LEAFHOPPER VECTORS, EPIDEMIOLOGY, AND CONTROL OF PEACH X-DISEASE

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ABSTRACT

LEAFHOPPER VECTORS, EPIDEMIOLOGY, AND CONTROL OF PEACH X-DISEASE

By

David A. Rosenberger

X-disease, a stone-fruit disease of assumed mycoplasma etiology, has caused extensive losses in Michigan peach (Prunus persica Batsch) orchards during recent years. This three-part study of the X-disease problem in Michigan included (1) detecting and analyzing disease spread in peach orchards, (2) determining vector-disease relationships in the field, and (3) testing various tetracycline treatments for their ability to induce symptom remission in X-diseased peach trees.

I. The percentage of X-diseased trees in 10 peach orchards surveyed between 1973 and 1976 ranged from 2 to 75%. The rate of disease spread was determined by calculating the infection rate, QR, where QR is the slope of the regression of $\log_e 1/(1-X)$ against time and X is the proportion of diseased plants. The lowest incidence of X-diseased trees (2-13%) and the lowest infection rates (QR = 0-0.02 per year) occurred in orchards where no X-diseased chokecherries (Prunus virginiana L.) existed within 500 m of the orchards. The proportion of X-diseased trees increased most rapidly (QR = 0.12-0.22) where diseased chokecherries were located within 300 m of the orchards.

However, X-disease continued to spread where diseased chokecherries within 200 m were eradicated in 1973. The apparently random spread of X-disease in orchards isolated from X-diseased chokecherries by 200 to 500 m suggests that X-disease is spreading from diseased peach trees or from more distant inoculum sources. Leafhopper trapping showed large populations of the X-disease vectors Paraphlepsius irroratus (Say) and Scaphytopius acutus (Say) were present in orchards.

II. The X-disease organism (XDO) was transmitted during June, July, and August to peach and chokecherry indicator plants exposed for 5-week periods beside X-diseased chokecherry in the field. Twenty-six percent of 387 indicator plants exposed in 1974 developed X-disease symptoms compared to 5% of 359 plants exposed in 1975 and 3% of 273 plants exposed in 1976. Transmission to indicator plants was not directly related to the numbers of P. irroratus, S. acutus, and Colladonus clitellarius (Say) captured on sticky-boards at the exposure sites. Paraphlepsius irroratus accounted for 87% of 9,986 specimens of X-disease vector species trapped in orchards during three years and was common from June to November. Thirty-seven percent of 331 P. irroratus leafhoppers, but only 26% of 153 S. acutus leafhoppers transmitted the XDO from diseased celery to celery test plants in greenhouse transmission trials. Four of 15 Orientus ishidae (Mat.) and eight of 44 Scaphoideus, tentatively identified as S. carinatus Osb. and as S. diutius De L. & M. or S. melanotus Osb., transmitted the XDO to celery. These new vector species were not abundant in cultivated orchards. Paraphlepsius irroratus is

considered the most important X-disease vector in Michigan because of its abundance and good transmission efficiency.

III. X-diseased peach trees with 9- to 17-cm trunk diameters were treated at various times during the growing season with five rates of oxytetracycline-HCl (OTC). Injections of 1.25, 2.5, and 3.75 g OTC per tree in September induced remission of symptoms for one year, whereas spring, summer, or fall injections of 0.5 or 0.9 g OTC per tree were less effective. Injections of 1.25 and 2.5 g OTC per tree in October and November were phytotoxic. Injections of dilute OTC by infusion and by pressure, and concentrated OTC pipetted directly into holes drilled in the trunks, all provided remission of foliar symptoms for one year. Terramycin-like activity was greatest in leaves from trees injected by infusion. Injections of concentrated OTC was the most rapid and convenient method tested, but 2.5 g OTC in concentrated form caused some necrosis around the injection holes in the tree trunks.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

PART III

APPENDIX B

APPENDIX C

APPENDIX D

LIST OF FIGURES

GENERAL INTRODUCTION AND LITERATURE REVIEW

X-disease first appeared in Michigan in 1939 (3) and persisted as a minor stone-fruit disease for many years. The incidence of X-disease in peach orchards began increasing in the late 1960s, and by 1971, X-disease was one of the major peach disease problems in southern Michigan. Extensive losses from X-disease lead to removal of entire peach orchards and to grower reluctance to replant peaches. X-disease has been found in tart cherry orchards in Michigan, but usually fewer trees are affected than in X-diseased peach orchards. Other disease and cultural problems in tart cherry orchards often make visual identification of X-diseased cherry trees difficult.

Because of the increasing incidence of X-disease in Michigan, an X-disease research program was begun in 1972. The objectives were (1) to document the rate of X-disease spread in Michigan peach orchards and investigate possible reasons for increased incidence of X-disease; (2) to determine the important disease-vector relationships by monitoring leafhopper populations, testing vector efficiencies, and determining the natural transmission season in the field; and (3) to determine the effectiveness of tetracycline chemotherapy for obtaining symptom remission in X-diseased peach trees. These three areas of study are described in the three parts of this

dissertation. The history of X-disease spread in United States and literature pertinent to the etiology, epidemiology, and vector relationships of X-disease are reviewed in the remainder of the introduction.

The causal agent of X-disease was assumed to be a virus until mycoplasmalike bodies (MLB's) were found in the phloem cells of X-diseased plants (7, 14, 17) and in infectious leafhoppers (18). The MLB's were spheroid to elongate with diameters of 120-360 nm and lengths up to 5000 nm. Further evidence for the mycoplasma etiology of X-disease includes the symptom remission achieved with tetracycline treatments (1, 21, 33) and the eradication of the pathogen in budwood with moderate heat treatment (35). Attempts to culture the causal agent in artificial media have failed (19, 20).

At least 20 Prunus species are susceptible to X-disease when experimentally inoculated (5, 8, 34), but peach, nectarine, Japanese plum, and sweet and tart cherry are the only known cultivated species susceptible to natural infection (4, 26). Symptoms of X-disease have been well described (8, 24, 34, 35). Leaves on infected peach trees develop large, chlorotic, water-soaked spots during late June. These spots later turn red and sometimes separate from healthy leaf tissue so that infected leaves appear shot-holed or tattered. During July and August, diseased branches defoliate starting from the base until only a "horsetail" of young leaves remains at the apex of twigs. Fruit on infected branches usually drops before maturing, and any fruit remaining at harvest is small and has a bitter flavor. Only

one branch of a tree may be affected during the first year of symptom expression, but the entire tree is usually affected within 2-4 years.

Wild chokecherry (Prunus virginiana L.) is considered the only wild host of X-disease in northeastern United States, and the western chokecherry (Prunus demissa [Nutt.]) is the wild host in the west. When infected with X-disease, chokecherry plants of both species develop brilliant red or yellow foliage during mid-summer. The wild black cherry (Prunus serotina Ehrh.) is common in hedgerows around orchards but is not susceptible to X-disease. Pin cherry (Prunus pensylvanica L.) may become infected following bud inoculation but remains symptomless and does not appear important in the spread of X-disease (26).

X-disease was first noted in eastern United States in 1933 when Stoddard (34) found it in a Connecticut peach orchard. X-disease was found in California infecting sweet cherry in 1928 and peach in 1932 (27, 36). Richards and Cochran (32) suggest X-disease was present in Utah as early as 1910, but the first report of X-disease in Utah was made in 1940 (31). X-disease is now found in at least 26 states and three Canadian provinces (4) in a range corresponding to the range of chokecherry in North America. Other names for X-disease included in the literature are yellow-red virosis, western X-disease, western X, cherry X-wilt and decline, cherry buckskin, western-X little cherry, small bitter cherry, and cherry pinkfruit (8, 15, 29, 32, 43). The peach yellow leaf roll disease found in California in 1951 (22) is a severe strain of X-disease (12).

The eastern and western forms of X-disease were at first considered distinct diseases. Hildebrand (8) found some differences in symptoms between eastern and western strains of X-disease, but these differences were no greater than differences between the several strains which have since been described within each geographic area (5, 28). X-disease is now considered a highly variable disease which is probably caused by a group of closely related organisms (4, 6, 32).

The major difference between X-disease in eastern and western United States is its relationship to wild hosts. In the northeastern United States chokecherry has always been found near diseased orchards and supplies the inoculum for infecting orchard trees (26, 35). X-disease does not appear to spread from diseased to healthy peach trees in the northeast, and economic control of X-disease has been obtained by eradicating chokecherry around commercial orchards (4, 16, 26). The western chokecherry may act as a bridge for carrying infections between orchards in the western United States (32), but it plays no important role in the spread of X-disease within orchards once infection is established there (28). X-disease control in the western United States depends on roguing diseased trees since the disease spreads from tree to tree in the orchard (30).

X-disease also developed along different host lines in the east and the west. X-disease was first found on peach trees in the east and the disease was not reported on cherry trees until 1947 (25), almost 17 years after the disease appeared on peach. By contrast, western X-disease was first reported on cherry trees and the first diseased peach orchard was adjacent to diseased cherry orchards (27, 36).

Some of the differences in the epidemiology of X-disease in the east and in the west may be due to differences in the vector species present in these areas (6, 32). The X-disease organism (XDO) is vectored by seven species of leafhoppers in the west (2, 9, 10, 42), and six vector species have been identified in New York (6).

The XDO has an incubation period of from 20 to more than 50 days in its insect vectors (6, 42). Whitcomb et al. (38) showed that the concentration of the XDO increases slowly in vectors for 20 to 30 days, then declines rapidly. Leafhoppers infected with the XDO show reduced fecundity and have shortened lifespans (11, 13), and cyto-pathological studies have shown that MLB's are present in neural, salivary, adipose, and connective tissues of infective vectors (37, 39, 40, 41). The X-disease pathogen thus parasitizes both its plant hosts and its insect vectors. Oman (23) has discussed the possible phylogeny of organisms with such dual parasitic abilities.

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PART I

SPREAD OF X-DISEASE IN MICHIGAN PEACH ORCHARDS

INTRODUCTION

X-disease of stone fruits is leafhopper-transmitted and is probably caused by a mycoplasma (3, 6). In the eastern United States, chokecherry, Prunus virginiana L., is a wild host for X-disease and its eradication within 152 meters of peach and cherry orchards is a recommended control (1, 10). Because chokecherry eradication has effectively stopped X-disease spread in peach orchards in the eastern United States, X-disease spread between peach trees has been considered rare or nonexistent (1, 10). Recently, however, infected peach trees were found more than 180 m from diseased chokecherry suggesting spread from peach to peach in the orchard (5). In light of this finding and because of the increasing incidence of X-disease in Michigan peach orchards, a survey was conducted to determine (1) the association of X-diseased chokecherry with X-disease in orchards, (2) the rate and pattern of disease spread, and (3) if the rate of disease spread is related to the vector frequency and distribution in the orchards.

METHODS

Ten Michigan peach orchards were surveyed during a 4-year period. Three were surveyed annually from 1973 to 1976, five were surveyed in 1975 and 1976 only, and two were surveyed in 1976 only. Six orchards were in southwestern Michigan, two were in southeastern Michigan, and one was in the central part of the state. Surveys were conducted during August and September when foliar symptoms were acute. During 1973, diagnoses based on foliar symptoms were verified by electron microscopy studies (4) and by observing symptom development on Halford peach seedlings inoculated with buds from diseased trees. Trees recorded as diseased were counted as such in subsequent surveys even if they were dead, removed, or replaced. Areas surrounding each orchard were carefully checked for chokecherry plants, and records of chokecherry eradication were obtained from growers.

The rate of disease spread in orchards surveyed 2 years or more is expressed as the infection rate, QR, where QR is the slope of the regression of $\log_e 1/(1-X)$ against time in years and X is the proportion of diseased trees observed each year (13). The quantity QR represents the amount of inoculum (Q) multiplied by the basic infection rate (R) and is convenient for comparing spread of disease where the inoculum is constant within each season (13).

Five yellow sticky-boards (12) per orchard were used in 1973, 1974, and 1975 to sample leafhoppers in two survey orchards and in one peach orchard outside the study area. The boards were suspended from branches 0.9 to 1.5 m high, were changed at about weekly intervals from 15 May to 21 October, and were examined for Paraphlepsius irroratus (Say) and Scaphytopius acutus (Say), the most common vectors of X-disease in Michigan orchards (12).

RESULTS

The proximity of chokecherries provided the basis for dividing the survey orchards into three groups. Group I orchards (orchards A, B, and C, Table 1) had no chokecherries, diseased or healthy, within 500 m. Orchard A was surrounded by other peach blocks which contained only two widely-separated X-diseased trees. Orchards B and C were planted in 1975 and 1973, respectively, in areas where chokecherry had been eradicated, but orchard B was bordered by peach blocks with more than 20% X-diseased trees and orchard C was bordered by peach and Montmorency cherry blocks each containing at least 25% infected trees.

X-diseased chokecherries were found 150 to 300 m away from orchards D, E, and F (Group II), but these orchards were separated from the chokecherries by an X-diseased peach orchard, an apple orchard, and a woodlot, respectively. One to six diseased chokecherries were growing with 30 m of orchards G, H, and I in Group III but were eradicated during the summer of 1973. Subsequent surveys showed areas within 200 m these orchards were chokecherry-free. Orchard J had X-diseased chokecherries adjacent to it until the summer of 1975.

Group I orchards had the lowest proportion (0-13%) of X-diseased trees. Orchards in Groups II, III, and IV contained 22-75%

Table 1.--X-disease incidence, yearly infection rates in 1976, and proximity of diseased chokecherry in 10 Michigan peach orchards.

Orchard	1975 Survey[a]		1976 Survey		QR[b] (per year)	Chokecherry		
	Total trees	No. of trees w/symptoms	Trees with new symptoms	Total percent w/symptoms		Number of diseased plants	Date removed	Distance from orchard (meters)
Group I[c]								
A	463	10	0	2.2	0	None		
B	412	0	10	2.4	0.02	None		
C[d]	493	-	66[e]	13.4	-	None		
Group II								
D	179	89	21	61.5	0.27	1	1975	180
E[d]	370	-	52[e]	14.1	-	15-20	1974	240
F	175	107	25	75.4	0.46	15-20	1974	200
Group III								
G	306	132	22	50.3	0.14	3	1973	15-23
H	284	8	55	22.2	0.22	6	1973	9

Table 1.--Continued.

Orchard	1975 Survey[a]		1976 Survey		QR[b] (per year)	Chokecherry		
	Total trees	No. of trees w/symptoms	Trees with new symptoms	Total percent w/symptoms		Number of diseased plants	Date removed	Distance from orchard (meters)
Group III (continued)								
I	327	172	18	58.1	0.12	2	1973	9
J	481	122	51	36.0	0.15	?[f]	1975	30-60

[a]Orchards were surveyed during July and August in 1975 and 1976.

[b]Yearly infection rates, QR, were calculated from Van der Plank's (13) equation:

$$QR = \log_e 1/(1-X_2) - \log_e 1/(1-X_1)$$

where X_1 and X_2 are the proportions of diseased trees in 1975 and 1976, respectively.

[c]X-diseased chokecherries were not found within 500 m of Group I orchards or within 150 m of Group II orchards but were found within 60 m of Group III orchards.

[d]Orchards were not surveyed prior to 1976.

[e]This number represents the total number of trees with symptoms, not all of which were new infections in 1976.

[f]Chokecherries were eradicated by the grower a few weeks before our survey.

X-diseased trees (Table 1) except for orchard E which was only three years old and contained 14% diseased trees.

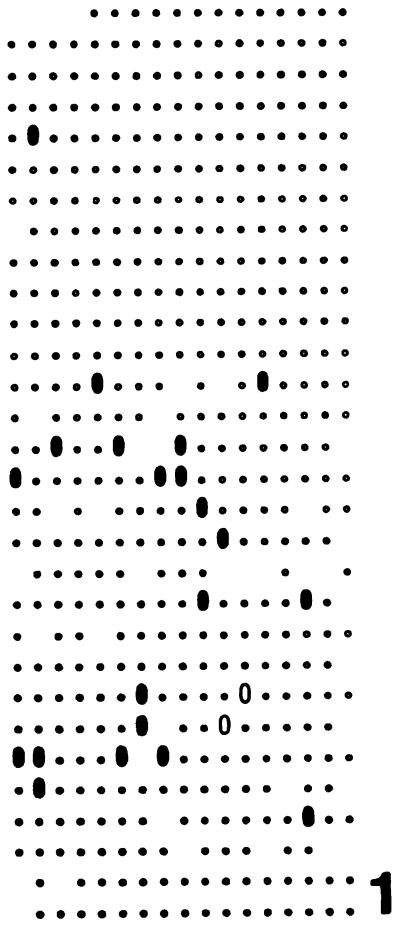
Infection rates were lowest, less than 0.02 per year, in Group I orchards and were highest, 0.27 and 0.46 per year, in Group III orchards (Table 1). The infection rates for Group III orchards were still 0.12 to 0.22 per year, three years after all chokecherries near orchards G, H, and I had been eradicated. In orchard J, QR was 0.15 per year despite the presence of chokecherry inoculum in 1975.

X-disease infections were not limited to areas immediately adjacent to X-diseased chokecherries or previous chokecherry sites (Figure 2, 4). X-diseased trees appeared by inspection to be randomly distributed except in orchard J (Figure 4) where most of the diseased trees were initially close to diseased chokecherries, and in orchard C (Figure 3) where diseased trees were concentrated in low areas of the orchard. Concentrations of X-diseased trees were also noted in low areas of some orchards not included in this survey.

Orchards A, G, and H were surveyed for four years. Only A showed a consistently low infection rate (now new infections during 1974 and 1976 and only two new infections in 1975, Figure 1). In orchard G, QR was 0.17 in 1974, 0.02 in 1975, and 0.14 in 1976 (Figure 2). In orchard H, QR was less than 0.02 per year in 1974 and 1975 but jumped to 0.22 in 1976.

Only 352 P. irroratus and 31 S. acutus were trapped in orchard A over three years compared to 975 P. irroratus and 264 S. acutus for orchard G and 1,548 and 76 of the respective species for the third peach orchard. Leafhoppers were often unequally

Fig. 1-4.--Orchard maps showing locations of X-diseased chokecherry (C), healthy peach trees (°), and peach trees with X-disease symptoms in 1973 (●), 1974 (@), 1975 (O), and 1976 (X). The dotted line (Figure 3) encloses low areas in the orchard. Orchards A (Figure 1) and G (Figure 2) were first surveyed in 1973, Orchards C (Figure 3) and J (Figure 4) in 1976 and 1975, respectively.



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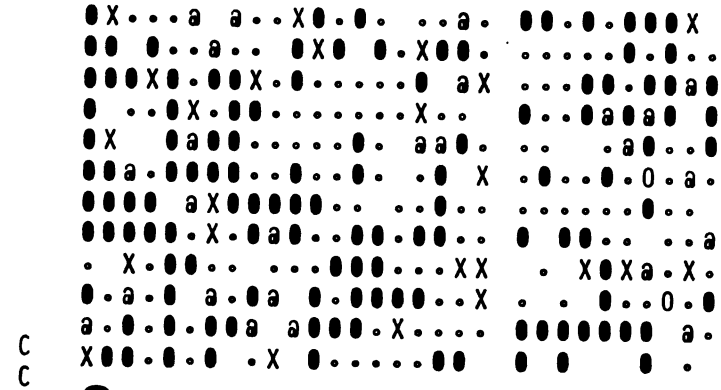

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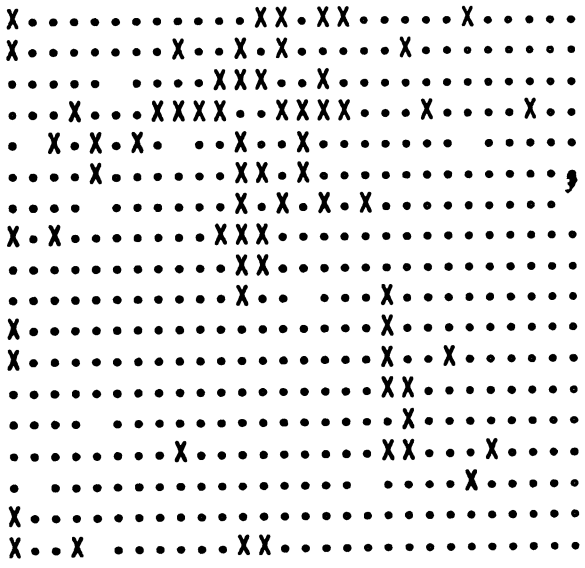

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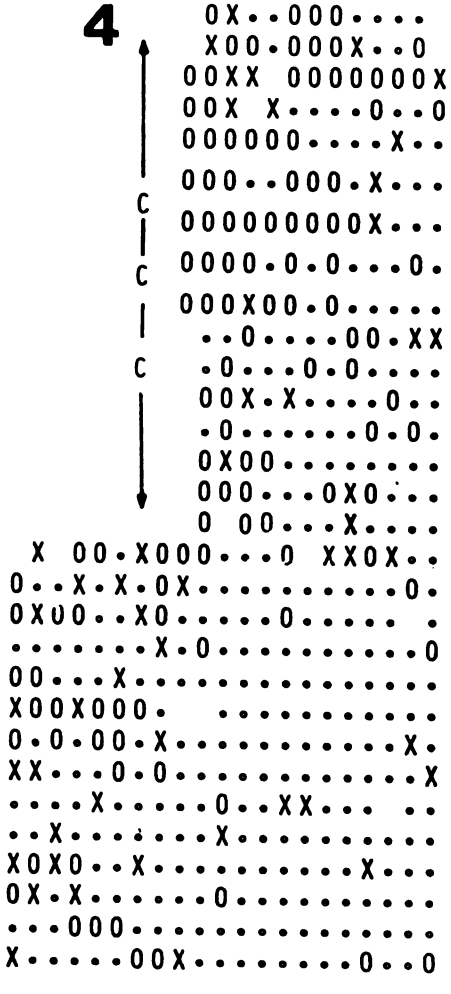

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distributed within orchards. In two orchards, each with five traps, single traps located in low areas accounted for 49% and 36% of the total number of P. irroratus captured in 1974. The succulent ground cover commonly found in lower, wetter areas of orchards may attract leafhoppers, particularly during late summer when vegetation elsewhere has stopped growing. Leafhopper preference for succulent vegetation could account for the concentration of X-diseased peach trees in low areas of some orchards (Figure 3).

DISCUSSION

X-disease is causing extensive tree losses in Michigan peach orchards. Four orchards had more than 50% diseased trees and 14% of the trees in 3- and 4-year old orchards were showing disease symptoms. Yearly infection rates for X-disease in Michigan orchards were comparable to those calculated from data reported by Palmiter and Hildebrand (9) in New York. The QR-values for three orchards they surveyed from 1939 to 1942 were 0.20, 0.08, and 0.34 per year. In a peach orchard surveyed for three years by Gilmer et al. (2), QR was 0.27 per year. But in these orchards and in those surveyed by Lukens et al. (5), infection rates decreased following chokecherry eradication, whereas in our survey, the eradication of chokecherry within 150 of the orchards had little apparent effect on infection rate. The incubation period for X-disease may be two years in mature peach trees (see Part II), but even with a 2-year incubation period, chokecherries eradicated in 1973 could not have provided inoculum for infections appearing in 1976. Moreover, X-diseased trees in Michigan orchards were not usually confined to areas near infected chokecherry as in New York (2, 10) and Connecticut (5).

The incidence and infection rates of X-disease were lowest in orchards where no chokecherries were found within 500 m. Where chokecherries were present, their number and location had no apparent

effect on the infection rate. Orchard J with numerous diseased chokecherries on its windward side had a lower infection rate than orchard D where a single diseased chokecherry was found 180 m away.

The prevalence of vectors may be just as important as presence of chokecherry inoculum. Good vector control probably contributed to the low rate of spread in orchard A. The association of unusually high disease incidence (Figure 3) with increased vector activity in low areas of orchards supports the importance of vector-disease relationships.

Michigan growers now use organic phosphate (O-P) insecticides instead of the more persistent heavy-metal and chlorinated-hydrocarbon insecticides. The commonly used O-P insecticides are relatively ineffective against leafhoppers, and have a short residual activity. Under current insecticide programs, vector populations peak in autumn when orchards are no longer sprayed (12). Late season transmission which continues through late August or early September in Michigan (see Part II), may be a significant factor in disease spread. The residual activity of the chlorinated hydrocarbon insecticides probably suppressed insect populations later into the autumn.

The extensive use of perennial grasses in orchards may furnish a food source and protection from insecticides for X-disease vectors. Both P. irroratus and S. acutus feed on grasses and legumes (2, 8) and Palmiter and Adams (7) suggested that S. acutus survived in the ground cover in sprayed peach orchards in New York. Clean-tilling orchards, however, will not necessarily decrease the rate of X-disease spread. Orchard D, the only clean-tilled orchard in our survey, had one of the

highest rates of spread. Clean tilling in the absence of good vector control may force vectors which are preferential grass feeders to move into trees and transmit X-disease more frequently.

The spread of X-disease in young orchards isolated from chokecherries and in older orchards where chokecherries were eradicated suggest the X-disease pathogen is carried into the orchards from distant chokecherry sources or is transmitted from diseased to healthy trees within orchards. Long distance spread is possible because X-diseased chokecherries are prevalent in southwest Michigan, and the most abundant vector, P. irroratus, is capable of flying considerable distances. However, spread of X-disease within orchards cannot be ruled out. It occurs in the field in western United States (11) and has been demonstrated in the greenhouse with a Scaphytopius species in Washington (14) and with Fieberiella florii (Stal) in New York (2). Leafhoppers can also acquire X-disease from diseased tart cherry trees (2, 14), but diseased tart cherries were found beside orchard C only.

This study suggests X-disease is spreading in the absence of local chokecherries. The disease may move from diseased chokecherries more than 200 m from orchards or from tree to tree within orchards. Eradication of chokecherries near orchards is important because this wild host still appears to be a major source of X-disease inoculum. However, growers should also remove X-diseased peach and cherry trees or treat them with oxytetracycline (see Part III) in order to reduce inoculum within orchards. Effective vector control is essential, and eliminating leafhopper habitat by clean-tilling or by using different grass species for ground cover may be advantageous.

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PART II

LEAFHOPPER VECTORS OF PEACH X-DISEASE AND SEASONAL TRANSMISSION FROM WILD CHOKECHERRY

INTRODUCTION

X-disease affects stone fruits in the northeastern and western areas of the United States and is probably caused by a mycoplasma (8, 11, 12). Earlier workers maintained a distinction between the eastern and western forms of X-disease, but the numerous strains of this disease, including those in Michigan, are now referred to as "X-disease" (4).

Leafhoppers are the only known vectors of the X-disease organism (XDO) in established orchards. Species capable of transmitting the XDO include Euscelidius variegatus Kirsh. (9) and Scaphytopius delongi Young (26) in addition to the ten species listed in a recent review (4). The most important vector species are Colladonus montanus (Van D.) in the west (4), Scaphytopius acutus (Say) in New York (5), and Paraphlepsius irroratus (Say) in Michigan (27).

In the eastern United States, X-disease vectors acquire the XDO primarily from chokecherry (Prunus virginiana L.) and are believed to transmit it from about June 15 to July 15 (7, 25). Spread of X-disease from diseased to healthy peach trees has been considered unimportant and eradication of chokecherry within 210 meters of orchards has provided satisfactory X-disease control (4, 19). However, X-disease spreads from diseased to healthy peach trees in the western

United States (20), and recent studies have suggested that the same thing is now occurring in the eastern United States (10, see Part I).

The rapid spread of X-disease in peach orchards since the late 1960s, including orchards where chokecherries were removed (see Part I), stimulated research on the leafhopper vectors and on their relationship to disease spread in Michigan. The objectives of this study were to determine (1) when X-disease transmission occurs in the field, (2) if the size and the seasonal fluctuation of vector populations are related to transmission in the field, (3) the transmission efficiencies for the most common vector species in Michigan, and (4) if other leafhopper species vector X-disease.

MATERIALS AND METHODS

Transmission to Indicator Plants in the Field

Successive groups of peach and chokecherry indicator plants were exposed beside clumps of X-diseased chokecherry bushes. Indicator plants were exposed at two sites near East Lansing and at two sites in southwestern Michigan (Berrien and Van Buren counties) in 1974, 1975, and 1976 except that only one East Lansing site was used in 1976. The sites were located in abandoned meadows, near railroad right-of-ways, and along road embankments (see Appendix C). Indicator plants were taken to the sites 20 May 1974, 8 May 1975, and 18 May 1976. They were changed on 17 June, 19 July, and 23 August in 1974; on 16 June, 25 July, and 28 August in 1975; and on 21 June, 26 July, and 28 August in 1976. The exposure periods ended 27 September 1974, 30 September 1975, and 2 October 1976.

The indicators were planted in six-inch diameter tins, were fertilized periodically to maintain growth until early September, and were held in a lath house before and after exposure. They were at least 35 cm tall at the time of exposure. Groups of about 25 indicator plants per exposure period were placed at each site. In 1974, each group included ten "Baby Gold" peach trees, seven Halford peach seedlings, and eight chokecherry seedlings, except three Halford seedlings were substituted for three chokecherry seedlings during the

fourth exposure period. Each group included 15 Halford and ten chokecherry seedlings in 1975, and 13 Halford and 13 chokecherry seedlings in 1976.

To prevent water stress when exposed, the tins containing the indicator plants were placed in shallow trenches lined with plastic, were mulched with sawdust and woodchips, and were watered weekly. Weeds and grass around the tins were controlled with a contact herbicide. In 1975 and 1976, yellow ribbon was placed over each group of plants as an insect attractant.

Exposed indicators were sprayed with insecticide when returned to the lath house to eliminate resident insects. One hundred indicator plants were maintained in the lath house each year to check for possible transmission in the house. All indicator plants were placed in a cooler at 3 C from October until January and then moved to the greenhouse and observed for symptom development.

Leafhopper Trapping

Sticky-board traps (27) were used from 1974 through 1976 to monitor leafhopper populations at the sites where indicator plants were exposed and in two tart cherry and in three peach orchards (two in 1976) in southwestern Michigan. Only known or suspected X-disease vector species were identified and counted during this study.

From descriptions provided by Bierne (2) and Delong (3) and from experience gained during a previous project (27), we were able to identify specimens of *Paraphlepsius irroratus* (Say), *Scaphytopius acutus* (Say), *Colladonus clitellarius* (Say), *Norvellina seminuda* (Say), *Norvellina chenopodii* (Osb), and *Fieberiella florii* (Stal.). J. P.

Kramer, Smithsonian Institute, Washington, D.C., D. E. Barnett, Department of Entomology, University of Kentucky, and O. Taboada, Department of Natural Science, Michigan State University, assisted in identifying other species counted on traps and used in transmission tests.

Transmission Tests with Paraphlepsius irroratus and Scaphytopius acutus

Paraphlepsius irroratus and S. acutus were tested under controlled conditions to determine their capabilities as X-disease vectors. Our colony of S. acutus originated from insects collected in Nebraska, was sent to us by Carol Musgrave in 1973, and was maintained on red and ladino clovers under a 16-hour photoperiod. The P. irroratus were field captured adults. Initially, they were collected with a sweepnet, but later an aspirator was used to collect adults attracted to yellow 60-watt lights on warm calm evenings.

The acquisition access period (AAP) varied from 5 hours to 20 days. Groups of 10 to 50 leafhoppers were caged together during the AAP and incubation period. Leafhoppers were tested individually starting about 20 days after their initial exposure to X-disease inoculum and were moved to new test plants at regular intervals.

Periwinkle (Vinca rosea L.), celery (Apium graveolens cv. Utah 52-70), chokecherry seedlings, and Halford peach seedlings were used as acquisition host plants and as test plants. Celery was used extensively because it was the most suitable host for the insects tested, it develops high titers of mycoplasmalike bodies in its phloem

cells (8, 12), and it developed distinctive X-disease symptoms 4-8 weeks after inoculation.

Transmission studies were conducted in controlled environment chambers at 22-24 C with a 16-hour-per-day photoperiod except for a few experiments where diseased peach or chokecherry plants growing in the field were the acquisition hosts. In the latter cases, insects were held in flexible screen cages tied over branches on diseased plants.

Following inoculation feedings, celery and periwinkle plants were held at 20-25 C in a greenhouse for 8 and 15 weeks, respectively, and were observed regularly for X-disease symptoms. After inoculated peach and chokecherry seedlings had stopped growing, they were stored in a cooler for about 4 months and were observed for symptom development during their next growth cycle in the greenhouse.

Transmission Tests with Other Leafhopper Species

Other leafhopper species collected from around yellow lights were caged for 4-14 days on X-diseased chokecherry or celery plants and then were tested individually on celery test plants. White silica sand was spread over the soil under the test plants so that dead insects could be recovered for identification.

RESULTS

Occurrence of X-Disease on Field Exposed Prunus Seedlings

Twenty-six percent of 387 peach and chokecherry indicator plants exposed in 1974, 5% of 355 exposed in 1975, and 3% of 273 exposed in 1976 developed X-disease symptoms (Table 1). Thirty percent, 4%, and 6% of the peach seedlings, and 18%, 7%, and 0.7% of the chokecherry seedlings exposed during 1974, 1975, and 1976, respectively, developed X-disease. For each exposure period, the proportions of infected peach and chokecherry indicators were compared using the chi-square test applied to results arranged in a 2 x 2 contingency table. The only significant difference in infection between the two plant species occurred during the first exposure period in 1974 when 48% of the peach indicators but only 3% of the chokecherry indicators developed X-disease.

Thirty-seven percent of the total transmission to indicator plants during the two years occurred during the third exposure period in July and August, 21% occurred during the second period, and 17% occurred during the fourth period. Nearly half of the peach indicators exposed during the first period in 1974 developed X-disease, but none of the peach or chokecherry indicators exposed from 8 May to 19 June 1975 or from 18 May to 21 June 1976 developed X-disease (Table 1).

Table 1.--Incidence of X-disease in peach and chokecherry indicator plants exposed beside naturally infected chokecherry bushes during 1974, 1975, and 1976.

Year	Indicator Plant	Exposure Period[a]				Yearly Total
		1	2	3	4	
1974	Peach	31/65[b] 48%[c]	17/65 26%	20/68 30%	12/73 17%	80/271 30%
	Chokecherry	1/32 3%	4/32 13%	14/32 44%	2/20 10%	21/116 18%
1975	Peach	0/37 0%	2/61 3%	5/58 9%	2/58 3%	9/210 4%
	Chokecherry	0/36 0%	0/34 0%	4/39 10%	6/36 17%	10/145 7%
1976	Peach	0/28 0%	4/31 13%	4/31 13%	0/39 0%	8/129 6%
	Chokecherry	0/37 0%	0/39 0%	1/34 3%	0/31 0%	1/144 0.7%

[a]Exposure periods one to four began 20 May, 17 June, 19 July, and 23 August in 1974, 8 May, 16 June, 25 July, and 28 August in 1975 and 18 May, 21 June, 26 July, and 28 August in 1976. The first three exposure periods ended with the beginning of the subsequent periods. Period 4 ended 24 September 1974, 30 September 1975, and 2 October 1976.

[b]The numerator is the number of indicator plants with X-disease; the denominator is the total number observed for symptom development. The results are totals for exposures at four sites.

[c]The percentage of indicator plants with X-disease.

X-disease symptoms never developed on any of the control plants kept in the lath house each summer. Plants which did not develop symptoms the first year following field exposure in 1974 remained healthy when observed for a second season. Five peach seedlings from the first exposure period in 1974 developed X-disease symptoms in 1974, but none of the plants exposed during subsequent years developed symptoms during that same year.

Vectors Trapped in Orchards and in Chokecherries

Of the leafhopper species surveyed, only P. irroratus, S. acutus, and C. clitellarius were common in peach and cherry orchards: 8,662, 803, and 402 specimens of the respective species, were trapped in orchards during three years (Table 2). Only 19 Novellina seminuda and 21 N. chenopodii were captured in orchards, although 107 and 242 specimens of the respective species were trapped in chokecherries in southwestern Michigan. Seventy-nine Orientus ishidae were trapped in orchards during 1975 and 1976.

All vector species were more common in orchards in late summer and autumn than in spring and early summer. Of the total of each species trapped in orchards during three years, 74% of the P. irroratus, 88% of the S. acutus, and 49% of the C. clitellarius were captured after August 25.

Three traps were placed in chokecherry bushes at each of two East Lansing sites from 1974 to 1976. The X-disease vector populations at these sites were similar in size and species distribution to the populations in chokecherry in southwestern Michigan except that Orientus ishidae was unusually abundant at a chokecherry site in

Table 2.--Numbers of the three most common X-disease vector species captured on yellow sticky-board traps in peach and tart cherry orchards and in unsprayed chokecherry bushes in southwestern Michigan from 1974-1976.

Leafhopper Species	Year	Total number of insects captured in:		
		Tart cherry[a]	Peach[b]	Chokecherry[c]
Paraphlepsius irroratus	1974	3,358[d]	1,564	477
	1975	1,171	859	319
	1976	1,134	576	399
Scaphytopius acutus	1974	52	126	426
	1975	87	94	275
	1976	353	91	140
Colladonus clitellarius	1974	122	54	84
	1975	59	19	60
	1976	124	24	99

[a]Leafhoppers were trapped in two tart cherry orchards on a total of ten traps in 1974 and 1975 and five traps in 1976.

[b]Leafhoppers were trapped in three peach orchards on a total of 15 traps in 1974 and 1975 and on five traps deployed in two peach orchards in 1976.

[c]Two traps per site were used at two sites in 1974 and 1976 and at three sites in 1975.

[d]The totals represent the numbers of insects counted on traps and have not been adjusted to compensate for the smaller numbers of traps used in 1976.

East Lansing where 407 individuals were captured during 1976. Adult O. ishidae first appeared in early July. The population peaked in mid-July, then slowly declined during August and early September.

Seasonal Population Trends of Paraphlepsius irroratus

Leafhopper trapping data from 1974-1976 were used to establish seasonal population trends for P. irroratus in peach and tart cherry orchards sprayed with insecticides and in unsprayed chokecherry sites. To compensate for varying trap exposure periods and for varying numbers of traps between sites, the data are presented as the number of P. irroratus captured per trap per day. The average number of insects captured per trap was divided by the number of days traps were exposed. The resulting daily values were averaged with values for the preceding three days and the following three days to produce a rolling average which was plotted against time (Figures 1, 2).

The timing of seasonal fluctuations in populations of P. irroratus in orchards and in chokecherry was similar to that reported for 1972-1973 (28). Each year, early and late season populations peaked at about the same levels in chokecherries (Figure 2), but in orchards, the number of insects captured per trap after August 1 was about four times the number captured before August (Figure 1). Populations of adult P. irroratus began to increase earlier in tart cherry orchards than in peach orchards, probably because cherry orchards were sprayed with insecticides until early July while peach orchards were sprayed through mid-August. The numbers of P. irroratus captured per trap were higher in 1974 and 1976 than 1975.

Fig. 1.--Average numbers of Paraphlepsius irroratus (Say) adults captured per trap per day in peach and tart cherry orchards in southwestern Michigan during three years.

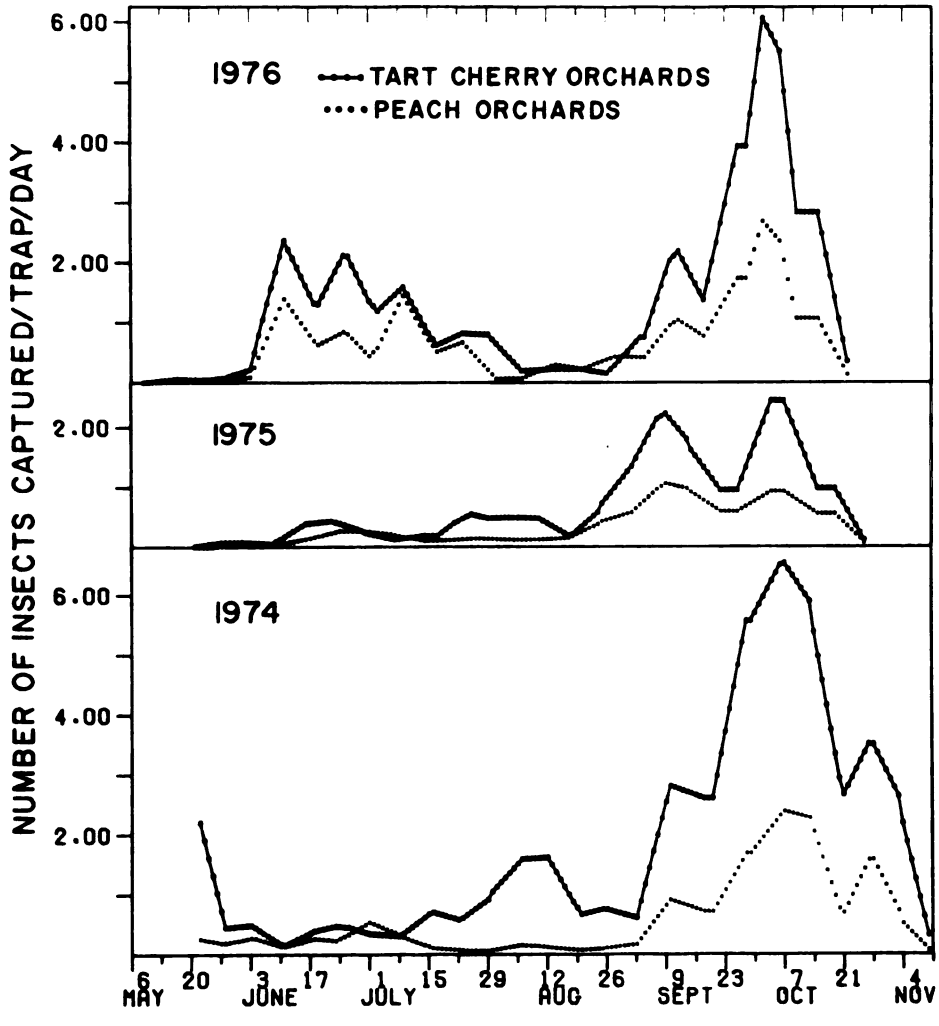
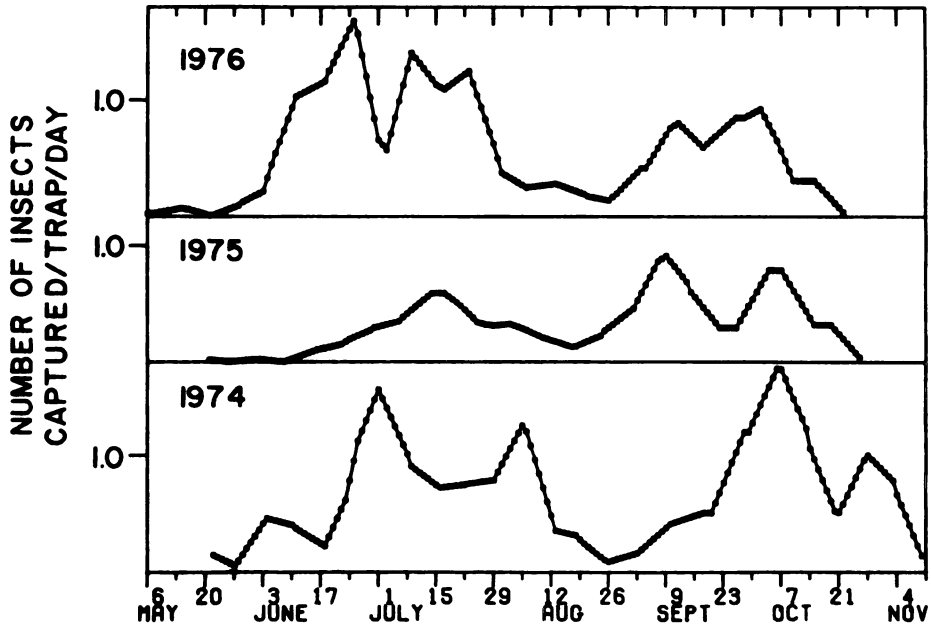

1976
TART CHERRY ORCHARDS
PEACH ORCHARDS
1975
1974
NUMBER OF INSECTS CAPTURED/TRAP/DAY
6.00
4.00
2.00
2.00
6.00
4.00
2.00
6 20 3 17 1 15 29 12 26 9 23 7 21 4
MAY JUNE JULY AUG SEPT OCT NOV

Fig. 2.--Average numbers of Paraphlepsius irroratus (Say) adults captured per trap per day in chokecherry in southwestern Michigan during three years.


NUMBER OF INSECTS
CAPTURED/TRAP/DAY
1976
1975
1974
1.0
1.0
1.0
6
20
3
17
1
15
29
12
26
9
23
7
21
4
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

Transmission by Paraphlepsius irroratus and Scaphytopius acutus

Over 50% of the leafhoppers present at the start of transmission tests died during the AAP and incubation period, so only results from leafhoppers surviving more than 20 days from the beginning of each experiment are reported. Both P. irroratus and S. acutus transmitted the XDO from celery to celery and from chokecherry to celery (Table 3), but only S. acutus transmitted from periwinkle to celery. None of 53 P. irroratus and 94 S. acutus transmitted after feeding for 7-14 days on X-diseased peach trees. In one experiment with 11 insects, S. acutus transmitted the XDO from celery to chokecherry seedlings, and two of 15 S. acutus transmitted from celery to small peach seedlings.

Thirty-seven percent of 331 P. irroratus and 26% of 153 S. acutus transmitted XDO after feeding on X-diseased celery (Table 3), and this difference in the proportions of transmitting insects was significant ($P < 0.05$) when tested against the chi-square distribution. Only 22% of the P. irroratus and 9% of the S. acutus transmitted after acquisition feedings on X-diseased chokecherry, but S. acutus survived poorly during AAP's on chokecherry. The ability of S. acutus to transmit the XDO after feeding on diseased chokecherry was calculated from two experiments in which one of 19 and three of 27 insects transmitted after AAP's of one and four days.

Thirty-eight percent of the P. irroratus and 27% of the S. acutus transmitted the XDO after AAP's of 4-8 days compared to 34% and 22% for the respective species after AAP's of 9-20 days. The difference in transmission after the shorter AAP compared to

Table 3.--Transmission of the X-disease organism by the leafhopper vectors Scaphytopius acutus (Say) and Paraphlepsius irroratus (Say) following acquisition access periods on four species of X-diseased plants.

Leafhopper vector species	Acquisition host plants[b]			
	Celery	Choke-cherry	Peach	Periwinkle
Scaphytopius acutus				
Positive[a]/total number of trials	45/62	2/7	0/9	1/5
Total no. of insects surviving 20 days	984	95	94	33
No. of individual insects tested in positive trials	153	46		
Fraction of tested individuals which transmitted	26%	9%		Groups of 5 and 8 insects transmitted
Paraphlepsius irroratus				
Positive/total number of trials	27/32	5/8	0/6	0/3
Total no. of insects surviving 20 days	433	60	53	14
No. of individual insects tested in positive trials	331	46		
Fraction of tested individuals which transmitted	37%	22%		

[a]A trial refers to the process of testing transmission by insects caged together on the same acquisition host plant. In positive trials, at least one insect transmitted the X-disease organism to at least one test plant.

[b]Acquisition access periods were 4-20 days.

transmission after longer AAP's was not significant ($P > 0.05$) for either species when tested against the chi-square distribution. In two additional trials, one of nine and three of 31 S. acutus transmitted after AAP's of 5 and 26 hours, respectively.

Male and female P. irroratus were equally effective as vectors. In a group of 83 adults, 39% of the males and 40.5% of the females transmitted the XDO from diseased to healthy celery.

Field captured S. acutus were tested to determine if the Michigan population of S. acutus was similar to the Nebraska strain in its ability to transmit the XDO. Three of 32 adults transmitted the XDO (Table 6), but 14 of the insects died 20-25 days after beginning the AAP and may not have survived long enough to transmit. Eighteen percent of the insects surviving beyond 25 days transmitted the XDO, and this proportion did not differ significantly ($P > 0.05$) from the proportion of the Nebraska strain which transmitted the XDO.

X-disease symptoms never developed in control plants exposed regularly in the greenhouse, in growth chambers, or to groups of S. acutus taken directly from the colony.

Frequency of Daily Transmission By Paraphlepsius irroratus

To determine the consistency of transmission by infective P. irroratus, 76 adults were transferred to new celery test plants at 1-4 day intervals starting 20-30 days after the beginning of a 7-day AAP on diseased celery. Twenty-one insects transmitted at least once during the test period (Table 4). Transmission skips of

Table 4.--Transmission of the X-disease organism by individual Paraphlepsius irroratus (Say) during frequent transfers on celery test plants after 7-day acquisition access periods on X-diseased celery.

Insect number	Sex	Days after beginning of the acquisition access period								
		20	25	30	35	40	45	50	55	60
1	F[a]	0----X00X-X-X000[b]								
2	F	X---000X0-								
3	F	0------XX-								
4	F	0--000000-0-X0								
5	F	X------X0-								
6	F	X--0								
7	F	0----0-X-0-								
8	F	0----0--0-X-0---								
9	M	0--000000-X-000X-0-00X0								
10	M	0----X000-0-00								
11	M	0---00000-0-000X-0-0X00								
12	M	0----X-0-0-00-0-0-0-								
13	M	0----0-X-0-0								
14	M	0----0--0-X-0---								
15	M			[c]0X-0-X-0X-X-0-0X--0---						
16	M			00-X-0-0						
17	F			00-0-0-0X-						
18	M			0X-X-0-X0-0-0-00--0---X--X--0-0-0---						
19	M			X--0-0-0---						

Table 4.--Continued.

Insect number	Sex	Days after beginning of the acquisition access period								
		20	25	30	35	40	45	50	55	60
20	M		0--0-0-X---X--0----							
21	F		0--0-0-0---0--0----------X------							

[a]M = male, F = female.

[b]Celery test plants developing X-disease symptoms (X) and those remaining healthy (0) are followed by dashes for each consecutive day that vectors fed on the same test plant. Thus, the first transmission by insect #5 may have occurred on day 26 although the X designating the diseased plant is shown under day 20.

[c]Insects 15-21 were members of groups which transmitted X-disease prior to individual tests shown here.

4-5 days were not uncommon, and one insect failed to transmit for 14 days between its last two transmissions.

Results of 1-, 2-, 3-, and 4-day exposures were totaled for 20 infective insects (insect #21, Table 4, produced no usable data). Counting from the day each insect first transmitted the XDO, the 20 insects were on test plants a total of 173 days. The expected numbers of daily transmissions for the 2-, 3-, and 4-day exposures were calculated using the probability equation $P_1 = 1 - (1 - P_n)^{1/n}$ where P_1 is the expected proportion of diseased plants from daily exposures and P_n is the proportion of diseased plants observed after exposures of n days (28). These calculations showed that transmission probably occurred on 42 days, or on 24% of the total exposure days (Table 5).

Transmission Trials with Other Leafhopper Species

At least two species not previously reported as X-disease vectors transmitted the XDO in our trials (Table 6). Four of 15 Orientus ishidae transmitted to celery test plants following a 7-day AAP on diseased celery. Seven of 43 Scaphoideus transmitted the XDO to celery test plants following AAP's of 7-13 days on X-diseased celery, and one Scaphoideus transmitted to celery after a 9-day AAP on diseased chokecherry. The transmitting Scaphoideus were females and could not be identified with certainty because identification of Scaphoideus species is based on characteristics of the male genitalia (2, 3). One of the two males in the group of 43 specimens was identified as S. diutius De L. & M. and the other as S. melanotus Osb. The female which transmitted X-disease from chokecherry was

Table 5.--Calculated frequency of daily transmission of X-disease by infective Paraphlepsius irroratus (Say) leafhoppers during 1- to 4-day inoculation access periods on celery test plants.

Inoculation access period (days)	Number of test plants		Number[a] of plant-exposure days	Calculated[b] number of transmission days
	Exposed	Infected		
1	35	10	35	10.0
2	44	19	88	21.7
3	6	5	18	8.1
4	8	2	32	2.2
		Totals	173	42
	Percent of plant-exposure days on which transmission occurred			24.3%

[a]Plant-exposure days are equal to the number of plants exposed multiplied by the length of the inoculation access period.

[b]The calculated number of transmission days were determined from the equation

$$P_1 = 1 - (1 - P_n)^{1/n}$$

where P_1 is the number of days on which transmission is expected and P_n is the fraction of days on which transmission occurred following inoculation access periods of n days.

Table 6.--Results of X-disease transmission tests with nine species of leafhoppers following acquisition access periods of 5-13 days on X-diseased celery or chokecherry plants.

Leafhopper[a] species	Acquisition Host	Number of insects[b]	
		Tested	Transmitting
Osbornellus auronitens	celery	11	0
Norvelina seminuda	celery	3	0
	chokecherry	7	0
Texananus majestus	celery	16	0
Prescottia lobata	celery	8	0
Scaphytopius acutus	celery	32	3 (9.4%)
Orientus ishidae	celery	15	4 (26.7%)
Gyponana species	celery	7	0
Scaphoideus species[c]			
(S. diutius)	celery	43	7 (16.2%)
(S. carinatus)	chokecherry	1	1

[a]All leafhoppers were field-captured adults.

[b]Only results from insects surviving 20 days after their first exposure to X-disease inoculum are reported.

[c]The transmitting Scaphoideus could not be identified with certainty because they were females.

larger than those transmitting from celery, and may be the species S. carinatus Osb.

Texananus majestus, Prescottia lobata, and Osbornellus auronitens failed to transmit the XDO (Table 6). Texananus majestus was occasionally trapped in orchards and was tested because other Texananus species transmit California aster yellows (22). Prescottia lobata is rare in Michigan orchards (27) but could not be distinguished from Scaphoideus species until the specimens were examined and identified after the transmission tests. Osbornellus auronitens was tested because O. borealis (De L. & M.) is a vector of X-disease in the western United States (4). However, O. borealis and O. auronitens represent two distinctly different groups of Osbornellus species (13), and O. auronitens feeds primarily on ferns (2, 13).

Norvellina seminuda and Gyponana lamina vector X-disease in New York (5) but the N. seminuda and Gyponana we tested failed to transmit the XDO in limited trials.

Minimum Incubation Periods in the Leafhopper Vectors

The shortest period between first access to X-disease inoculum and transmission of the XDO was 23 days in S. acutus, 22 days in P. irroratus, and 20 to 35 days in O. ishidae and the Scaphoideus species. No symptoms developed on plants where S. acutus adults were removed before the 23rd day after initial access to inoculum. A single P. irroratus (insect #6, Table 4) transmitted the XDO 22 days after first access to inoculum. One O. ishidae transmitted to a

test plant between days 28 and 35, and the earliest transmission with Scaphoideus occurred 20-27 days after initial access to inoculum.

With all vector species tested, some infective individuals failed to transmit for more than 30 days after first access to inoculum. The S. acutus given AAP's of 5 hours and 26 hours on X-diseased chokecherry did not transmit for at least 32 days, and one P. irroratus did not transmit for 41 days after the end of the AAP (insect #21, Table 4).

Natural Infectivity in Field Captured Vectors

Insects captured at lights about 40 m from three infected chokecherry bushes were tested for field-acquired infectivity in 1975 and 1976. One hundred and fifteen P. irroratus captured in 1975, and 150 P. irroratus, 10 N. seminuda, and 7 C. clitellarius captured in 1976 were placed, five to nine insects per plant, on celery test plants and were transferred to new test plants at 7- to 14-day intervals. One group of nine P. irroratus collected in 1975 transmitted the XDO to celery 38 to 47 days following capture and four remaining insects from this group transmitted to another celery plant 30 days later. None of the control insects tested in 1976 transmitted the XDO up to 30 days after capture.

DISCUSSION

Our results support an earlier suggestion (27) that P. irroratus is a major X-disease vector in Michigan, although it is not considered as such in other fruit-growing areas (4). Paraphlepsius irroratus accounted for 87% of the vectors trapped in orchards, was common from June to November, and occurs in virtual swarms under certain environmental conditions (15, 24). This species could account for long distance spread of X-disease since adults have been trapped at altitudes of 450 feet (16) and more than 9 miles from land (23). Individual P. irroratus acquired and transmitted the XDO more efficiently than the other vector species tested.

Nymphs and adults of P. irroratus generally stay hidden in orchard ground cover where they feed at the base of herbaceous plants (Rosenberger and Jones, unpublished). Adults appear to feed on woody plants primarily during the evening when they are particularly active. If adults acquire the XDO while feeding in trees, they must survive a 20- to 30-day incubation period before they can transmit the disease agent. These factors suggest P. irroratus should be less efficient in vectoring X-disease than vector species which feed on woody plants for extended periods as nymphs and adults (5, 18). In orchards, however, vector species that prefer woody hosts would be exposed directly to insecticide sprays whereas P. irroratus is

probably somewhat protected by the ground cover. Scaphytopius acutus also feeds and breeds on herbaceous plants (3, 15) and may have survived DDT sprays in perennial ground cover in New York orchards (17). Only a small proportion of a vector population feeding primarily in the ground cover is likely to acquire the XDO, but the ability of large populations to survive in sprayed orchards could compensate for the low probability of transmission by any given individual.

Paraphlepsius irroratus and S. acutus failed to acquire X-disease from diseased peach trees in our experiments, but too few insects may have been tested to detect low levels of transmission. Titers of the X-disease pathogen are lower in diseased peach trees than in diseased chokecherry (see Appendix B) and very few of the vectors feeding on diseased peach trees would be expected to encounter the XDO during feeding probes. If the XDO was acquired by 0.5% of a vector species feeding on diseased peach trees, significant peach to peach spread might occur because of the large vector populations in orchards. Detecting this level of transmission in greenhouse tests would require testing as many as a thousand insects and would be especially difficult given the possibility that field-captured insects might occasionally acquire the XDO prior to capture.

Undetermined factors other than the size of vector populations apparently affected X-disease transmission to indicator seedlings exposed beside diseased chokecherries. Similar numbers of vectors were trapped at the chokecherry sites in 1974 and in 1976, but 26% of the indicator plants developed X-disease after 1974 exposures compared to only 3% after 1976 exposures. Furthermore, the number of

indicators developing X-disease during the various exposure periods could not be related to population fluctuations of the vector species we counted except that the large number of diseased peach indicators resulting from the first period exposure in 1974 may have been related to the unusually early appearance of P. irroratus in May of 1974 (Figure 1).

Our results from three years suggest that the period of X-disease transmission may vary from season to season but is longer than the June 15 to July 15 period reported by Hildebrand (7) and Stoddard (25). X-disease transmission appears to occur from at least early June (the latter part of the first exposure period) through late August (the early part of the last period).

The transmission of X-disease from late August to early September suggests that significant transmission of X-disease may occur in orchards during autumn. The incidence of X-disease in indicator plants was not significantly greater in the last exposure period than in earlier periods, but the indicators were exposed near chokecherries where vector populations in autumn were lower than in peach orchards.

Surveys of Michigan peach orchards showed many trees developed X-disease symptoms in 1976 whereas few new infections were noted in 1975 (see Part I). Both the orchard surveys and the indicator plant exposures were made in the same area of Michigan. Because transmission to indicator plants and leafhopper vector populations in peach orchards were greater in 1974 than in 1975, we suspect that the increase in disease incidence noted in peach orchards in 1976 resulted

from natural inoculations made in 1974. Thus mature peach trees naturally inoculated with X-disease may not develop X-disease symptoms for more than 20 months after inoculation. Based on X-disease development in young, nonbearing orchards, Stoddard (25) suggested that naturally-inoculated peach trees develop X-disease symptoms about one year after inoculation. However, the XDO might reach high titers more rapidly in small than in large, mature trees.

Orientus ishidae and the Scaphoideus vector species are probably of minor economic importance because they are far less abundant in orchards than P. irroratus. Because they feed primarily on woody plants and occur in the same ecological niche as wild chokecherry, they may be important in spreading X-disease in chokecherry. Although no Scaphoideus species had previously been reported as an X-disease vector, Scaphoideus luteolus Van D. and Scaphoideus littoralis Ball were known to transmit elm phloem necrosis and flavescence doree of grape, respectively (1, 21).

Orientus ishidae was introduced from Japan during the early 1900s at about the same time that F. florii was introduced from Europe (13). Oman (14) has suggested that X-disease may have evolved with F. florii in Europe, but the discovery that O. ishidae is an X-disease vector introduces the possibility that X-disease originated in Japan.

Results of this study indicate that X-disease control measures should include more effective leafhopper control in orchards as well as the traditional chokecherry eradication programs. The fact that X-disease is transmitted during late summer means growers should be

concerned about the large vector populations which develop in orchards during late summer. Because the major vector, P. irroratus, apparently benefits from perennial ground cover in orchards, eliminating ground cover or planting orchards to a grass species less acceptable to leafhoppers might increase a grower's ability to control X-disease vectors in peach orchards.

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PART III

SYMPTOM REMISSION IN X-DISEASED PEACH TREES AS AFFECTED BY DATE, METHOD, AND RATE OF APPLICATION OF OXYTETRACYCLINE-HCl

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Symptom Remission in X-Diseased Peach Trees as Affected by Date, Method, and Rate of Application of Oxytetracycline-HCl

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ABSTRACT

ROSENBERGER, D. A., and A. L. JONES. 1977. Symptom remission in X-diseased peach trees as affected by date, method, and rate of application of oxytetracycline-HCl. Phytopathology 67: 277-282.

X-diseased peach trees with 9- to 17-cm trunk diameters were treated at various times during the growing season with five rates of oxytetracycline-HCl (OTC). Injections of 1.25, 2.5, and 3.75 g OTC per tree in September induced remission of symptoms for one year, whereas spring, summer, or fall injections of 0.5 or 0.9 g OTC per tree were less effective. Injections of 1.25 and 2.5 g OTC per tree in October and November were phytotoxic. Injections of dilute OTC by infusion and by pressure, and concentrated OTC pipetted directly into holes drilled in the trunks, all provided remission of foliar symptoms for one year. Terramycinlike activity (TLA) was greatest in leaves from trees injected by infusion. Injection of concentrated OTC was the most rapid and convenient method tested, but 2.5 g OTC in concentrated form caused some necrosis around the injection holes in the tree trunks. Increasing solution concentration by reducing the volume of solution injected did not reduce TLA activity in leaves except for the most concentrated treatment, 1.25 g OTC injected in 10 ml of solution. Terramycinlike activity in leaves declined rapidly following September injections and TLA in fruit from September-treated trees was below a desired residue tolerance of 0.1 μg/g fruit tissue.

Additional key words: mycoplasma, *Prunus persica*.

Although Stoddard reported suppression of symptoms of X-disease of peach using chemical treatments (20), control currently depends on eradicating infected chokecherry plants (*Prunus virginiana* L.) near commercial orchards (6, 10, 16). In 1967, mycoplasma-like organisms (MLO) were reported in phloem cells of plants affected by several "yellows" diseases (1), and tetracycline antibiotics caused remission of symptoms in one of these diseases (8). Subsequently, MLO's were found in phloem cells of peach trees affected by both X-disease and western X-disease (3, 9, 11, 12), and tetracycline treatments produced remission of symptoms (13, 18).

Methods for experimental applications of tetracycline reviewed by Schwarz (19) include root dips, sprays, infusions, pressure injections, and the application of concentrated pastes. Because tetracycline sprays generally proved to be ineffective (15), various methods of trunk injection have been used to treat diseased trees. In large field trials, tetracycline infusions were effectively used to control pear decline (14). Lethal yellowing of coconut palms has been controlled with similar treatment (7). Tetracyclines currently are used commercially for control of both pear decline and lethal yellowing.

Although X-diseased peach trees respond to tetracycline treatments, optimum chemical rates and treatment dates have not been defined fully and application methods have not been compared. The objectives of this study were to determine: (i) the most practical and effective method for treating X-diseased peach trees, (ii) the amount of chemical necessary to achieve symptom remission for at least one year, and (iii) the best timing for treatment. A preliminary account of these findings has been published (17).

MATERIALS AND METHODS

Two wettable powder formulations of oxytetracycline-HCl (OTC) containing the equivalent of 20% oxytetracycline base were used. The formulation for treating pear decline (EPA Reg. No. 1007-79) was tested in 1973, 1974, and 1975, whereas that for treating lethal yellowing of coconut palms (EPA Reg. No. 1007-80) was tested only in 1975. Rates of OTC are given in grams of active ingredient injected per tree.

In 1973, peach trees (*Prunus persica* Batsch 'Glohaven') with X-disease were sprayed weekly for 5 weeks starting 3 May, about 6 weeks before symptoms usually appear. About 15 liters per tree of OTC solution (100 μg/ml) were applied using a handgun. Other trees were injected with 0.5 g OTC per tree on 10 May, 22 June, or 19 July, or with 0.5, 0.9, 1.25, or 2.5 g OTC per tree in early September.

Trees were 6-10 years old with 9- to 17-cm diameter trunks.

Injections were made by the gravity infusion method of Nyland and Moller (14) into three holes per tree, drilled 4-cm deep with a 7-mm diameter bit, and spaced equally around the trunk, about 30 cm above the ground. Location of scaffold limbs was not considered in hole placement, but we avoided dead, sunken, or flattened areas in the trunk.

During autumn 1974, 16 treatments (Table 1) were applied to X-diseased Red Haven peach trees in a 7-year-old orchard, and in September 1975, six treatments were applied in another Red Haven orchard. Each treatment was replicated on four trees. Trees were selected and data were analyzed in blocked design based on the diameter of the tree trunks 30 cm above the ground.

Application methods in 1974 were gravity infusion, pressure injection, and injection of concentrates. Infusions were applied as described for 1973 treatments. Pressure injections were made at 2.8 kg/cm^2 (40 psi) through three holes in the trunk with a Model 102-C pressure injector from the Elm Research Institute, Harrisville, New Hampshire. The pressure and infusion methods were tested at rates of 1.25, 2.5, and 3.75 g OTC per tree. For concentrated injections (18) of 1.25 and 2.5 g OTC per tree, seven holes 10 mm in diameter were drilled at a downward angle of 45 degrees and in a spiral pattern around the trunk. Several milliliters of a concentrated OTC solution were pipetted into each hole. After uptake of solution, injection sites were sealed with wound dressing amended with benomyl.

Treatment dates were 12 and 13 September, 10 October, and 5 November. The effect of solution concentration was tested in 1974 using pressure injections of 1.25 and 2.5 g OTC per tree in 1.89, 3.79, and 7.58 liters of water and in 1975 using infusion of 1.25 g OTC per tree in final volumes of 10 ml and 0.94 and 3.79 liters. The two formulations of OTC were compared in 1975 using infusions of 1.25 g OTC per tree.

Treatments were compared for phytotoxicity, suppression of symptoms, and terramycinlike activity (TLA) in leaves. For assay of TLA, samples of 25 leaves per tree were collected weekly for 4 weeks after treatment on 12 September 1974. Samples of 50 leaves per tree were collected 23 May and 19 June from all October and November treatments and from September infusion treatments. Samples of 40 leaves per tree were collected 10 and 17 September and 23 October from the 1975 treatments. Leaves were taken at random from the center and the periphery of all trees. Samples were held at −20 C until assayed.

Terramycinlike activity was determined by the agar diffusion method (4) using paper assay disks (2). Weighed leaf samples were blended in phosphate buffer (pH 4.5) and vacuum-filtered through Whatman No. 2 filter paper. Filtrates were adjusted to pH 6.8 with 5 N NaOH. Assay disks impregnated with filtrate were placed on agar seeded with *Bacillus cereus* var. *mycoides*. Each time samples were tested, technical OTC (92.7%) was added to extract from healthy leaves to give standard concentrations of 0.1, 0.16, 0.32, 0.63, 1.25, 2.5, 5.0, and 10.0 μg OTC per milliliter of extract. The diameters of

TABLE 1. Oxytetracycline-HCl (OTC) treatments applied to mature X-diseased peach trees in 1974, with results of leaf assays and 1975 ratings for phytotoxicity to foliage

Treatment number	Application method	OTC rate (grams a.i. per tree)	Solution injected (liters)	Treatment date (1974)	Foliage phytoxicity rating[a]	Terramycinlike activity in leaves (μg/g)	
						Autumn[b]	Spring[c]
1	Infusion[d]	1.25	3.79	9/12	1.4	14.74	0.41
2	Infusion	2.50	3.79	9/12	2.4	26.98	0.68
3	Infusion	3.75	3.79	9/12	2.9	35.24	
4	Pressure	1.25	3.79	9/12	1.1	13.21	
5	Pressure	2.50	3.79	9/12	2.1	15.63	
6	Pressure	3.75	3.79	9/12	2.7	26.21	
7	Pressure	1.25	7.58	9/12	1.1	12.12	
8	Pressure	2.50	7.58	9/12	1.8	18.34	
9	Pressure	1.25	1.89	9/12	1.0	13.16	
10	Pressure	2.50	1.89	9/12	1.1	21.24	
11	Concentrate	1.25	10 ml	9/12	1.0	9.63	
12	Concentrate	2.50	17 ml	9/12	1.2	19.33	
13	Infusion	1.25	3.79	10/10	3.7		1.35
14	Infusion	2.50	3.79	10/10	4.8		3.58
15	Infusion	1.25	3.79	11/5	3.2		3.14
16	Infusion	2.50	3.79	11/5	3.9		3.68
17	Control	0	0	...	1.4	<0.30	<0.30

[a] Phytotoxicity to foliage was rated 23 May and 15 June 1975: 1.0 = normal foliage development; 2.0 = yellow foliage; 3.0 = yellow foliage with approximately one-half of tree showing stunted foliage development; 4.0 = foliage development severely stunted throughout the tree; and 5.0 = severely stunted foliage with some death of limbs. Ratings are means of four trees and two observation dates.

[b] Autumn leaf samples consisted of 25 leaves per tree collected 19 September, 24 September, 3 October, and 10 October 1974 from each of four replicates.

[c] Spring leaf samples consisted of 50 leaves per tree collected from each of four replicates on 23 May and 19 June 1975.

[d] Pressure injections (2.8 kg/cm^2) and infusions were applied through three holes 7 mm in diameter drilled 4 cm into the trunk 30 cm above ground. For concentrated injections, solution was pipetted directly into seven 10-mm diameter holes drilled 4 cm into the trunks.

inhibition zones produced by the standard concentrations were measured and an equation relating inhibition zone to $\log_{10}$ OTC concentration was derived by linear regression. This equation was used to convert the inhibition zones of sample extracts to micrograms of TLA per milliliter of extract. For samples with activity exceeding 10 μg/g of leaf tissue, extracts were diluted with buffer to allow measurement in the 0.16-10.0 μg/ml range of standard concentrations. The final activity in samples was expressed in micrograms TLA per gram of fresh leaf weight. The minimum detectable level of activity with this technique was 0.16 μg/ml of extract or 0.30 μg/g of leaf tissue. No zones of inhibition were produced by extracts from untreated healthy or diseased trees.

Fruit samples (approximately 2.2 kg) for residue analysis were collected at harvest from most 1973 and 1974 treatments. These samples were held at −20 C until the soluble solids were extracted by homogenizing and straining 100-g subsamples. Clear supernatant solution containing the soluble solids was freeze-dried. Samples later were dissolved in 0.1 M phosphate buffer (pH 6.8) to a final volume of 25 ml, and this solution was analyzed for TLA in the laboratories of Pfizer Inc. by the method of Grove and Randall (4).

The 1974 treatments were rated in 1975 for symptom remission, damage to the tree trunks, and toxicity to foliage. Trees were checked for X-disease symptoms in early September and a tree was considered diseased if symptoms occurred on any branch.

Each tree was examined for possible trunk damage at least twice and those showing severe damage were noted. Control trees were not rated since holes were not drilled in them.

Toxicity to foliage was rated using a scale of 1.0 (= no phytotoxicity) to 5.0 (= severe phytotoxicity). Ratings were made on 23 May and again on 5 June, and the results were averaged.

RESULTS

Trees sprayed with a 100 μg/ml OTC solution during May 1973 developed X-disease symptoms 2 weeks after symptoms appeared on untreated trees. By September, sprayed trees did not differ from controls. Infusion of 0.5 g OTC per tree on 10 May delayed the appearance of leaf symptoms for 4 weeks and decreased the rate of symptom development during the remainder of the season. Infusions in June and July checked further symptom development that season, but none of the 1973 spring or summer treatments affected symptom development in 1974.

Injections of 0.5 g OTC per tree in September 1973 delayed the onset of X-disease symptoms by several weeks in 1974, whereas 0.9 g delayed symptom onset about 7 weeks. Trees injected with 1.25 or 2.5 g OTC per tree in September 1973 developed no X-disease symptoms during 1974. Trees treated during September 1973 were treated again with 1.25 or 2.5 g OTC in September 1974 and remained symptomless through 1975.

All 16 treatments applied in autumn 1974 gave remission of foliar symptoms through 1975, whereas control trees exhibited leaf symptoms in July and extensive defoliation in September. Fruit on treated trees was similar in size to fruit on healthy trees; control trees produced small fruit which usually dropped before ripening.

The time required for infusion of the solution varied with weather conditions and with time of year. In 1973, uptake of 3.79 liters of solution required about 2 weeks in May, 5-7 days in June and July, and 1-3 days in September. September infusions in 1974 and 1975 required up to 3 days, although during periods of 29 C temperatures in 1974, uptake was completed within 8 hours. In October and November, infusions were completed within 4 days, although in November the trees were defoliated. The time required for pressure injection

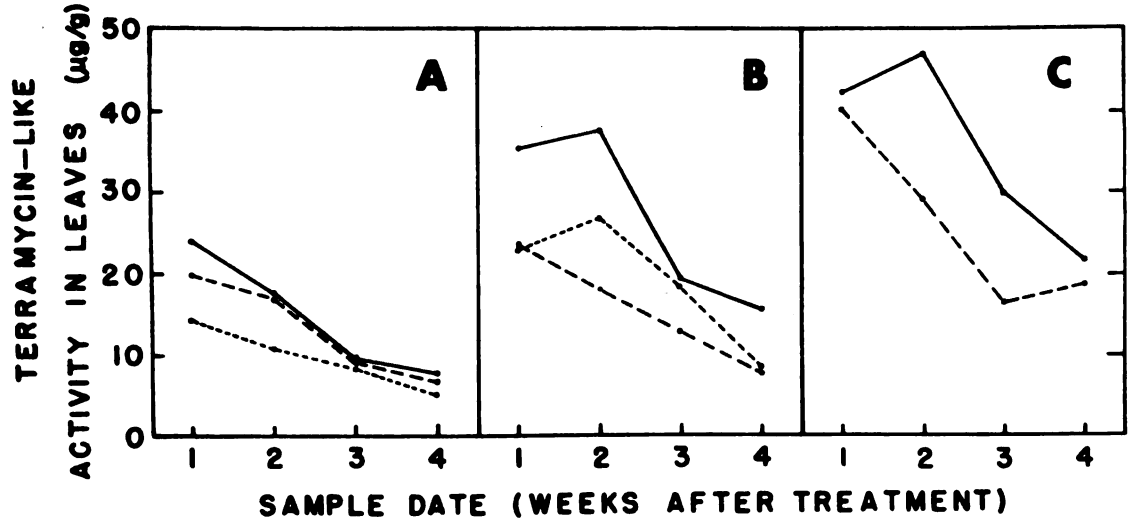

Fig. 1.-(A to C). Decline of terramycinlike activity (μg/g) in peach leaves following injections of: A) 1.25 g oxytetracycline-HCl (OTC) per tree; B) 2.5 g OTC per tree; and C) 3.75 g OTC per tree. Treatments were applied 12 to 13 September by infusion (●——●), by pressure injection (●– – –●), and as OTC concentrate (●—— —●).

varied considerably from tree to tree and usually exceeded 4 hours.

The foliage of injected trees turned slightly yellow for several weeks after treatment. Injections of 2.5 or 3.75 g OTC per tree in September also caused reddening of leaf veins after treatment and some dwarfing and yellowing of foliage the following spring (Table 1). September injections of 1.25 g OTC per tree caused a slight yellowing of leaves after treatment, but little or no yellowing appeared on spring foliage. Trees treated in October and November exhibited some death of branches the following spring, and many of the new leaves were chlorotic, strap-shaped, and small. Phytotoxicity ratings (Table 1) ranged from 3.2 to 4.8 for October and November treatments compared to 1.0 to 2.0 for most other treatments. Usually the chlorosis disappeared from treated trees by mid-June, but some trees treated in October and November remained stunted through early August.

Decline of TLA in leaves during the 4 weeks following treatment on 12 to 13 September was nearly linear for trees pressure-injected with 2.5 or 3.75 g OTC per tree and for trees injected with 1.25 g OTC by any method [Fig. 1-(A to C)]. For trees treated with 2.5 g OTC by infusion or concentrate (Fig. 1-B), or with 3.75 g OTC by infusion (Fig. 1-C), TLA in leaves did not decline until after the second sampling period. Most treatments had no detectable TLA in leaves after about 40 weeks (19 June 1975). For example, mean TLA in leaves of trees treated with 2.5 g OTC by infusion was 35.5, 15.1, 1.0, and 0.33 μg g after 1, 4, 37, and 40 weeks, respectively.

The mean TLA in leaves during autumn and/or spring was determined for each treatment (Table 1), and also for each rate, date, and method of application (Table 2 and 3). Effects and interactions of treatment factors were determined using several 3 × 2 split-plot factorial analyses in which the main treatment factors were split across four fall or two spring leaf-sampling dates. Treatment numbers from Table 1 are used to refer to the treatments included in the following statistical analyses.

In a comparison of infusion and pressure injection at 1.25, 2.5, and 3.75 g OTC per tree (treatments 1-6), high TLA in leaves was produced by high OTC rates (Table 2). The infusion method, when compared across all three rates, resulted in significantly ($P = 0.05$) greater TLA in leaves than did pressure injections. No rate × method interaction was detected.

In comparisons among treatment dates (treatments 4, 5, and 13-16), trees treated with 1.25 or 2.5 g OTC in September or with 1.25 g OTC in October showed low TLA in spring foliage (0.41-1.35 μg/g), whereas trees treated in November or with 2.5 g OTC in October showed higher levels (3.14-3.68 μg/g) (Table 3). The rate × date interaction was not significant.

The effects of solution concentration were tested by using three volumes of solution for pressure injections of two rates of OTC (treatments 4, 5, and 7-10). No significant differences in TLA were found among treatments involving 1.89, 3.79, and 7.58 liters of solution. When compared across the three volumes, trees treated with 2.5 g of OTC had significantly ($P = 0.05$) higher TLA in leaves than trees treated with 1.25 g of OTC.

The effect of solution concentration was tested again in 1975 when both the pear and the palm formulations of OTC were applied as concentrate or by infusion at 1.25 g/tree in final volumes of 10 ml, 0.94 liters, and 3.79 liters. These volumes resulted in mean TLA of 5.6, 12.8, and 10.9 μg/g, respectively, with LSD ($P = 0.05$) = 3.17. Formulation did not affect activity in leaves of 1975 treatments.

Residue analysis of fruit samples showed 0.032 and 0.030 μg TLA/g of fresh fruit for 1.25- and 2.5-g OTC treatments applied September 1973. Most of the September 1974 treatments, including those applied to trees also treated in 1973, resulted in no detectable fruit residue (<0.0125 μg/g). Infusion of 3.75 g OTC in September resulted in the highest level of TLA that was detected in fruit, 0.0255 μg/g.

The majority of treated trees showed no external signs of trunk damage. One year after treatment most holes had healed although small *Cytospora* infections occasionally were observed. However, trees treated with 2.5 g OTC as a concentrate (17 ml) showed extensive necrosis extending above and below some injection holes, and incidence of

TABLE 2. Terramycinlike activity (TLA) in peach leaf samples as influenced by two injection methods and three rates of oxytetracycline-HCl (OTC)

Rate of OTC (g/tree)[a]	Application method		Mean TLA for rates (μg/g)[c]
	Infusion	Pressure	
1.25	14.74[b]	13.21	13.97
2.50	26.98	15.63	21.31
3.75	35.24	26.21	30.72
Mean TLA for methods (μg/g)[d]	25.66	18.35	

[a]With both methods, all rates of OTC were applied in 3.79 liters of water on 9 September 1974.

[b]Each treatment mean represents the average TLA (μg/g) in leaves from four replicates sampled on four dates. LSD ($P = 0.05$) = 11.12.

[c]Least significant difference between means for application rates ($P = 0.05$) = 7.86.

[d]Least significant difference between means for application methods ($P = 0.05$) = 6.42.

TABLE 3. Terramycinlike activity (TLA) in peach leaf samples collected in spring 1975 as influenced by injection date and rate of oxytetracycline-HCl (OTC) applied

Treatment date[a] (1974)	Rate of OTC (g/tree)		Mean TLA for dates (μg/g)[c]
	1.25	2.50	
13 September	0.41[b]	0.68	0.55
10 October	1.35	3.58	2.46
5 November	3.14	3.68	3.41
Mean TLA for rates (μg/g)[d]	1.63	2.65	

[a]All treatments were applied by gravity infusions of 3.79 liters of solution.

[b]Each treatment mean represents the average TLA (μg/g) in leaves from four replicates sampled on two dates. LSD ($P = 0.01$) = 1.75.

[c]Least significant difference between means for dates ($P = 0.01$) = 1.24.

[d]Least significant difference between means for rates ($P = 0.01$) = 1.01.

Cytospora canker was higher than for other treatments.

DISCUSSION

This study indicates that a single injection of 1.25 or 2.5 g OTC per tree in September will give remission of X-disease for 1 year in medium-size peach trees. The failure of injections of 0.5 and 0.9 g OTC per tree to give year-long remission of symptoms is consistent with the results of a previous study (18) in which rates approaching 1.0 g OTC per tree gave a maximum of 77% X-disease symptom remission. Nyland (13) reported year-long remission of X-disease symptoms with less than 0.5 g OTC per tree, but he treated trees in both autumn and spring.

We assayed TLA in leaves because the potential effectiveness of various treatments for X-disease control should be reflected by their relative residual activity. Terramycinlike activity in leaves reflects, among other factors, how effectively the chemical is translocated into tree crowns. However, TLA in leaves could not be related quantitatively to symptom remission because most of the rates we tested provided a high degree of remission. Leaf residues were related to phytotoxicity in that residues and phytotoxicity increased together. Based on TLA in leaves, infusion was the most effective method for introducing OTC into infected peach trees.

The injection methods differed in ease of application. Infusion required 2-7 days to complete. With the pressure system, establishing pressure-tight connections was a problem, a significant amount of solution sometimes was lost by exudation through wounds and pruning cuts, and injections were not always finished in 1 day. Application of concentrated OTC required less equipment, and treatments were applied and holes sealed during one visit to the orchard.

Except for concentrated 10 ml injections, the volume of solution injected by infusion or pressure, did not significantly affect levels of TLA in leaves. By using higher solution concentrations and less volume per tree, treatment time may be reduced with no loss of effectiveness.

Treatment with concentrated OTC at the lower rate (1.25 g/tree in 10 ml) resulted in the lowest TLA of any treatment. But 2.5 g OTC per tree in 17 ml caused unacceptable damage to the tree trunks. Sands and Walton (18) did not mention trunk damage, but tested only 7 and 10% OTC solutions. They also used a different formulation which may have been less phytotoxic than those used in our study.

The relation of tree size to amount of OTC required has been defined to some extent. A dose of 1.25 g OTC produced symptom remission for 1 year in trees with trunk diameters up to 17 cm, and 2.5 g OTC was not damaging to trees with trunk diameters as small as 9 cm. In treatments not reported here, objectionable toxicity to foliage resulted from September infusion of 2.5 g into a tree of 7.5-cm trunk diameter, and trees with trunks of about 20-cm diameter developed a few symptoms 1 year after treatment with 1.25 g OTC. To be assured of year-long symptom remission in trees with trunk diameters greater than 17 cm, our experience indicates they should be treated with 2.5 g OTC using four or five injection sites.

The most unexpected result was the severe phytotoxicity of some October and all November treatments. Tetracyclines inhibit protein synthesis (5). Possibly some of the chemical injected in late autumn was stored in the tree and, in spring, moved to the new growth where even the low concentrations of OTC were detrimental to synthesis and development of new leaves. Trees were no longer growing when treated in September and the foliage could tolerate OTC concentrations 10 times greater than those which caused phytotoxicity in spring foliage. Chemical residues in leaves initially were high following September injections, but declined to below toxic levels by the following spring.

Another explanation for the toxicity of late autumn treatments is that trees treated at or after leaf fall may have concentrated the OTC in dormant buds where it damaged proplastids. The resultant production of defective plastids could explain the persistance of toxic symptoms on leaves after TLA no longer was detectable. Assays for TLA in dormant buds would have been helpful in assessing this theory.

Another advantage of September or postharvest treatments was that the fruit were not harvested for 9 to 11 months, thereby reducing the likelihood of unacceptable fruit residues. Residues were not detected in fruit from trees treated with 1.25 g OTC the previous September. Moreover, the level of residue in fruit from trees treated with 3.75 g was well below the desired tolerance level of 0.1 μg/g.

Because most treatments we tested were effective, the final choice of OTC rate and method for treating X-diseased peaches depends on equipment available, on tree size, and on preferences of the applicator. For best results, we suggest that injections be made after harvest but before normal leaf activity declines and at a rate of 1.25 g OTC per tree for all but small (possibly younger than 4 years old) and large trees. Trees with trunk diameters exceeding 17 cm may be injected with 2.5 g OTC per tree. For infusions or pressure injections the chemical should be mixed to apply 0.89 to 3.79 liters of solution per tree, and at least three holes per tree should be used. More holes are required to apply 1.25 g in 10-15 ml with the concentrate method. Even with appropriate treatment, trees infected with X-disease for several years require 1-2 years for new growth to replace the fruit-bearing wood killed by the disease.

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APPENDICES

APPENDIX A

TRANSMISSION OF MYCOPLASMALIKE BODIES FROM MILKWEEDS FOUND NEAR X-DISEASED STONE FRUIT TREES

APPENDIX A

TRANSMISSION OF MYCOPLASMALIKE BODIES FROM MILKWEEDS FOUND NEAR X-DISEASED STONE FRUIT TREES

Abstract

Milkweeds (Asclepias syriaca L.) with small chlorotic leaves, numerous axillary shoots, and severe rosetting were observed near X-diseased peach and tart cherry trees in seven orchards in southwest Michigan. The milkweed disease agent was transmitted by dodder (Cuscuta compacta Juss.) from diseased milkweeds collected in three orchards to healthy milkweeks and to periwinkle, but not to tomato, tobacco, or celery. The X-disease agent was transmitted by dodder to periwinkle and celery, but not to milkweed. Transmission electron microscopy showed mycoplasmalike bodies were present in phloem cells of milkweeds and periwinkle affected by the milkweed disease. However, symptoms in periwinkle infected with the milkweed disease agent were distinctly different from symptoms of X-disease in periwinkle. Based on symptom and host range differences, the disease in milkweeds is considered different from X-disease. Milkweeds do not appear important as an X-disease host in Michigan orchards.

Introduction

X-disease infects a wide range of Prunus species (2) and is probably caused by a mycoplasma (3, 5). Under experimental conditions, X-disease has been transmitted to numerous herbaceous hosts including periwinkle, annual mum, China aster, radish, cauliflower, turnip, filaree, strawberry, coriander, and carrot (4). However, milkweed is the only herbaceous host found naturally infected with X-disease in the field (1). Milkweed is becoming more prevalent in orchards because herbicides used in orchards often fail to control it. The purpose of this study was to determine if X-diseased milkweeds were prevalent in southwestern Michigan orchards.

Methods

Milkweeds (Asclepias syriaca L.) observed in seven orchards in southwest Michigan in 1972 appeared to have X-disease (1). They were severely rosetted, had small chlorotic leaves, and had numerous axillary shoots developing along the main stem. A total of 14 diseased milkweed plants were collected near X-diseased trees in two peach and two tart cherry orchards, were potted in 6-inch tins, and were maintained in the greenhouse. Because herbicides commonly used in orchards sometimes affect the growth habit of milkweeds, milkweeds were collected only from areas apparently free of herbicides.

To determine whether a transmissible agent was present, dodder (Cuscuta compacta Juss.) transmission from infected milkweed to periwinkle (Vinca rosea L.), tomato (Lycopersicon esculentum Mill. cv. 'Rutgers'), tobacco (Nicotiana tabacum L. cv. 'Burley' and 'Xanthi'), celery (Apium graveolens L. cv. 'Utah 52-70'), and healthy milkweed

was attempted. Dodder was allowed to parasitize either the diseased milkweed or the healthy host, and the dodder shoots produced were used to form a bridge between healthy and diseased plants. Dodder bridges were maintained 45 to 60 days. The same method was used to transmit the X-disease organism from diseased peach (Prunus persica Batsch) to periwinkle and celery, and to transmit the aster yellows organism from diseased asters (Callistephus chinensis [L.] Nees) to periwinkle.

Thin sections of secondary leaf veins from infected milkweed and from periwinkle infected with the milkweed disease were observed under the electron microscope and examined for viruslike particles or mycoplasmalike bodies (MLB). Small sections of leaf veins were fixed in glutaraldehyde and osmium tetroxide and embedded in Spurr's resin (6). Following thin sectioning, sections were mounted on grids, stained with uranyl acetate and lead citrate, and examined on a Phillips 300 transmission electron microscope.

Results and Discussion

The disease agent from infected milkweeds was dodder-transmitted to periwinkle and to healthy young milkweeds, but not to tomato, tobacco, or celery. The X-disease organism was dodder-transmitted from peach to celery and periwinkle but not to milkweeds, and the aster yellows organism was transmitted by dodder from aster to periwinkle.

Milkweeds from three orchards were used as source plants for transmitting the disease to periwinkle, and all of the periwinkle plants developed similar disease symptoms. Infected periwinkle showed

reduced growth, a one-third reduction in flower size, a fading of flower color, a slight overall chlorosis of leaves, and an increased number of small axillary shoots.

Periwinkle infected with the milkweed disease developed different symptoms. X-disease in periwinkle caused a greater reduction in growth rate, more reduced flower size, production of fewer flowers, and less axillary shoot production than did the milkweed disease. X-diseased periwinkle developed cupped leaves with bright yellow margins and normal green centers whereas a slight but uniform chlorosis was noted in foliage of periwinkle plants infected with the milkweed disease.

Preliminary comparisons of aster yellows-infected periwinkle and periwinkle infected with the milkweed disease suggest that the agent transmitted from milkweeds may be the aster yellows organism, but further host range and insect transmission tests are required to confirm this possibility.

Mycoplasmalike bodies were observed in the phloem cells in leaves from milkweeds and periwinkle plants infected with the milkweed disease when thin sections of leaf veins were examined under the electron microscope (Figure 1). The MLB's could not be distinguished from those observed in X-diseased periwinkle and in X-diseased peach and cherry trees (3, 5). MLB's were not found in phloem cells in leaves from healthy milkweed or periwinkle used as controls.

Although this study has shown that the "yellows" disease affecting milkweeds in southwest Michigan orchards is probably caused by a mycoplasma, the disease syndrome in periwinkle indicates the

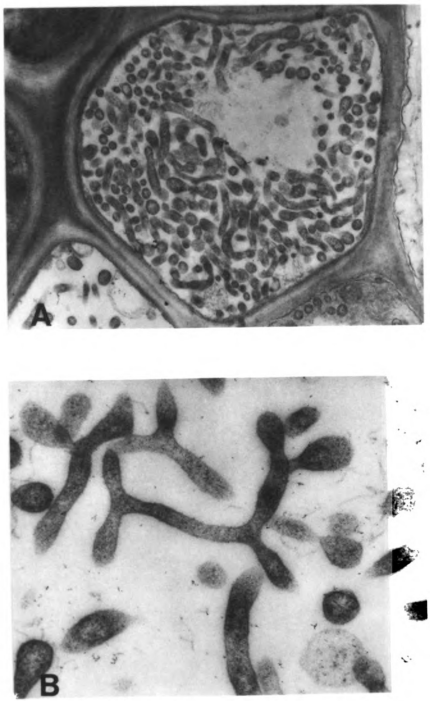

Fig. 1.--Mycoplasmalike bodies in sieve tube elements of lateral leaf veins from milkweed (A) and periwinkle (B) infected with the disease originating in milkweeds.

milkweed disease is distinct from X-disease. This distinction is substantiated by our failure to transmit the milkweed agent to tomato, tobacco, and celery, three common herbaceous hosts for X-disease (1, 4). We were unable to confirm that naturally infected, X-diseased milkweeds occur in orchards and conclude that milkweed is not important in the epidemiology of X-disease in southwest Michigan.

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APPENDIX B

SEASONAL VARIATION IN INFECTIVITY OF INOCULUM FROM X-DISEASED PEACH AND CHOKECHERRY PLANTS

APPENDIX B

SEASONAL VARIATION IN INFECTIVITY OF INOCULUM FROM X-DISEASED PEACH AND CHOKECHERRY PLANTS

Abstract

Actively-growing healthy peach and chokecherry seedlings were inoculated with X-diseased peach or chokecherry buds collected at various times of year. Individual seedlings were budded with three buds from diseased plants of the homologous species. Fifteen of 26 chokecherry and 4 of 48 peach seedlings inoculated between 24 February and 1 June developed symptoms. The proportion of inoculated chokecherry seedlings developing X-disease increased from 20% for March to 100% for may inoculations. Infection of peach seedlings rose from 8% for May to 100% for June inoculations, then declined to 82%, 32%, and 20% for August, September, and October inoculations, respectively. X-disease pathogen apparently survives winter in a low percentage of peach buds since three of 14 peach seedlings inoculated with buds taken in March and one of ten seedlings inoculated in December were infected.

Introduction

X-disease of stone fruits, a disease of assumed mycoplasma etiology (3, 7, 8), is leafhopper-transmitted in the field (1) and can be transmitted by budding. Hildebrand (4) found 17 of 25 peach buds collected from diseased orchard trees were infective during early July but only four of 25 buds were infective in August. X-disease could not be transmitted from peach budwood collected during winter (4, 10), but Stoddard (10) transmitted X-disease from peach root tissue. He then suggested that the X-disease pathogen overwinters only in the peach roots and moves upward with the sap flow each spring. Gilmer et al. (2) found that X-disease overwintered both in roots and in buds of X-diseased chokecherry plants.

Seasonal variation in the distribution and titer of the X-disease pathogen in diseased trees may affect the frequency of vector transmission. In this study, variations in the infectivity of inoculum were followed in diseased peach and chokecherry trees.

Methods

Budwood was collected periodically from visibly infected chokecherry bushes, peach seedlings, and peach trees and was used to inoculate actively growing indicator seedlings maintained in the greenhouse. Occasionally, infected peach seedlings or chokecherry bushes were dug in the field and taken to the greenhouse where tissues from the trunk above the soil line and from the root system were removed and used as inoculum.

Trunks of indicator seedlings were inoculated at three sites by T-budding with buds or rootchips, or by patch-budding with bark

patches. Inocula from peach trees and from chokecherry bushes were used to inoculate indicator seedlings of the same species. A supply of actively-growing seedlings was maintained by regularly moving dormant seedlings from a cooler to the greenhouse for approximately eight weeks (up to 12 weeks in winter) before inoculation. After inoculation the indicator plants were maintained in the greenhouse until growth slowed, placed in a cooler for four months, and observed for X-disease symptoms during the subsequent growth cycle in the greenhouse.

Results

Fifteen of 26 chokecherry seedlings inoculated during winter and spring of 1974 developed X-disease symptoms. The percentage of seedlings developing X-disease was consistently higher for winter-inoculated chokecherry seedlings than for peach seedlings inoculated during the same period. The proportion of infected chokecherry seedlings rose from 20% for March 18 inoculations to 40% for April 17 and 100% for May 20 inoculations (Table B1).

Three X-diseased chokecherry bushes were dug on 6 February 1975 after January temperatures had dropped to -31 C. Twenty-five chokecherry seedlings were inoculated with buds and 12 with root chips. Both buds and root chips formed good unions with the seedlings and many of the buds produced shoots. Seventy-two percent of the bud-inoculated and 75% of the root chip-inoculated seedlings developed X-disease symptoms. Apparently the X-disease pathogen was equally distributed in buds and in root tissue of chokecherry plants during the coldest part of winter.

Table B1.--Transmission of X-disease to Halford peach and chokecherry seedlings with buds taken from X-diseased peach trees and chokecherry plants at various times of year.

Date of Inoculation[b]	Indicator Plants[a]			
	Peach		Chokecherry	
	Number inoculated	Percent infected	Number inoculated	Percent infected
February 24	7	0	5	20
March 18	14	21	5	20
April 17	15	0	5	40
May 20	12	8	7	100
June 1	-	-	4	100
June 26	6	100		
August 11	11	82		
September 15	34	32		
October 24	5	20		
December 19	10	10		

[a]Indicator plants were observed under greenhouse conditions for X-disease symptoms for up to one year after inoculation. Peach buds were used to inoculate peach seedlings and chokecherry buds were used for chokecherry seedlings.

[b]Peach seedling inoculations were made from August 1972 through June 1974 and data from the two years has been combined. All chokecherries were inoculated in 1974.

Peach seedling inoculations were made from August 1972 through June 1974; data from the two years were similar. X-disease symptoms developed on 26 of 51 peach seedlings inoculated with buds collected from June through September, but on only six of 61 peach seedlings inoculated with buds collected from October through May (Table B1). All peach seedlings inoculated on June 26 developed X-disease symptoms, but the proportion of seedlings developing symptoms declined to 82%, 32%, and 20% for August 11, September 15, and October 24 inoculations, respectively. The four transmissions from December 19 and March 18 inoculations showed that the X-disease pathogen overwinters in a low percentage of peach buds, but the infectivity of peach buds remained low until June when foliar symptoms of X-disease usually appear on infected peach trees.

It was difficult to obtain unions on peach indicator seedlings with root chips and bark patches. Only five of 51 indicators inoculated with root chips and 10 of 38 indicators inoculated with bark patches developed X-disease. Root-chip and bark-patch transmissions were scattered throughout the year.

Discussion

Our results confirm earlier reports that the X-disease pathogen overwinters both in buds and roots of diseased chokecherry plants (2), and that the infectivity of peach buds declines during late summer and fall (4). However, X-disease transmissions from peach budwood collected during December and March do not support the suggestion that the X-disease pathogen overwinters only in roots of diseased peach trees (10). The failure of earlier workers to

transmit X-disease from dormant buds probably resulted from poor bud unions as Hildebrand (4) has suggested.

Transmission of X-disease was more erratic with peach than with chokecherry buds, possibly because only low titers of the X-disease mycoplasmalike organisms (MLO) are present in infected peach trees during most of the year. Jones et al. (5) found that few phloem cells in X-diseased peach leaves contained MLO, and, when MLO were present, they were usually few in number. The MLO were abundant in phloem cells of X-diseased chokecherry leaves, in phloem cells of tart cherry fruit stems, and in phloem cells of leaves from peach trees with peach yellows and little peach diseases (5, 6). Peach trees infected with western strains of X-disease apparently develop high titers of MLO's since workers on the west coast report no problems in detecting X-disease MLO's in peach phloem cells (7, 8).

The decrease in MLO titer in X-diseased peach trees begins well before the onset of cold temperatures and may be due to high sensitivity of peach to X-disease infection. During winter, the X-disease pathogen may die out completely in some peach trees since diseased trees and parts of trees have occasionally recovered from X-disease after overwintering (9, 10). The natural decline in X-disease pathogen titers during late summer and fall may contribute to the effectiveness of fall tetracycline injection treatments (see Part III).

The ability of vectors to acquire X-disease from infected peach and chokecherry depends in part on their chances of encountering MLO's during their feeding probes into the phloem cells. Lower

pathogen titers in X-diseased trees in the eastern than in the western United States could explain why transmission of X-disease from diseased to healthy peach trees has not been important in the east but is common in the west (1). Peach to peach transmission, if it does occur in the east (see Part I), would most likely occur during late June and early July when pathogen titers are highest in peach trees.

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APPENDIX C

RESULTS OF LEAFHOPPER TRAPPING AND INDICATOR PLANT EXPOSURES AT X-DISEASED CHOKECHERRY SITES IN THE FIELD

APPENDIX C

RESULTS OF LEAFHOPPER TRAPPING AND INDICATOR PLANT EXPOSURES AT X-DISEASED CHOKECHERRY SITES IN THE FIELD

Methods used for trapping leafhoppers and for exposing indicator plants are given in Part II. The exposure sites are described below, and results for 1974, 1975, and 1976 are presented on Tables C1, C2, and C3.

Site 1 was located about 400 m west of the Botany and Plant Pathology field laboratory on the Michigan State University Farms south of the university campus in East Lansing. Several X-diseased chokecherry bushes 1.5-3 m tall were found in an unmowed meadow between two small woodlots. The bushes used for exposures and trapping were about 20 meters from an experimental orchard.

Site 2 was located along the spur line of the Penn-Central railroad on the Michigan State University campus directly east of Trowbridge road in East Lansing. The X-diseased clumps of chokecherry at this site were surrounded by brambles and other species of shrubs.

Site 3 was located in an overgrown meadow south of Hinchman Road and about one-half kilometer west of Hinchman in Berrien County. The single, X-diseased chokecherry bush used at this site was about

2 m tall and was surrounded by grass on the south and by other species of shrubs on the north.

Site 4 was located on 62nd Street about 2.5 kilometers south of Interstate 94 in Van Buren County. The small X-diseased chokecherry bush at this site was growing with other shrubs on the road embankment. The surrounding fields were planted with corn and soybeans.

Site 5 was located south of Meadowbrook Road about 2.4 kilometers east of Interstate 94 in Berrien County. The X-diseased chokecherry bush at this site was growing in a wet, grassy area bordering a woodlot and was partially shaded by larger trees.

Insect counts presented in the tables represent the number of insects trapped on three sticky boards at sites 1 and 2 and on two sticky boards at sites 3, 4, and 5.

APPENDIX D

RESULTS OF INDIVIDUAL TRANSMISSION TRIALS WITH SCAPHYTOPIUS ACUTUS (SAY) AND PARAPHLEPSIUS IRRORATUS (SAY)

Table C1.--Proportions of peach and chokecherry indicator seedlings which developed X-disease following exposures at four chokecherry sites during 1974, and numbers of various leafhopper species trapped at those sites during the exposure periods.

Site Number	Exposure Period[a]	Proportion of diseased indicators		Total Percent Diseased Indicators	Leafhopper Species[b]					Total leaf-hoppers captured
		Peach	Choke-cherry		P. irroratus	S. acutus	C. cli-tellarius	N. seminuda	N. chenopodii	
1	1	4/15[c]	0/8	17.4	60	0	1	0	0	61
2	2	5/16	1/8	25.0	165	67	12	1	0	245
	3	4/16	4/8	33.3	132	10	0	0	0	142
	4	4/18	0/5	17.4	133	76	1	0	0	210
2	1	8/17	1/8	36.0	20	0	0	0	0	20
	2	2/15	3/8	21.7	62	24	6	0	0	92
	3	6/19	4/8	37.0	104	2	3	0	0	109
	4	1/19	1/5	8.3	53	12	0	0	0	65
3	1	7/15	0/8	30.4	2	1	0	0	0	3
	2	4/16	0/8	16.7	7	61	11	0	0	79
	3	7/16	2/8	37.5	0	12	1	0	0	13
	4	3/16	0/5	14.3	1	36	0	0	0	37
4	1	12/18	0/8	46.2	27	0	3	0	0	30
	2	6/18	0/8	23.1	115	26	15	0	3	159
	3	3/17	4/8	28.0	87	2	3	3	6	101
	4	4/20	1/5	25.0	39	60	8	5	26	138

[a]Exposure periods 1-4 began 20 May, 17 June, 19 July, and 23 August, respectively, and the fourth period ended 24 September.

[b]The insect species counted were Paraphelpsius irroratus (Say), Scaphytopius acutus (Say), Colladonus clitellarius (Say), Norvellina seminuda (Say), and Norvellina chenopodii (Osb.).

[c]The numerator is the number of plants which developed X-disease; the denominator is the number of plants observed for symptom development.

Table C2.--Proportions of peach and chokecherry indicator seedlings which developed X-disease following exposures at chokecherry sites during 1975, and numbers of various leafhopper species trapped at the sites during the exposure periods.

Site Number	Exposure Period[a]	Proportion of diseased indicators		Total Percent Diseased Indicators	Leafhopper Species[b]						Total Leafhoppers captured
		Peach	Chokecherry		P. irroratus	S. acutus	C. clitellarius	N. seminuda	N. chenopodii	O. ishidae	
1	1	0/12[c]	0/9	0	6	0	2	0	0	0	8
	2	1/15	0/8	4.3	166	14	7	3	1	1	192
	3	1/15	2/10	12.0	36	2	0	2	0	13	53
	4	0/13	1/10	4.3	41	5	2	1	0	1	50
2	1	0/12	0/9	0	0	0	0	0	0	0	0
	2	0/15	0/9	0	2	0	2	0	4	8	16
	3	0/14	1/10	4.2	0	4	0	0	1	47	52
	4	0/15	1/10	4.0	3	6	1	1	0	1	12
3	1	0/10	0/9	0	1	0	0	0	0	0	1
	2	0/15	0/7	0	1	9	7	0	0	0	31
	3	0/12	0/11	0	0	7	0	0	6	0	13
	4	0/15	2/6	9.5	3	38	0	0	0	0	41
4	1	-[d]	-	-	7	0	0	0	0	0	7
	2	-	-	-	78	11	12	2	27	0	130
	3	-	-	-	46	6	1	2	5	0	60
	4	-	-	-	91	44	3	0	24	0	162
5	1	0/3	0/9	0	0	0	3	0	0	0	3
	2	1/16	0/10	3.8	2	1	14	0	0	0	17
	3	4/17	1/8	20.0	2	2	7	0	0	5	16
	4	2/15	2/8	17.4	12	10	5	2	1	2	32

[a]Exposure periods 1-4 began 8 May, 16 June, 25 July, and 28 August, respectively, and the fourth period ended 30 September.

[b]The insect species counted were Paraphlepsius irroratus (Say), Scaphytopius acutus (Say), Colladonus clitellarius (Say), Norvellina seminuda (Say), Norvellina chenopodii (Osb.), and Orientus ishidae (Mat.).

[c]The numerator is the number of plants which developed X-disease; the denominator is the number of plants observed for symptom development.

[d]Indicator plants were not exposed at Site 4 during 1975.

Table C3.--Proportions of peach and chokecherry indicator seedlings which developed X-disease following exposures at chokecherry sites during 1976, and numbers of various leafhopper species trapped at the sites during the exposure periods.

Site Number	Exposure Period[a]	Proportion of diseased indicators		Total Percent Diseased Indicators	Leafhopper Species[b]						Total Leafhoppers captured
		Peach	Chokecherry		P. irroratus	S. acutus	C. clitellarius	N. seminuda	N. chenopodii	O. ishidae	
1	1	0/12[c]	0/12	0	62	7	4	0	0	0	73
	2	0/11	0/12	0	321	12	11	3	0	8	355
	3	0/12	0/12	0	67	31	1	2	0	17	118
	4	0/12	0/12	0	227	72	5	1	0	1	306
2	1	-[d]	-	-	3	7	0	0	1	0	11
	2	-	-	-	14	19	1	0	3	242	265
	3	-	-	-	3	19	0	0	2	145	168
	4	-	-	-	62	103	5	1	6	20	197
4	1	0/12	0/12	0	38	22	9	5	11	0	85
	2	1/12	0/12	4.2	135	10	21	10	26	1	203
	3	4/11	1/12	21.7	33	5	7	12	22	0	79
	4	0/12	0/12	0	73	46	3	22	45	0	189
5	1	0/11	0/12	0	37	2	23	0	0	0	62
	2	3/12	0/12	12.5	31	4	12	0	0	2	49
	3	0/12	0/12	0	1	5	12	0	0	14	32
	4	0/12	0/12	0	16	7	5	0	0	0	28

[a]Exposure periods 1-4 began 18 May, 21 June, 26 July, and 28 August, respectively, and the fourth period ended 2 October.

[b]The insect species counted were Paraphlepsius irroratus (Say), Scaphytopius acutus (Say), Colladonus clitellarius (Say), Norvellina seminuda (Say), Norvellina chenopodii (Osb.), and Orientus ishidae (Mat.).

[c]The numerator is the number of plants which developed X-disease; the denominator is the number of plants observed for symptom development.

[d]Indicator plants were not exposed at Site 2 during 1976.

APPENDIX D

RESULTS OF INDIVIDUAL TRANSMISSION TRIALS WITH

SCAPHYTOPIUS ACUTUS (SAY) AND

PARAPHLEPSIUS IRRORATUS (SAY)

Table D1.--Results of X-disease transmission trials with the leafhopper vector Scaphytopius acutus (Say).

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the transmitting groups
			Tested[d]	Transmitting	
Acquisition host plant: celery Test Plant: celery					
3 March 1974	N	7	3	+[e]	group of 15
18 March	A	8	3	1	
11 May	N	20		+	groups of 6, 21
2 July	N	6	22	7	
12 July	N	12	14	5	
26 February 1975	N	14	18	+	groups of 3, 4, 11
12 March	N	16	10	+	group of 4
19 March	N	14	8	+	group of 8
3 April	N	12	2	0	
8 April	N	9	13	1	
20 May	N	14	3	+	group of 3

Table D1.--Continued.

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the transmitting groups
			Tested[d]	Transmitting	
2 June	N	4	27	+	group of 7
9 June	N	4	15	3	
9 June	N	8	16	4	
1 July	N	8	1	0	
11 July	N	5	10	0	
30 July	N&A	3	14	+	group of 14
8 August	N	18	4	+	group of 4
18 August	N&A	8	4	1	
20 August	N&A	20	23	+	groups of 10, 13, 23
20 August	N&A	14	5	+	group of 2
20 August	N&A	14	28	+	groups of 10, 18
26 August	N	3	5	+	group of 5
30 August	A	10	42	+	2 groups of 14
15 September	N	(5 hrs)	9	1	
22 September	N	(26 hrs)	31	3	
28 September	N&A	8	17	+	groups of 2, 15, 17
6 October	N&A	9	30	+	groups of 9, 10, 15
9 October	N&A	6	9	5	
9 October	N&A	8	7	+	group of 7
13 October	N&A	9	2	0	

Table D1.--Continued.

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the trans-mitting groups
			Tested[d]	Trans-mitting	
22 October	N	6	7	0	
11 November	N	7	14	3	
24 November	N	4	12	0	
28 November	N	8	18	+	3 groups of 6
6 December	N	7	5	+	group of 2
24 December	N&A	7	5	1	
31 December	N&A	7	34	+	2 groups of 4
31 December	N&A	7	44	+	2 groups of 4 and 2 groups of 5
16 January 1976	A	6	3	0	
16 January	N	6	4	0	
16 January	A	6	7	3	
5 February	A	5	4	1	
5 February	N	5	2	1	
23 March	A	7	24	0	
23 March	A	7	10	0	
6 April	A	8	3	0	
6 April	A	8	8	2	
19 April	N&A	7	18	+	2 groups of 2
3 May	N&A	7	12	1	
14 May	N&A	7	2	0	
14 May	N	7	8	0	

Table D1.--Continued.

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the transmitting groups
			Tested[d]	Transmitting	
17 May	A	4	8	+	2 groups of 2
24 May	N&A	7	21	+	group of 3
31 May	N&A	7	6	0	
31 May	N&A	7	5	1	
11 June	A	6	12	+	group of 2
9 August	N&A	7	19	+	group of 4
9 August	N&A	7	15	0	
24 August	N&A	9	38	+	groups of 6, 12, 14
24 August	N&A	6	30	0	
Acquisition host plant: celery Test plant: chokecherry					
19 March 1974	N	7		+	group of 11
Acquisition host plant: celery Test plant: peach					
16 July 1975	N	14	15	2	
Acquisition host plant: chokecherry Test plant: periwinkle					
10 April 1973	A	7	5	0	
Acquisition host plant: chokecherry Test plant: celery					
24 June 1974	N	8	4	0	
12 May 1975	N	9	15	0	
16 July	N	4	27	3	

Table D1.--Continued.

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the transmitting groups
			Tested[d]	Transmitting	
23 February 1976	N&A	1	19	1	
1 March	N&A	2	16	0	
3 May	A	4	9	0	
Acquisition host plant: peach Test plant: periwinkle					
24 September 1973	A	8	5	0	
2 October	A	6	7	0	
Acquisition host plant: peach Test plant: chokecherry					
24 January 1974	A	6	20	0	
5 February	A	18	8	0	
Acquisition host plant: peach Test plant: celery					
20 December 1973	A	17	6	0	
30 January 1974	A	12	9	0	

Table D1.--Continued.

Date[a]	Insect[b] growth stage	AAP[c] (days)	Number of Insects		Number of Insects in the transmitting groups
			Tested[d]	Transmitting	
5 February	A	18	8	0	
5 February	N	18	16	0	

[a]Dates are given for the day insects were first placed on acquisition host plants.

[b]The growth stage of insects placed on acquisition host plants at the beginning of experiments: N = fourth and fifth instar nymphs, A = adults, N&A = mixture of nymphs and adults. Statistical analysis (chi-quare applied to a contigency table) of data from experiments in which individual insects were tested showed that variations in the growth stages of insects at the beginning of the experiments did not significantly affect the percentage of insects which acquired and transmitted the disease.

[c]Acquisition access period.

[d]Only insects surviving 20 days after initial access to X-disease inoculum are included in these results.

[e]The number of insects in groups which transmitted X-disease are given where insects were not tested individually.

Table D2.--Results of X-disease transmission trials with field-captured, adult Paraphlepsius irroratus (Say).

Date[a]	AAP[b] (days)	Number of Insects	
		Tested[c]	Trans-mitting
Acquisition host plant: celery Test plant: celery			
18 August 1974	17	12	4
6 September	15	25	5
19 September	4	7	3
18 June 1975[d]	7	4	0
18 June[d]	7	4	1
19 June	8	27	12
21 June[d]	7	39	8
21 June[d]	7	17	2
1 July	8	4	1
30 July	9	2	2
30 August	10	25	8
2 September	8	2	1
11 June 1976	6	27	8
12 June	7	5	0
14 June	7	26	4
19 July	7	8	1
20 July	11	8	1
24 July	9	22	8
26 July	7	7	0
30 July	7	8	2

Table D2.--Continued.

Date[a]	AAP[b] (days)	Number of Insects	
		Tested[c]	Trans-mitting
11 August	7	5	4
11 August	7	3	0
12 August	13	11	7
12 August	7	3	2
18 August	7	15	5
18 August	7	8	4
23 August	4	3	0
23 August	7	31	16
26 August	6	20	(2 groups of 3 insects transmitted)
2 September	20	22	9
13 September	16	19	6
Acquisition host plant: chokecherry Test plant: celery			
26 June 1974	9	3	2
8 July	8	5	1
19 June 1975	8	8	0
22 June	9	3	1
15 June 1976	8	30	5
19 July	7	5	1
23 July	10	1	0
24 July	9	5	0

Table D2.--Continued.

Date[a]	AAP[b] (days)	Number of Insects	
		Tested[c]	Trans-mitting
Acquisition host plant: periwinkle Test plant: periwinkle			
22 August 1973	9	3	0
31 August	5	5	0
5 September	3	6	0
Acquisition host plant: peach Test plant: celery			
24 September 1974	14	6	0
12 June 1975	9	1	0
2 July	15	11	0
3 July	14	7	0
9 July	14	8	0
9 July	14	20	0

[a]Dates are given for the day insects were first placed on acquisition host plants.

[b]Acquisition access period.

[c]Only insects surviving 20 days after initial access to X-disease inoculum are included in these results.

[d]These experiments were lost 25 to 28 days after insects were first placed on acquisition host plants. A higher percentage of insects might have transmitted if the test period had been longer.