Internal Movement of *Erwinia amylovora* Through Symptomless Apple Scion Tissues into the Rootstock

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ABSTRACT

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Shoot tips of potted Empire and Golden Delicious trees on the susceptible dwarfing rootstock M.26 in the greenhouse were injected with inoculum containing 5×10^9 CFU/ml *Erwinia amylovora*. At intervals after inoculation, trees were sampled at increments between the shoot tip and the roots by excising stem segments. Segments were ground in phosphate buffer and assayed for the presence of *E. amylovora* by plating on semi-selective medium and by a polymerase chain reaction (PCR)-based detection method. Eleven days after inoculation, *E. amylovora* was detected by PCR in symptomless scion tissue >50 cm below the shoot-tip in Empire and Golden Delicious, and in 2-year-old tissue in Golden Delicious. By 21 days, *E. amylovora* was detected in the M.26 rootstock of Empire trees, and by 41 days in the M.26 rootstock of Golden Delicious. In a similar experiment the following year, Empire trees on M.26 rootstock were inoculated with *E. amylovora* at early (16 May), mid- (11 June), and late (6 July) phenophase of shoots. Three weeks after inoculation, *E. amylovora* was detected by PCR from M.26 rootstocks of five of six plants inoculated at the late phenophase, compared to zero of six plants inoculated at the early or mid-phenophase. Late-season fire blight infections of the scion may be particularly hazardous for the health of the rootstock.

Fire blight caused by *Erwinia amylovora* (Burrill) Winslow et al. is a serious, often devastating disease of apples, pears, and many members of the family Rosaceae. It can kill dwarf apple trees by girdling susceptible rootstocks or interstems. It is an increasing problem in North America in high-density apple orchards planted on M.9 and M.26 rootstocks, which are both very susceptible to fire blight (15,22). Since it is virtually impossible to eradicate an infection once it has penetrated the rootstock, prevention is crucial. Hypotheses for infection of rootstocks include spread through infected rootstock suckers; direct infection of rootstocks through burrknot cracks and wounds caused by mechanical, insect, or freeze injuries; and internal spread of bacteria from infections in the scion. Although some infections of rootstocks are clearly attributable to extension

of infection from suckers, in many instances of rootstock infection suckers were not present. Furthermore, rootstock infection frequently occurs on trees with no visible evidence of direct infection through a growth crack, wound, or burrknot.

Gowda and Goodman detected *E. amylovora* 70 cm below the stem apex 2 weeks after inoculation, and bacteria were found well in advance of visible symptoms (7). *E. amylovora* was recovered from symptomless side shoots of 20% of Red Jonathan-M.7 trees grown in the greenhouse for about 10 months after inoculation (6). *E. amylovora* can also be resident in symptomless apple and pear tissue, which may explain the unexpected appearance of fire blight disease in nurseries, young plantings, and bearing orchards (10). Although these and other studies have documented the presence of *E. amylovora* in symptomless tissues, they have not attempted to follow the spread of *E. amylovora* through symptomless scion tissue into the rootstock. More recently, sensitive and species-specific polymerase chain reaction (PCR) methods have been developed that make it feasible to detect low internal populations of *E. amylovora* (3,4,12). Combining PCR with biological amplification, or enrichment, has been used to reduce or eliminate the detection of nonviable bacterial cells and dilute PCR inhibitors that may be present in plant samples (20). The objective of the present research

was to determine if cells of *E. amylovora* could move systemically through healthy scion tissue to the rootstock, and if time of scion infection relative to tree phenology (phenophase of shoot) affects internal movement of the bacteria. Preliminary results of this work have been reported (14,16).

MATERIALS AND METHODS

Bacterial strains and inoculum. *E. amylovora* strain Ea273 (21) and its nalidixic acid-resistant mutant, Ea273Nal^R (17), were used in these studies. Inoculum consisted of 18-h-old cultures grown in Kado 523 broth (9) at 28°C, and contained approximately 5×10^9 CFU/ml *E. amylovora*. The concentration of the inoculum was estimated by absorbance at 620 nm.

Plant material, inoculation, and sampling. Internal downward movement of *E. amylovora* was studied in potted trees grafted on M.26 rootstock that were grown in the greenhouse and trained to a single shoot. To avoid external contamination of rootstocks with *E. amylovora* from shoot infections, each pot was carefully watered from below. Pesticides were applied to prevent aphid and mite infestation. In 1995, the surfaces of M.26 rootstocks were monitored for the presence of epiphytic *E. amylovora* using a cotton swab method (1,2). Ten randomly chosen plants were monitored 1 day before the last assay date (15 weeks after inoculation) of each inoculation time treatment. Swabbing samples, as well as negative and positive controls of phosphate buffer and *E. amylovora*, respectively, were plated directly on modified Miller-Schroth medium (MMS; 5) plus nalidixic acid medium, and assayed for the presence of *E. amylovora* by PCR.

Shoot tips of trees were inoculated by inserting a 0.46-mm-diameter (26-gauge) hypodermic needle through the stem just above the youngest unfolded leaf. Sufficient inoculum was introduced to fill the wound, leaving drops at each end of the wounds when the needle was removed (18).

In 1994, potted Empire and Golden Delicious trees on M.26 rootstock in the greenhouse were inoculated with strain Ea273. Most of the shoots were at a late stage of growth and were not growing vigorously at the time of inoculation. Three inoculated and three non-inoculated shoots were sacrificed and assayed for *E. amylo-*

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vora 5, 11, 21, and 41 days after inoculation. Scion wood was assayed at 10, 25, and 50 cm below the shoot tip of <1-year-old wood, at 1 cm above the margin of 2-year-old wood, at the mid-point of 2-year-old wood, and at the mid-point of 3-year-old wood. The rootstock tissue was assayed 1 cm below the graft union.

In 1995, Empire trees on M.26 rootstock were grown in pots in the greenhouse with either a normal or a high level of nitrogen fertilization. Normal nitrogen fertilization consisted of 23.5 g 17:6:12 (N:P:K) slow-release fertilizer (Sierra-Scotts, Marysville, OH) incorporated into the soil of each 22-cm-diameter pot; high nitrogen fertilization consisted of the same amount of the slow-release fertilizer plus 5 g nitrogen in the form of calcium nitrate. Shoot tips were inoculated with Ea273Nal^R at three phenophases: early (16 May), mid- (11 June), and late phenophase (6 July). Six inoculated and three non-inoculated trees were sacrificed and assayed for the presence of *E. amylovora* at 3, 6, and 15 weeks after inoculation. Scion wood was assayed at the mid-point of symptomless 2-year-old wood, and the rootstock was assayed 1 cm below the graft union.

Detection of *E. amylovora*. Segments of shoots (4 cm long) were surface-sterilized with 0.5% NaOCl for 2 min and washed three times with tap water. Segments were then trimmed to 1 cm in length by removal of both ends of the shoot. Tissues external to the hard wood were shaved off and ground (Polytron, Brinkman Instruments, Westbury, NY) in 5 ml sterile potassium phosphate buffer (0.05 M, pH 6.5) three times for 5 s under sterile conditions. The polytron was sterilized between grindings by dipping the head in 70% ethanol and flaming. In 1994 tests, 100- μ l aliquots of grindate were plated on MMS and incubated at 28°C and 75% relative humidity for 48 h. In 1995, aliquots were plated on MMS amended with nalidixic acid (50 mg/liter). When typical colonies (orange-yellow colonies with deep orange centers and clear margins) were observed, one

representative colony per plate was subjected to PCR for confirmation of the identification.

For enrichment, 100- μ l aliquots of grindate also were plated on nutrient agar (NA), and incubated for 48 h at 28°C. NA plates were washed with 2 ml sterile water. Washings were assayed by PCR (8) using two 17-mer oligonucleotides (primer A: 5'-CGGTTTTTAACGCTGGG; primer B: 5'-GGGCAAATACTCGG-ATT; 4), which amplify a 1-kilobase (kb) fragment (12) of a 29-kb plasmid common to most strains of *E. amylovora*. PCR was carried out in a total volume of 100 μ l containing 50 pmol of each primer (A and B), 2.5 U *Taq* DNA polymerase (Promega Corp., Madison, WI), 20 μ l DNA template (bacterial cells washed from NA were subjected to direct PCR), 0.4 mM each of dATP, dCTP, dGTP, and dTTP (Boehringer-Mannheim Biochemicals, Indianapolis, IN), reaction buffer (10 mM TrisHCl, 50 mM KCl, and 0.1% TritonX-100), and 2.5 mM MgCl₂ under three drops of mineral oil. The PCR was performed in a Hybaid Omni Gene

DNA Thermal Cycler (National Labnet Co., Middlesex, UK) or PTC-100 Programmable Thermal Controller (MJ Research, Inc., San Francisco). Initial denaturation was done at 94°C for 2 min. *Taq* DNA polymerase was added to the reaction tubes during denaturation (hot start). Subsequent cycles were as follows: denaturation at 94°C for 1 min, annealing at 52°C for 2 min, and extension at 72°C for 2 min. After 30 cycles, final extension (1 cycle) was at 72°C for 6 min (13). The PCR products were separated on a 1% agarose gel, stained with ethidium bromide, and photographed under UV light. Three replicates each of phosphate buffer and *E. amylovora* served as negative and positive controls, respectively, in all PCR assays. A signal at 1 kb on the agarose gel identified the presence of *E. amylovora* in the tissue tested.

Southern blot analysis was performed on all positive PCR samples from the 1995 test. DNA from PCR analysis was transferred from the gel to GeneScreen Plus (NEN Research, Boston, MA) by capillary

Table 1. Sensitivity of detection of *Erwinia amylovora* by direct plating and by enrichment on nutrient agar (NA) followed by polymerase chain reaction (PCR) with primers A and B

Anticipated CFU/ml ^a	Detected CFU/ml ^b	DNA fragment ^c
3×10^3	450	+
3×10^2	130	+
3×10	30	+
3	0	-

^a Concentration calculated by spectrophotometry from a standard curve. Apple tissues were ground with known *E. amylovora* CFU/ml.

^b Detected by direct plating on Miller-Schroth medium. Mean of two experiments.

^c Detection by NA enrichment and PCR. (+) indicates the presence of a 1-kilobase diagnostic DNA fragment on agarose gel after PCR and (-) indicates its absence.

Fig. 1. Detection of *Erwinia amylovora* in inoculated Empire/M.26 and Golden Delicious/M.26 apple plants by direct plating on modified Miller Schroth medium and identification confirmed by polymerase chain reaction. Typical *E. amylovora* colonies (black) detected or (white) not detected; A, B, and C are three different replicate plants, which are different for each sampling date. Scion wood was assayed at 10, 25, and 50 cm below the shoot tip of <1-year-old wood, at 1 cm above the margin of 2-year-old wood, at the mid-point of 2-year-old wood, and at the mid-point of 3-year-old wood. M.26 rootstock tissue was assayed 1 cm below the graft union (1994 experiment).

transfer under alkaline conditions as described by Sambrook et al. (19), except that the transfer solution was 1 M NaOH, 3 M NaCl. The membrane was hybridized with labeled probe of the 1-kb DNA frag-

ment of *E. amylovora* Ea273. The probe was synthesized by PCR from a pure culture of strain Ea273 using primers A and B (4). The PCR product was run on an agarose gel, excised, purified with Prep-A-

Gene (Bio-Rad Laboratories, Richmond, CA), and DIG-labeled with the Genius System (Boehringer GmbH, Mannheim, Germany) according to manufacturer's instructions.

In order to determine and compare the sensitivity of direct plating on MMS and enrichment on NA followed by PCR for detecting *E. amylovora*, noninfected Empire shoot tissue was ground in sterile potassium phosphate buffer (0.05 M, pH 6.5) amended with *E. amylovora* to approximate concentrations of 3×10^3 , 3×10^2 , 3×10 , or 3 CFU/ml. Detection was attempted twice using both methods.

RESULTS

Preliminary tests indicated that direct plating on MMS medium and enrichment followed by PCR were of approximately equal sensitivity in detecting *E. amylovora*, and the approximate limit of detection was 150 cells per tissue sample or 30 cells/ml (Table 1). However, in plant material inoculated in the greenhouse, *E. amylovora* was detected much more frequently by enrichment followed by PCR than by direct plating (Fig. 1 and Table 2 versus Fig. 2 and Table 3).

In 1994, the length of necrotic lesion was 1 to 4 cm for Empire and 1 to 5 cm for Golden Delicious. *E. amylovora* was seldom detected by direct plating in tissue 10 cm below the necrosis margin in symptomless current season's shoot growth of Empire or Golden Delicious trees (Fig. 1). It was detected by direct plating in symptomless 3-year-old scion and rootstock tissue of one Empire tree 5 days after inoculation. Although these isolates were confirmed to be *E. amylovora* by PCR, they appeared different from the inoculated strain, Ea273, in that no ooze was produced in an immature pear fruit test. No *E. amylovora* was detected in non-inoculated trees by direct plating.

Using enrichment followed by PCR, *E. amylovora* was detected 11 days after inoculation throughout symptomless current season's shoot growth of both Golden Delicious and Empire trees and in symptomless 2-year-old tissues of Golden Delicious (Fig. 2). *E. amylovora* was detected throughout Empire trees 21 days after inoculation in symptomless current season's tissue, in 2- and 3-year-old scion tissue, and in symptomless M.26 rootstock tissues; however, it was only detected in the current season's and 2-year-old symptomless tissues of Golden Delicious. By 41 days after inoculation, *E. amylovora* was detected in the M.26 rootstock of Golden Delicious trees, as well as the 2- and 3-year-old wood, all of which were symptomless. In Empire trees, *E. amylovora* was not detected in symptomless 2- or 3-year-old scion tissues, or in M.26 rootstock tissues 41 days after inoculation. *E. amylovora* was detected in two of three non-inoculated Empire trees in the 21-day assay

Table 2. Frequency of detection of *Erwinia amylovora* in inoculated Empire-M.26 apple plants assayed 3, 6, and 15 weeks after inoculation by direct plating on Miller-Schroth medium amended with nalidixic acid (1995 experiment)

Phenophase ^a	Weeks after inoculation	Date assayed	Scion: Empire ^{b,c}	Rootstock: M.26 ^{c,d}
Early	3	6 Jun	0/6	0/6
	6	27 Jun	0/6	0/6
	15	30 Aug	1/6	1/6
Mid	3	3 Jul	0/6	0/6
	6	26 Jul	1/6	1/6
	15	25 Sep	0/6	0/6
Late	3	27 Jul	2/6	2/6
	6	18 Aug	0/6	0/6
	15	18 Oct	0/6	0/6

^a Phenophase of shoot at inoculation: early, inoculated 16 May, mid, inoculated 11 June; late, inoculated 6 July.

^b Scion wood was assayed at the mid-point of symptomless 2-year wood.

^c *E. amylovora* strain Ea273 NaI^R was not detected from non-inoculated control trees.

^d Rootstock was assayed 1 cm below the graft union.

Fig. 2. Detection of *Erwinia amylovora* in inoculated Empire/M.26 and Golden Delicious/M.26 apple plants by enrichment on nutrient agar followed by polymerase chain reaction (PCR). Black indicates the presence of 1-kilobase diagnostic DNA fragment on an agarose gel after PCR and white indicates its absence; A, B and C are three different replicate plants, which are different for each sampling date. Scion wood was assayed at 10, 25, and 50 cm below the shoot tip of <1-year-old wood, at 1 cm above the margin of 2-year-old wood, at the mid-point of 2-year-old wood, and at the mid-point of 3-year-old wood. M.26 rootstock tissue was assayed 1 cm below the graft union (1994 experiment).

and in one non-inoculated Golden Delicious tree in the 41-day assay. However, *E. amylovora* was never detected in the 2- or 3-year-old scion tissues or M.26 rootstock tissues of any non-inoculated trees. Three replicates of phosphate buffer and *E. amylovora* tested negative and positive, respectively, throughout the PCR tests.

In 1995, in tests using strain Ea273Nal^R, trees receiving different nitrogen fertilization treatments did not differ significantly in the amount of shoot blight resulting from inoculation or in the presence of internal *E. amylovora* (data not shown). Leaf analysis indicated foliar nitrogen levels at 2.81 and 2.84% NH₃-N in low- and high-nitrogen treatments, respectively. Due to the lack of detectable difference between nitrogen treatments, data between treatments were combined for analysis.

The detection of *E. amylovora* in M.26 rootstock in 1995 was more closely associated with the phenophase of plants than with the amount of time after inoculation, regardless of the method of detection used (Tables 2 and 3). For example, detection in rootstocks of plants inoculated at the early phenophase occurred 15 weeks after inoculation; in contrast, in plants inoculated at the late phenophase, detection of *E. amylovora* in the M.26 rootstock occurred only 3 weeks after inoculation. Using enrichment followed by PCR, *E. amylovora* was detected in the rootstocks of five of six plants inoculated at the late phenophase, compared with one of six and two of six plants inoculated at the early and mid-phenophases, respectively.

Detection of *E. amylovora* in M.26 rootstock was usually associated with detection in the 2-year-old wood of the Empire scion. However, 6 weeks after inoculation of mid-phenophase shoots, *E. amylovora* was detected by PCR in the rootstock without corresponding detection in the scion (Table 3). In contrast, 3 and 6 weeks after inoculation of early-phenophase shoots and 6 weeks after inoculation of late-phenophase shoots, *E. amylovora* was

detected by PCR in the scion without corresponding detection in the rootstock.

As with the 1994 tests, phosphate buffer and *E. amylovora* were included as negative and positive controls, respectively, in all PCR tests (three of each) and consistently gave the expected results. In addition, necrotic shoot tissue resulting from inoculation were assayed by PCR in 1995 and consistently indicated the presence of *E. amylovora* (Ea273Nal^R). *E. amylovora* (Ea273Nal^R) could not be detected by either direct plating on MMS plus nalidixic acid medium or PCR on the surface of M.26 rootstocks of early-, mid-, or late-phenophase trees 15 weeks after inoculation after swabbings.

To confirm that PCR samples were the result of amplification of *E. amylovora* DNA and were not artifact bands, Southern analysis of 1995 PCR positive samples was conducted using authentic *E. amylovora* DNA as probe. In all cases, PCR-positive samples hybridized with the probe, indicating that the PCR bands were due to amplification of *E. amylovora* DNA (Fig. 3).

DISCUSSION

These data indicate that *E. amylovora* can move systemically from infections in the scion through symptomless tissue into rootstock tissue. *E. amylovora* was isolated or detected by enrichment on NA followed by PCR in the symptomless woody scion tissues and, most importantly, in M.26 rootstock tissues in 2 years of greenhouse tests. There was limited evidence in these experiments of contamination of greenhouse trees with *E. amylovora* from other sources. However, our inability to detect *E. amylovora* in the rootstock tissues, 2-year-old tissues, or 3-year-old tissues of any non-inoculated trees in the 1994 experiment, and the large differences between detection in inoculated and non-inoculated trees in 1995, indicate that the observed movement into the rootstock could not be explained by contamination. In addition,

our inability to isolate *E. amylovora* from the surface of greenhouse trees supports the hypothesis of internal movement of bacteria. The detection of *E. amylovora* in non-inoculated trees usually occurred late in the progress of the experiment and may have resulted from transmission by insects.

Downward systemic movement of *E. amylovora* from shoot tip infections through healthy shoot tissue into the M.26 rootstock was demonstrated in two different scion cultivars, Empire and Golden Delicious. The rate of movement through Golden Delicious and Empire tissue appeared to differ. In Golden Delicious, significant movement was detected within 5 days, but *E. amylovora* was not detected in the rootstock until 41 days after inoculation. In contrast, systemic movement in Empire was not detected until 11 days after inoculation, but *E. amylovora* was detected in rootstock tissues within 21 days of inoculation of shoot tips in 1994 and 1995. Gowda and Goodman (7) reported that the movement of *E. amylovora* in Jonathan proceeded the greatest distance from the point of inoculation (up to 70 cm) in the first 14 days. The presence of *E. amylovora* in apparently healthy shoots 5 to 11

Table 3. Frequency of detection of *Erwinia amylovora* in inoculated Empire-M.26 apple plants assayed 3, 6, and 15 weeks after inoculation by direct plating on nutrient agar followed by polymerase chain reaction (PCR; 1995 experiment)

Phenophase ^a	Weeks after inoculation	Date assayed	Scion: Empire ^{b,c}	Rootstock: M.26 ^{c,d}
Early	3	6 Jun	2/6	0/6
	6	27 Jun	1/6	0/6
	15	30 Aug	2/6	1/6
Mid	3	3 Jul	0/6	0/6
	6	26 Jul	0/6	2/6
	15	25 Sep	0/6	0/6
Late	3	27 Jul	4/6	5/6
	6	18 Aug	2/6	0/6
	15	18 Oct	0/6	0/6

^a Phenophase of shoot at inoculation: early, inoculated 16 May, mid, inoculated 11 June; late, inoculated 6 July.

^b Scion wood was assayed at the mid-point of symptomless 2-year wood.

^c *E. amylovora* was detected in some non-inoculated control trees of the late phenophase assay; and from one scion and one rootstock tissue of the 27 July assay and one rootstock tissue of the 18 Aug assay. In all PCR assays, no bacteria were detected in negative control buffers.

^d Rootstock was assayed 1 cm below the graft union.

Fig. 3. (A) Polymerase chain reaction (PCR) analysis and (B) corresponding Southern blot hybridized with authentic *Erwinia amylovora* DNA of grindates of stem segments from trees inoculated with *E. amylovora* 3 weeks earlier at the late phenophase. Origin of samples and (+) detection or (–) no detection of *E. amylovora*: 1, 2-year-old Empire scion of inoculated tree (+); 2 and 3, M.26 rootstock of inoculated tree (+); 4, 2-year-old Empire scion of inoculated tree (–); 5, M.26 rootstock of inoculated tree (–); 6, M.26 rootstock of non-inoculated tree (–); 7, phosphate buffer control (–); 8, necrotic lesion from inoculated tree (+); 9, pure culture of Ea273Nal^R (+); and M, PCR marker (Promega Corp., Madison, WI).

days after inoculation and in rootstocks 21 days after inoculation has important implications for orchard pruning practices, especially on small trees. Bacteria are probably present beyond where most pruning cuts are made, even when infections are removed shortly after the appearance of symptoms.

Although these data indicate that *E. amylovora* can move systemically from scion infections into rootstock tissues, they do not establish that the presence of bacteria leads to necrotic infection of the rootstock. In both experiments, after initial detection of *E. amylovora* in rootstock tissues, we were unable to detect the bacteria at later sampling dates. Similar results were reported by Gowda and Goodman (7), who observed that the population of bacteria decreased at distances greater than 20 cm 4 and 6 weeks after inoculation. This implies that *E. amylovora* populations quickly decreased in rootstock tissues. Perhaps physiological conditions, such as phenology, nutrient status, or other predisposing factors, are required for necrotic infection to occur after ingress of bacteria. Alternatively, a resident population of bacteria below the detection limits may become established in the rootstock and result in necrotic infection after some predisposing event occurs, such as winter injury. The factors determining whether necrotic infections develop from the detected populations will be investigated in future research.

The internal movement of *E. amylovora* from shoots to rootstocks appeared to be dependent on phenophase. In 1995, internal movement to the rootstock was detected in the later stages of shoot growth regardless of when the trees were inoculated (Tables 2 and 3). In addition, Empire trees inoculated at the late phenophase had the greatest amount of internal spread of *E. amylovora* to the rootstock, and it was detected soonest after inoculation. Although plants in the 1994 test were inoculated at only one phenophase, it was at a late phenophase when terminal buds were beginning to form. Lewis and Goodman (11) reported that *E. amylovora* spread more rapidly in actively growing shoots than in hardened-off shoots, but they were studying the upward movement of the bacteria. Late-season fire blight infections of the scion may be particularly hazardous for the health of the rootstock.

The recovery frequencies on the semi-selective medium MMS are inversely related to the original concentration of the bacterial suspension tested. The rate is high when the initial bacterial concentration is low. This might be due to single colony

formation by more than one bacteria with higher concentrations (Table 1).

The reported limit of sensitivity for detecting *E. amylovora* from plant tissue by first-round PCR with primers A and B has ranged from 50 up to 1,000 cells (3,12). The method used in this work combined viable cell enrichment on NA with first-round PCR to limit detection to viable cells, improve sensitivity, and reduce the inhibitory effect of PCR inhibitor such as polyphenols without attempting DNA extraction (20). Detection of *E. amylovora* by PCR after enrichment on NA plates appeared far more sensitive than direct plating on MMS medium in the greenhouse tests but not in controlled laboratory (in vitro) tests. Because of the enrichment step prior to PCR detection, this difference is not likely to be due to the detection of nonviable *E. amylovora* cells in greenhouse trees. The enrichment procedure resulted in a 5,000-fold dilution from grindate to PCR mixture (2% of grindates used for enrichment $\times$ 1% of enrichment used for PCR), which should have eliminated the possibility of detecting DNA from nonviable *E. amylovora* cells. It may be possible to increase detection sensitivity by the use of nested PCR (12,20).

Previous studies demonstrated that *E. amylovora* could spread rapidly in phloem tissue (7,11). Although the research reported here did not address the tissues in which systemic movement occurred, the results do support previous findings of rapid internal movement of *E. amylovora* in woody tissues, and indicate that this spread not only occurs in scion tissue but also allows for movement of the bacteria into rootstock tissue.

LITERATURE CITED

- Beer, S. V., and Norelli, J. L. 1977. Fire blight epidemiology: factors affecting release of *Erwinia amylovora* by cankers. *Phytopathology* 67:1119-1125.
- Beer, S. V., and Oppenorth, D. C. 1976. *Erwinia amylovora* on fire blight canker surfaces and blossoms in relation to disease occurrence. *Phytopathology* 66:317-322.
- Bereswill, S., Pahl A., Belleman, P., Berger, F., Zeller, W., and Geider, K. 1993. Efficient detection of *Erwinia amylovora* by PCR analysis. *Acta Hort.* 338:51-58.
- Bereswill, S., Pahl A., Belleman, P., Zeller, W., and Geider, K. 1992. Sensitive and species-specific detection of *Erwinia amylovora* by polymerase chain reaction analysis. *Appl. Environ. Microbiol.* 58:3522-3526.
- Brulez, W., and Zeller, W. 1981. Seasonal changes of epiphytic *Erwinia amylovora* on ornamentals in relation to weather conditions and the course of infection. *Acta Hort.* 117:37-43.
- Ge, Q., and van der Zwet, T. 1996. Persistence and recovery of endophytic *Erwinia amylovora* in apparently healthy apple tissues. *Acta Hort.* 411:29-33.
- Gowda, S. S., and Goodman, R. N. 1970. Movement and persistence of *Erwinia amylovora* in shoot, stem, and root of apple. *Plant Dis. Rep.* 54:576-580.
- Innis, M. A., Gelfand D. H., Sninsky J. J., and White T. J. 1990. *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., San Diego, CA.
- Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology* 60:969-976.
- Keil, H. L., and van der Zwet, T. 1972. Recovery of *Erwinia amylovora* from symptomless stems and shoots of Jonathan apple and Bartlett pear trees. *Phytopathology* 62:39-42.
- Lewis, S. M., and Goodman, R. N. 1965. Mode of penetration and movement of fire blight bacteria in apple leaf and stem tissue. *Phytopathology* 55:719-723.
- McManus, P. S., and Jones, A. L. 1995. Detection of *Erwinia amylovora* by nested PCR and PCR-dot-blot and reverse-blot hybridizations. *Phytopathology* 85:618-623.
- Momol, E. A., Momol, M. T., and Hacıoglu, E. 1994. A PCR approach for early and specific detection of *Erwinia amylovora* in monitoring studies in Turkey. (Abstr.) *Phytopathology* 84:1087.
- Momol, M. T., Momol, E. A., Norelli, J. L., Gustafson, H. L., Cummins, J. N., and Aldwinckle, H. S. 1994. Internal spread of *Erwinia amylovora* through symptomless apple scion tissues into the rootstock. (Abstr.) *Phytopathology* 84:1373.
- Momol, M. T., Norelli, J. L., Breth D. I., and Aldwinckle, H. S. 1997. Rootstock blight of apple caused by *Erwinia amylovora*: Case studies from New York State. (Abstr.) *Phytopathology* 87:S67.
- Momol, M. T., Norelli, J. L., Piccioni, D., and Aldwinckle, H. S. 1996. Effect of growth stage of apple shoots on the internal spread of *Erwinia amylovora* into the rootstock. (Abstr.) *Phytopathology* 86:S16.
- Norelli, J. L., Aldwinckle, H. S., and Beer, S. V. 1988. Virulence of *Erwinia amylovora* strains to *Malus* sp. Novole plants grown in vitro and in the greenhouse. *Phytopathology* 78:1292-1297.
- Norelli, J. L., Aldwinckle, H. S., Beer, S. V., and Lamb, R. C. 1987. The effects of virulence of *Erwinia amylovora* on the evaluation of fire blight resistance in *Malus*. *Phytopathology* 77:1551-1555.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Schaad, N. W., Cheong, S. S., Tamaki, S., Hatziloukas, E., and Panopoulos, N. J. 1995. A combined biological and enzymatic amplification (BIO-PCR) technique to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seed extracts. *Phytopathology* 85:243-248.
- Seemüller, E. A., and Beer, S. V. 1976. Absence of cell wall polysaccharide degradation by *Erwinia amylovora*. *Phytopathology* 66:433-436.
- van der Zwet, T., and Miller, S. S. 1987. Occurrence of severe fire blight in Malling 26 rootstock. (Abstr.) *Phytopathology* 77:990.