Recovery of Erwinia amylovora from Symptomless Stems and Shoots of Jonathan Apple and Bartlett Pear Trees

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Accepted for publication 12 July 1971.

ABSTRACT

Highly virulent *Erwinia amylovora* was readily isolated from the internal tissues of symptomless side shoots on artificially inoculated apple and pear trees in the greenhouse. These shoots did not exist at the time of inoculation, but developed from axillary buds immediately below the base of the cankers when blight progress ceased. Bacteria were easily recovered from the symptomless lower stem below the blighted portion also, and the pathogen survived in both types of tissues for periods up to 6 months. In addition, fire blight bacteria were isolated from 60% of apparently healthy suckers from blighted Bartlett trees in the orchard. Of these, 93% showed bacteria in the upper two-thirds of the suckers. Virulent bacteria were also recovered from symptomless shoots of resistant Magness pear buds and from branches of two other pear cultivars, all collected from orchard trees without any record of visible fire blight. Thus, *E. amylovora* seems to be a resident in symptomless apple and pear tissue, which may explain the frequent, unexpected appearance of the disease in nurseries, young plantings, and well-managed bearing orchards.
Phytopathology 62:39-42.

Additional key words: Pyrus communis, Malus sylvestris.

No adequate explanation has been given for sudden, severe epiphytotics of fire blight (*Erwinia amylovora*) in young orchards or established plantings where the disease had not been observed for 1 to several years (4, 6, 15). It has been suggested that bacterial ooze from hold-over cankers was solely responsible for blight infections the following year, even though only a small fraction of cankers oozed in surveyed orchards (3, 11, 12, 13, 14). Thus, there remains a lack of understanding regarding survival and dissemination of *E. amylovora* and epidemiology of the fire blight disease.

Usually, after blight ceases to progress in a branch or shoot, the first axillary bud, and sometimes the second below the blighted portion, begins to develop. In the greenhouse, we observed hundreds of newly developed shoots from such buds, but never saw blight symptoms. This paper presents the evidence that virulent *E. amylovora* may exist in symptomless host tissues of apple and pear. A short account of this work was reported previously (7).

MATERIALS AND METHODS.—In greenhouse experiments we inoculated young, tender whips of apple (*Malus sylvestris* Mill. 'Jonathan') or pear (*Pyrus communis* L. 'Bartlett') trees with an aqueous suspension (10^6 cells/ml) of *E. amylovora*. The suspension was injected into the apex of the shoot with a 25-gauge hypodermic needle. The bacteria were grown for 24 hr on slants of nutrient yeast dextrose agar (NYDA). Blight developed in the inoculated whips, and usually progressed for about 30-40 cm before it stopped. The axillary buds in the first and second nodes below the base of the blighted portion started to grow in 14-20 days, and developed into apparently healthy side shoots or branches. Such shoots and symptomless stems, developed below the blighted portion, were removed from the plants about 30 days after inoculation, surface-sterilized for 3 min in 0.5% sodium hypochlorite, rinsed 3 times in sterile water, and divided into pieces 2-5 cm long. Two segments, about 3 mm thick, were cut from the end of each piece, one plated on NYDA and the other placed into nutrient yeast dextrose broth (NYDB) in tubes. Both were incubated at 26 C for 3 days. Bacterial colonies usually developed on NYDA around the segments, whereas NYDB became turbid.

In addition, attempts were made to isolate *E. amylovora* with a selective medium (10). After 48-hr incubation, one loop (2 mm) of the above turbid broth was streaked on plates containing this special medium. After 2-5 days, *E. amylovora* was readily distinguished from other bacterial colonies by a characteristic colony morphology and red color.

Also after 2 days' incubation of the NYDB containing the segments, pathogenicity of the bacteria was determined by injecting the suspension with a hypodermic needle into the apex of young, tender whips of Jonathan apple or Bartlett pear trees. The plants were incubated in a moist chamber at 25 C and ca. 100% relative humidity for 5 days, then returned to the greenhouse for an additional 7 days and examined for blight symptoms.

Suckers from Bartlett pear trees growing in the field were also examined for the presence of internal bacteria. Two symptomless suckers, 75 cm tall, were selected at random from each of 25 blighted trees. These suckers were usually attached to the base of the trunk or large scaffold limbs at least 1.5-2.0 m from any recent active blight. First blight observations in 1970 in these trees were made 13 May, and blighted twigs removed weekly. Pruning cuts were made up to 25 cm below visible blight symptoms with shears disinfected in 10% Clorox (sodium hypochlorite) between each cut. Symptomless suckers were collected 28 June, surface-sterilized, and sectioned as described before. Pieces, 3 mm thick, were plated on NYDA. Bacterial isolates from these pieces were subcultured to NYDA slants. After 24 hr of growth, the bacteria were suspended in 9 ml sterile water; and 0.25 ml of this suspension were used to inoculate the cut surface of green Bartlett pear fruit to test pathogenicity.

TABLE 1. Isolates of *Erwinia amylovora* from apparently healthy tissues of two Jonathan apple trees A and B, obtained by using different media

Sample site[a]	Isolation and growth in vitro						Pathogenicity in vivo[e]	
	NYDA[b]		NYDB[c]		M-S[d]		NYDB[c]	
	A	B	A	B	A	B	A	B
Side shoot I								
a	+	+	+	+	+	+	+	+
b			+					
c	+	+	+	+	+			
d				+				
e		+						
f								
g			+	+	+	+	+	+
Side shoot II								
h	+	+	+	+	+	+	+	+
i	+		+	+	+		+	
j	+		+		+		+	
k								
l	+							
m			+					
n			+		+		+	
o	+		+		+		+	
p	+		+		+		+	
Lower main stem								
q	+		+	+	+	+	+	+
r	+	+	+	+		+	+	+
s	+	+	+	+	+	+	+	+
t	+	+	+	+	+	+	+	+
u		+		+		+		+
v	+	+		+		+		+
w				+		+		+
x								
y		+		+				

[a] Samples 3 mm thick taken at 2.5-cm increments.
[b] Isolation on nutrient yeast dextrose agar (NYDA); + = bacterial growth around stem section; blank = no growth.
[c] Isolation in nutrient yeast dextrose broth (NYDB); + = turbidity indicating bacterial growth; blank = no turbidity.
[d] Isolation on Miller-Schroth selective medium (M-S); + = characteristic red coloration of colonies; blank = no coloration.
[e] + = blight symptoms in Jonathan whips following artificial inoculation; blank = no symptom.

RESULTS.–*Shoots from axillary buds.*–Bacteria were recovered from about 50% of the sections of the apparently healthy side shoots and lower portions of the main stem (Table 1). The Miller-Schroth medium appeared to be more selective than the standard agar and broth media for recovering pathogenic bacteria from symptomless shoots. Seventy-six per cent of 29 isolates from NYDB produced fire blight symptoms on Jonathan trees. We have no explanation for the failure of the remaining isolates to induce symptoms in the inoculated plants.

Virulent fire blight bacteria were recovered from the base and apex only of apparently healthy side shoots (Fig. 1). It is possible that the organism was uniformly present in the shoots, but was not recovered. Bacteria seemed to be present predominantly in the main stem immediately below the blighted portion and in the base of the two side shoots. In these plants, the downward movement of bacteria in the stems was detected in apparently healthy tissues only a short distance (9-18 cm) below visible blight symptoms. In other experiments, however, we recovered virulent *E. amylovora* from symptomless stems and side shoots up to 75 cm from the visible canker of three Jonathan apple and five Bartlett pear trees and three pear seedlings.

Two Jonathan and four Bartlett trees, inoculated in the apex with *E. amylovora*, remained on the greenhouse bench for 6 months, including the hot summer months. Apparently healthy side shoots (75-90 cm), which developed from axillary buds after visible blight stopped progressing in the main stem, were brought into the laboratory in October; and virulent fire blight bacteria were isolated from sections of the basal third of these shoots. In addition, pathogenic bacteria were isolated from a similar side shoot of a Bartlett tree held in the greenhouse for 1 year.

Tree suckers and other field material.–Erwinia amylovora was recovered from apparently healthy suckers from 76% of 25 sampled orchard trees, and from 60% of 50 symptomless suckers collected from

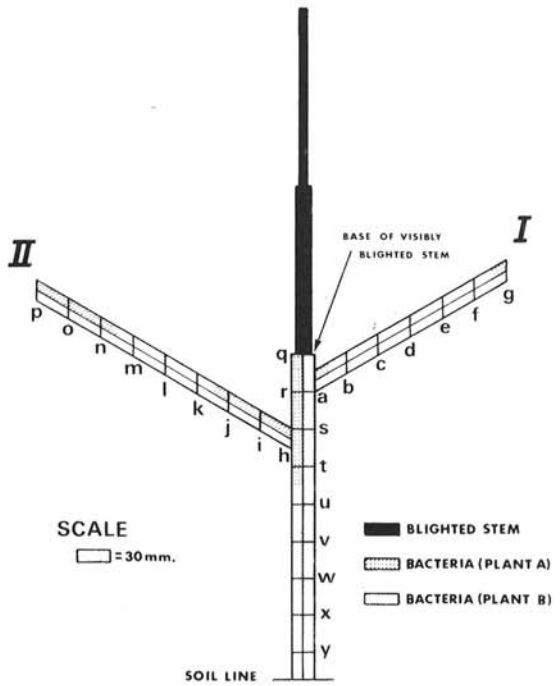

Fig. 1. Schematic presentation of two Jonathan apple trees (A and B) (whips), each showing two apparently healthy side shoots I and II, developed from axillary buds after visible blight stopped progressing in the main stem. Letters indicate sites tested for presence of internal *Erwinia amylovora*.

TABLE 2. Distribution of virulent *Erwinia amylovora* in symptomless suckers of blighted orchard Bartlett pear trees

Portion of sucker plated[a]	Frequency of recovery
Top	16
Middle	24
Base	2

[a] Fifty symptomless suckers (75 cm long) from 25 blighted trees (two suckers/tree). Each sucker was divided into three equal portions (25 cm long), then each portion was cut into pieces 5 cm long, and 3-mm segments were plated from the end of each piece.

these trees. These bacteria were recovered predominantly from the upper two-thirds of these suckers (Table 2). Surprisingly, 26% of the suckers showed bacteria in the middle third only and an additional 20% in both the middle and upper thirds. Of the 30 suckers with bacteria, 93% showed their presence in the upper two-thirds, and an additional 3% in all parts of the sucker.

In 1969, at Beltsville, Md., budwood was collected from a 6-year-old blight-resistant pear (*P. communis* 'Magness') tree which had never shown visible blight symptoms. Buds were propagated in July on Bartlett seedling understock, and subsequently potted and forced in the greenhouse. The newly developed whips (7.5-15 cm) were removed in January 1970, surface-sterilized, cut lengthwise, and placed on NYDA with the cut surface down. Virulent bacteria were recovered from 3 of 16 symptomless whips. In addition, we isolated *E. amylovora* from the internal tissues of a Magness pear fruit and dormant branches of two pear (*P. communis* 'Moe' and 'Chasset') trees. All were collected from field trees with no record of visible fire blight.

DISCUSSION.—Our data indicate that, after fire blight bacteria enter a tree naturally or artificially, they apparently may reside there for periods of from 1 to 6 months and move into newly developed shoots with or without producing blight symptoms. Recovery of *E. amylovora* from symptomless shoots was successful with all three media used. Pathogenicity of the broth cultures correlated more highly with typical colony characteristics of *E. amylovora* on the Miller-Schroth medium than with those on NYDA. These recoveries seem remarkable, especially from one Bartlett tree kept in the greenhouse for 1 year, considering that temperatures exceeded 38 C for many days during the summer.

In the past, the presence of fire blight bacteria in symptomless tissue of apple and pear has rarely been mentioned (9, 13). More recently, the movement and persistence of *E. amylovora* in Jonathan apple tissue from leaf to apex or from blighted to existing healthy tissue has been demonstrated (5, 8). Our research, however, has proved that fire blight bacteria move into apparently healthy shoots that did not exist at the time of infection, but developed after visible blight ceased to progress. In addition, the recovery of *E. amylovora* from shoots developed from buds collected from resistant pear trees without any record of visible blight is most surprising and significant.

Our attempts to induce fire blight symptoms in trees containing internal resident bacteria have failed. Apparently healthy side shoots, as described above, were cut off, sandblasted, or needle-injected with sucrose into the apex, in order to expose or effect multiplication of the organism. It is possible that sandblasting did not cause injury deep enough to expedite bacterial multiplication in the exposed internal tissues. However, under field conditions, internal *E. amylovora* bacteria in the tissues are potentially capable of causing fire blight, which may explain the frequent development of the disease after the blighted parts of the tree have been removed by pruning, or when healthy branches have been severely damaged by hail. A serious outbreak of fire blight in 4-year-old pear trees of the resistant cultivar Magness in an isolated planting in northern Arkansas, following a severe hailstorm, strongly suggested that the causal organism was present internally (15).

Fire blight research and field observations in general indicate today that the causal organism is present in or on apparently healthy pear and apple buds, shoots, and fruit. This contention was recently supported by Baldwin & Goodman (2), who isolated bacteria from dormant Jonathan apple buds in Missouri which gave positive reactions when typed against *E. amylovora* phages. Furthermore, in California, fire blight bacteria were isolated from

beneath small, water-soaked lesions in apparently healthy Bartlett pear fruit which developed after holding them for 6 days at ripening temperatures (1).

We suggest that *E. amylovora* exists as a natural resident in its host tissue, as proved with many other bacteria in human and animal systems. It thus appears that complete protection of trees from fire blight can be achieved only by developing a more effective, nonphytotoxic, systemic bactericide which will kill the internal organism.

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