Differential Host × Pathogen Interactions Among Cultivars of Apple and Strains of *Erwinia amylovora*

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ABSTRACT

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The virulence of 19 strains of *Erwinia amylovora* was compared by inoculating several apple cultivars in orchard tests conducted during two growing seasons. The virulence of six strains was compared on five cultivars during both growing seasons. Virulence was measured by determining the percent of current season's shoot length that developed fire blight symptoms. There was a significant cultivar × strain interaction. *E. amylovora* strain 266 was differently virulent to plants of apple cultivar Quinte in both seasons of orchard tests. Greenhouse tests confirmed the differential virulence of this strain for Quinte.

Additional key words: apple breeding, disease resistance, *Malus pumila, Malus domestica*, phytopathogenic bacteria.

Erwinia amylovora (Burr.) Winslow et al, the incitant of fire blight of apple and pear, has been reported to infect more than 130 species of plants in 39 genera of the family Rosaceae (27). Generally, strains of *E. amylovora* are not host species-specific. For example, a strain of *E. amylovora* isolated from apple (*Malus pumila* Miller) is pathogenic on pear (*Pyrus communis*) or other rosaceous ornamentals (3). However, strains of *E. amylovora* isolated from *Rubus* species have been reported to be host specific (21,24).

Differences in virulence among strains of *E. amylovora* have been reported by several researchers (3,19,23). However, in those tests virulence was evaluated by using a single cultivar or by using seedlings of apple or pear. There has been little characterization of strains of *E. amylovora* for differences in cultivar-specific virulence (22). Differential virulence to specific host cultivars could affect the evaluation of host resistance. The purpose of this study was to determine if differential virulence to specific apple cultivars could be detected among strains of *E. amylovora*.

Differential virulence has been reported for species of host-specific phytopathogenic bacteria. Based upon qualitative differences in reaction to inoculation of a series of differential host cultivars, several races of *Pseudomonas syringae* pv. *glycinea* (9,25), *Xanthomonas campestris* pv. *malvacearum* (15), and *X. campestris* pv. *vesicatoria* (8) have been identified. Differential interactions among cultivars of rice and strains of *X. campestris* pv. *oryzae* have been reported (10,11,14,18). Based upon quantitative disease reactions on differential rice cultivars with specific resistance genes, Japanese scientists have identified five virulence groups of *X. campestris* pv. *oryzae*, and scientists at the International Rice Research Institute have identified four virulence groups. A comparative study of both differential systems indicated that there are nine distinct virulence groups of *X. campestris* pv. *oryzae* (14).

Recently Quamme and Bonn (20) found no evidence of significant cultivar × strain interaction when the virulence of nine strains of *E. amylovora* was compared on four cultivars of pear. In this paper we present evidence for the occurrence of specific interactions among apple cultivars and strains of *E. amylovora*.

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MATERIALS AND METHODS

Bacterial strains and inoculum. The nineteen strains of *E. amylovora* used for inoculation originated from various areas in North America and western Europe and were isolated from several cultivars of apple and pear (Table 1). All strains are maintained in the Cornell University Collection of Phytopathogenic Bacteria in lyophilized form. Broth cultures were started from a single colony from a freshly revived lyophilized culture. Inoculum consisted of 18- to 20-hr-old cultures grown in Kado 523 broth (16) at 25 ± 3 C and contained ~10^{10} colony-forming units of *E. amylovora* per milliliter.

Orchard tests. The virulence of strains of the selected *E. amylovora* was compared by inoculating shoots of several apple cultivars in an orchard at Geneva, NY, during two growing seasons. Trees were planted in a completely randomized design on the M.7 rootstock and were 5 and 6 yr old in 1980 and 1981, respectively, when inoculated. The cultivars inoculated in each growing season are indicated in Tables 2 and 3. Ten shoots of each cultivar were inoculated with each strain of *E. amylovora*. The ten shoots of a particular combination occurred on 8–10 trees. Inoculations were conducted on 25 June 1980 and 2 and 3 June 1981.

Greenhouse tests. The virulence of strains Ea 224, Ea 273, and Ea 266 of *E. amylovora* was compared on cultivars Delicious, Idared, McIntosh, and Quinte in a greenhouse test. Trees were grown from bench grafts on seedling rootstocks in the greenhouse and trained to single shoots by methods previously described (2). Fifteen shoots of each cultivar were inoculated with each strain on 8 August 1980.

Inoculation and disease measurement. Vegetative shoots at least 15 cm in length and in a stage of vigorous growth were selected for inoculation on orchard and greenhouse trees. 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 and leave visible drops at both ends of the wound. The lengths of fire blight lesions and of current season's shoot growth were recorded after all lesions had ceased to extend, as determined by the formation of a determinate margin between diseased and healthy tissue. The mean percentage of the current season's shoot length that was necrotic was calculated. Lesions that extended into previous season's growth were recorded as 100%. Differences among susceptibility of cultivars, among virulence of strains, and the interactions among cultivars and strains were analyzed by a two-way analysis of variance after angular transformation of data. For the treatment combinations included

TABLE 1. Strains of *Erwinia amylovora* used in comparison of strain virulence on 5- and 6-yr-old trees of apple cultivars growing in the orchard

Strain	CUCPB[a] no.	Year tested (19-)	Origin		Isolator	Isolator's original designation
			Host	Location		
Ea 109	009	80, 81	pear	California	S. V. Thomson	WEO 7
Ea 224	036	80, 81	apple (Rome Beauty)	New York	S. V. Beer	···
Ea 242	282	80	pear	England	J. E. Crosse	P42
Ea 243	050	81	apple (Jonathan)	Illinois	S. M. Ries	Apple #1
Ea 263	284	80	apple (Jonathan)	S. Carolina	E. I. Zehr	···
Ea 265	070	81	apple (Jonathan)	Ontario	W. G. Bonn	E2002A
Ea 266	071	80, 81	apple (R. I. Greening)	Ontario	W. G. Bonn	E4001A
Ea 267	072	81	pear (Bartlett)	Ontario	W. G. Bonn	E4003P
Ea 269	074	80, 81	crab apple	Saskatchewan	W. G. Bonn	E7001M
Ea 270	075	81	crab apple	Alberta	W. G. Bonn	E7002M
Ea 273	273	80, 81	apple (R. I. Greening)	New York	S. V. Beer	27-3
Ea 280	084	81	apple (Jonathan)	Michigan	D. Ritchie	122 wt
Ea 284	087	81	crab apple	Michigan	D. Ritchie	137 wt
Ea 307	138	80, 81	apple (McIntosh)	Michigan	E. J. Klos	Mac 715
Ea 339	377	80	apple (Delicious)	New York	J. L. Norelli	···
Ea 340	382	80	pear (Passe Crassane)	France	J. P. Paulin	1314-1
Ea 342	384	80	apple (Reine des Reinettes)	France	J. P. Paulin	1342-2
Ea 344	386	80	pear (Passe Crassane)	France	J. P. Paulin	1379-9
Ea 356	476	81	apple (James Grieve)	W. Germany	W. Zeller	Ea 1/79

[a]Cornell University Collection of Phytopathogenic Bacteria.

TABLE 2. Severity[a] of fire blight symptoms on apple cultivars inoculated with strains of *Erwinia amylovora* in 1980 orchard tests—including a summary of analysis of variance

Cultivar	Strain of *E. amylovora*												Cultivar mean
	242	109	263	340	342	344	307	339	273	224	266	269	
Monroe	6[a]	51	57	52	89	99	77	87	70	71	100	96	71
Idared	6	53	67	69	71	76	86	68	70	79	91	96	69
McIntosh	2	6	0	3	5	6	28	61	28	76	26	24	22
Delicious	4	11	8	5	5	7	16	9	34	30	43	39	18
Jerseymac	1	4	1	3	1	3	10	28	7	28	47	46	15
Golden Delicious	0	3	5	4	8	7	15	10	9	26	36	42	14
Prima	4	1	3	2	7	1	4	16	9	15	29	25	10
Empire	2	4	2	4	3	5	4	18	9	9	16	8	7
Quinte	0	0	1	0	1	0	1	4	1	4	43	32	7
Strain mean	3	15	16	16	22	23	27	33	27	38	48	45	26

Analysis of variance Sources of variation	Degrees of freedom	Mean squares[b]	F
Cultivars	8	16.667	
Strains	11	3.730	
Cultivars × strains	88	0.305	3.85**
Error	953	0.079	

[a]Values are expressed as percent of current season's shoot length that was necrotic and represent the mean of 10 replicates.
[b]Calculated from arc-sine $\sqrt{(x)}$, in which x = the proportion of the current season's shoot length that became necrotic. Asterisks (**) denote statistical significance, $P = 0.01$.

TABLE 3. Severity[a] of fire blight on apple cultivars inoculated with strains of *Erwinia amylovora* in 1981 orchard tests and summary of analysis of variance

Cultivar	Strain of *Erwinia amylovora*													Cultivar mean
	243	284	109	307	356	273	224	266	269	267	270	280	265	
Idared	17[a]	10	40	19	47	54	73	79	79	80	84	90	77	58
Delicious	9	12	23	20	41	60	67	79	62	95	89	95	91	57
Holly	14	34	21	7	19	88	85	57	63	80	85	76	85	54
Melrose	14	14	19	17	40	49	62	63	80	74	71	81	100	51
McIntosh	8	16	6	44	28	64	71	63	52	49	40	67	62	44
Empire	11	21	23	28	14	34	73	41	52	33	53	62	100	41
Quinte	4	13	2	4	7	13	24	74	19	24	54	57	63	29
NY 212	0	0	13	6	2	0	21	10	9	6	4	8	25	8
Strain mean	10	15	18	18	25	44	59	58	51	52	58	66	72	42

Analysis of variance Sources of variation	Degrees of freedom	Mean squares[b]	F
Cultivars	7	7.493	
Strains	12	7.161	
Cultivars × strains	84	0.361	1.70**
Error	787	0.213	

[a]Values are expressed as percent of current season's shoot length that became necrotic and represent the mean of 10 replicates.
[b]Calculated from arc-sine $\sqrt{(x)}$, in which x = the proportion of the current season's shoot length that was necrotic. Asterisks (**) denote statistical significance, $P = 0.01$.

in both growing seasons, data were combined after testing for homogeneity of error over growing season. The effect of environment on cultivar × strain interaction was then analyzed by testing for cultivar × growing season, strain × growing season, and cultivar × strain × growing season interactions. For analysis of greenhouse tests, the sums of squares from the analysis of variance were partitioned into single-degree-of-freedom orthogonal contrasts (7).

RESULTS

Orchard tests. In 1980 orchard tests, both cultivar and strain affected the severity of fire blight infection (Table 2). Several of the strains (eg, Ea 109, Ea 263, Ea 273, and Ea 307) were similar in pattern of virulence to the apple cultivars tested (Table 2). They caused a large amount of disease on susceptible cultivars Monroe and Idared, a lesser amount of disease on moderately resistant cultivars McIntosh and Delicious, and little disease on resistant cultivars Empire and Quinte. Strains Ea 266 and Ea 269 appeared to differ from the other strains in pattern of virulence; they caused a greater amount of disease on the resistant cultivar Quinte. In 1980, the overall mean percent shoot length infected on Quinte for all strains was 7%, but on Quinte inoculated with Ea 266 and Ea 269 the mean percents of shoot infected were 43 and 32%, respectively. Unlike the other strains tested, strains Ea 266 and Ea 269 caused more disease on Quinte than on McIntosh.

In 1981 orchard tests (Table 3), strain Ea 266 again exhibited a differential virulence for Quinte. The overall mean percent shoot length infected on Quinte for all strains was 29%, but on Quinte inoculated with Ea 266 the mean percent shoot length infected was 74%. Strains Ea 224 and Ea 266 caused similar overall mean percent shoot length infection (59 and 58%, respectively) but the amount of disease they caused on Quinte was 24 and 74%, respectively. Although strain Ea 265 also caused a large amount of disease on Quinte (63%), it caused greater infection on all cultivars (72%) than did Ea 266 (58%). Strain Ea 265 also caused more disease on the other resistant cultivars, Empire and NY 212. Strains Ea 270 and Ea 280 caused a large amount of disease on Quinte, but their differential response to Quinte was not as large as that of strain Ea 266. Strain Ea 269 did not exhibit a differential virulence for Quinte in 1981 as it had in the 1980 orchard tests.

Two-way analysis of variance of 1980 and 1981 orchard inoculation data indicated a significant cultivar × strain interaction for the severity of fire blight infection (Tables 2 and 3). The combined 1980 and 1981 data for treatment combinations in both growing seasons indicated that there was not a significant cultivar × strain × growing season interaction. Errors were nonhomogeneous over growing seasons. Under such circumstances, tabulated *F*-values frequently produce excess significant results. Since, in this case, the *F*-value for interaction was not significant, we concluded that there was no cultivar × strain × growing season interaction.

Greenhouse tests. In greenhouse test inoculations, Ea 266 exhibited a differential virulence for Quinte (Fig. 1). Strains Ea 273, Ea 224, and Ea 266 were chosen for greenhouse tests since Ea 273 has been used previously as a standard strain in test inoculations (2,4,12), Ea 224 had appeared nondifferentially more virulent than Ea 273, and Ea 266 had appeared differentially more virulent than Ea 273 in orchard tests. Idared, McIntosh, and Delicious were chosen as nondifferentially susceptible, moderately resistant and resistant cultivars, respectively, and Quinte was chosen as the differentially resistant cultivar. In greenhouse tests, the virulence of Ea 266 and Ea 224 was similar on plants of cultivars Delicious, Idared, and McIntosh, but Ea 266 was more virulent than either Ea 224 or Ea 273 on plants of Quinte. Results of two-way analysis of variance of greenhouse inoculations also indicated a significant cultivar × strain interaction for the severity of fire blight infection. When the single-degree-of-freedom contrast for the interaction between Quinte and strain Ea 266 was partitioned from the total interaction sums of squares, its effect was highly significant and accounted for nearly all of the cultivar × strain variability (Table 4). The cultivar × strain interaction for the remaining five degrees of freedom was no longer significant, indicating that the significant cultivar × strain interaction was caused by the reaction of Quinte to strain Ea 266 as compared to the other strains.

TABLE 4. Summary of analysis of variance for severity of fire blight on apple cultivars inoculated with strains of *Erwinia amylovora* in greenhouse tests

Source of variation	Degrees of freedom	Sum of squares[a]	F
Cultivars	3	9.302	
Strains	2	3.103	
Cultivars × strains	6	2.264	6.5**
Quinte × Ea 266	1	2.057	35.72**
Remaining	5	0.207	0.72
Error	168	9.677	

[a] Calculated from arc-sine $\sqrt{(x)}$, in which x = the proportion of the current season's shoot length that was necrotic. Asterisks (**) denote statistical significance, $P = 0.01$.

DISCUSSION

In these studies the virulence of strains of *E. amylovora* was evaluated based on the severity of fire blight infection caused on apple cultivars, not on the incidence of infection. A severe inoculation technique was used in order to reliably establish infection and then to quantify its severity by determining the percent of the shoot length that became infected.

Our data clearly indicate that differential interactions occur between cultivars of apple and strains of *E. amylovora*. A significant cultivar × strain interaction in the two-way analysis of variance is an indication of differential cultivar-specific virulence (26). The differential virulence of strain Ea 266 for Quinte was clearly demonstrated by two seasons of orchard tests and by greenhouse tests. Other differential interactions occurred in the

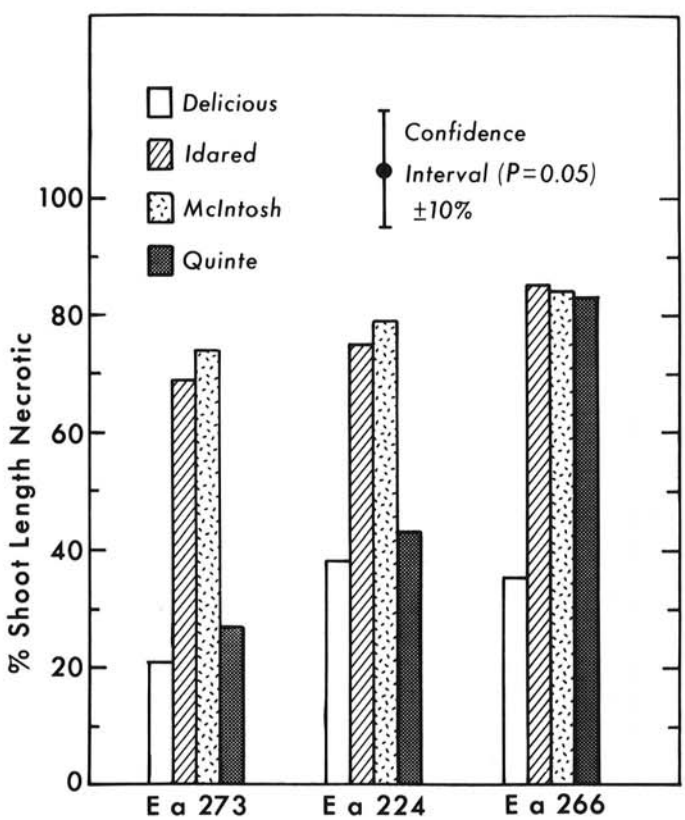

Fig. 1. Severity of fire blight on four apple cultivars inoculated with three strains of *Erwinia amylovora* in a greenhouse test. The disease severity value is expressed as percent of current season's shoot length that was necrotic and represents the mean of 15 replicates.

orchard trials, such as the McIntosh × Ea 224 interaction, but these were much smaller. All trees were at approximately the same growth stage when inoculated and differences in tissue succulence would not have accounted for these interactions.

Aldwinckle and Preczewski (2) evaluated 92 apple cultivars for their relative susceptibility to fire blight, based upon the severity of the infection, by means of controlled inoculation using a single standard virulent strain of *E. amylovora* (Ea 273). In general, the evaluation of cultivar susceptibility from these controlled inoculations agreed with susceptibility observed in the orchard (4). One notable exception has been the cultivar Quinte. Severe fire blight infection has been reported to occur on Quinte in the orchard (R. L. Norton, *personal communication*) although Quinte appeared resistant in controlled inoculation tests. There are several possible explanations for the orchard infection of a resistant cultivar, including growth under extremely favorable environmental conditions for disease development, increased susceptibility as a consequence of horticultural practices, or by growth on a particular rootstock (1). However, the results of the studies reported here indicate that severe infections in the orchard might be due to strains of *E. amylovora* with specific virulence for Quinte. Unfortunately, no strains of *E. amylovora* have been isolated from infections of Quinte. It is interesting to note that both Ea 266 and Ea 273 originated from the cultivar Rhode Island Greening (Table 1) and that, unlike some other diseases studied (5,6,13), there was no association between host cultivar of origin and differential virulence.

There are several possible explanations for the disagreement between our results on apple and the results of Quamme and Bonn (20) on pear concerning the existence of differential virulence in *E. amylovora*. The four cultivars they used may have been insufficient to detect differential virulence. A more likely explanation may be differences in the genetics of fire blight resistance in apple and pear. Resistance to *E. amylovora* in *Pyrus communis* and other *Pyrus* species is reported to be polygenically controlled (17). Gardner et al (12) found that fire blight resistance in apple could be conditioned polygenically or oligogenically. Although the inheritance of resistance in Quinte has not been studied in detail, the resistance of other highly resistant cultivars of *Malus* appears to be controlled by a few dominant genes (12). Differential virulence to host cultivar is more likely with oligogenic than with polygenic resistance (26).

Our results indicate that the evaluation and selection of fire blight resistance may be affected by the virulence of the strains of *E. amylovora* used in screening. In resistance breeding, steps must be taken to ensure that progenies are challenged by strains representative of the complete range of differential virulence in *E. amylovora*.

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