determine the effect of strain and cross on the proportion of the shoot length that became necrotic. There was no significant strain by cross interaction in this test, indicating that specific strains were not differentially causing more disease on the progeny of specific parents. Specific strains did alter the observed segregation of resistance within the progeny of a cross, but this effect was not cross specific. This suggests that the overall aggressiveness of the strain has a greater effect on the observed segregation of resistance to *E. amylovora* than does differential virulence. The aggressiveness and differential virulence of the *E. amylovora* strains used in this investigation had been determined previously (12,13). None of the parents of the field-grown progeny are known to be differentially susceptible to the *E. amylovora* strains used (Table 2). The differential virulence of an individual strain may have had a greater effect on the observed segregation of resistance if parents of these progeny had been differentially susceptible to the strains used.

Both aggressiveness and differential virulence of *E. amylovora* strains are important considerations when selecting strains used in screening seedlings for fire blight resistance. When field-grown progenies were inoculated with several strains of *E. amylovora*, more aggressive strains resulted in a higher percentages of the progeny being evaluated as susceptible (Fig. 1). Ideally, a highly aggressive strain of *E. amylovora* should be used for the initial screening of greenhouse-grown seedlings. However, determining the aggressiveness of strains is time consuming, and new strains obtained from fresh isolations or other locations require continued checking. A mixture of four or five strains protects against using inoculum of low aggressiveness. The components of the mixture of strains in the inoculum should be adjusted with time. As more aggressive strains are identified, they should replace less aggressive strains in the mixture.

Although our data indicate that a mixture of strains is effective for preliminary evaluation of fire blight susceptibility, differential susceptibility to specific strains of *E. amylovora* can be detected only by inoculation with individual strains (Table 3). For differentially resistant selections, evaluation depends on the differential virulence of the strains used. Inoculation of field-grown selections should be done with several individual strains that are highly aggressive and are known to be differentially virulent to specific cultivars (12,13).

Reduced disease severity resulting from mixed inocula has been reported for the following host-pathogen combinations: tobacco: *Pseudomonas solanacearum* (17); tobacco, tomato, and eggplant: *P. solanacearum* (3); cherry: *Psyringia pv. mors-prunorum* (4); and apple: *E. amylovora* (8). Although these reports did not deal with cultivar-specific virulence, the results are similar to those reported here. The presence of incompatible cells prevented or reduced the ability of compatible cells to infect disease even though compatible cells were present in sufficient numbers to infect disease if introduced alone into the plant.

Parlevliet (14) has argued that using mixtures of races of a pathogen does not enhance recognition of horizontal resistance. He suggested using several single races having the broadest spectrum of virulence to suppress the expression of as many resistance genes as possible. We concur on the use of single strains for selection of resistance to *E. amylovora* in advanced selections, which allows for multiple inoculations of trees. However, we suggest using mixed inoculum for screening young seedlings.

Quamme et al (15) concluded that greenhouse inoculation of young pear seedlings could be used to identify progeny resistant to fire blight. Their tests were conducted at two locations: Beltsville, MD, where a single strain of *E. amylovora* was used for inoculation, and Harrow, Ontario, Canada, where a mixture of strains was used. No specific effect of using mixed inoculum was
noted. In general, disease severity was greater in the Harrow trials, but this could be caused by factors other than inoculum. Consistent with our results, Quamme et al (15) observed significant correlations between the Harrow greenhouse evaluations (mixed inoculum) and field scores, but not between the Beltsville greenhouse evaluations (single strain) and field scores. There were, however, several differences between the studies of Quamme et al (15) and those reported here. Field evaluation of progenies was based on natural infections in their studies, not artificial inoculation. Correlations calculated by Quamme et al (15) were based on mean response for an entire progeny, whereas correlations reported here were based on single plant response. Our correlation ratios suggested that evaluation of greenhouse-grown seedlings with mixed inoculum was a better predictor of field susceptibility than evaluation with single strains. Correlations were based on greenhouse evaluations done in two different years. Therefore, environment, as well as the use of a mixture of strains in the inoculum, could affect the correlations observed. The observation of a significant correlation between greenhouse evaluations done in Harrow and Beltsville (15), despite different growing and inoculation conditions, suggests that environment might not have a major effect in comparing greenhouse evaluations.

Our results indicate that evaluation for resistance to *E. amylovora* can be influenced by the virulence of strains used in screening. Highly aggressive strains are most effective in identifying susceptible genotypes. Because there are differentially virulent strains of *E. amylovora* (12,13), apple cultivars that are highly resistant to some strains may be susceptible to others. When evaluating apple seedlings for resistance to *E. amylovora* they must be challenged by strains representative of the complete range of virulence of this bacterium.

**LITERATURE CITED**