Short-Term Population Dynamics of *Erwinia amylovora* in Succulent Pear Tissue

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ABSTRACT

Erwinia amylovora was isolated in greater numbers from inoculated apple shoots than from pear shoots when sand was used to facilitate grinding of tissues. Chromatographic grade alumina substituted for the sand increased recovery of *E. amylovora* from pear shoots but not to the level obtained for apple shoots.

When *E. amylovora* was inoculated into wounded tissue, the frequency of recovery of the pathogen decreased progressively with time after inoculation from 0 to 60 min. Multiplication of the bacteria apparently was negligible up to 24 hr but increased thereafter so that within 32 to 56 hr the number of bacteria approached the concentration in the inoculum. The variation in time required for the bacterial cell number in individual shoots to again reach the concentration of bacteria in the inoculum may account for the variations observed in time for symptom development observed in different shoots.

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Gowda & Goodman (1) described the migration of *Erwinia amylovora* (Burr.) Wilson et al. from wounds that were inoculation sites in apple shoots. However, we did not find any reports in the literature of the

TABLE 1. The number of *Erwinia amylovora* colonies isolated from 'Bartlett' pear and 'Jonathan' apple twigs when sand was used as an abrasive during grinding of the tissue[a]

Trial	Bartlett		Jonathan	
	0 hr[b]	1 hr	0 hr	1 hr
1	7.0×10^2	0	3.0×10^4	1.4×10^4
2	5.0×10^2	2×10	2.6×10^4	1.8×10^4
3	1.4×10^3	0	3.4×10^4	2.2×10^4

[a] Inoculum consisted of a 5-μliter drop containing 10^7 bacterial cells/ml.
[b] Isolations were attempted at 0 time and 1 hr after inoculation.

immediate effect on *E. amylovora* cells placed in wounded tissue of either apple or pear. Since injury to pear tissue has been associated with production of phenolics (7) that are toxic to the fire blight bacterium (2, 3, 4, 5) we investigated the rates of multiplication of the bacterium after ingress via wounded tissue to gain more insight into the epidemiology of fire blight.

MATERIALS AND METHODS.—The host material used in this study consisted of expanding shoots of *Pyrus communis* L. 'Bartlett' and *Malus sylvestris* Mill. 'Jonathan' excised from plants in the field. The cut ends of the shoots were placed immediately in water and the shoots were maintained in water at 23 to 27 C throughout the investigation.

The isolate of *E. amylovora* was originally obtained from diseased pear tissue collected from an orchard near Bridgeport, Washington. A strain of this isolate resistant to 1,000 ppm of streptomycin was selected. Although this strain appeared vigorous, three transfers a week were required to maintain viability. The use of this isolate helped insure that data were based solely on the bacterium inoculated into the host. This culture was maintained and all isolations were made on SNYDA, a medium containing 23.0 g Bacto nutrient agar, 5.0 g Bacto yeast extract, 1.5 g dextrose and 5.0 g streptomycin sulfate (21.2% Merck) in 1 liter of water.

A puncture wound was made diagonally through each shoot just above the axil of the youngest expanded leaf. A 5 μliter-drop of inoculum containing ca. 10^7 cells/ml *E. amylovora* was applied to each wound with a micropipet. The inoculum suspension was prepared by washing 36- to 48-hr-old slants of bacteria with 0.05 M phosphate buffer (pH 6.5) and diluting the resulting suspension to the desired concentration with the same buffer. Inoculum concentration was determined turbidimetrically with a Coleman 124 spectrophotometer and periodically confirmed by dilution plating.

The isolation procedure was a modification of that used by Gowda & Goodman (1). The sample from which each isolation was made consisted of a 1-cm section of shoot centered on the point of inoculation. The sample was ground in 0.5 ml phosphate buffer (pH 6.5), and then diluted to 5 ml with additional buffer. This suspension was serially diluted to 0.0001 of the original suspension and 0.25 ml was streaked on each of four replicate plates of SNYDA. Time elapsed for this procedure was ca. 5 min. Counts were made 48 hr after streaking.

RESULTS.—Gowda & Goodman (1) ground apple shoots in a saline solution using sand as an abrasive. Nevertheless, with that technique we were unable to isolate substantial numbers of bacteria from the inoculated tissue of pear 1 hr after inoculation. In Table 1, the efficiency of the technique for isolating *E. amylovora* from apple and pear stems are compared.

The possibility that the arbutin-hydroquinone complex reported by Hildebrand & Schroth (2, 3, 4) was a factor responsible for reducing the number of bacteria below the point of detection was examined by testing each of several reducing agents; e.g., ascorbic acid, methionine, sodium thiosulfate, sodium oxalate, and ferric sulfate at 0.025 M and 0.25 M as a component of the grinding medium. The results were either erratic or no isolations resulted.

Alumina very strongly adsorbs phenolic compounds (6). Therefore, 1 cc of chromatographic grade adsorption alumina, 80-200 mesh, was substituted for the sand in the grinding process. Phosphate buffer at pH 6.5 was substituted for the

TABLE 2. The number of viable cells of *Erwinia amylovora* isolated at various times following inoculation of 'Bartlett' pear twigs, using activated alumina as a grinding abrasive during grinding of the tissue[a]

Trial	Numbers of colonies at intervals (min) after inoculation					
	0	1	10	20	30	60
1	1.4×10^4	2.8×10^3	1.0×10^3	9.0×10^2	0	0
2	3.8×10^4	1.7×10^3	6.0×10	2.0×10	2.0×10	0
3	3.2×10^4	5.8×10^3	1.4×10^3	1.4×10^3	9.0×10^2	0
4	2.0×10^3	2.0×10	0	3.0×10^2	0	0
5	8.0×10^3	2.4×10^3	6.0×10^2	0	2.0×10	0
6	1.2×10^4	2.2×10^3	1.0×10^2	2.0×10	0	2.0×10
7	6.0×10^2	2.0×10^2	0	1.5×10^3	0	2.0×10^2
8	4.3×10^3	7.0×10^2	3.2×10^2	4.0×10	6.0×10	0
9	4.8×10^3	1.3×10^3	6.0×10	0	0	0

[a] Inoculum consisted of a 5-μliter drop containing 10^7 bacterial cells/ml.

TABLE 3. The number of viable cells of *Erwinia amylovora* isolated from 'Bartlett' pear twigs at various times following inoculation, using activated alumina as an abrasiving during grinding of the tissues[a]

Trial	Number of colonies/interval (hr) after inoculation				
	8	24	32	48	56
1	4.0×10	4.0×10	1.4×10^2	3.3×10^3	9.8×10^3
2	0	0	0	2.0×10	6.1×10^3
3	2.4×10^2	6.6×10^2	1.8×10^3	3.9×10^3	3.8×10^5

[a] Inoculum consisted of a 5-μliter drop containing 10^7 bacterial cells/ml.

saline solution. An average of 10^5 viable bacteria were isolated from 1 cm of inoculated pear shoots ground in alumina, whereas no bacteria were isolated from similar shoots ground in sand.

Isolations were attempted when tissues were ground with alumina 0, 1, 10, 20, 30, and 60 min after inoculation. The results of this experiment are presented in Table 2. There was a steady decrease in the number of viable bacteria isolated with time between inoculation and grinding. The technique made recovery of about 10% of the bacteria possible immediately after inoculation. Considerable variation also occurred between shoots at any given time. Finally, the apparent loss of all viable bacteria at 60 min after inoculation is incorrect since detached pear shoots resulted in 90-100% of the shoots blighted (symptoms appeared over a 5- to 9-day period).

To determine at what time *E. amylovora* increased to a detectable number of cells, isolations were made at longer intervals. Samples taken at 1, 2, 4, and 6 hr following inoculation yielded no bacteria (Table 3). At 8, 24, and 32 hr only a few bacteria were found. By 48 hr the numbers had increased and at 56 hr the populations were about equal to those at 0 time. In another experiment, higher counts were noted as early as 32 hr after inoculation; the shoots in that experiment appeared slightly more vigorous.

DISCUSSION.—The presence in pear tissue of a bacterial inhibitor, possibly the arbutin-hydroquinone complex (2, 3, 4, 6), complicates population and migration studies. To be sure that most of the bacteria in the tissue are isolated, some destructive technique such as grinding must be used. This presumably releases such large quantities of the inhibitor that most, if not all, of the bacteria are destroyed. The use of alumina in grinding the tissues largely overcomes the difficulty.

We conclude from the data that the number of cells of *E. amylovora* that reach the site of a wound is greatly decreased shortly after ingress. At some point after 32 hr, the host-pathogen balance apparently shifts in favor of the pathogen, which then multiplies sufficiently to cause symptom expression. A delay in initial multiplication may be reflected in delayed symptom expression. In one greenhouse test, symptom expression varied from 5-9 days when similar Bartlett pear shoots were inoculated with the same inoculum. Quantitative differences between members of the arbutin-hydroquinone complex have been reported within, and between, pear varieites (3, 4). These differences may account for the variations in rate of multiplication of *E. amylovora* in wounded pear shoots, the time difference in symptom expression and the variation noted between shoots in the short term experiments reported in this paper. It remains to be determined whether the inhibitor of *E. amylovora* which is produced by wounded pear tissue is significantly related to host resistance to this bacterium.

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