Population of *Erwinia amylovora* on External and Internal Apple Fruit Tissues

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ABSTRACT

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Injury of apple fruit at time of dip inoculation with *Erwinia amylovora* led to increased disease when compared to injury after or before inoculation. Few fruit expressed symptoms when injured 96 hr after inoculation. Fruit of Delicious, a relatively resistant cultivar, and Rome Beauty, a susceptible one, became infected more frequently when injured with bruising or large punctures than with small punctures. Blight developed on 4% of Rome Beauty fruit that were punctured but not artificially inoculated. Moreover, only 1% of 125 surface-disinfested Rome Beauty fruit, collected from apparently healthy trees, developed blight symptoms during a 4-mo cold storage period, compared to as much as 15% of fruit taken from blighted trees. No *E. amylovora* was detected on or inside 210 Delicious fruit that had been obtained from two orchards without blight in Washington state. Endophytic populations of *E. amylovora* were recovered from apples located within 30 cm from blighted shoots but not from those 60 or 200 cm away. The bacterium was not detected in core tissues of 280 apples sampled from four cultivars collected from apparently healthy trees grown in four regions of North America. Thus, chances for the dissemination of *E. amylovora* to areas or countries without fire blight is extremely unlikely when undamaged Delicious fruit are harvested a minimum of 100 cm from visible blight symptoms or, preferably, from apparently healthy trees located in orchards free of fire blight.

Additional keywords: detection, export, quarantine

Erwinia amylovora (Burr.) Winslow et al has been readily isolated from symptomless leaves (17,33), flowers (2,25,33), twigs (6,10,15,17), buds (1), and fruit (5,8,22) from pear (*Pyrus communis* L.) and apple (*Malus domestica* Borkh.) orchards in the United States and Canada, especially in areas where fire blight is prevalent. Recently, *E. amylovora* was isolated from apparently healthy pear scion wood that had been collected from blighted trees and was propagated on symptomless rootstock at the Appalachian Fruit Research Station (AFRS) in Kearneysville, West Virginia (28,30). Aerial strands (16,23) and aphids (19) have also been suggested as possible forms by which this bacterium may be disseminated to shoots and fruit.

Very little information is available on populations of *E. amylovora* on or in pear or apple fruit. This pathogen can infect the fruit and cause extensive oozing on pear but considerably less on apple. Borden and Thomas (3) reported that Bartlett pear trees in California sprayed with a summer oil had 17% of the fruit infected with *E. amylovora*, compared to only 0.5% of fruit infected on trees not sprayed with oil. In 1965, 30–50% of fruit in a shipment of apparently healthy Bartlett pears that originated from blighted orchards in California had developed fruit blight symptoms upon arrival in Hawaii (26). In 1969, the Ministry of Agriculture, Fisheries and Food in Great Britain reported apparent internal disease in pear fruit that appeared healthy externally (11). Pockets of diseased tissue up to 2 cm in diameter were found in the center of the fruit, connected by thin threads of blighted tissue to small bruises approximately 2 mm in diameter on the fruit surface.

Epiphytic populations of *E. amylovora* were not found on mature symptomless Wealthy apples harvested from naturally infected trees in Canada (8). In West Virginia, however, the bacterium was readily recovered from the surface of immature cv. Rome Beauty apples collected from a severely blighted orchard (33). Preliminary attempts to isolate the bacterium from the surface of Delicious apples that had been shipped from Washington in October and stored for 3 mo at 1 C were negative (van der Zwet, *unpublished*).

The objectives of this research were to determine if various fruit injuries predispose apple fruit to attack by *E. amylovora* and to determine if the bacterium survived on the surface of or inside the fruit. Preliminary results from fruit injury experiments were reported (34), and the bacterium was not isolated from core tissues of apples harvested from apparently healthy trees (32).

MATERIALS AND METHODS

Apple cultivars. The highly susceptible cultivars used were Jonathan, Rome Beauty, York, and Idared, and the normally resistant Delicious was used as the principal fruit considered for export. Experiments were performed during the 1984–1986 growing seasons, which included one year (1985) when fire blight was very severe in the part of West Virginia in which the study took place.

Selective culture media. Routine isolations were made on nutrient-yeast-dextrose agar (NYDA). Additionally, three selective media for *E. amylovora* were used: Miller-Schroth (MS) (18), Crosse-Goodman (CG) (7), and Ishimaru-Klos (CCT) (12). Although other microorganisms can grow on these media, colonies of *E. amylovora* were tentatively identified as a yellow-orange colony coloration with a deep orange center on MS, a characteristic cratering of colonies on CG, and near hyaline colonies with light purple centers and cratering on CCT. The buffer (pH 6.5) used for preparation of inoculum, recovery of bacteria, or dilution plate analyses was a combination of 70 ml of monobasic potassium phosphate (0.2 M), 30 ml of dibasic potassium phosphate (0.2 M), and 300 ml of distilled water.

Pathogenicity tests. All presumptive cultures of *Erwinia*-like bacteria recovered from the various fruit tissues were tested for pathogenicity on small (2-cm-diam.) green pear fruit. Fruit were thoroughly washed and placed in large trays. A thin slice of fruit was removed, a loopful of bacteria from a representative colony of the bacterium was placed on the cut surface, and the fruit was incubated for 3 days at 16 C. Production of ooze at wound sites was considered positive for *E. amylovora*. A known strain of *E. amylovora* served as a

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positive control. This pathogenicity test was used to verify all strains reported as *E. amylovora*.

Fruit injury and inoculation. In two laboratory experiments, 252 Rome Beauty and 252 Delicious apples harvested in August and September were submerged for 3 min in 0.65% of sodium hypochlorite, rinsed in deionized water, and dried. At the time of inoculation, they were immersed for 1 min in 10^8 colony-forming units (cfu) of *E. amylovora* per ml of buffer. Following inoculation, fruit were incubated for 14 days on large plastic trays maintained at 21–27 C and 80–90% RH. At intervals of 0, 24, 48, and 96 hr before or after inoculation, 36 fruit were punctured or bruised mechanically. Three degrees of injury were obtained by pressing the side of the fruit against a wooden block with two small nails (4D, finishing), two large nails (8D, coated), or two screw heads (#8, flat head). The nails caused punctures that were 3–5 mm deep and spaced 1.6 cm apart, whereas the flat head screws caused a severe bruise but did not break the skin. The injury block was disinfested with 70% of ethanol between treatments. There were 12 fruit per treatment and control fruit were not disinfested.

Fruit in storage. In 1984, 375 mature Rome Beauty apple fruit were harvested from spurs located 1–3 or 60–120 cm from blighted shoots and were stored for 1–4 mo at 1 C. A control sample of 375 fruit was randomly harvested from blight-free orchards in the same vicinity. Half of the fruit within each sample were treated to eliminate surface bacterial populations (immersed for 3 min in .65% of sodium hypochlorite and rinsed three times in distilled water), whereas the remainder were not. On 21 November (37 days in storage), 18 December (64 days), 16 January (93 days), and 13 February (121 days), fruit were examined for evidence of external and internal disease symptoms. On each date, 20 fruit were individually soaked in 200 ml of phosphate buffer for 15 min and sonicated (Branasonic 527, Shelton, CT) for 30 sec (55,000 cps). Samples (0.2 ml) of the wash water were plated on MS medium.

A similar storage test was performed on a shipment of 210 Delicious fruit collected from two orchards near Wenatchee, Washington. Both were visibly blight-free, but one was located near a moderately blighted Bartlett pear orchard. Twenty fruit from each orchard were examined and tested for populations of *E. amylovora* on the dates described above. After the wash procedure, the fruit were cut into four quarters and examined for internal blight symptoms.

In a third storage experiment, 25 fruit each of Red Rome, York, Winter Banana, Smoothee, Top Red, Red Prince, and Delicious were harvested between 9 September and 6 November 1984 from blight-free trees at AFRS. Fruit were stored for 1 wk at 1 C and then sampled for epiphytic populations of *E. amylovora* as described previously.

Correlation among developing fruit, blight source, and *E. amylovora* populations. During the growing seasons of 1984 and 1985, fruit were examined for internal populations of *E. amylovora* in relation to fruit proximity to blighted shoots. Fruit of Red Rome were collected from points up to 400 cm from blighted shoots. The fruit were surface-disinfested and core sections were removed aseptically with a #6 cork borer. The stem and calyx ends (5 mm) were removed and discarded. The central core was divided into five to eight sections which were plated directly on NYDA. The plates were incubated for 2–3 days at 26 C and examined for bacterial growth.

In 1986, Red Rome fruit were sampled at points of 0, 15, 60, and 200 cm from blighted shoots on the tree (Fig. 1B–E) and washed. Samples of the wash water were plated on selective media. For controls, blighted and apparently healthy fruit were sampled from diseased and symptomless trees, respectively (Fig. 1A, F). Fruit from apparently blight-free trees of York, Delicious, and Golden Delicious served as additional controls.

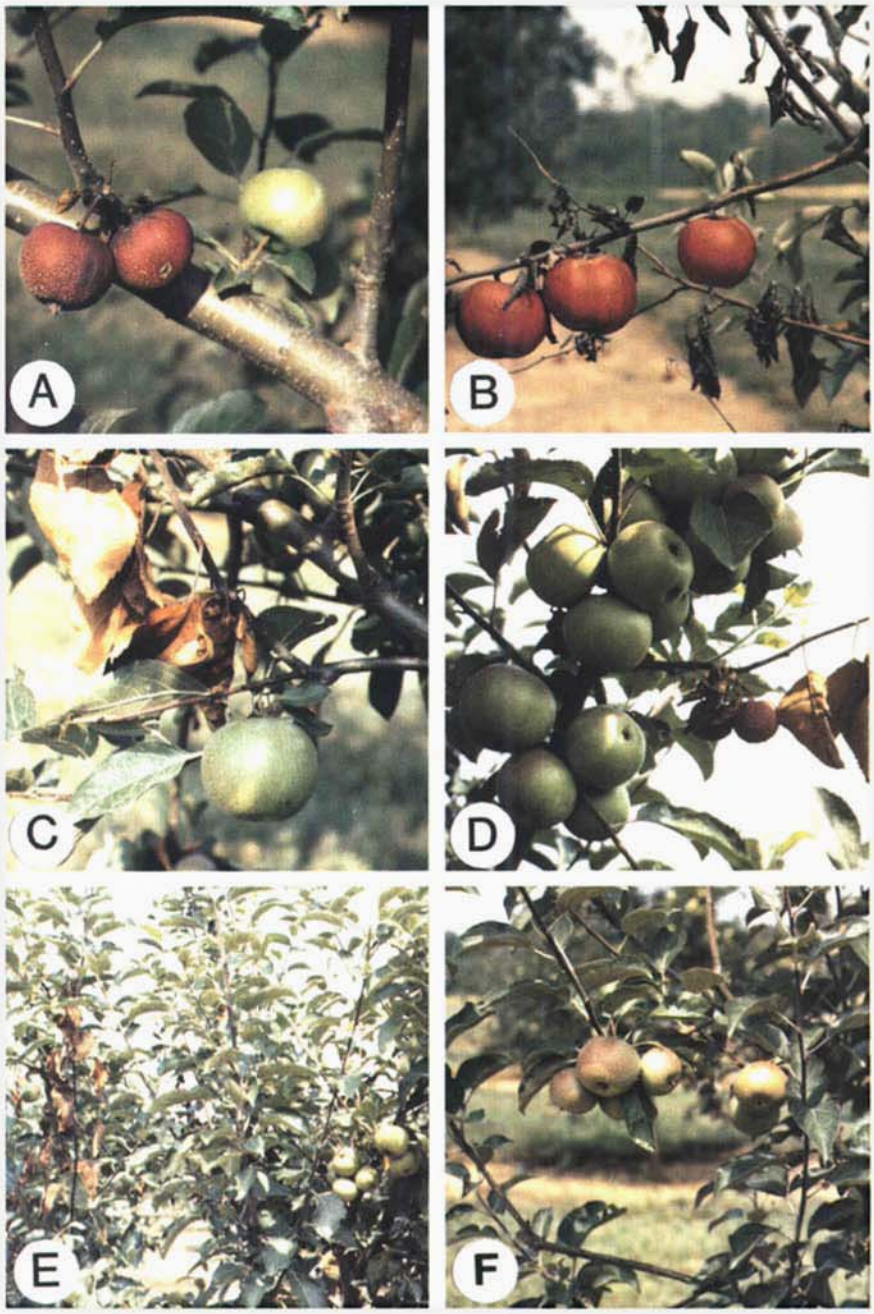

Fig. 1. Location of Red Rome apple fruit on trees in relation to proximity to shoots infected with *Erwinia amylovora*: **(A)** infected fruit following delayed blossom blight infection; **(B)** apparently healthy fruit attached at the base of a blighted shoot; **(C, D and E)** apparently healthy fruit attached to branches within 15 cm, 60 cm, and 200 cm respectively, from blighted shoots; and **(F)** apparently healthy fruit located on tree free of fire blight. Note premature red coloration of infected fruit in A and B.

Clean, disposable gloves were used in harvesting the individual fruit. A new glove was used for each fruit, and care was taken not to rub the fruit unnecessarily. The fruit were placed on new styrofoam trays in a cooler, transported to the laboratory within 2 hr, and analyzed for epiphytic populations as described previously.

Four parts of each fruit were examined for *E. amylovora*: 1) calyx region with remains of pistils and stamens and the adjacent (3 mm) core tissue attached, 2) the stem plus adjacent core tissue (3 mm), 3) the fruit surface, and 4) the internal core without the calyx and stem tissues. The first two parts were removed with a sterile cork borer and placed in a test tube with 1.0 ml of sterile saline containing 0.85% of NaCl. The tube was vortexed for 1 min, and 0.1 ml of the wash suspension was spread onto MS and CCT media. The two open ends of the fruit were then covered with cold petrolatum. The entire fruit was washed in 5 ml of sterile saline in a sealable plastic bag (16 × 16 cm) and 0.1 ml of wash water from the fruit surface was plated as above. Following removal of the petrolatum, the fruit was surface-disinfested in 0.65% of sodium hypochlorite plus 1 ml/l of 95% ethanol for 3 min and allowed to drain.

For the internal tissues, the core (part 4) was removed with a flamed cork borer. Different size cork borers were used, from #6 (1 cm) in late July to #12 (2 cm) in late September. To eliminate the possible spread of bacteria by coring, the core was rinsed in ethanol, flamed, and divided into three equal portions, designated upper, center, and lower core. Each section was finely diced with a flamed scalpel and added to 2 ml of sterile saline in a test tube. The tube was vortexed for 1 min, and 0.5-ml samples were removed and spotted three times on MS and CCT media. Whole and split seeds from some fruit were also plated directly on the media. The plates were incubated for 2–3 days at 26 C and examined for colonies of *E. amylovora*.

Geographic survey. In 1985 and 1986, fruit were sampled from orchards in West Virginia, Utah, Washington, and Ontario, Canada, and tested for populations of *E. amylovora*. At each location, 10 apparently healthy fruit were collected from a susceptible apple cultivar and from Delicious, both from a blighted or near a blighted orchard and from a disease-free orchard.

West Virginia. Fruit of York were collected from severely blighted trees, and fruit of Delicious were collected from a mini-block of 36 trees surrounded by large orchards with severely blighted trees. Fruit of both cultivars were also collected from a disease-free orchard 30 km away.

Utah. Fruit of Rome Beauty were collected from trees with 50–200 blighted shoots per tree. Fruit were taken within 30 cm of infected shoots. Fruit of Delicious were collected from branches on healthy trees located 1–2 m from severely blighted Jonathan trees with more than 200 blighted shoots per tree. Apparently healthy fruit of both cultivars were collected from orchards without obvious symptoms of fire blight infections.

Washington. Fruit of Delicious were collected from an orchard with some blighted blossoms on Golden Delicious trees, located adjacent to a moderately blighted pear orchard. Apparently healthy fruit was collected from a remote orchard located 1 km from any active source of fire blight. No susceptible apple cultivar was available.

Ontario. Fruit of Idared and Delicious were collected from alternate rows in an orchard that contained trees with severe fire blight symptoms. Samples of healthy fruit of the same two cultivars were collected from a similar orchard without any history of fire blight, except for one young replant tree that first showed symptoms in 1986.

At each of the four locations, fruit were processed for the detection of populations of *E. amylovora* as described earlier. In 1985, samples were collected at harvest, whereas in 1986, the trees were sampled in late July, August, and September. The CCT selective medium was used with samples from all four locations, whereas MS was also used at Utah, West Virginia, and Ontario. In 1986, CG was used in Ontario instead of MS.

RESULTS

Fruit injury experiments. Significantly more fruit developed blight symptoms when injury accompanied inoculation than when the fruit were injured before or after inoculation (Table 1). Rome Beauty fruit were more susceptible than Delicious fruit, especially when injured 24 hr after the dip inoculation. However, very few fruit of either cultivar developed symptoms when injured 96 hr after inoculation. More disease was observed on bruised than on punctured fruit, especially when the injury accompanied the time of inoculation. When uninjured Rome Beauty fruit were dip inoculated, 17% developed symptoms. Only 3 of 72 uninoculated and non-disinfested fruit developed blight symptoms—all through injury in the puncture treatment.

Fruit in storage. Considerably more disease developed among the surface-disinfested Rome Beauty fruit than among the non-disinfested ones after 4 mo in storage at 1 C (Table 2). However, after the first 2 mo of storage, only 1% of fruit collected from apparently healthy trees developed symptoms. Internal fruit blight symptoms were difficult to distinguish from other fruit rots. Random sampling from the surface of blighted fruit in storage resulted in recovery of *E. amylovora* mainly from fruit collected at or directly below blighted shoots.

Table 1. Percentage fire blight in Rome Beauty (RB) and Delicious (DE) apple fruit injured, immersed in aqueous cell suspension of *Erwinia amylovora*, and incubated at 21–27 C for 14 days

Timing of injury	Type of fruit surface injury[x]						Means for injury timing
	Bruise		Large puncture		Small puncture		
	RB	DE	RB	DE	RB	DE	
Before inoculation[y]							
96 hours	41.7 b[z]	37.5 b	58.3 ab	41.7 b	12.5 b	20.8 bc	35.4 b
48 hours	37.5 b	25.0 bc	33.3 bc	4.2 c	16.7 b	0.0 c	19.4 c
24 hours	33.3 bc	20.8 bc	33.3 bc	8.3 c	20.8 b	4.2 c	20.1 c
At inoculation	87.5 a	95.8 a	83.3 a	75.0 a	54.2 a	54.2 a	75.0 a
After inoculation							
24 hours	45.8 b	33.3 bc	83.3 a	33.3 bc	50.0 a	33.3 ab	46.5 b
48 hours	8.3 cd	16.7 bc	37.5 bc	20.8 bc	16.7 b	4.2 c	17.4 c
96 hours	0.0 d	0.0 c	4.2 c	4.2 c	0.0 b	4.2 c	2.1 d
Means for injury type	34.5 a		37.2 a		20.8 b		

[x]Total of 12 fruit (4 × 3 replications) used per variety in August and September 1984. Fruit injury was accomplished by pressing one side of fruit against wooden block to affect punctures with small (4D) or large (8D) nails or #8 flat head screws.

[y]Dip inoculated for 1 min in buffered bacterial suspension (10^8 cfu/ml) of *E. amylovora*.

[z]Values with different letters within columns and between three types of injury means are significantly different based on Duncan's multiple range test ($P = 0.05$).

After 1 mo of storage, as much as 15% of the disinfested fruit blighted (presumably from endophytic bacteria), and only 4% of the non-disinfested fruit developed symptoms.

Blight was not observed at any of the four storage examination dates on the Delicious fruit from Wenatchee. The pathogen was not detected in washings of these fruit. Only 10 colonies of *E. amylovora* were recovered from 25 fruit each of the seven apple cultivars collected from apparently blight-free trees. These colonies were recovered from three fruit of Red Rome, one of York, and one of Winter Banana. The bacterium was not recovered from Smoothee, Top Red, Red Prince, or Delicious.

Table 2. Incidence of Rome Beauty fruit blight in cold storage, relative to distance between fruit and shoot blight prior to harvest

Date examined	Days in storage (1C)	Disease incidence (%)[a]					
		Non-disinfested fruit			Disinfested fruit[b]		
		A_1	B	C	A_2	B	C
November	37	4	1	3	15	8	1
December	64	0	1	0	10	4	1
January	93	3	3	0	13	7	0
February	121	0	2	0	14	3	0

[a]Based on 125 fruit harvested from each of three locations (A, B, and C) on trees in blighted orchards: A_1 = located on branch directly below (15–30 cm) a blighted shoot; A_2 = attached within 1–3 cm to the base (canker) of a blighted shoot; B = located within 60–120 cm of a blighted shoot; and C = collected from tree free of fire blight.
[b]Fruit were surface-sterilized for 3 min in 0.65% of sodium hypochlorite before storage.

Table 3. Recovery of *Erwinia amylovora* from core sections of apple fruit collected at specific distances from visible shoot blight symptoms on the tree

Apple cultivar	Distance from blighted shoot (cm)	Percent tissues sections[a]			
		Erwinia-like bacteria[b]		*Erwinia amylovora*	
		July	August	July	August
Red Rome	Blighted fruit	21 (14)	7 (3)	4 (3)	3 (1)
Red Rome	0	35 (19)	26 (14)	21 (14)	5 (8)
Red Rome	15	11 (9)	9 (5)	6 (4)	2 (2)
Red Rome	60	15 (13)	9 (6)	0 (0)	0 (0)
Red Rome	200	4 (4)	5 (2)	0 (0)	0 (0)
Red Rome	control[c]	1 (1)	5 (4)	0 (0)	0 (0)
York	control	11 (5)	4 (3)	1 (1)	0 (0)
Delicious	control	12 (2)	8 (1)	0 (0)	0 (0)
Golden Delicious	control	4 (3)	4 (4)	0 (0)	0 (0)

[a]Twenty fruit were examined in each treatment; results based on five core sections in each of four fruit, each with five replications. Figures in parenthesis indicate number of fruit.
[b]*Erwinia*-like bacteria indistinguishable from colonies of known strain of *E. amylovora*.
[c]Control fruit were collected from apparently symptomless trees.

Correlation between fruit location and blight source. *Erwinia amylovora* was recovered from up to 21% of the core sections of apple fruit that had been harvested from within 15 cm of visibly blighted shoots (Table 3). However, the bacterium was not detected when fruit were harvested from points 60 cm or more from blight symptoms. Moreover, control fruit of Delicious and Golden Delicious were apparently free of the pathogen, and it was found in washes of only one York control apple.

Geographic survey. Cultures of *E. amylovora* were recovered from only 24 of 320 Delicious fruit sampled from orchards in four growing regions (Table 4). Twenty-two of the positive fruit had been harvested from severely blighted trees in an orchard in Utah and two from an apparently healthy orchard in West Virginia. The positive isolations in West Virginia were found in the calyx of apples taken from a blight-free orchard in 1985, when severe fire blight was present in the area. The population exceeded 1,000 cfu/fruit. The pathogen was also detected on the surface of one York apple but only 5 cfu/fruit. In Utah, the bacterium was detected in the calyx, stem end, and core of one, two, and two of five Rome Beauty fruit, respectively. Also, it was recovered from the surface, calyx, stem end, and core of eight, one, one, and 12 of 22 Delicious fruit, respectively, collected from severely

Table 4. Detection of *Erwinia amylovora* in external and internal fruit tissues of various apple cultivars harvested at four locations in North America

Location Orchard type[b]	Apple cultivar[c]	*Erwinia amylovora* in sampled fruit tissue[a]					
		Calyx	Stem	Surface	Upper core	Center core	Lower core
Utah							
Blighted	Rome Beauty	+(1)	+++(2)	–	+++(2)	–	–
	Delicious	+(1)	+++(1)	+++(8)	++(4)	+(5)	+(3)
Healthy	Rome Beauty	–	–	–	–	–	–
	Delicious	–	–	–	–	–	–
West Virginia							
Blighted	York	+(1)	+(2)	+(1)	–	–	–
	Delicious	–	–	–	–	–	–
Healthy	York	–	–	+(1)	–	–	–
	Delicious	+(2)	–	–	–	–	–
Washington							
Blighted	Delicious	–	–	–	–	–	–
Healthy	Delicious	–	–	–	–	–	–
Ontario							
Blighted	Idared	–	–	–	–	–	–
	Delicious	–	–	–	–	–	–
Healthy	Idared	–	–	–	–	–	–
	Delicious	–	–	–	–	–	–

[a]Colony count: + = 1–50; ++ = 51–299; +++ = 300 cfu/plate. Figures in parenthesis indicate number of fruit.
[b]For a description of blighted and healthy orchards, see text.
[c]Forty fruit of each cultivar were collected from each blighted and healthy orchard. Number of fruit is based on 10 fruit examined in September 1985 and 10 fruit each in July, August, and September 1986.

blighted orchards. The populations generally exceeded 1,000 cfu/fruit section.

Delicious fruit collected from orchards with disease in West Virginia, Washington, and Ontario were free of any detectable levels of the pathogen. The pathogen was not recovered from any fruit examined in Washington and Ontario or from the internal tissues of fruit harvested from apparently healthy orchards in Utah and West Virginia. However, *E. amylovora* was recovered from the calyx of two Delicious fruit and the surface of one York fruit collected from a blight-free orchard in West Virginia. Most of the successful isolations of *E. amylovora* occurred in 1985, when both areas experienced very severe fire blight. The bacterium was never recovered from any whole or split apple seeds. The CCT selective medium appeared somewhat more effective for recovery of *E. amylovora* than MS.

DISCUSSION

Fresh wounds in apple fruit were successful infection courts for *E. amylovora*. In most cases, the incidence of fruit blight decreased 50% or more when the interval between inoculation and wounding increased from 24 to 48 hr. This is in agreement with earlier findings by Brooks (4) and Plurad et al (19). Moreover, considerably more fruit became diseased when injured at or within 24 hr of dip inoculation than at greater intervals. Bruising, especially at time of inoculation, led to more disease than puncturing, as was also reported previously with pears (31). However, the unusually high concentration of bacterial inoculum used may have overstated the predispositon associated with the injuries. Only few uninjured fruit became infected when dip inoculated for up to 10 min (van der Zwet, *unpublished*). All uninjured Rome Beauty fruit that did become diseased developed initial blight symptoms in the skin surface immediately around the stem. Wright (27) made similar observations in Washington when pear fruit were washed in water contaminated with *E. amylovora*.

A few uninoculated fruit of Rome Beauty stored at 1 C developed fire blight. Thus, asymptomatic fruit of a susceptible cultivar, harvested from blighted trees, may develop fire blight during commercial storage. Also, a low incidence of blight was observed among stored fruit that had been harvested from apparently healthy trees. The recovery of *E. amylovora* from asymptomatic fruit harvested from three susceptible cultivars grown in West Virginia is evidence that populations of *E. amylovora* may exist epiphytically in extremely small numbers in orchards with blight. Minute numbers of bacteria may remain hidden on old flower parts inside the calyx cavity (24). However, our failure to recover *E. amylovora* from 100 fruit of four resistant cultivars grown in West Virginia as well as from 210 Delicious fruit collected near Wenatchee, Washington, supports the recent findings by Roberts et al (21) who could not detect the organism in fruit harvested from blighted trees of seven apple cultivars grown in five locations in Washington. Moreover, all information is indicative that fruit on asymptomatic trees at harvest time are not likely to be infested with the bacterium.

The bacterium was recovered more frequently from fruit located within 15 cm of blighted shoots than from those located 60–200 cm away. However, under severe blight conditions in West Virginia, only one of 40 York fruit and two of 40 Delicious fruit, harvested from apparently healthy trees, possessed detectable external epiphytic populations, and none possessed internal populations. Because *E. amylovora* was recovered in Utah from internal parts of apple fruit located within 30 cm of blighted shoots, a distance of 100 cm (or about 1 yd) appears to be a safe and practical distance to harvest fruit without internal populations of *E. amylovora*. However, the positive recovery of endophytic *E. amylovora* from 14 apples of two cultivars in Utah requires caution and may partially explain the observations of fruit blight symptoms on pear shipments to Hawaii (3,26) and England (11).

The geographic survey of the presence of *E. amylovora* on the surface and internal parts of apple fruit confirmed preliminary observations (32) that the bacterium is usually not present when fruit are collected from apparently healthy orchards. The recovery of *E. amylovora* from blighted orchards in Utah proved to be a good check because the trees were severely blighted and the sampled fruit were attached within 30 cm of blighted shoots. Also, the recovery of *E. amylovora* from the calyx of two Delicious fruit in West Virginia in 1985 was not unexpected because this fruit region experienced very severe blight that year and the bacterium apparently survived on the dry pistils in the calyx of these fruit, reportedly the most predominant tissue for *E. amylovora* survival (24). However, the bacterium was not recovered from internal tissues of Delicious and three other susceptible apple cultivars collected from nonblighted trees in the four geographic regions.

We have demonstrated that although *E. amylovora* may be disseminated from one area to another via fruit, such dissemination is highly unlikely when sound fruit without injury are harvested from trees and orchards free of fire blight. Although small populations may have escaped detection, such populations would have to be exposed to optimum conditions for disease development in order to reach proper infection courts to cause disease to be expressed.

For additional action to ensure uninfested or uncontaminated fruit, especially because bacteria can survive on old stigmas in the calyx, fruit may be treated with gamma radiation (14), chlorine-based chemicals (13,20), citrate buffer (20), or acetic acid (22,29). The latter supports earlier findings by Dueck (9) in Canada. All treatments provide an extra security remedy to fulfill quarantine requirements for export of apples to countries without fire blight.

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