Pear Decline in Connecticut and Response of Diseased Trees to Oxytetracycline Infusion

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We thank M. Finkbeiner, M. Reisner, P. Sasaki, and R. Schlesinger for technical assistance.
Accepted for publication 12 March 1979.

ABSTRACT

McINTYRE, J. L., H. SCHNEIDER, G. H. LACY, J. A. DODDS, and G. S. WALTON. 1979. Pear decline in Connecticut and response of diseased trees to oxytetracycline infusion. Phytopathology 69:955-958.

Pear decline in Connecticut orchards is evidenced by symptomatology, graft transmission of symptoms to indicator hosts, mycoplasmalike organisms in sieve tubes of leaf tissue from diseased trees, and symptom remission after infusion of diseased trees with oxytetracycline (OTC) for two consecutive years. Of 5,318 trees observed in 18 orchards throughout Connecticut, 33% had symptoms of pear decline. Connecticut and California sources of pear decline could not be differentiated by symptoms on indicator pear cultivars. After 1 and 2 yr of treatment, trees infused with OTC showed significant remission of foliar symptoms. After 2 yr of treatment, tree yield (kg/cm^2 trunk area at 30 cm above the soil) and weight of individual fruit were significantly increased. Tree vigor, evidenced by increased trunk circumference and shoot growth, also improved significantly, so fruit yield should increase in the future.

We reported a disorder of pear trees in Connecticut with symptoms similar to the "tree decline" type of pear decline common in western but previously unreported in northeastern orchards of North America (6). The disorder was observed in 15 orchards surveyed in 1977, and 29% of 4,850 trees examined had symptoms. We observed in Connecticut that 42% of 1,696 Bartlett trees and 20% of 2,173 Bosc trees showed symptoms. These observations agree with a previous report (11) that the cultivar Bartlett is more severely affected by pear decline than the cultivar Bosc.

Mycoplasmalike organisms (MLO) in sieve tubes of leaves from diseased trees and remission of foliar symptoms 1 yr after trees were infused with oxytetracycline suggested pear decline (6). Graft transmission studies, however, had not been done.

We now report, on the basis of graft transmission studies and 2 yr of observing symptom remission after oxytetracycline treatment, that pear decline is present in Connecticut orchards. Because diseased trees live for years and produce crops annually, tree productivity and vigor after oxytetracycline infusions also were studied.

MATERIALS AND METHODS

Orchard survey. To extend the range of the 1977 survey, three orchards in the west-central region of Connecticut were surveyed on 21 September 1978 as previously described (6).

Transmission studies. On 14 October 1977, 1- and 2-yr-old scion wood was collected from trees with pear decline symptoms in an 8-yr-old orchard (cultivars Bartlett, Bosc, Magness, and Moonglow). Leaves were removed and the twigs were wrapped in moist paper towels, enclosed in plastic bags, and sent by airmail to California. Single leader indicator hosts (cultivars Precocious, Magness, and Chojuro) were grafted on 19 October 1977 with three scions from different bud sticks of a single cultivar (sources were Connecticut or California). A tip graft was placed at 76 to 100 cm above the soil, a side graft 10 to 15 cm below the tip graft, and another side graft 15 to 20 cm below that. The grafted trees were placed in a greenhouse. Growth from buds on the scions (shoots varied from whorls to 15 cm) had ceased by 1 December 1977, and the trees were placed in a cold room at 3 C. The trees were returned to the greenhouse on 7 February 1978. By 6 March 1978, all scions had produced shoots, some of which were 60 cm long. Since growth of the scions was predominant and caused reduced growth of the indicators, the tip and upper side grafts were removed on 10 March 1978. Final observations of foliar symptoms were made on growth from both the scion and indicator trees on 6 July 1978.

Transmission electron microscopy. Leaf tissue collected from pear trees in the orchards was prepared for transmission electron microscopy as previously described (6).

Symptom remission with oxytetracycline. On 7 October 1976 and 23 September 1977, 20 11-yr-old *Pyrus communis* L. 'Bartlett' pear trees were rated for disease severity and infused with 0.1 g active ingredient (a.i.) of oxytetracycline (OTC) (Terramycin Tree Injection Formula, Pfizer Inc., New York, NY 10016) per tree (3,5,6). Because the incidence of pear decline symptoms in this orchard was high and reliable disease-free control trees were unavailable, OTC-infused trees were compared with 20 untreated trees with symptoms. Trees were rated for symptom remission on 23 September 1977 and 27 September 1978. All trees were rated in the field as previously described, and each tree was photographed to

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enable comparisons of year-to-year changes in symptom severity (6).

Tree productivity and vigor. Pears were harvested on 5 September 1978 and the total number of fruits per tree (standardized to fruit/cm² trunk area at 30 cm above the ground), total weight of fruit per tree (kg/cm² trunk area), and average weight of individual fruit (g/fruit) were determined. Increase in trunk girth, a parameter for tree vigor, was obtained by measuring the circumference at 30 cm above the ground at the time of infusion and at fruit harvest. Tree vigor was also assessed by measuring shoot growth on 21 September 1978. Terminal bud scale scars were used to assess growth for 1977, and 3–10 shoots on the main terminal branch of the first scaffold limb of each tree were measured.

RESULTS

Orchard survey. Of the 468 pear trees (cultivars included Bartlett, Bosc, Seckel, and Devoe) observed in the three orchards surveyed, 80% showed pear decline symptoms. Trees ranged from 2 to ≥40 yr old, and trees of all ages and cultivars had symptoms. The incidence ranged from 38% in one orchard to over 90% in the other two orchards. The greatest number of trees observed were of the cultivars Bartlett (284) and Bosc (131), and 93 and 62% of these trees, respectively, showed pear decline symptoms.

Transmission electron microscopy. We previously reported MLO in sieve tubes of field-collected pear leaves (6). Figure 1 shows the tissue location and abundance of MLO in field-collected leaves from trees of two cultivars with pear decline symptoms.

TABLE 1. Expression of pear decline symptoms in inoculating scions and indicator cultivars[a]

Inoculating scion cultivar[b]	Source[c]	Indicator cultivar	Tests (no.)	Symptoms in:	
				Scion	Indicator
Bosc	CT	Precocious	2	1	2
Bosc	CT	Magness	1	0[d]	1
Moonglow	CT	Precocious	2	2	2
Moonglow	CT	Magness	1	1	1
Magness	CT	Precocious	4	2	2
Magness	CT	Magness	2	2	2
Magness	CT	Chojuro	1	1	1
Bartlett	CT	Precocious	4	4	4
Bartlett	CT	Magness	2	2	1[e]
Precocious	CA	Precocious	1	1	1
Magness	CA	Precocious	1	1	1
Precocious (healthy)	CA	Precocious	1	0	0
Magness (healthy)	CA	Precocious	1	0	0

[a]On 14 October 1977, 1- and 2-yr-old wood for scions from trees in Connecticut with symptoms was collected from an 8-yr-old orchard and sent immediately to California, where twigs were tip- and side-grafted onto indicator cultivars. Symptoms were read on 6 July 1978.
[b]Unless otherwise noted, all scions were from trees with pear decline symptoms.
[c]CT = Connecticut, CA = California.
[d]Only one whorl of two leaves was formed.
[e]The shoot of the indicator tree was broken.

TABLE 2. Remission of pear decline symptoms in Bartlett pear trees infused with oxytetracycline (OTC)[a]

Date of rating	Treatment and average rating (± $SE_{\bar{x}}$)[b]		t Value[c]
	Infused	Untreated	
7 October 1976	1.60 ± 0.15	1.55 ± 0.15	0.23
27 September 1978	0.46 ± 0.11	1.18 ± 0.18	3.26*
t Value[c]	5.89*	1.06	

[a]On 7 October 1976 and 23 September 1977, 20 11-yr-old Bartlett pear trees with pear decline symptoms were infused with 0.1 g a.i. of OTC per tree; 20 untreated trees with symptoms were used as controls.
[b]Ratings: 0 = no visible symptoms; 1 = 10%, 2 = 50%, 3 = 90% of leaves red and curled; 4 = tree dead.
[c]Student's standardized *t* test. Values significant at $P \leq 0.01$ (*).

Transmission studies. Pear decline symptoms, including leaf roll and brittleness and swelling of major veins (9), were evident on growth from scions and indicators on 23 March 1978. Symptoms at the time of final reading included browning of the adaxial mid and major veins of Precocious, browning of the abaxial minor veins of Magness, and browning of the smallest abaxial minor veinlets of Bosc (Bartlett and Moonglow scions). Positive transmission occurred in 84% of the indicator trees (Table 1), and symptoms of Connecticut and California pear decline were similar.

Symptom remission with oxytetracycline. Significant remission of foliar pear decline symptoms was evident 1 yr after trees were infused with OTC (6). After 2 yr of chemotherapy, trees remained significantly improved, compared with initial disease ratings (Table 2). Initial ratings of untreated trees did not differ significantly from those made 2 yr later.

Tree productivity and vigor. In 1978, both total and individual weights of fruit from infused trees were significantly greater than those from untreated trees (Table 3). Infused trees produced more fruit than untreated trees, but the difference was not significant. Shoot growth and trunk enlargement of infused trees were also significantly increased. Shoot growth of treated trees was 71% greater in 1978 than in 1977 ($P \leq 0.01$), compared with a 4% improvement in shoot growth in untreated trees during the same period.

DISCUSSION

Our conclusion that pear decline is present in the northeastern United States is based on graft transmission of symptoms of Connecticut pear decline to indicator hosts, field symptomatology, MLO in sieve tubes of leaf tissue collected from diseased trees, and symptom remission after treatment with OTC for 1 and 2 yr.

The incidence of pear decline in Connecticut (33% of 5,318 trees observed in 1977 and 1978), its presence in each of 18 orchards surveyed, MLO in 13% of the sieve tubes observed (6), and the high incidence of positive transmissions to indicator pear cultivars (84%) indicate that pear decline is widespread in Connecticut. Because symptoms of Connecticut and California pear decline were similar in indicator cultivars, we cannot assume differences in the causal agent between these two areas.

Pear decline was first reported in western North America in 1948 (4), yet it is only now reported in the northeastern United States. Pear decline is known to be transmitted by grafting (10) and by the vector *Psylla pyricola* (2), an insect introduced to North America via Connecticut in 1832 (8). The high incidence of pear decline in 2 to 10-yr-old trees suggests that pear decline may have been introduced to Connecticut only recently, and the vector has spread it to older trees (6). Symptoms of pear decline, however, may have been

TABLE 3. Fruit yield and tree vigor of Bartlett pear trees infused with oxytetracycline (OTC)[a]

	Average values (± $SE_{\bar{x}}$)		
	Infused	Untreated	t Value[b]
Total fruit weight (kg/cm²)	0.53 ± 0.06	0.36 ± 0.07	2.16**
Weight/fruit (g)	114.61 ± 6.84	87.57 ± 4.98	3.19***
Total fruit/cm²	4.94 ± 0.60	4.13 ± 0.51	1.03
△ Circumference (cm)[c]	2.23 ± 0.16	1.63 ± 0.10	2.21**
Shoot growth (cm)[d]	24.65 ± 2.91	17.47 ± 2.51	1.87*

[a]On 7 October 1976 and 23 September 1977, 20 11-yr-old Bartlett pear trees with pear decline symptoms were infused with 0.1 g a.i. of OTC per tree; 20 untreated trees with symptoms were used as controls. Fruit were harvested on 5 September 1978. Total fruit weight per tree and total fruit number per tree were standardized to trunk area at 30 cm above the ground.
[b]Student's standardized *t* test. Values significant at $P \leq 0.10$ (*), 0.05 (**), or 0.01 (***).
[c]Trunk circumference was measured at 30 cm above the ground on 7 October 1976, 25 August 1977, and 5 September 1978. The value for 1978 represents changes in trunk girth from 1977 to 1978.
[d]Shoot growth was measured on 21 September 1978.

observed in Connecticut in 1960 (1), and the higher incidence of pear decline in younger trees may be a reflection of less severe symptoms in older trees (7).

Foliar symptoms of pear decline remitted in trees treated with OTC by the end of the following growing season (6). The rating of pear decline symptoms after two yearly infusions continued to decrease. However, untreated trees also were rated lower in 1978 than in 1977. Foliar symptoms in 1977 may have been more intense than in 1978 because the summer of 1977 was exceptionally dry, whereas rainfall exceeded normal in 1978. In a dry year, phloem blockage symptoms, such as those caused by pear decline, would be expected to be more severe. However, there were no significant differences in ratings for untreated trees when 1977 or 1978 values were compared to 1976 values, whereas trees infused in 1976 and 1977 showed significant improvements in both 1977 and 1978.

One year after trees were infused with OTC, fruit size was the only significant improvement compared with untreated trees; the increase was not due to thinning of fruit on the tree (6). After two consecutive years of OTC treatment, infused trees showed significant improvements in tree productivity, including adjusted total fruit weight per tree and weight per fruit. Results of a consumer preference taste test showed that fruit from OTC-treated trees was preferred in three of four tasting sessions (L. Hankin, G. Lacy, and J. McIntyre, *unpublished*).

An increase in fruit yield of 47% was realized after two yearly infusions with OTC. On the basis of increased tree vigor, as indicated by increases in trunk girth and shoot growth, even greater improvements in fruit yield can be predicted.

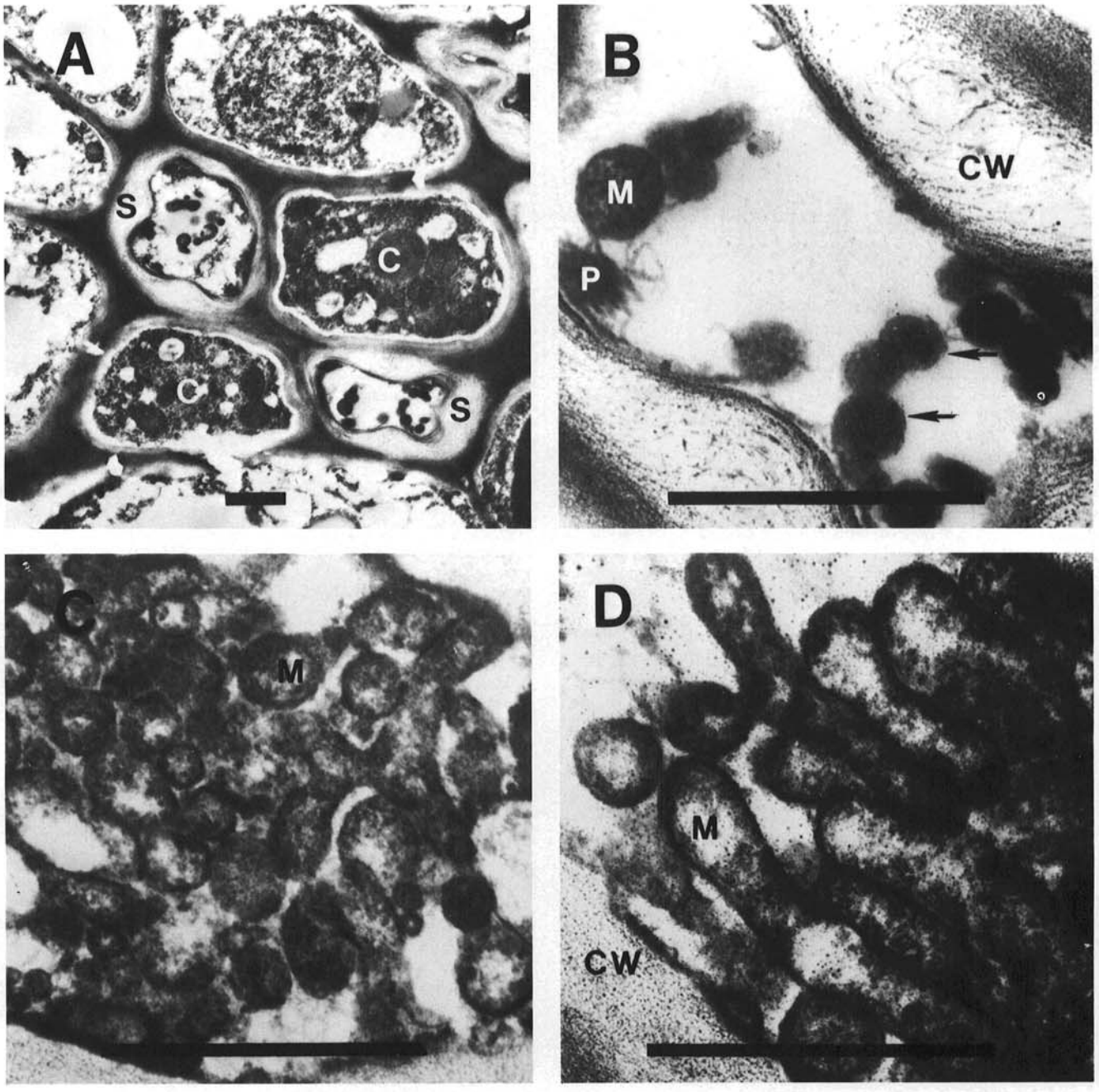

Fig. 1. Electron micrographs of mycoplasmalike organisms (MLO) in sieve tubes of leaves collected from Bartlett and Magness pear trees with pear decline symptoms. **A,** Two sieve tubes with MLO in a small region of phloem (Bartlett). **B,** One of the tubes at higher magnification showing P-protein and MLO; a membrane can be distinguished around some MLO (arrows). **C,** High concentration of MLO in a sieve tube (Magness). **D,** Elongate MLO (Magness). C = companion cell, CW = sieve tube cell wall, M = MLO, P = P-protein, S = sieve tube. Bar represents 1μm.

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Techniques

Practical Nuclear Staining Procedures for Rhizoctonialike Fungi

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Approved for publication as Journal Article 98-78 of the Ohio Agricultural Research and Development Center.
Accepted for publication 21 March 1979.

ABSTRACT

HERR, L. J. 1979. Practical nuclear staining procedures for Rhizoctonialike fungi. Phytopathology 69:958-961.

An HCl-Giemsa staining procedure and two rapid direct staining methods were used to stain nuclei of multinucleate and binucleate Rhizoctonialike fungal isolates. Isolates were cultured on thin layers of Difco potato dextrose agar (DPDA) in plastic petri plates. Disks cut from the cultures were transported through the HCl-Giemsa staining process in tissue carrier mounts. Stained disks were mounted in dark corn syrup. The DPDA cultures also were stained directly in the plates with either 0.5% aniline blue or 0.05% trypan blue in lactophenol. In either case, the mycelium to be stained first was wetted with an acidified wetting agent to facilitate staining. Cover slips were added, and the stained hyphae were microscopically examined in the plates with a dry × 40 objective. Of the staining methods tried HCl-Giemsa consistently gave better results.

Determination of the numbers of nuclei in vegetative cells aids in distinguishing between *Rhizoctonia solani* Kühn and Rhizoctonialike fungi in the absence of the perfect state (6–8). Parmeter et al (8) investigated Rhizoctonialike cultures and separated them into groups with: (i) multinucleate vegetative cells and (ii) binucleate cells. In agreement with Flentje et al (4) all *R. solani* isolates were multinucleate. Parmeter and Whitney (7) reviewed much of this literature.

Collectively, the procedures for staining nuclei of *R. solani* and Rhizoctonialike fungi with HCl-Giemsa (1,2,4,8,10,13) seemingly have little interrelationship. Some of the procedures involve permanent mounting of stained preparations for detailed cytologic studies; for simple enumeration purposes, schedules can be less complex.

Other nuclear stains, as well as phase contrast microscopy, also have been used with *R. solani*, but reference will be limited to two rapid staining procedures suitable for doliform pore detection and nuclear staining: (i) an aniline blue staining technique reported by Tu and Kimbrough (12) and (ii) the method of Sanders et al (11) based on trypan blue staining. Burpee et al (3) recently published an expanded account of the latter method.

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The objective of this study was to make these staining procedures, especially HCl-Giemsa, more practical and reliable for routine nuclear staining as an aid to identification of *R. solani*.

MATERIALS AND METHODS

General. Rhizoctonialike isolates were cultured on Difco (Difco Laboratories, Detroit, MI) potato dextrose agar (DPDA) in 9-cm diameter plastic petri plates containing 7 ml of the medium. Transfers were made to one side of plates and, when growth was sufficient, a disk was cut in the colony with an 11-mm diameter cork borer. The disk then was transferred with a curved section lifter to a Tims® (Lab-Line Inc., Melrose Park, IL) deep-bottom tissue carrier (2.54 × 2.54 × 0.64 cm). The holes in the carriers were enlarged to 0.4 mm diameter to speed drainage. Groups of four or five carriers (number fitting readily into a 9-cm petri dish bottom) could be taken conveniently through the staining schedule together. The culture plates from which disks were removed were retained for subsequent rapid, direct staining of isolates in the plates with aniline blue and trypan blue stains.

HCl-Giemsa schedule. Culture disks were fixed in a 3:1 mixture of 95% ethanol and glacial acetic acid in a wide-mouth jar (9 × 11 cm) with lid for 10 min (volume was sufficient to cover the carriers completely, ie, 4–16 culture disks), transferred to (i) 95%