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Symptom remission of peach X-disease using Mauget microinjection of oxytetracycline /

Julianne T. Schieffer
University of Massachusetts Amherst

SYMPTOM REMISSION OF PEACH X-DISEASE USING MAUGET MICROINJECTION OF OXYTETRACYCLINE

A Thesis Presented

By

JULIANNE T. SCHIEFFER

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JULIANNE T. SCHIEFFER

Approved as to style and content by:

Terry A. Tattar, Chairperson of Committee

Daniel R. Cooley, Member

Mark S. Mount, Member

Mark S. Mount, Department Head
Plant Pathology

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ABSTRACT

Oxytetracycline (OTC) has a history of successfully inducing symptom remission of X-disease in peach. However, traditional techniques of injecting the antibiotic tend to be labor intensive, cumbersome, and cause wound damage to trees. Mauget microinjection, used to deliver chemicals to shade trees with little injury, was considered for applications of OTC in commercial peach orchards. In September 1986, peach and nectarine trees in 4 Massachusetts orchards were rated on a 0 - 4 scale according to severity of X-disease symptoms. In October 1986, pressurized capsules (one 4 ml capsule / 5 cm trunk diameter) were used to deliver 4% OTC to selected trees. One year later, symptoms on OTC-treated trees were absent or less severe while untreated controls either remained unchanged or became worse when compared to the previous year's ratings. Fruit yields on OTC-treated trees were significantly higher than for untreated trees. Injections were most effective for trees with symptoms that occurred on 50% or less of the crown. Mauget microinjection of OTC was found to be an easy, effective technique for management of X-disease in commercial peach orchards.

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CHAPTER I

INTRODUCTION

A destructive and mysterious disease of peach (*Prunus persica* Batsch.) was initially discovered at a Connecticut orchard in 1933 (Stoddard, 1934). The name "X-disease" was given to this new disorder to denote its unknown nature. Subsequent transmission studies indicated that this disease was caused by a virus (Stoddard, 1938) and the disease also became known as yellow-red virosis or Eastern X-disease (Hildebrand, 1953). The first reported case of X-disease in Massachusetts came from Worcester and Middlesex counties in 1938 (Boyd).

In 1967, Doi, et al. found that mycoplasma-like organisms (MLOs) were responsible for aster yellows disease, thereby leading to more studies on diseases of supposed virus origin. By 1970, MLOs in the phloem of X-infected trees were found to be associated with peach X-disease (Nasu, et al., 1970; Granett & Gilmer, 1971; Jones, et al., 1974). The MLOs were spherical, oval or filamentous, approximately 5,400 mu long and 120-360 mu in diameter, and bound by a unit membrane with dense ribosome-like granules and DNA-like fibrils (Huang & Nyland, 1970; Macbeath, et al., 1972; Sinha & Chiykowki, 1980). Evidence also exists that spiroplasmas may be part of the X-disease etiology (Kloepper, 1983; Raju, et al., 1984; Thomson, et al., 1978).

Transmission of the MLOs occur most commonly from wild chokecherry hosts (*Prunus virginiana* L.) to peach and nectarine (*Prunus persica* var. *nectarine* (Ait.) Maxim)

(Stoddard, 1938) through leafhopper vectors (Wolfe, et al., 1951; Rosenberger & Jones, 1978). The most common vectors for the eastern United States include the common orchard inhabitants *Collandonus clitellarius* Say, *Fieberiella florrii* Stal., *Gyponarra lamina* Delong, *Norvellina seminuda* Say, *Paraphlepius irroratus* Say, and *Scaphytopius acutus* Say (Gilmer & Blodgett, 1976; McClure, 1980). The range of this disease extends over much of the northeastern and midwestern United States plus adjoining Canada (Gilmer & Blodgett, 1976).

Closely related strains of the disease appear on peach and cherry in the western United States (Rawlins & Thomas, 1941; Granett & Gilmer, 1971; Gilmer & Blodgett, 1976). These strains are known most commonly as western X-disease, peach yellow leaf roll, or cherry buckskin (Gilmer & Blodgett, 1976).

A tree infected with X-disease is asymptomatic when it breaks out of dormancy. After seven to eight weeks, sections of the tree, usually the scaffolds or suckers nearest the tree base, begin to show a diffuse chlorosis. Soon the leaves become brittle, develop red spots, tatter, and curl upward (Palmiter & Hildebrand, 1943; Stoddard, 1947). The oldest diseased leaves fall, often leaving a rosette of chlorotic, dwarfed leaves at the tip of each infected branch. This produces a "rat tailing" effect as the terminal bud breaks prematurely. Diseased trees have reduced growth and lower fruit set and yields (Lacy & McIntyre, 1980; Douglas, 1986a). Asymptomatic scaffolds within the same tree may continue to grow and bear, but the fruit often lacks flavor or is bitter (Stoddard, 1938). Death of the tree, or winter kill of affected sections, often follows within one to five years (Gilmer & Blodgett, 1976).

Histopathological observations show that the phloem of an X-infected tree is killed. The sieve tubes and companion cells become necrotic and collapse in the older parts of affected phloem, eventually filling with wound gum. The cambium then produces a wide ring of secondary phloem, which soon becomes necrotic (Schneider, 1945).

Attempts to control this terminal disease began soon after its discovery. Pruning X-diseased scaffolds is often unsuccessful as the disease may reappear the following season (Stoddard, 1938). Stoddard (1947) found that soaking X-infected buds in aqueous solutions of various sulfa compounds, quinhydrone, 8-hydroxyquinoline deritives, p-nitrophenol, or resorcinol reduced transmission of X-disease in subsequent grafted bud unions. But it also caused severe damage to the trees, so studies were discontinued (Stoddard, 1938; 1947).

Currently, the recommended procedures for management of X-disease are eradication of chokecherries within 500 feet of orchards, immediate removal of all X-diseased trees, eliminating ground cover, and managing leafhopper populations (Stoddard, 1938; Palmiter & Hildebrand, 1943; Parker, et al., 1963; Butkewich, et al., 1987; Douglas & McClure, 1988). Antibiotic therapy can cause remission of

X-disease symptoms and in some cases may be able to cure the disease (Butkewich, et al., 1987; Douglas & McClure, 1988; D. Rosenberger, personal communication).

Antibiotic therapy for X-disease grew from the discovery that tetracyclines suppressed the symptoms of MLO-caused mulberry dwarf (Ishiie, et al., 1967). In 1971, Nyland first reported symptom remission using a gravity infusion technique to treat scaffolds of X-infected peach and declining pear. Two liters of tetracycline hydrochloride (TC) solution (34 ug/ml) were placed in a reservoir bottle which was suspended over a network of tubes leading to the scaffold injection sites. In 1975, Sands and Walton compared the effects of TC versus oxytetracycline hydrochloride (OTC). They filled small holes drilled under X-diseased scaffolds with approximately 2 ml of a 7.5% or 10% (w/v) concentration of each chemical. Autumn injections of OTC appeared more effective in producing symptomless branches in the following year. In 1977, Rosenberger and Jones compared OTC application equipment, concentrations, and timing. Injections of 1.25, 2.5 and 3.75 g per tree in September induced remission of symptoms for one year. Spring, summer, or late fall injections of 0.5 or 0.9 g OTC per tree were less effective. When they compared spraying, pressure injection, gravity infusion, and pipetting techniques, they concluded that injection of concentrated materials was the most rapid and convenient application technique, but concentrated 2.5 g of OTC caused necrosis around injection sites. In 1978, Allen and Davidson induced remission of X-disease symptoms with 100-2400 mg OTC per tree, using gravity infusion or a syringe to fill drilled holes. Also in 1978, Pearson and Sands compared over 2 years the responses on X-diseased trees from a commercial formulation of OTC, and analytical grades of TC and OTC. Fifteen percent, 10%, and 7.5% active ingredient (a.i.) per tree was delivered to drill holes under diseased scaffolds. The analytical tetracycline formulations showed greatest X-symptom remissions while trees treated with the commercial formulation of OTC over 2 consecutive years showed the greatest fruit yields. Table 1 summarizes the chemotherapy techniques, chemicals, and results of treatment for symptom remission of various MLO-caused fruit diseases.

In 1982, Lacy compared all the available injection methods in terms of efficacy, cost, and damage to the trunk. He reported that trees infused by gravity (500-2000 mg a.i./1000 ml) and by pipettes (100-300 mg a.i.) were damaged less than ones infused by the (no longer available) Mauget cup (100-300 mg a.i./10-15 ml), concentrated OTC (100 mg a.i./1.0 ml), or pressure injection (500-2000 mg a.i./1000 ml). The least elaborate and lowest cost technique was pipette injection. However, trees treated by pipette showed incomplete symptom remittance in scaffolds of some trees, indicating limited transport of OTC.

Injected antibiotics rarely enter the phloem directly. This is partly because of the positive pressure which exists in phloem cells, and because the act of wounding quickly results in sealing off of exposed cells (Crafts & Yamaguchi,

Table 1. Comparison of chemotherapy used to produce symptom remission of MLO-caused fruit tree diseases.

Disease	Method	Chemical	Concentation	Comment	Reference
XD	top injection soil watering	sulfa compounds misc. phenols misc. others	varied	trunk damage ineffective	(73)
XD PD	gravity infusion	TC	34 ug/ml in 2 l 100 mg/ml in 3 l	successful	(33)
PD	gravity infusion	OTC	6-8 qts of 100 ug/ml	successful 2 trtmts best	(47)
XD BS	fill holes with concentrate	TC OTC	7.5%, 10%, 15% (w/v) soln	successful	(62)
PR	spraying modified gravity	TC CTC* OTC-dihydrate	10 ml of 500 mg/ml	ineffective " "	(30)
XD	spraying gravity infusion concentrate pressure inject. gravity infusion	OTC	15 l of 20% 0.5, 0.9g ai. 2.50 g ai. 1.25-3.75 g ai. 1.25-3.75 g ai.	ineffective " necrosis successful "	(58)
XD	gravity infusion " syringe concentrate	OTC	100 mg 200 mg 800-2400 mg	ineffective more effective extensive damage	(1)
PD	Mauget cups	OTC	0.1 g ai./tree	successful	(43,44)
XD	fill holes with concentrate	TC (analytical) OTC (analytical) OTC (commercial)	0.6-1.0 g ai./tree	most effective " more fruit	(51)

1 X-disease

2 Pear decline

3 Bacterial spot

4 Peach rosette

*chlorotetracycline

ai. = active ingredient

Continued on next page.

Table 1. (Continued) Comparison of chemotherapy used to produce symptom remission of MLO-caused fruit tree diseases.

Disease	Method	Chemical	Concentration	Comments	Reference
XD	pipette	OTC	0.1, 0.5 g 1.0, 2.0 g ai./tree	needs 2 yr trtmt trunk damage	(36)
XD CB	pressure inject.	OTC	2-3g/tree	successful	(48)
		Ca-Afn	3-6g/tree	"	
		TC-199	"	"	
		chlorampehnicol	"	"	
		erythromycin	"	"	
		tylan	"	"	
XD	concentrate	OTC	100 mg/ml in 1 ml	trunk damage	(34)
	pipette		10 mg/ml in 10 ml	partial remission	
	pipette		20 mg/ml in 15 ml	"	
	Mauget cup		12 mg/ml in 50 ml	most damage	
	gravity flow		0.5 mg/ml in 1 l	least damage	
	pressure inject.		0.25mg/ml in 1333ml	trunk damage	
	pipette	OTC	10 mg/ml in 10 ml	most successful	
	"	erythromycin	500-1000 mg/10-15ml	ineffective	
	"	spectinomycin	"	killed tree	
	"	sulfanilamide	"	ineffective	
	"	streptomycin	500 mg/ml in 10 ml	killed tree	
	"	taurolin	300-2000 mg/ 15 ml	ineffective	
	"	salt	2000 mg/ 1000 ml	ineffective	

1 X-disease

2 Cherry buckskin

ai. = active ingrediant

1964; Shigo & Marx, 1977; Shigo & Wilson, 1982). Instead, a chemical enters the xylem where it is translocated in the transpiration stream to eventually act on MLOs within the phloem (Chaney, 1978; Sachs, et al., 1978). Tetracyclines inhibit protein synthesis (Hash, 1972) and have been shown to disrupt unit membranes and condense the cellular contents of MLOs within the phloem (Wolanski, et al., 1971). Because of the extensive contact with the host during transport of the antibiotic from the entry site to the target area, a method which minimizes chemical damage to host tissue is desirable. Also, many factors such as operator technique, chemical formulation (Shigo, et al., 1977), and weather (Shigo, 1981; Clifford, et al., 1984) determine uptake and the resulting length of host-chemical contact.

Currently, the treatment methods used to deliver the antibiotic are inefficient (Lacy, 1982; Kostka, et al., 1985), cumbersome (Sands & Walton, 1975; Reil & Beutel, 1976), time consuming (Sands & Walton, 1975; Rosenberger & Jones, 1977b), and cause trunk damage (Rosenberger & Jones, 1977b; Keil & Civerolo, 1979; Lacy, 1982). Table 2 evaluates the available techniques used for symptom remission in MLO-caused diseases. A relatively inexpensive, yet easy and efficient OTC delivery system could quickly gain grower acceptance.

In 1977, Rosenberger and Jones, after comparing various OTC concentrations using the different application techniques, stated that an injection method using higher solution concentrations of OTC and less volume per tree,

Table 2. Evaluation of injection techniques available for delivery of tetracyclines for symptom remission of MLO-caused diseases. (See references 1, 7, 32, 34, 36, 42, 47, 51, 55, 58, and 62).

	TECHNIQUE			
CHARACTERISTICS	Gravity Infus.	Pressure Inject.	Pipette	Fill Holes
Hole Size	1/4"	3/8"	3/8"	3/16"
Hole Depth	1 1/4 - 1 1/2"	1 - 3"	2"	2"
Uptake Time	30 min.- 48 hrs	3 - 8 min.	1 min.	.5 - 2 min.
Efficiency	+++	+++	++	++
Labor (trees/hr)	15 - 25	2 - 3 +	100	100
Trunk Damage	minimal	gum, extensive	mod-severe	extensive
Problems	slow, cumbersome	leaks	leaks	damage
Cost*	$2 - $5	$43 - $500	.15 - .40	$1

1 Recommended for use on peach X-disease in New York state. Recommended rate is 6.4 oz OTC / 7.5 gallons water (D. Rosenberger, personal communication).

2 Currently the method of choice for large scale commercial pear orchards in California. Recommended rate for symptom remission of pear decline is 1 qt of 600 ppm Terramycin (OTC) (Reil & Beutel, 1976; McCoy, 1982).

3 Currently used in the state of Connecticut for symptom remission of peach X-disease. Recommended dose is 6.6 oz OTC / 1 gallon water (S. Douglas, personal communication).

4 Used in research by Sands & Walton, 1975.

* Cost does not include labor or the chemical, only equipment.

+++ = very effective for remission of MLO-caused symptoms.

++ = partial remission of MLO-caused symptoms possibly due to incomplete translocation of the antibiotic (Lacy, 1982).

would reduce treatment time without loss of effectiveness. The Mauget microinjection technique meets these criteria.

Mauget microinjection is used widely to deliver chemicals effectively with little injury to shade trees. A small pressurized Mauget capsule, containing concentrated 4% OTC in 4 ml of solution, is attached to a feeder tube and plugged into a small 3/16" drilled hole. This self-contained, disposable capsule may have the potential to become an effective, easy technique for symptom remission of X-disease. The objectives of this research were as follows:

1. To evaluate the efficiency of Mauget microinjection as a method for delivery of OTC for remission of X-disease in a commercial peach orchard;

2. To establish the X-disease symptom threshold at which a peach tree in a commercial peach orchard can be most effectively treated;

3. To determine the wound reaction from Mauget microinjection of OTC.

CHAPTER II

MATERIALS AND METHODS

Field plot design

Four commercial peach orchards in Massachusetts were selected for experimental OTC chemotherapy in September of 1986. The orchards and locations (Figure 1) were as follows: Hamilton's Orchards in New Salem; Westward Orchards in Harvard; Bolton Springs in Bolton; and Green Acres in Wilbraham. The peach and nectarine trees within all the orchards were grouped as one population of approximately 155 trees. Standard peach management practices of fertilizing, pruning, and spraying for brown rot, leaf curl, and various insects were followed for all orchards. Tree variety differed greatly between the orchards.

Peach and nectarine trees within Hamilton's orchards bordered woodlands with X-diseased chokecherry on two sides, and plum (*Prunus americana* Marsh), and sour cherry (*Prunus cerasus* L.) on the other sides. The trees had been established for 7-8 years, had had extensive brown rot problems in the past, and high weed cover beneath each tree. Stock came from Hilltop Nurseries in Michigan.

Westward Orchards was characterized by older peach trees averaging between 16 to 20 years of age. Many of the trees suffered from Cytospora canker infections. Two sides of the orchard were wooded with few chokecherry, while the other two sides were bordered by apple (*Malus* spp.) orchards. Trees were set somewhat in a depression and had a

Figure 1. Location of orchards within Masachusetts for peach X-disease research investigating Mauget microinjection of oxytetracycline (OTC).

high grass understory. Stock came from Hillsdale Nurseries in New York.

Peach trees at Bolton Springs grew at a higher elevation than other orchards and were pruned more heavily. The peach block was surrounded by an apple orchard and had one border of woodland (never surveyed for chokecherry). The peach orchard was approximately 12-15 years old. Stock was of unknown origin.

Green Acres had two separate blocks of peach and nectarine trees. One block of older trees (more than twenty years old) were in a state of decline and surrounded by a larger apple orchard. The other block (12 years old), was bordered by woodland with chokecherry on one side, a road and fence row on another side, and a pond with residential areas on the other two sides. Herbicides were used to keep bare soil beneath all trees. Drip irrigation was used for the peach blocks, which resulted in rapid, heavy growth. Stock came from Hilltop Nurseries.

Rating system

Trees were rated once and flagged accordingly in mid September, 1986. The rating system used was based upon the outward symptoms of X-disease, modified from one developed for pear decline (McIntyre, et al., 1979). Care was taken to prevent confusion of X-disease with symptoms of mineral deficiencies (Cullinan, 1951), peach borer damage, Cytospora canker, bacterial spot, or chemical injury. Foliage that was chlorotic, red spotted, tattered, brittle, curled upward, or twigs that had a tufted appearance from the defoliation of the oldest leaves, were considered the basic symptoms of X-disease. The rating on the live crown of each tree was determined according to the severity and extent of the X-disease symptoms throughout that crown, and was as follows:

0 = apparantly healthy; no X-disease symptoms as described.

1 = foliar symptoms as described previously on 10% or less of the crown.

2 = foliar symptoms and defoliation as described previously on more than 10% but less than 50% of the crown.

3 = foliar symptoms and defoliation to the extent that more than 50% but less than 90% of the tree is affected.

4 = over 90% of the tree affected or dead.

The number of trees found in each rating class within each orchard is shown in Table 3.

Mauget Microinjection

The Mauget capsules, each containing 4 ml of 4% OTC solution, were supplied by the J. J. Mauget Company, 2810 No. Figueroa St., Los Angeles, CA 90065. The OTC solution has a pH of 1.0 - 1.6 with the Terramycin base altered so there is less sugar. The carrier is glycerol. Each capsule was attached to a beveled feeder tube. Pressure was applied by compressing the capsule top with a mallet to achieve 10-12 psi (according to J.J. Mauget Co.). A battery powered drill, operating at 600-800 rpm, was used to drill one or more evenly spaced 3/16" diameter holes at the tree base. These holes were angled downward at 45 degrees and were

Table 3. Number of X-diseased peach and nectarine trees of each disease rating class and treatment, in four Massachusetts orchards in 1986.

	RATING										
	Zero		One		Two		Three		Four		Total
	OTC	Con.	OTC	Con.	OTC	Con.	OTC	Con.	OTC	Con.	
Bolton	2	5	1	1	7	5	3	4	0	1	29
Harvard	2	4	4	2	4	4	4	2	2	2	30
New Salem	6	9	16	13	15	11	6	3	0	0	79
Wilbraham	1	1	0	1	5	5	2	1	1	0	17
Total	11	19	21	17	31	25	15	10	3	3	155

*Con. = untreated control trees.

approximately 1/2" deep. The capsules were inserted immediately and tapped with the mallet. This broke the capsule's internal membrane and seated it within the hole, thereby forcing the OTC solution into the xylem of the tree. Figure 2 portrays the Mauget capsules on a peach trunk. The appropriate dose rate was one capsule per two inches of trunk diameter, with most trees receiving 2 or 3 capsules. The contents of most capsules were taken up by the tree within a few hours. Empty capsules were removed within a few days to two weeks.

Approximately half the peach population in each rating class was injected in 1986 after fruit harvest while the other half served as untreated controls. Injection dates for each orchard were as follows: Harvard and Bolton on September 23; New Salem on October 2; and Wilbraham on October 5. Care was taken to inject at sites of healthy, live bark to enhance chances of fast uptake. Soil and air temperatures were taken at each orchard to give an indication of tree transpiration.

The same trees were rated again September 18 or 19 one year later to determine the change in X-disease symptoms due to the OTC treatment or to disease progression. The live crown present at the time of rating was used to classify the symptom severity. For each disease rating class, a 2 X 3 Chi-square analysis was conducted on the data to compare rating changes between the OTC-treated and untreated control trees. A least-squares analysis of variance and a regression analysis, which weighted the means by taking into

Figure 2. Mauget capsules on the trunk of a peach tree.

account the unequal numbers within each rating class, were also performed on the data (Harvey, 1977; Damon & Harvey, 1987).

Field observations

Treated and untreated trees were observed the following growing season for injection wound reactions, general tree health and death, blooming and fruit set, and onset of X-disease symptoms. Observations began before bloom in April and continued approximately bi-weekly until October. Instructions were given to growers so study trees would not be pruned until the degree of winter injury could be determined on formerly X-diseased scaffolds.

Microinjections of OTC and water

A preliminary study was conducted to determine the wounding reactions of OTC and water microinjections. Eight healthy peach trees were injected on July 24, 1987 at the University of Massachusetts Horticultural Research Center, located in Belchertown. Each tree was injected with three Mauget capsules as described previously under the section Mauget Microinjection. Four of the peach trees received Mauget capsules filled with distilled water while the other four trees had Mauget capsules with OTC applied.

In January of 1988, the peach tree trunks were cut down and the bark removed. Measurements were taken of the widest horizontal and vertical discolorations associated with each injection hole to determine area (Lacy, et al., 1980) of the wound reaction. The three wound reaction areas per tree were then averaged to give a mean wound area per treatment.

Fruit yields on X-diseased trees

As many trees as possible were qualitatively rated according to the amount of marketable fruit produced to determine if OTC microinjection or the disease had influenced fruit yields. The simple rating system was as follows:

- = no fruit produced.

+ = few fruit (3-10) produced by the tree when compared to a healthy tree.

++ = half the crown bore fruit; approximately half a normal fruit crop.

+++ = full fruit crop; normal yield.

Fruit yields were rated once in July or August of 1987 at the time of ripening for that particular variety of peach or nectarine. For data analysis, the - and + fruit yield classes were combined as a "low fruit" class, and the ++ and +++ fruit classes became the "high fruit" class. This minimized the effects of thinning on the fruit yield ratings. For each disease rating class, the high and low fruit yields were compared using a 2 X 2 Chi square analysis to determine OTC microinjection effect on the fruit yields of X-diseased peach and nectarine trees.

MLO detection in peach tissue

Three twig samples were obtained from each treated and untreated tree within the study population to confirm the presence of MLOs. An effort was made to collect samples with severe X-disease symptoms, or if the disease was not outwardly noticeable, the suckers nearest the tree base. The twigs and leaf petioles of each sample were fixed in 5%

glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7 (Douglas, 1986b). Samples were collected on May 28-29, July 1-2, and August 6-7 of 1987 to target high MLO populations (Jones, et al., 1974; Rosenberger & Jones, 1977a; Douglas, 1986b).

The fixed tissue from each tree was hand sectioned (Hiruki & da Rocha, 1986) and stained on a slide with a few drops of 2% 4'-6' Diamidin-2-Phenylindol, 2 HCl (DAPI) for 20 minutes in order to detect the presence of MLO DNA (Russell, et al, 1975; Douglas, 1986b). The slides were then viewed with a Zeiss fluorescent microscope using a number 2 blue filter. Results were tabulated as either positive or negative depending on whether there was a fluoresence reaction emanating from the phloem sieve tubes.

Antibiotic assay on peach tissue

Leaf samples were collected on April 17, 1987 from a few OTC-treated trees in Green Acres to detect OTC residues from the treatment the previous year. Also, starting August 7, 1987, and continuing every two weeks for a total of three times, leaves and fruit of every tree injected with OTC or water was sampled. The leaves and fruit were frozen for use later in an antibiotic bioassay. The assay (Carr, 1986; Grove & Randall, 1955; Rosenberger, 1977) uses the tetracycline sensitive Bacillus cereus subsp. mycoides (Flugge) Smith, et al. as the detecting organism.

CHAPTER III

RESULTS

Mauget microinjection

Rainy conditions at time of OTC treatment were not optimum for chemical uptake in the orchards of Bolton, Harvard, and New Salem. Soil and air temperatures for the Harvard orchard were 17.6 and 19.2 C respectively and the Bolton orchard's temperatures were 18.8 and 20 C. New Salem had an air and soil temperature of 17.6 C. The orchard in Wilbraham was very windy and overcast at the time of injection with an average of 18.5 and 18.2 C for the respective soil and air temperatures.

It took approximately 3 - 7 minutes per tree for Mauget microinjection, depending on the number of people drilling, the layout of the orchard, and the distance between diseased trees. The entire contents of all capsules were taken up within 24 hours.

Effect of OTC microinjection on X-disease symptom rating

Figure 3 depicts two peach trees, one treated with OTC and the other untreated. Originally, both trees had the same disease rating of 1. One year later, the treated tree was asymptomatic and rated 0, while the untreated tree developed more X-disease symptoms and was rated 3. A peach tree with a rating 2 (Figure 4), would typically appear at least half affected by X-disease before treatment with OTC. Figure 5 shows that after treatment, and one year later, the same peach tree could appear healthy and bear fruit.

Figure 3. Two X-diseased nectarine trees that illustrate the effects of microinjections of oxytetracycline (OTC) on symptomology. Both trees had an X-disease rating of one in 1986. The tree on the right was treated with OTC microinjection while the tree on the left was an untreated control. One year later, the OTC-treated tree was asymptomatic and received a rating of zero, while the untreated tree became worse and received a disease rating of three.

Figure 4. Typical peach tree with the X-disease rating of two.

Figure 5. One year later, an OTC-treated tree previously in the disease rating class two, is asymptomatic and bears fruit. This tree would now be classified as a rating zero.

Out of the original population of 155 trees, 6 trees had to be eliminated from the study because of extraneous problems which would have complicated the evaluation of X-disease. This left 149 trees for analysis. Over the season, 9 trees out of this population died as a result of the effects of X-disease. Out of these 9, the 5 OTC-treated trees that died were all seriously affected by X-disease as indicated by their original ratings of 3 or 4. The other 4 trees were untreated controls of varied rating classes.

Figures 6 - 11 represent the change in disease rating class over one year due to treatment with OTC or to progression of X-disease. An increased rating change indicates a tree whose X-disease symptoms have increased over one year, from one to four rating classes higher than its original rating. Likewise, a tree that has shown a decrease in X-disease symptoms has improved, and its rating class lowered one to four classes. The no change column represents the percentage of trees that have remained in the same disease rating class from one year to the next.

Most OTC-treated trees (91%) with an original rating of 0, indicating no apparent X-disease symptoms, did not change disease rating as a result of treatment (Figure 6). However, of the originally asymptomatic trees, 47% of the untreated and 9% of the OTC-treated trees exhibited X-disease symptoms in the second year. All of the healthy trees that became X-diseased were located either at Hamilton's Orchards in New Salem, or Bolton Springs Orchard in Bolton. Untreated or OTC-treated trees rated 0 could not

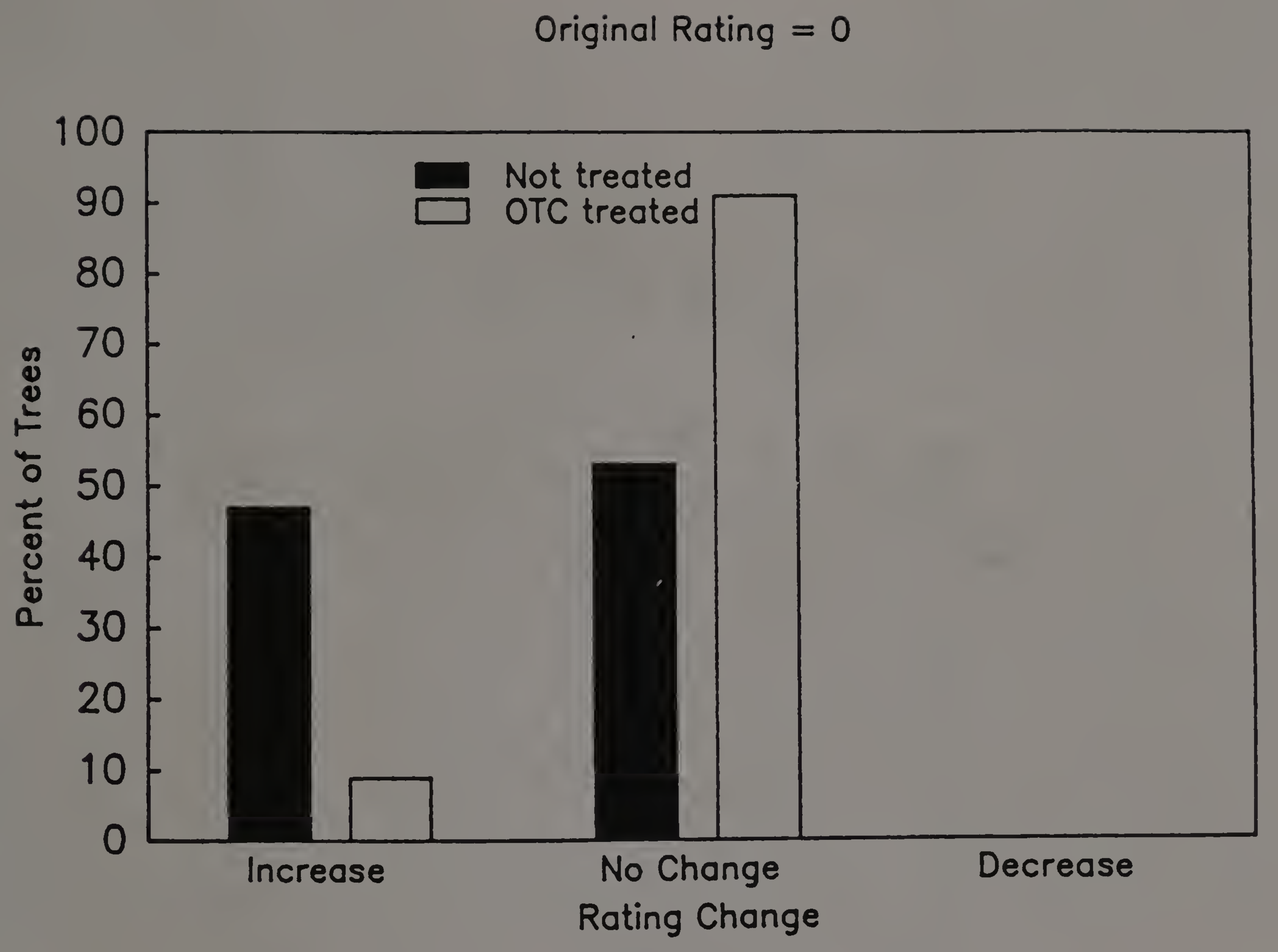

Figure 6. Percent of OTC-treated and untreated control peach and nectarine trees with rating 0 that changed X-disease rating class over one year. This bar graph represents 30 trees. No significant difference ($P<0.250$) between treated and untreated trees as determined by Chi square analysis.

improve since this rating class was already the healthiest. Nonsignificant differences ($P<0.25$) exist between treated and untreated trees as determined by Chi square analysis.

Of the trees originally rated 1, 5% of the OTC-treated and 73% of the untreated trees became worse over the year (Figure 7). Some of these trees (19% of the treated and 13% untreated) did not exhibit a change in disease rating at all. The OTC-treated trees in this group generally showed a lower disease rating with 76% improving. Only 13% of the untreated trees exhibited improved symptomology without treatment. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

None of the trees which originally had a rating 2 became worse after treatment (Figure 8). Untreated trees generally remained unchanged (75%) or became worse (17%), with only 8% of them improving. In contrast, 80% of the trees treated with OTC had improved ratings and 20% remained unchanged. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

Seventy nine percent of the trees which were more than half infected (original rating 3) also showed general improvement in symptom ratings following OTC treatment (Figure 9). By comparison, most untreated trees (70%) remained at the same severe rating of X-disease, with only 20% improving, and 10% getting worse. Those trees that became worse, either died or were more than 90% affected.

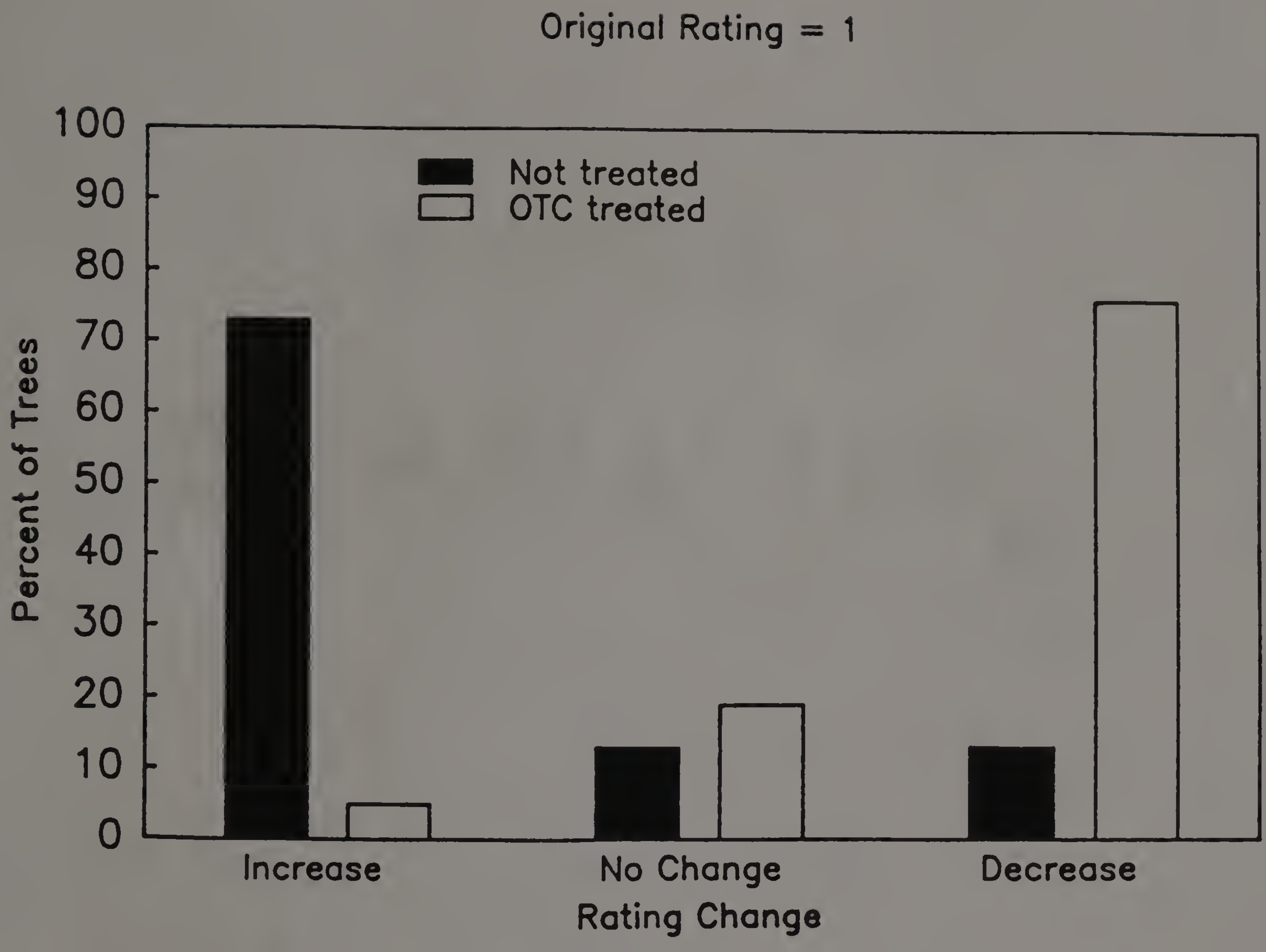

Figure 7. Percent of OTC-treated and untreated control peach and nectarine trees with rating 1 that changed X-disease rating class over one year. This bar graph represents 36 trees. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

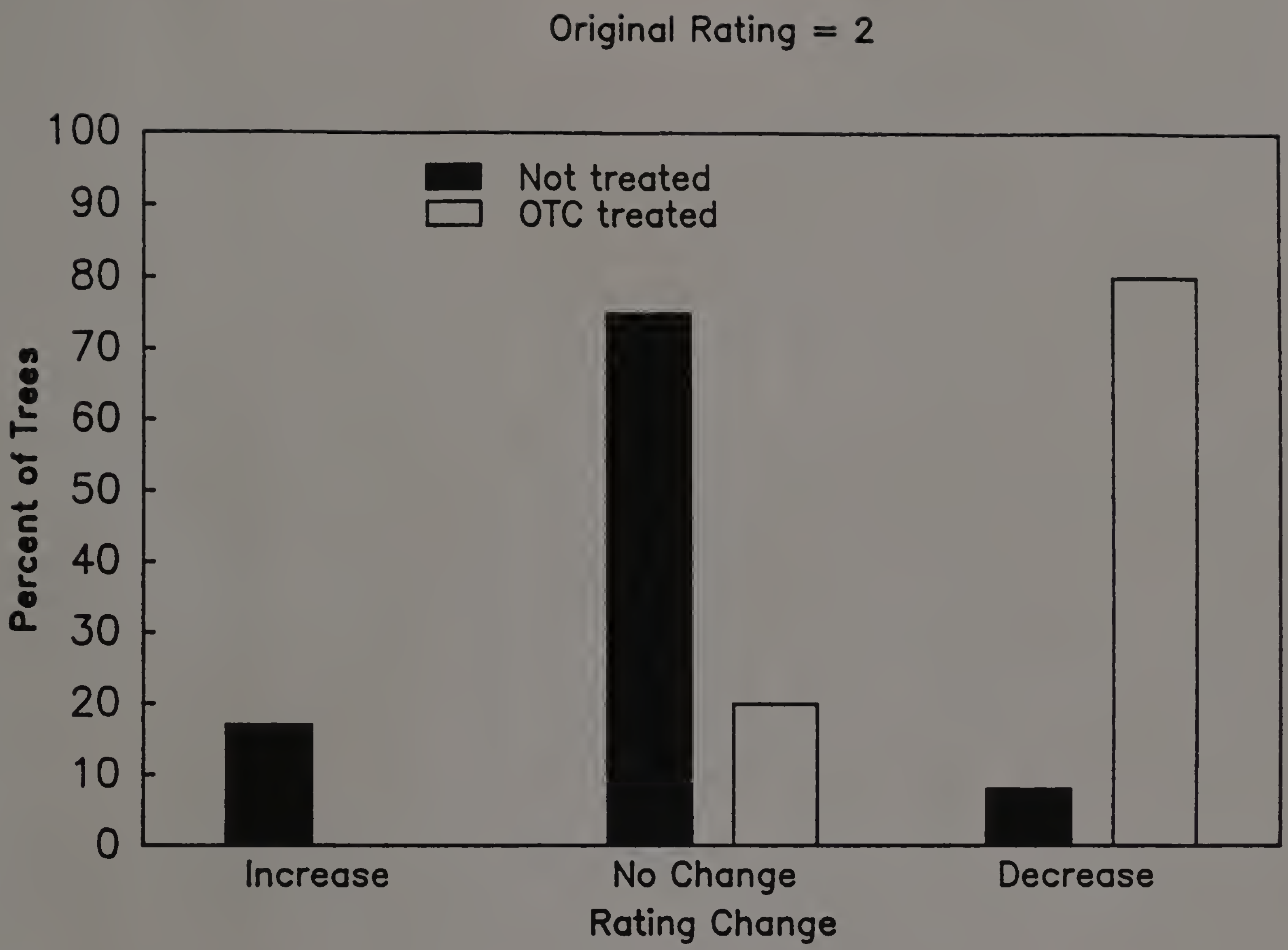

Figure 8. Percent of OTC-treated and untreated control peach and nectarine trees with rating 2 that changed X-disease rating class over one year. This bar graph represents 54 trees. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

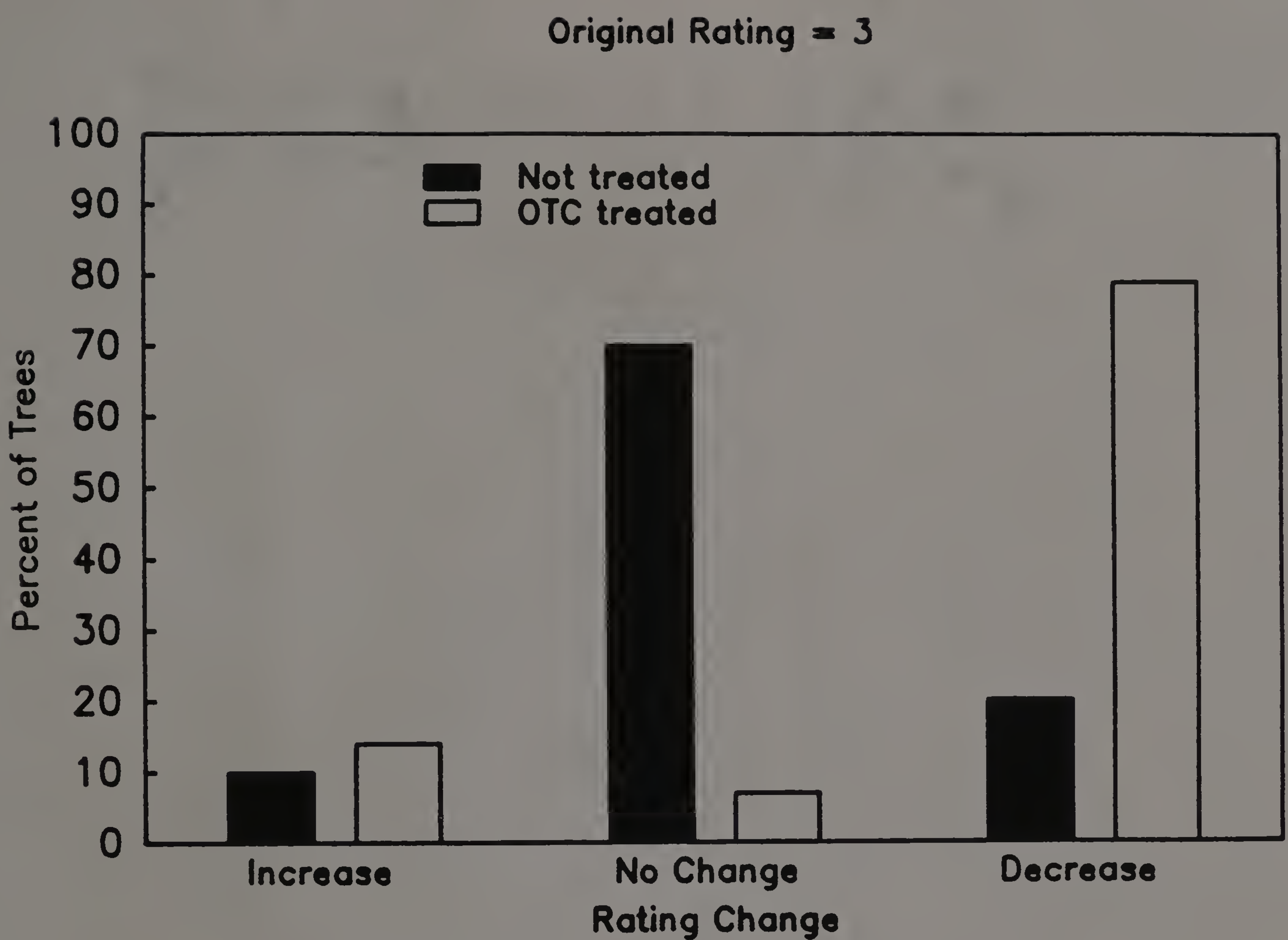

Figure 9. Percent of OTC-treated and untreated control peach and nectarine trees with rating 3 that changed X-disease rating class over one year. This bar graph represents 24 trees. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

Of the treated trees, 7% remained as diseased as before and only 14% of the trees became worse. Highly significant differences (P<0.005) exist between treated and untreated trees as determined by Chi square analysis.

Trees which had more than 90% of the crown affected by X-disease could not possibly be rated higher at the second rating. Figure 10 depicts 33% of the OTC-treated trees as improved. The remaining 67% of the treated trees remained unchanged. All of the untreated trees remained unchanged. The rating class 4 did not have enough trees to conduct a Chi square analysis.

Figure 11 compares OTC-treated trees versus the untreated control trees across all rating classes. Most (38%) of the untreated trees either had a higher disease rating or remained unchanged (57%) in the second year. Most (66%) of the OTC-treated improved or remained unchanged (29%). Only 7% of the untreated trees have improved, and only 5% of the treated trees deteriorated. Highly significant differences (P<0.005) exist between treated and untreated trees as determined by Chi square analysis.

Regression analysis

The results, as analyzed by least-squares analysis of variance (Damon & Harvey, 1987), showed a highly significant difference among the OTC-treated and untreated control trees of each rating class. There was no significant interaction between treatment and rating classes. Figure 12 compares the change in disease rating in each class for OTC-treated trees and the untreated controls. The predication equations

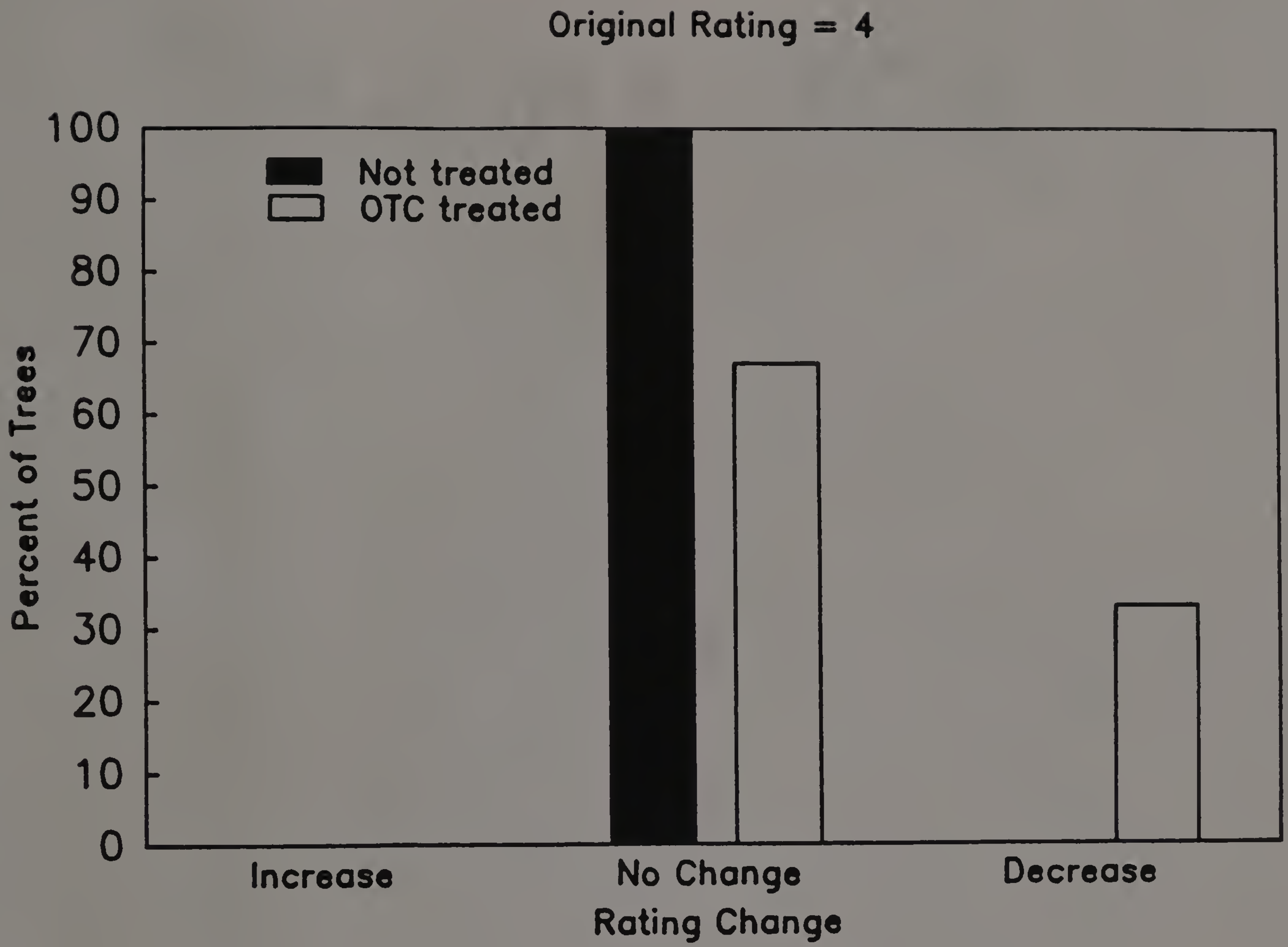

Figure 10. Percent of OTC-treated and untreated control peach trees with rating 4 that changed X-disease rating class over one year. This bar graph represents 5 trees. Chi square analysis not performed because of insufficient tree population.

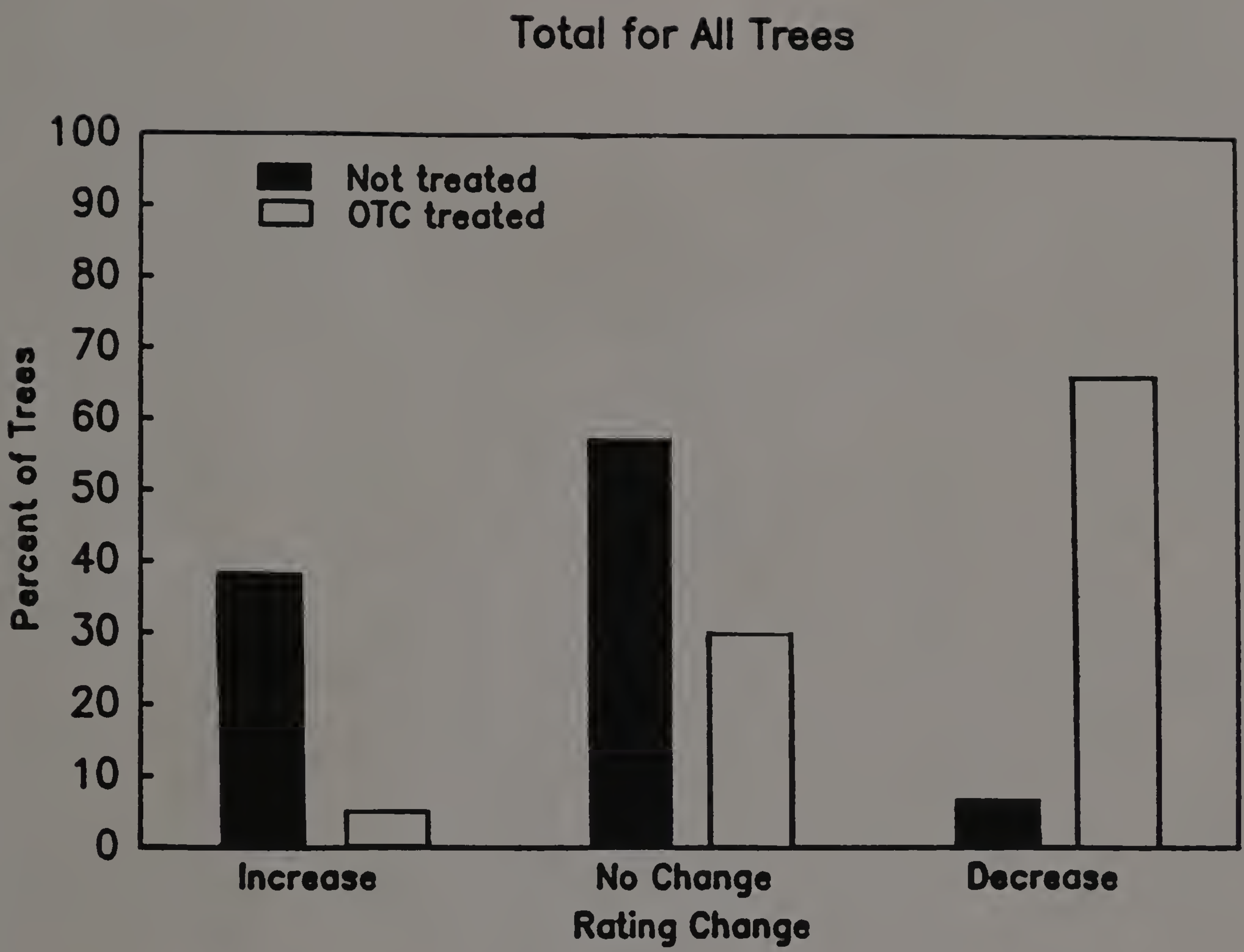

Figure 11. Percent of OTC-treated and untreated control peach and nectarine trees of all rating classes that changed X-disease rating class over one year. This bar graph represents a total of 149 trees. Highly significant differences ($P<0.005$) exist between treated and untreated trees as determined by Chi square analysis.

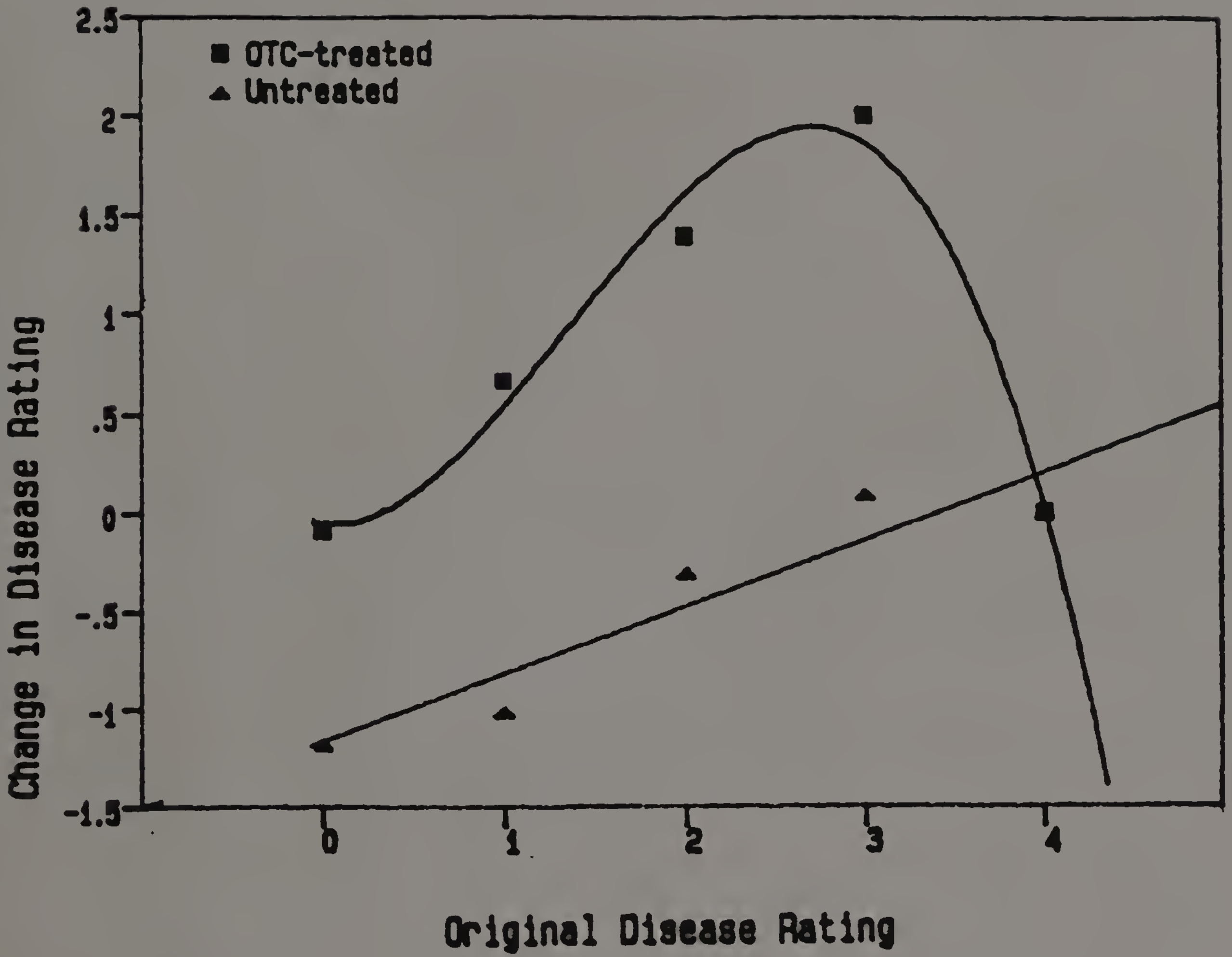

Figure 12. Comparison of the response between OTC-treated ($y = 0.8913 + 0.08254X1 - 0.003593X2 - 0.00019X3$; $r = 0.551$; $P<0.0100$) and untreated ($y = 0.1625 + 0.0413X1$; $r = 0.551$; $P<0.0100$) peach and nectarine trees in all rating classes as measured by change in disease rating class after one year.

for OTC-treated and untreated trees are y = 0.8813 + 0.88254X1 - 0.003593X2 - 0.00019X3 and y = 0.1625 + 0.0413X1, respectively. For both equations, the R value is 0.551 with 0.752 as the regression coefficient. Highly significant differences existed between OTC-treated and untreated trees for all rating classes.

Field observations

For the most part, the portions of a tree that were affected by X-disease one year did not recover fully the following year despite treatment with OTC. Those X-diseased scaffolds became more susceptible to winter injury, as evidenced by the many dead branch tips and branches on the affected scaffolds the next spring. Trees with the highest disease rating of 3 or 4, especially, were killed back and did not produce much foliage or fruit. Table 4 gives the approximate percentage of dead crown, fruit yields, initial rating and second rating of individual OTC-treated trees in the 3 or 4 disease rating classes. OTC-treated trees within the 0 - 2 rating classes had, at the most, approximately 20% of their crowns winter killed and generally normal fruit yields. In comparison, even though many treated trees rated 3 or 4 showed great response to the OTC, many died or had an average of 66% dead crown and poor fruit yields.

Observations noting the fruit varieties affected by X-disease did not detect any varieties that were more or less resistant. Typically, the trees that became X-diseased were located on orchard edges or in small groups within the orchard. The nectarine varieties noted as affected with X-

Table 4. Percent dead canopy and fruit yields of individual OTC-treated peach trees within the X-disease rating classes of 3 and 4 in four Massachusetts orchards in 1987.

Location	1986 rating	1987 rating	% Dead canopy	Fruit
Bolton	3	0	50%	+++
Bolton	3	1	40%	++
Harvard	3	0	55%	++
Harvard	3	0	66%	++
Harvard	3	0	63%	+
Harvard	3	1	90%	+
Harvard	4	0	70%	+
Harvard	4	4	100%*	-
New Salem	3	0	60%	+
New Salem	3	0	40%	**
New Salem	3	0	20%	+
New Salem	3	1	20%	-
New Salem	3	3	75%	-
New Salem	3	4	100%*	++
Wilbraham	3	0	80%	+
Wilbraham	3	4	100%*	-
Wilbraham	4	4	100%*	-

* tree died during the study

** fruit data not collected

\- = no fruit produced.

\+ = a few (3 - 10) fruit produced.

++ = half of a normal fruit crop produced.

+++ = full, normal crop of fruit produced.

disease were Harko, Nectared #4 & #6, and Sweet Sue. X-infected peach varieties were Blake, Cresthaven, Early White Giant, Elberta, Garnet Beauty, Glohaven, Golden East, Hale, Harmony, Jersey Queen, Loring, Redhaven, Redskin, Reliance, Sunhi, and Sweethaven.

No OTC phytotoxicity was observed on the foliage of treated trees the following spring. Also, there were no observed differences between the blooming times of healthy and X-diseased peach trees. Treatment with OTC appeared to delay symptom onset. Untreated control trees exhibited X-disease symptoms sooner than the OTC-treated trees which showed symptoms.

Wound reactions associated with injection site

Careful inspection of the injection holes revealed trunk injury in the form of gummosis, cracks, fungal decay, and the lack of callus formation (see Figures 13 - 15). A few injection sites had splits in the bark reaching up and down the entire trunk where the path of the OTC uptake could be expected to travel. Many holes had both gummosis and cracks associated with the injection site. The unidentified decay fungi that had invaded some of the holes were tooth or small bracket fungi of the basidiomycetes class. Only two holes out of the 190 viewed had Cytospora canker invasion.

Table 5 summarizes the classification and occurrence of the various wound reactions and trunk injury found associated with the microinjections of OTC. An average of nearly 15% of the holes had callused over a year and a half

Figure 13. Typical uncallused injection site of most OTC-injected peach and nectarine trunks more than one year later. A drip irrigation tube can be seen at lower right.

Figure 14. Gummosis on a peach trunk associated with the injection site of OTC microinjection more than one year later.

Figure 15. Cracking on a peach trunk associated with the injection site of OTC microinjection more than one year later.

Table 5. Classification and percent occurrence of wound reactions found on peach and nectarine trees microinjected in 1986 with oxytetracycline (OTC) in four Massachusetts orchards. Total number of injection sites is 190.

		PERCENT WOUND RESPONSE				
Orchard	No.holes	Healed	Unhealed	Gummosis	Cracks	Fungi
Bolton	31	3.2	29.0	22.6	51.6	9.7
Harvard	45	6.6	44.4	24.4	33.3	17.8
New Salem	87	25.3	28.7	32.2	29.9	14.9
Wilbraham	27	7.4	66.6	25.9	7.4	----
Average percent for all holes		14.7	37.9	27.9	31.1	12.6

after treatment, whereas nearly 38% of the holes did not have any callus, gummosis, cracks, or fungi associated with it. Approximately 28% showed gummosis and 31% exhibited cracks associated with the injection site. Some holes (13%) had decay fungi growing within or surrounding the hole.

Preliminary studies of microinjection injury revealed that healthy, water-injected trees had callused over the injection holes after 6 months. In contrast, peach trunks injected with OTC had no callus present and exhibited small amounts of gummosis and cracks associated with the injection site. After removal of the bark, it was clear that the cambium surrounding the injection site had been killed back on OTC-injected trunks. Peach trees injected with water did not show this reaction. Measurements of this necrotic area gave a mean wound reaction area of 5.38 square centimeters for the OTC injection sites and 0.72 square centimeters for the water-injected trunks. Further removal of the sapwood on OTC-injected trunks revealed long streaks of brown discoloration which were measured to be 32 cm to 45 cm and sometimes longer. Water-injected trunks showed thinner streaks of brown discoloration (from 13 to 15 cm long) and callus wood.

<u>Fruit yields on X-diseased trees</u>

Table 6 shows the numbers of trees within the high and low fruit yield classes of all disease rating classes. Highly significant differences, as determined by Chi square analysis, exist between OTC-treated and untreated trees in the disease rating classes 1 and 2.

Table 6. Comparison of peach and nectarine yields for OTC-treated and untreated trees within each X-disease rating class in four Massachusetts orchards in 1987.

		YIELD		
Rating Class	Treatment	Low Fruit	High Fruit	Total
Zero	OTC	0	10	10
	Control	3	11	14
One*	OTC	4	14	18
	Control	8	5	13
Two**	OTC	4	17	21
	Control	11	7	18
Three	OTC	8	5	13
	Control	7	1	8
Four***	OTC	3	0	3
	Control	1	1	2
Totals**	OTC	19	46	65
	Control	30	25	55

*significance at X 0.05.

**highly significant at X 0.01.

***tree population not large enough for Chi square analysis.

MLO detection in peach tissue

Due to storage problems, during which most of the samples became dehydrated, histological detection of MLOs with DAPI staining was unsuccessful.

Antibiotic assay on peach tissue

All fruit and foliage samples were destroyed due to mechanical breakdown of the storage freezer, and therefore, the assay could not be conducted.

CHAPTER IV

DISCUSSION

Applications of OTC microinjection to X-disease management

X-diseased trees within all the rating classes responded positively to Mauget microinjections of OTC when compared to the untreated control trees. The highly significant differences found between the OTC-treated and the untreated controls of all rating classes indicates that Mauget microinjection of OTC for X-diseased trees is very effective compared to no treatment. The simple equipment requirements (basically a power drill and the self contained, disposable Mauget capsules), the ease of application, and the quickness of the system appealed to growers, who felt the technique offered an efficient way to manage X-disease.

It also may be relatively inexpensive to apply OTC to X-diseased trees with the Mauget microinjection technique. Besides direct economic losses from X-disease in fruit yield and tree health, growers have the costs of removing and replacing dead or severely diseased trees and the 3 - 4 year wait for the replacements to reach commercial bearing. With 2 - 3 Mauget capsules per tree at approximately $1.00 per capsule, productivity of an X-diseased tree could be prolonged. Also, the ideal time for OTC application is after peach harvest, which often coincides with the extremely busy apple harvest. The Mauget microinjection technique is ideal since it is convenient and not labor intensive.

Mauget microinjection of OTC could have the potential to prevent X-disease in healthy trees. The ten healthy trees that became diseased over the course of this study were found only in the Bolton and New Salem orchards where disease pressure was high, based on evaluation of X-infected chokecherry and sour cherry in the areas. There is the possibility that plum trees present could also contribute to disease pressures by becoming symptomless hosts for X-disease as it is in other MLO-caused diseases of peach yellows, peach rosette, and western X-disease (Stoddard, et al., 1951; Jensen, 1971; Pine and Gilmer, 1976; KenKnight, 1976). However, because of preliminary data and other work indicating injurious effects associated with the trunk injections of OTC (Rosenberger & Jones, 1977b; Keil & Civerolo, 1979; Lacy, 1982; Carr, 1986), recommendations for prophylactic use could only be for situations where there is high disease pressure.

Peach trees within the severely X-diseased rating classes of 3 and 4 had higher mortality, appeared more susceptible to winter injury (Gilmer & Blodgett, 1976), and had less fruit than trees in the lower rating classes. Despite the improvement in tree health gained from OTC treatment, these trees did not recover a full canopy of foliage or have sufficient fruit yields even after treatment. This makes it uneconomical to treat or keep X-diseased trees in the 3 and 4 rating classes.

In 1975, McCoy found that coconut palms severely affected by lethal yellows did not respond as positively to

Mauget microinjections of OTC as did trees less diseased. In this study, the severely X-diseased rating classes of 3 and 4 also continued to decline and have low fruit yields even after OTC treatment. Therefore, a symptom threshold for management of X-disease is proposed at the disease rating class 2. The highly significant differences that exist between the fruit yields of OTC-treated and untreated trees for disease rating classes 1 and 2 also supports rating class 2 as an economic threshold. It becomes, therefore, most cost effective to microinject peach and nectarine trees not more than half affected by X-disease. In spite of the trunk injury observed with OTC microinjection, fruit would still be produced on OTC-treated X-diseased trees whereas untreated trees may continue to decline and fruit yields diminish.

In the preliminary wound reaction study, the large size differences between wounds created by microinjections of water and OTC indicates that it is the OTC that causes the injury, not the technique. Others have reported similar injury after the use of concentrated OTC (Rosenberger & Jones, 1977b; Allen & Davidson, 1978; Pearson & Sands, 1978; Keil & Civerolo, 1979; Lacy, et al., 1980; Lacy, 1982; Carr, 1985). Since xylem tissue normally has a pH from 5 - 7 (Clifford, et al., 1984), it is likely that the extremely low pH required to keep the OTC in solution is responsible for some of the trunk injury. Other possible factors are the high concentration of OTC within the capsule or the glycerol carrier. Therefore, reducing chemical contact with host tissue by increasing the speed of chemical uptake, or by changing the OTC formulation or concentration, is desirable (Costinis, 1980).

Proper microinjection depends on operator technique, the chemical and method of delivery, time of injection, site and condition of tree at time of injection, and the genetic capacity of the tree to respond to the wound and chemical (Shigo, 1981; Clifford, et al., 1984). Small, shallow, clean edged holes placed on the root flare are recommended for most rapid uptake of chemicals and "healing" over of holes (Shigo & Campana, 1977; Shigo, 1978; Costinis, 1981). Uptake of chemicals is also most rapid on hot days of low relative humidity (Chaney, 1986). To treat trees in subsequent years, injection holes should not be made directly above former holes as xylem discoloration from previous treatments may inhibit efficient uptake (Shigo, 1978; Lacy, 1982).

Recommendations

For optimum symptom remission and fruit yields, Mauget microinjection of OTC is recommended for peach and nectarine trees that are not more than half affected by X-disease symptoms. Also, because of the injurious effects associated with trunk injections of OTC, prophylactic treatment of healthy trees should only be in situations where there is high disease pressure from X-diseased chokecherries or cherries.

To minimize the impact of the wounding reactions associated with OTC, small trees less than 3" in diameter should not be microinjected. Recommended dosage rate is one capsule per 5 cm trunk diameter. Small, shallow, clean edged holes made with a battery powered 600-800 rpm drill and 3/16" bit are recommended for most rapid chemical uptake. Injection should be evenly spaced and at sites of clean healthy bark. Trees are to be injected after fruit harvest from mid September to mid October (Rosenberger & Jones, 1977) when soil temperature is more than 14.7 C.

MLO detection in peach tissue

The difficulties in detection of the X-disease MLOs was not that unusual because of the pathogen's low titres and irregular distribution within the peach tree (Jones, et al., 1974; Rosenberger & Jones, 1977; Lahey, 1978; S. Douglas, personal communication). Staining technique, storage conditions, or the failure to recognize the MLOs could also have led to detection difficulties. Since MLOs have not been cultured, and therefore can not fulfill Koch's postulates, a positive response to an antibiotic treatment is one way to confirm a MLO cause for a disease (McCoy, 1982). The positive response of nearly all OTC-treated trees confirms a MLO etiology for the trees within this study.

Future research

Research should continue on the economics of OTC use in commercial fruit management. Factors such as how long OTC-treated X-diseased trees stay in remission would determine the necessary frequency of treatments. Also, more precise fruit yield and cost data would give a more accurate indication of the economic benefits of Mauget microinjections of OTC for MLO-diseased trees.

Efforts should continue to minimize the wounding caused by the OTC found within the Mauget capsule. Ways to reduce this injury might include changing the pH, concentration, or OTC formulation, while maintaining effectiveness. Mandatory grower training to ensure proper microinjection techniques could also reduce the injurious effects associated with OTC microinjection.

Because of the successful remission of X-disease symptoms found within this study, Mauget microinjection of OTC has great potential for decreasing symptoms and increasing yields of other MLO-caused fruit diseases such as pear decline, cherry buckskin, little peach, peach yellows, or peach rosette. Studies to determine effects, symptom thresholds, and fruit yield associated with microinjections of OTC could benefit growers with these problems.

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