# Distribution of *Xylella fastidiosa* Within Roots of Peach

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**ABSTRACT**

Aldrich, J. H., Gould, A. B., and Martin, F. G. 1992. Distribution of *Xylella fastidiosa* within roots of peach. Plant Dis. 76:885-888.

The distribution of *Xylella fastidiosa*, the bacterium that causes phony peach disease, was studied in roots of 10 Flordaking peach scions grafted on Nemaguard rootstock. *X. fastidiosa* was detected in all trees, whether symptomatic or asymptomatic for phony peach disease. Populations of *X. fastidiosa* in the root xylem fluid of symptomatic trees were high, and the distribution of these bacteria was uniform both along the length of roots and among roots within the root ball. In asymptomatic trees, the distribution of infected roots among the quadrants of the root ball was variable. The distribution of *X. fastidiosa* was not influenced by the distance the root sample was taken from the trunk or the diameter of the root piece sampled. The minimum sample size required to detect *X. fastidiosa* in the roots of asymptomatic peach trees, at a risk of 5%, was five to 10 roots.

Phony peach disease (PPD) is caused by a strain of the fastidious plant bacterium *Xylella fastidiosa* Wells et al (8) and is principally transmitted in the southeastern United States by the leafhopper *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae) (2,7). *Prunus persica* (L.) Batsch trees symptomatic for PPD appear dwarfed, with deep green leaves borne on limbs with shortened internodes (6). The onset of bloom and fruit set is earlier than normal, and fruit size is reduced by the disease, resulting in commercially nonproductive trees.

*X. fastidiosa* is a xylem-limited bacterium as shown by electron microscopy (5). Although the bacterium resides within the xylem of stems and roots of infected peach trees, populations of *X. fastidiosa* are much greater within roots than within stems (9). These bacteria are also present within the roots of asymptomatic trees (3,9).

Current sampling procedures for PPD incidence in the field include the removal of a limited number of root pieces from the root ball and extraction of bacteria from root xylem fluid (4). Evert et al (3) state that two to three roots should be examined for 95% confidence in detecting *X. fastidiosa* in asymptomatic trees. Wells et al (9) suggest that a minimum of three roots should be sampled. These researchers assume that *X. fastidiosa* is equally distributed along the length of a root. More precise knowledge of the distribution of *X. fastidiosa* within the root systems of symptomatic and asymptomatic peach trees is needed for a more confident assessment of disease incidence in the field. With confident assessment of PPD in an orchard, a grower could rogue even asymptomatic trees, and researchers could accurately evaluate potential PPD control measures. In this study, peach roots were thoroughly sampled in a destructive procedure. The objective of this procedure was to devise a sampling plan in which the probability of detecting the pathogen with minimal disturbance to the root systems of asymptomatic trees under field conditions is maximized.

Florida Agricultural Experiment Station Journal Series No. R-02006.

Accepted for publication 6 March 1992.



## MATERIALS AND METHODS

**Experimental material.** Nemaguard peach seedlings were budded with Flordaking peach scion in 1983 at the University of Florida North Florida Research and Education Center in Monticello. The 10 trees sampled in this study were part of a larger planting designed to assess the influence of rootstock on

the incidence of PPD. In February 1985, 65 Flordaking scions on three different rootstocks budded in 1983 or 1984 were transplanted into a 1.48-ha block, with soil type Dothan loamy fine sand. The trees received standard cultural practices of fertilization and pest control and were irrigated daily from April through October with 42 L per tree per day in 1985 and 1986 and 48 L per tree per day in 1987 and 1988.

**Sampling for *X. fastidiosa*.** The 10 trees (Flordaking on Nemaguard rootstock) sampled in this study were chosen to include trees both symptomatic and asymptomatic for PPD. These trees were naturally infected with *X. fastidiosa*. Each tree was hydraulically lifted from the ground with a tractor-mounted front-end loader. The exposed root ball was arbitrarily divided into quadrants, and one to 13 roots were removed from each quadrant. On average, one tree was uprooted and sampled every 2 wk, commencing on July 1989.

In the laboratory, the tissue exterior to the xylem was removed, and sections of root (approximately 4.0 cm long) were cut (using hand clippers surface-sterilized with 70% ETOH) every 15 cm along the length of each root sampled, commencing 15 cm from the trunk (section 1) and proceeding toward the root tip up to 91 cm (sections 2–7). The pieces of root from which xylem fluid was extracted were measured and classified into categories based on root diameter in increments of 0.25 cm, where A = root diameter $\leq$ 0.25 cm, B = 0.26–0.50 cm, C = 0.51–0.75 cm, D = 0.76–1.00 cm, and E = root diameter > 1.0 cm. Xylem fluid from each section was extracted with 0.5 ml of 0.1 M KOH, using the vacuum infiltration procedure of French et al (4). This collection procedure has been shown to extract approximately 82% of the total *X. fastidiosa* (1). Ten µl of the filtrate was examined under a phase contrast microscope at 400×. Ten randomly selected microscope fields were examined for *X. fastidiosa* (straight rods). A "positive" microscope field contained one or more bacteria. A "positive" section or root contained one or more positive microscope fields. Although bacteria were not cultured from the xylem fluid of these trees at the time of sampling, the presence of *X. fastidiosa* in the planting had been previously confirmed by isolation and culturing on periwinkle wilt media (*data not shown*). There are seasonal fluctuations in the titer of *X. fastidiosa* (9). However, since this study labels as "positive" a root with just one bacterium, then seasonal fluctuations are not relevant.

**Data analysis.** Pearson correlation analysis was utilized to examine the relationship between the distance a root sample was taken from the trunk (section number) and the diameter of the root piece sampled (root diameter category). Calculations of the minimum sample size required to successfully detect *X. fastidiosa* in asymptomatic trees ($P \leq 0.01$, 0.05, and 0.10) were based on the assumption that bacteria were randomly distributed in root tissue, and that 10 microscopic fields per root section were examined for the presence or absence of bacteria. Each tree was placed into one of three population categories (high, moderate, and low) based on the percentage of microscope fields that contained bacteria. The relationship between population category and root section or root diameter category was assessed utilizing analysis of variance.

**Table 1.** Detection of *Xylella fastidiosa* in roots of 10 naturally infected peach trees and corresponding bacterial population category for each tree

| Tree | Symptom expression | Number of bacteria/total (%) | | | Bacterial population category |
|---|---|---|---|---|---|
| | | Roots | Sections | Microscope fields | |
| 1 | Symptomatic | 32/32 (100) | 94/94 (100) | 915/940 (97.3) | High |
| 2 | Symptomatic | 28/30 (93.3) | 75/78 (96.1) | 692/780 (88.7) | High |
| 3 | Asymptomatic | 14/22 (63.6) | 20/57 (35.1) | 133/570 (23.3) | Moderate |
| 4 | Asymptomatic | 30/35 (85.7) | 87/124 (70.2) | 673/1,240 (54.3) | Moderate |
| 5 | Asymptomatic | 9/26 (34.6) | 23/87 (26.4) | 147/870 (16.9) | Moderate |
| 6 | Asymptomatic | 3/21 (14.3) | 3/80 (3.8) | 3/800 (0.4) | Low |
| 7 | Asymptomatic | 8/17 (47.1) | 12/59 (20.3) | 16/590 (2.5) | Moderate |
| 8 | Asymptomatic | 5/17 (29.4) | 6/71 (8.5) | 7/710 (1.0) | Low |
| 9 | Asymptomatic | 5/19 (26.3) | 5/77 (6.5) | 5/770 (0.6) | Low |
| 10 | Symptomatic | 24/24 (100) | 96/97 (99.0) | 888/970 (91.5) | High |

**Table 2.** Recovery of *Xylella fastidiosa* from peach tree roots according to bacterial population of tree, distance of sample from trunk (root section number), and root diameter, and relationship between population category and root section or diameter

| Bacterial population category[a] | *X. fastidiosa*-positive roots[b] (%) | |
|---|---|---|
| | Root section number[c] | Root diameter category[d] |
| Low | 5.6 | 1.1 |
| Moderate | 26.2 | 30.2 |
| High | 91.4 | 94.7 |
| Main effects | | |
| Population category | ***[e] | *** |
| Variable | NS[f] | NS |
| Interactive effects | | |
| Population category × variable | NS | NS |

[a] Trees naturally infected with *X. fastidiosa* were categorized as low (≤ 1% positive microscope fields), moderate (2–88%), or high (>88%).
[b] Containing one or more bacteria.
[c] Sections (approximately 4 cm long) were cut every 15 cm along the length of each root sampled, commencing 15 cm from the trunk and proceeding toward the root tip up to 91 cm.
[d] Pieces of root from which xylem fluid was extracted were measured and categorized by root diameter in 0.25-cm increments, from 0.25 to <1.25 cm.
[e] $P \leq 0.0001$ (analysis of variance).
[f] Not significant.

## RESULTS

**Symptomatology.** *X. fastidiosa* was detected in one or more roots of each tree examined (Table 1). The percentage of positive microscope fields for trees 1, 2, and 10 was greater than 88%. These trees were symptomatic for PPD and were classified in the high bacterial population category (Table 1). The percentage of positive microscope fields in trees 6, 8, and 9 was less than or equal to 1%, and these trees were placed in the low population category. The remaining trees were classified as moderate. Trees in the moderate and low categories were asymptomatic for PPD.

**Distribution of *X. fastidiosa* within roots.** The percentage of roots from which *X. fastidiosa* was recovered differed between trees in the low, moderate, and high population categories (Table 2). Within each population category, the percentage of positive roots was in-

**Table 3.** Actual risk ($\beta$) of failing to detect *Xylella fastidiosa* within asymptomatic infected peach trees while scanning 10 microscope fields for small values of $\delta$[a] and the number of microscope fields ($n$) that must be scanned to have at most a 5% risk of failing to detect bacteria at the same levels of $\delta$

| $\delta$ | $\beta$ | $n$ |
|---|---|---|
| 0.25 | 5.6 | 11 |
| 0.20 | 10.7 | 14 |
| 0.15 | 19.7 | 19 |
| 0.10 | 34.9 | 29 |
| 0.05 | 59.9 | 59 |
| 0.01 | 90.4 | 299 |

[a] Probability that *X. fastidiosa* is present in any given microscope field.

**Table 4.** Detection of *Xylella fastidiosa* in the first two adjacent sections of root sampled for 10 naturally infected peach trees

| Root infection[a] | Positive first section | Positive second section | Number of infected roots: Tree 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total roots |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Negative | No | No | 0 | 2 | 8 | 5 | 17 | 18 | 9 | 12 | 14 | 0 | 85 |
| Positive | No | No | 0 | 0 | 1 | 2 | 2 | 1 | 3 | 1 | 1 | 0 | 11 |
| Positive | No | Yes | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 7 |
| Positive | Yes | Yes/no | 32 | 30 | 12 | 27 | 6 | 1 | 5 | 3 | 2 | 24 | 142 |
| Total roots | | | | | | | | | | | | | 245 |

[a] Roots are considered negative if all microscope fields assessed for that root are negative for *X. fastidiosa*. In positive roots, one or more fields contained the pathogen.

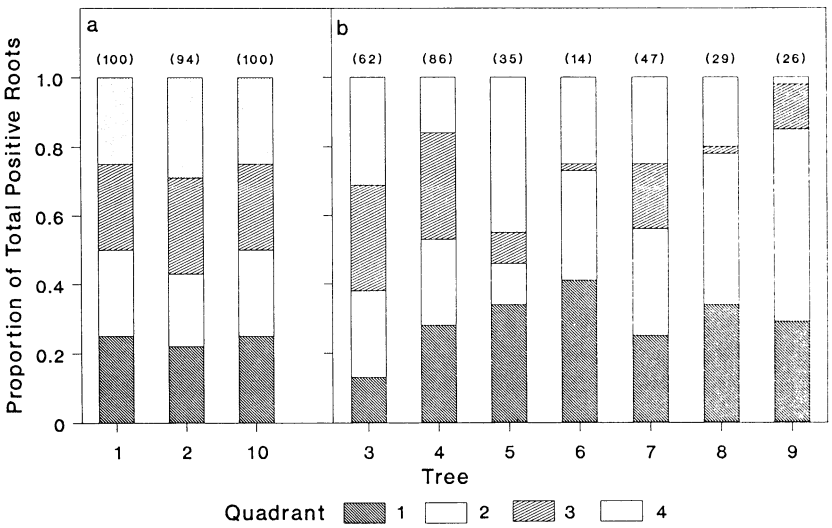


**Fig. 1.** The proportion of roots from which *Xylella fastidiosa* was detected within each quadrant of the root ball for **(A)** trees symptomatic for phony peach disease and **(B)** trees asymptomatic for phony peach disease. Numbers in parentheses represent the percentage of positive roots for each tree.

fluenced by neither the distance the sample was taken from the trunk (section number) nor the size of the root piece sampled (root diameter category) (Table 2). There was a weak, but significant, inverse relationship between section number and root diameter ($R = -0.26$, $P \leq 0.0001$, Pearson correlation coefficients).

**Distribution of *X. fastidiosa* among quadrants.** The proportion of positive roots in symptomatic trees was fairly uniform among the four quadrants of the root zone (Fig. 1A). However, in asymptomatic trees, the proportion of positive roots varied considerably among quadrants (Fig. 1B).

**Sampling strategy to maximize the probability of detecting *X. fastidiosa* within roots of asymptomatic trees.** The minimum number of microscope fields, sections, or roots required to successfully detect *X. fastidiosa* with an acceptable level of accuracy within asymptomatic infected trees is based on the assumption that the bacteria are randomly distributed within the roots.

*Microscope fields.* The minimum number of microscope fields that should be examined depends on the probability that bacteria are present in the section from which the xylem fluid was extracted. If it is assumed that bacteria are randomly distributed throughout the section, such that the probability that bacteria will be found in any given microscope field is $\delta$, then the probability of failing to detect bacteria scanning $n$ independent microscope fields is $(1 - \delta)^n$. When $\delta$ is greater than 0.26, there is less than a 5% chance of failing to detect bacteria in infected trees when $n = 10$. Therefore, for all trees in the high population category and for one tree in the moderate category, the risk of failing to detect bacteria in xylem fluid from an examination of 10 random fields from a single section is less than 5%. However, for probabilities less than 0.20, this risk increases rapidly (Table 3). The number of microscope fields that must be scanned to meet a desired risk $\beta$ can be estimated using the formula $n = \log \beta / \log (1 - \delta)$. This number increases rapidly with small $\delta$ (Table 3).

*Sections.* As the distribution of bacteria appeared fairly uniform along the length of the root (Table 2), the section from which the xylem fluid sample was taken had minimal influence on the probability of detecting bacteria within infected asymptomatic trees. Generally, there is little to gain by sampling more than two sections of any root. Of the 103 roots for which the first section sampled (closest to the trunk) was found to be free of bacteria, 96 (or 93.2%) of these roots were also free of bacteria in the adjacent section (Table 4). Had only the first section of the 245 total roots examined in this study been used to determine the presence of the bacterium, only 18 (or 7.3%) would have been misclassified as free of bacteria. If the second, adjacent section had also been used to determine the presence of the bacterium, the percentage of misclassified roots would have dropped to 4.5%. In no case would this have resulted in a tree being declared disease-free. Therefore, it is generally better to sample more roots than to sample additional sections along the same root.

*Roots.* The following calculations of the minimum number of roots that must be sampled to successfully detect bacteria within asymptomatic infected trees is based on the assumption that the first two sections of each root are sampled. If $n$ roots of the tree are randomly tested, then the probability $P$ of falsely stating the tree does not contain *X. fastidiosa* is given by $P = \sum_{k=0}^{k=n} \binom{n}{k} p^{n-k} (1 - p)^k q^k$, where $p$ is the probability that any given root is not infected, $n$ denotes $n$ objects taken $k$ at a time, and $k$ is the index of summation. The variable $q$ is the conditional probability of failing to detect bacteria given that the root is infected. In the above equation the variable $q'$ can be expressed as the probability of selecting an infected root and failing to detect the bacteria, and it is given by the equation $(1 - p)q$. The values for the probabilities $p$ and $q'$ are presented in Table 5. From the above equation, the minimal number of roots that must be sampled to detect bacteria in asymptomatic trees can be calculated (Table 6). The reasonable ranges for the probabilities $p$ and $q'$ are $0.15 \leq p \leq 0.85$ and $0.05 \leq q' \leq 0.08$. At all levels of risk, the minimum number of roots that must be sampled to detect the pathogen increases dramatically as the probability that the root is not infected increases (Table 6).

**Table 5.** Probability ($p$) that any given root of a naturally infected peach tree is not infected by *Xylella fastidiosa*, and the probability ($q'$) of selecting an infected root and failing to detect the bacterium for each asymptomatic tree[a]

| Tree | $p$ | $q'$ |
|---|---|---|
| 3 | 0.36 | 0.05 |
| 4 | 0.14 | 0.06 |
| 5 | 0.65 | 0.08 |
| 6 | 0.86 | 0.05 |
| 7 | 0.53 | 0.18 |
| 8 | 0.71 | 0.06 |
| 9 | 0.74 | 0.05 |

[a] Given that 10 microscope fields in the first two adjacent sections of root are assessed.

**Table 6.** Minimum number of roots that must be sampled to have a risk of less than 10, 5, and 1% of failing to detect *Xylella fastidiosa* in asymptomatic infected trees at a range of $q'$[a] and a range of $p$[b]

| | Minimum number of roots | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <10% risk | | | | <5% risk | | | | <1% risk | | | |
| | $q'$ | | | | $q'$ | | | | $q'$ | | | |
| $p$ | 0.05 | 0.06 | 0.07 | 0.08 | 0.05 | 0.06 | 0.07 | 0.08 | 0.05 | 0.06 | 0.07 | 0.08 |
| 0.15 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 |
| 0.20 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 |
| 0.25 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 5 | 5 |
| 0.30 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 5 | 5 |
| 0.35 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 6 | 6 | 6 | 6 |
| 0.40 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 6 | 6 | 7 | 7 |
| 0.45 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 7 | 7 | 8 | 8 |
| 0.50 | 4 | 4 | 5 | 5 | 6 | 6 | 6 | 6 | 8 | 8 | 9 | 9 |
| 0.55 | 5 | 5 | 5 | 5 | 6 | 7 | 7 | 7 | 10 | 10 | 10 | 10 |
| 0.60 | 6 | 6 | 6 | 6 | 7 | 8 | 8 | 8 | 11 | 12 | 12 | 12 |
| 0.65 | 7 | 7 | 8 | 8 | 9 | 9 | 10 | 10 | 13 | 14 | 15 | 15 |
| 0.70 | 9 | 9 | 9 | 10 | 11 | 11 | 12 | 13 | 17 | 17 | 18 | 19 |
| 0.75 | 11 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 21 | 22 | 24 | 25 |
| 0.80 | 15 | 16 | 17 | 19 | 19 | 20 | 22 | 24 | 29 | 31 | 34 | 37 |
| 0.85 | 22 | 25 | 28 | 32 | 29 | 32 | 36 | 42 | 44 | 49 | 56 | 64 |

[a] The probability of selecting a positive root and failing to detect the bacterium.
[b] The probability that a root is negative.

## DISCUSSION

Although seven of the 10 trees sampled in the study were not symptomatic for phony peach disease, varying levels of bacteria were detected in their roots. In a study conducted by Wells et al (9), the percentage of infected asymptomatic trees in several experimental plantings was related to the percentage of symptomatic trees. Practically speaking, if a substantial percentage of trees in an orchard were symptomatic, one would expect a higher proportion of the asymptomatic trees to be infected. As a result, the minimum number of root samples required to successfully detect bacteria in infected trees under this scenario would be lower than in an orchard with few symptomatic trees.

Sampling strategy becomes more critical in asymptomatic trees because bacterial populations are low and unevenly distributed. Sampling strategy, therefore, may be devised according to the number of symptomatic trees in the orchard. Wells et al (9) suggest that sampling from more than one region in the root ball in asymptomatic trees optimizes the chances of detecting *X. fastidiosa*. The data from this study are in agreement with this assessment. However, the position on the root from which the sample is taken, or the size of the root piece, appears to have little bearing on whether bacteria are detected in a given root.

The minimum number of roots and fields that must be assessed increases dramatically as the probability that a tree is infected decreases. Based on this study, current methods of sampling (e.g., taking a minimum of three roots per tree) would not detect the presence of bacteria in trees in which the probability of infection was less than 80% (at a risk $\beta = 0.05$). In fact, if the current sampling method had been applied to trees in this study, the number of infected trees would have been underestimated. Since the only suggested method for reducing the incidence of PPD at this time is the removal of infected trees, early and accurate detection of *X. fastidiosa* in asymptomatic peach trees is important. Practically, sampling two sections from five to 10 roots per tree ($\beta = 0.05$ and $q' = 0.05$) and scanning 10 microscope fields should provide a more accurate assessment of infection.


### ACKNOWLEDGMENT

We thank Philip Crampton for field and laboratory assistance.


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