SUPPRESSION OF BACTERIAL LEAF SCORCH SYMPTOMS IN AMERICAN ELM THROUGH OXYTETRACYCLINE MICROINJECTION

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Abstract. American elms with bacterial-associated leaf scorch (ELS) were injected with oxytetracycline (OTC) to determine if the antibiotic would cause a delay in symptom onset or a remission of symptoms. Degree of symptom expression and presence of fastidious, xylem-inhabiting bacteria were determined in August 1982. Eleven symptomatic trees were injected in August 1982 with OTC (40 mg a.i./cm dbh) delivered via Mauget capsules. OTC was injected at 40 mg a.i./cm dbh into 14 trees and at 80 mg a.i./cm dbh into 8 trees using a pipette injection technique. Ten untreated, symptomatic trees served as controls. In June 1983, all OTC treated trees were injected using prefilled Mauget capsules at dosage levels of 50 mg a.i./cm dbh or 80 mg a.i./cm dbh. By July, symptoms appeared in 7 of 10 untreated trees but only in 2 of the remaining 29 OTC-treated trees. By late August, symptoms were absent or reduced in 22 of 23 trees treated at the low OTC levels, and 3 of 6 trees treated at the high OTC level. Symptom remission was most apparent in trees which had less than 20% of the canopy leaf area affected with ELS prior to treatment. Delay of symptom onset and symptom remission support the role of the fastidious, xylem-inhabiting bacterium as the causal agent of ELS.

Symptoms of elm leaf scorch (ELS) develop by mid-July in the Washington, DC, area (Wester and Jyllka, 1959; Hearon *et al.*, 1980) and are characterized by an undulating, marginal necrosis bordered by a chlorotic halo. During the summer months symptoms progress acropetally, becoming more pronounced. Necrosis extends inward towards the midrib and leaves curl. Early leaf abscission commonly leaves a tuft of unaffected leaves at the branch apex. Affected trees may exhibit branch dieback (Hearon *et al.*, 1980) and have been reported more susceptible to *Ceratocystis ulmi,* the Dutch elm disease fungus, than are non-scorching trees (Wester and Jyllka, 1963). Fastidious, xylem-limited bacteria have been associated with elm and oak leaf scorch (Hearon *et al.*, 1980) and have been shown to be the cause of sycamore leaf scorch (Sherald *et al.*, 1983) and mulberry leaf scorch (Kostka *et al.*, 1983). Although the elm leaf scorch-associated bacterium has been cultured on artificial media, pathogenicity has not been demonstrated (Kostka *et al.*, 1981).

Tetracycline antibiotics block protein synthesis in prokaryotes and have been used to control diseases caused by mycoplasma-like organisms (McCoy, 1982). These materials, applied as a soil drench, have been used to demonstrate the involvement of fastidious, xylem-inhabiting bacteria in Pierce's disease of grape (Hopkins and Mortensen, 1971; Hopkins and Mollenhauer, 1973) and citrus young tree decline (Tucker *et al.*, 1974). Nyland (1979) demonstrated that oxytetracycline (OTC) injected into the xylem of almonds affected by leaf scorch could serve as a therapeutic control.

The objective of this study was to determine if injections of OTC into the xylem of leaf scorch-affected American elms would cause a remission of symptoms.

Materials and Methods

Naturally infected American elms *(Ulmus americana),* 2 to 6 m in height, were selected for treatment. All trees were growing in naturalized sites in Washington, DC. Symptom expression was initially evaluated in August 1982. Isolations were made from all selected trees by aseptically

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incubating excised wood chips in a modified PW broth (Sherald *et al.*, 1983) or by vacuum extracting bacteria from stem segments and confirming their presence using phase contrast microscopy (French *et al.*, 1977; Hearon *et al.*, 1980; Sherald *et al.*, 1983).

Two microinjection techniques were selected for introduction of OTC (Terramycin Tree Injection Formula, Pfizer Chemical Company): 1) Mauget capsules (McIntyre *et al.*, 1978; McCoy, 1982) and 2) the pipette injection technique (Lacy and McIntyre, 1978) developed for the introduction of antibiotics into pears with pear decline. OTC levels were established from the rates developed for control of elm phloem necrosis (Filer, 1976). Trees were treated in August 1982 utilizing both techniques, while in June 1983 treatments were made with Mauget capsules.

Eleven symptomatic trees (approx. 12 cm dbh) were injected in August 1982 with OTC, 40 mg a.i./cm dbh (low level), delivered via Mauget capsules (provided by J.J. Mauget Co.). OTC (Terramycin Tree Injection Formula) was injected at 40 mg a.i./cm dbh into 14 symptomatic trees and at 80 mg a.i./cm dbh (high level) into 8 trees using the pipette injection technique. Ten untreated, symptomatic trees served as controls. In June 1983, all OTC treated trees were injected using prefilled Mauget capsules at dosage levels of approximately 50 mg a.i./cm dbh (low level) or 80 mg a.i./cm dbh (high level).

The success or failure of treatments was based on the following observations: 1) presence or absence of symptoms in August 1983, 2) date on which first symptoms appeared, and 3) degree of symptom remission.

Results

Initial symptom development was observed in June of 1982. By August, symptoms were well developed. Symptoms in selected trees ranged from 10 to 90 percent of the total leaf area of the canopy. The presence of fastidious, xylem-inhabiting bacteria in symptomatic trees was confirmed by isolation and/or buffer extraction of stem segments and microscopic observation of bacteria in extracts.

Uptake times for antibiotic solutions were approximately 24 hours for the August 1982 treatments and less than 4 hours for the June 1983 treatments. The pipette injection technique was eliminated in 1983 because of leakage around the pipette and the excessive depth required for the injection hole to securely hold the pipette.

By mid-July 1983, 7 of 10 control trees were exhibiting leaf scorch symptoms (Table 1). Symptoms were extensive in two of the trees and limited in the remaining five. Symptoms were observed in 1 of 25 trees injected at the low OTC levels (40/50 mg a.i./cm dbh) using Mauget capsules. Of the six trees injected at the high OTC level (80 mg a.i./cm dbh) symptoms had only developed in one tree. Two trees were accidently removed for right-of-way clearance. Although symptoms developed in 2 of the treated trees, the majority were symptomless in July.

In August, 1983 all trees were rated for presence and severity of symptoms (Table 1). Percent total leaf area affected in untreated, control trees was equivalent to or increased above 1982 levels. Fourteen of the 23 scorch-affected elms treated at the low OTC levels showed a complete remission of symptoms. Of the remaining 9 trees, a reduction of symptoms was observed in 8, while increased symptoms were observed in 1. Two trees were removed because of Dutch elm disease. Results from trees treated at the high OTC level were inconsistent. Of the 6 trees remaining, 3 trees had substantially reduced symptoms and 3 were unchanged from 1982. A complete or near complete remission of symptoms was observed in treated trees which had 20 percent or less of the total leaf area exhibiting leaf scorch in 1982 (Table 2).

Discussion

When the rules of proof (Koch's postulates) cannot be fulfilled with a fastidious prokaryote (i.e., the fastidious, xylem-inhabiting bacterium), an important step in confirming the involvement of the organism in disease is demonstration of symptom remission through antibiotic treatment (Hopkins and Mortensen, 1971; Nyland, 1979; McCoy, 1982). Oxytetracycline injected into the xylem of American elms affected with bacterial leaf scorch delayed symptom onset and suppressed symptom development. Remission was

Table 1. Pre- and post-treatment comparison of percent elm leaf scorch-affected canopy area in American elms injected with oxytetracycline in August 1982 and June 1983.

Mauget injection
Low level [a]
% Leaf scorch

Tree #	8/82	7/83	8/83
1	10	0	0
2	10	0	≪10[d]
3	50	0	DED[f]
4	<10[e]	0	0
5	10	0	0
6	10	0	0
7	10	0	0
8	<10	0	0
9	95[g]	0	≪10
10	>20	0	<10
11	>80	0	30

Pipette/Mauget injection
High level [c]
% Leaf scorch

Tree #	8/82	7/83	8/83
26	25	0	10
27	<10	0	>10
28	>50	0	30
29	50	0	50
30	>90	removed[h]	
31	>50	removed	
32	75	0	<10
33	>30	30	>30

Pipette/Mauget injection
Low level [b]
% Leaf scorch

Tree #	8/82	7/83	8/83
12	>30	0	DED
13	20	0	0
14	>25	<10	<10
15	20	0	0
16	>10	0	<10
17	>10	0	≪10
18	>10	0	0
19	<10	0	0
20	10	0	0
21	10	0	0
22	>20	0	70
23	<10	0	0
24	20	0	≪10
25	20	0	0

Controls
% Leaf scorch

Tree #	8/82	7/83	8/83
34	>90	50	90
35	<10	10	>10
36	<10	10	40
37	<10	10	10
38	40	10	100
39	>50	10	80
40	75	80	100
41	>20	0	50
42	<10	0	10
43	80	0	100

a August, 1982; Mauget, 40 mg a.i./cm dbh.
June, 1983; Mauget, 50 mg a.i./cm dbh.
b August, 1982; pipette, 40 mg a.i./cm dbh.
June, 1983; Mauget, 50 mg a.i./cm dbh.
c August, 1982; pipette, 80 mg a.i./cm dbh.
June, 1983; Mauget, 80 mg a.i./cm dbh.
d 10 indicates negligible symptoms.
e = less than.
f Tree killed by Dutch elm disease.
g = greater than.
h Tree removed for right-of-way clearance.

Table 2. Percent leaf scorch symptoms in oxytetracycline-treated (low level) American elms before (1982) and after (1983) treatments. Trees are grouped in pre-treatment severity categories.

0-10% Leaf scorch

Tree #	1982	1983
1	10	0
2	10	<<10[a]
4	<10[b]	0
5	10	0
6	10	0
7	10	0
8	<10	0
19	<10	0
20	10	0
21	10	0
23	<10	0

11-20% Leaf scorch

Tree #	1982	1983
13	20	0
15	20	0
16	>10[c]	<10
17	>10	<<10
18	>10	0
24	20	<<10
25	20	0

21-50% Leaf scorch

Tree #	1982	1983
3	50	DED[d]
10	>20	<10
12	>30	DED
14	>25	<10
22	>20	70

50-100% Leaf scorch

Tree #	1982	1983
9	>95	<<10
11	>80	30

a 10 = Negligible symptoms
b 10 = less than 10%
c 10 = greater than 10%
d Tree killed by Dutch elm disease

most effective when 20% or less of the canopy leaf area was affected prior to treatment. Results in trees with more than 20% leaf scorch were inconsistent at either OTC dosages. The development of symptoms in treated trees was not unexpected based upon previous OTC injection studies of almond trees affected with almond leaf scorch (Nyland, 1979). Inconsistent symptom remission may be due to inadequate distribution or final concentration of the antibiotic in the xylem to inhibit bacterial growth.

The observed delay in the onset of symptoms and the complete or partial symptom remission that occurred in treated trees supports the role of an OTC-sensitive organism (i.e., the fastidious, xylem-inhabiting bacterium) as the causal agent of elm leaf scorch. Although OTC induced symptom remission, its applicability as a therapeutic control requires further study to determine long-term control and potential phytotoxicity of the antibiotic in treated trees.

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ABSTRACT

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When you're ready to plant a tree, carefully prepare the site. Good preparation includes digging a large-enough hole, removing any large rocks or debris from the fill soil, and providing a proper soil layer between the bottom of the hole and the bottom of the tree. Soil preparation is best done when the soil is moist but not wet. The hole you dig should be 12 inches wider than the present root ball of the tree, and 6 to 8 inches deeper. If soil conditions are poor (either very sandy or a heavy clay), dig a larger hole than would normally be required. Position the tree no deeper than the original soil line visible around the trunk. Spread the roots as evenly as possible, and fill the area around them with prepared soil. When three-quarters of the hole has been filled with soil, add water to eliminate all air pockets that would eventually dry out the roots. Then fill the rest of the hole with soil. After planting the tree, you should make a commitment to care for it. Most newly planted trees that die are the victimes of neglect.