Evaluation of an Abatement Program for Western X-Disease in Sweet Cherry

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ABSTRACT

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The components of a western X-disease (WXD) abatement program for sweet cherry in California were evaluated in nine cherry orchards from 1986 through 1990. Components of the program included removal of WXD-infected trees to reduce WXD inoculum in the orchards and post-harvest application of long-residual insecticides to reduce leafhopper populations. A stepwise linear regression of the percentage of new WXD infections against the percentage of WXD-infected trees remaining in the orchards from one year to the next and insecticide residual longevity showed that WXD-infected tree removal was primarily responsible for reduction in new WXD infections and that insecticide residual longevity contributed only a minor amount in suppression of new infections.

Additional keywords: *Colladonus montanus*, *Fieberiella florii*

Western X-disease (WXD) is a serious threat to sweet cherry (*Prunus avium* L.) production in California. It has eliminated most cherry production in Napa, Sonoma, and Solano counties (10,12) and threatens production in San Joaquin County, where the majority of sweet cherries are currently produced in California. The causal agent of WXD is a mycoplasma-like organism (XMLO) (5) that is transmitted by several leafhopper species (1,6).

In California, two leafhoppers, *Colladonus montanus* (Van Duzee) and *Fieberiella florii* (Stål), are the most important vectors of XMLO (8,9,11,17). *Colladonus montanus* is the most abundant and dispersive vector species found in cherry orchards and is an efficient vector of XMLO. However, *C. montanus* survives poorly on cherry trees and prefers herbaceous hosts. Important herbaceous hosts of *C. montanus* are curly dock (*Rumex crispus* L.), burr clover (*Medicago hispida* L.), which can be a source for XMLO inoculum, and other clovers (*Trifolium* spp.). *Colladonus montanus* develops on these and other herbaceous hosts, and then disperses to adjacent cherry orchards. Since *C. montanus* is highly dispersive and the most numerous vector found in cherry orchards, *C. montanus* is believed to be the most likely vector to introduce XMLO into healthy cherry orchards (9). *Fieberiella florii* is much less abundant than *C. montanus* in cherry orchards. However, *F. florii* can reproduce on cherry and is an efficient vector of XMLO (2,7). *Fieberiella florii* is apparently responsible for rapid tree-to-tree spread of XMLO within infected orchards because diseased cherry trees are a primary source of inoculum in California (3,13).

Visual diagnosis of WXD on Bing sweet cherry is based on fruit appearance and tree growth with the rootstock having a major influence on disease symptomatology (1). Trees on Mazzard rootstock, *P. avium*, infected with XMLO produce fruit that are small, conical in shape, pale red in color and have shorter and thicker pedicels than normal fruit. Leaves are smaller, slightly chlorotic, often with wavy margins. Trees in advanced stages of decline lack vigor and the canopy exhibits a general "see-through appearance." WXD-infected trees on Mazzard rootstock slowly decline in vigor and may survive for many years before dying (1,10). Trees on *Prunus mahaleb* L. rootstock infected with XMLO produce fruit and pedicel symptoms similar to those on Mazzard rootstock, but tree decline and death occur rapidly. This rapid tree death occurs because the XMLO causes a hypersensitive reaction at the *P. mahaleb*/*P. avium* graft union that girdles the scion. Trunk grafted "low-worked" WXD-infected trees decline and die uniformly. However, in scaffold limb grafted "high-worked" trees, a single scaffold limb may become infected with WXD and die in mid-season while the other high-worked scaffold limbs remain healthy. Visual symptoms of WXD occur approximately 1 year after the tree is infected with WXD (A. H. Purcell, *unpublished data*). Sole reliance on visual symptoms to provide positive identification of WXD trees can lead to false positive results. However, diagnostic tests such as enzyme-linked immunosorbent assay (ELISA) and DNA hybridization assays using cloned fragments of the XMLO chromosome (3,4) provide the means to unambiguously diagnosis XMLO trees.

An abatement program for WXD was developed by the University of California in 1985 to limit the spread of this disease in San Joaquin County. The program consists of removal of WXD-infected trees to reduce WXD inoculum in the orchard and post-harvest application of long-residual insecticides to reduce leafhopper populations (9). Identification of WXD-infected trees is based on symptomatic fruit and tree growth prior to harvest. Immediately after harvest, a long-residual insecticide is applied to eliminate leafhopper vectors and all WXD-infected trees are removed from the orchard. Thereafter, at approximately 6-week intervals, the orchard is retreated with a long-residual insecticide. This abatement program is now practiced in about 80% of bearing cherry orchards in San Joaquin County, although the second or third insecticide application is often omitted. Reported here is an evaluation of the effectiveness of the abatement program and its components (WXD tree removal and insecticide applications) in suppression of WXD.

MATERIALS AND METHODS

The study was conducted from 1986 through 1990 in mature Bing sweet cherry orchards located near Stockton, California. *Prunus mahaleb* was the principal rootstock, either scaffold limb or trunk grafted, while Mazzard (*P. avium*), Colt (*P. avium* × *Prunus cerasus* L.), or Stockton Morello (*P. cerasus*) were the principal replant rootstocks (Table 1). All cherry trees or tree positions in seven orchards (approximately 5 ha each) were visually inspected from 1986 though 1990. Two additional orchards were included in the study from 1987 through 1990. The orchards were inspected each year prior to harvest (mid-May to mid-June) and each tree or tree position was classified based on fruit appearance and tree growth as (i) healthy in appearance, (ii) replanted (a tree less than

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4 years old), (iii) WXD symptomatic, or (iv) other (dead or missing trees, or trees with the symptoms of crown or root rot caused by *Phytophthora* spp.).

All WXD-symptomatic trees from six orchards 1987 and all orchards 1988 through 1990 were tested using polyclonal ELISA and a DNA hybridization assay, both of which used cloned fragments of the XMLO chromosome as probes (4). Trees that were WXD symptomatic but tested negative in the above tests were classified as healthy. In addition, WXD symptomatic trees that were not tested by ELISA and DNA hybridization assays (all orchards in 1986 and three orchards in 1987) and were healthy the next year were classified as healthy. This procedure prevented false classification of WXD-infected trees.

Colladonus montanus and *F. florii* populations were monitored using yellow whitefly sticky traps (Seabright Enterprises, Emeryville, Calif.). Traps were placed about 1.8 m above the ground in six cherry trees in each orchard. The traps were placed a minimum of five trees and/or rows (30 to 40 m) apart in a circular pattern within the orchards. Traps were monitored weekly from 23 April though 7 October 1987 and from 8 April through 28 October 1988. Traps were replaced every month or when dirt or debris interfered with their effectiveness.

The number of insecticide applications and type of insecticides used post-harvest varied by orchard and year (Table 2). The following insecticides were used: esfenvalerate (Asana XL 0.66EC [emulsifiable concentrate] at 37 to 59 g a.i./ha; Dupont, E. I. de Nemours Inc., Wilmington, Del.); carbaryl (Sevin 80S [soluble powder] at 2.8 to 3.6 kg a.i./ha; Rhone-Poulenc, Research Triangle Park, N.C.); or diazinon (Diazinon 50WP [wettable powder] at 1.7 to 2.2 kg a.i./ha; Ciba-Geigy, Greensboro, N.C.). The residual activity for *C. montanus* mortality that was measured during a 12-h period (6:00 p.m. to 6:00 a.m.) is 1 day for diazinon, 21 days for carbaryl, and 42 days for esfenvalerate (14–16). Using these residual activities, the post-harvest insecticide residual longevity of the various control programs was calculated by multiplying the residual activity by the number of applications of that insecticide (Table 2).

Table 1. Rootstocks, number of trees or tree positions and hectares inspected annually for western X-disease in nine sweet cherry orchards near Stockton, California

Orchard	No. of ha	No. of trees inspected	Original rootstock[a]	Replant rootstock[b]
1	3.7	895	80% S. G. Mahaleb 20% T. G. Mazzard	S. G. Mahaleb
2	4.9	1,087	S. G. Mahaleb	Mazzard
3	4.0	1,195	T. G. Mahaleb	Colt
4	4.2	1,131	S. G. Mahaleb	Colt
5	6.4	1,525	S. G. Mahaleb	S. G. Mahaleb
6	3.7	1,002	T. G. Mahaleb	Mazzard
7	6.9	1,293	T. G. Mahaleb	Colt or Mazzard
8	5.4	797	S. G. Mahaleb	T. G. Mahaleb
9	5.0	1,345	T. G. Mahaleb	Stockton Morello

[a] Orchards were originally planted on these rootstocks, where S. G. = scaffold limb grafted, T. G. = trunk grafted, Mahaleb = *P. mahaleb*, Mazzard = *P. avium*.

[b] Stockton Morello = *Prunus cerasus* L, Colt = *P. avium* x *P. cerasus* and Mazzard rootstocks were all trunk grafted.

Table 2. Insecticide use pattern, residual longevity, and *Colladonus montanus* population in nine sweet cherry orchards near Stockton, California

	Insecticide use pattern and residual longevity (days)[a]				Mean no. of *C. montanus* per trap per season[b]	
Orchard	1986	1987	1988	1989	1987	1988
1	3 (3)	3 (3)	3 (3)	7 (84)	6.8	1.3
2	5 (42)	5 (42)	7 (84)	7 (84)	0.8	0.0
3	3 (3)	8 (126)	8 (126	8 (126)	4.8	2.0
4	1 (1)	7 (84)	7 (84)	7 (84)	4.5	1.5
5	9 (168)	9 (168)	9 (168)	9 (168)	2.7	0.3
6	3 (3)	3 (3)	3 (3)	3 (3)	0.5	0.7
7	2 (2)	6 (43)	6 (43)	7 (84)	8.5	0.3
8	. . .	0 (0)	0 (0)	0 (0)	6.5	2.0
9	. . .	4 (42)	4 (42)	4 (42)	13.3	2.8
Mean	3.7 (31.7)	5.0 (56.8)	5.2 (61.4)	5.8 (75.0)	5.4	1.2
+ SEM	2.4 (57.3)	2.7 (55.3)	2.7 (55.6)	2.7 (51.1)	3.8	0.9

[a] Insecticides used post-harvest: 0 = untreated; 1 = 1 diazinon application; 2 = 2 diazinon applications; 3 = 3 diazinon applications; 4 = 2 carbaryl applications; 5 = 1 esfenvalerate application; 6 = 1 esfenvalerate and 1 diazinon application; 7 = 2 esfenvalerate applications; 8 = 3 esfenvalerate applications; and 9 = 4 esfenvalerate applications.

[b] Six traps per orchard monitored from April to October. No *Fieberiella florii* were captured during the study.

The data were analyzed by multivariate linear regression using the MGLH module of SYSTAT (18). Percentage of trees newly infected with WXD was regressed as the dependent factor against the following two independent factors in a stepwise manner: (i) the percentage of WXD-infected trees remaining in the orchard from one year to the next year, i.e., the number of WXD-infected trees the first year and also infected the second year divided by the number of WXD-susceptible trees in the second year; and (ii) the post-harvest insecticide residual longevity (in days) of the insecticide program in the same year that the WXD-infected trees remained in the orchard transformed to the inverse of residual longevity + 1. Susceptible trees excluded trees less than 4 years old, dead or missing trees, or trees with symptoms of Phytophthora crown or root rot. Trees less than 4 years old are WXD asymptomatic. The minimum tolerance for entry of a independent factor in the regression analysis was $P < 0.01$.

The percentage of trees newly infected with WXD was calculated as the number of WXD-infected trees in the second year that were healthy the previous year divided by the number of WXD-susceptible trees in the second year with a 1-year lag. The 1-year lag of new WXD infections was used because appearance of visual symptoms of WXD occurs approximately 1 year after the tree is infected with XMLO. In inoculations of field-grown cherry trees by *C. montanus*, symptoms of WXD appeared the following summer, regardless of whether inoculations were made during the spring or summer (A. H. Purcell, *unpublished data*). Acquisition from cherry and transmission of the pathogen by *C. montanus* is most likely during June through September (13).

RESULTS AND DISCUSSION

The WXD abatement program, which consisted of diseased tree removal and applications of long-residual insecticides, gained in grower acceptance during the study period. Mean percent WXD-infected trees remaining in the orchards decreased from 1.7% in 1986 to 1987 to 0.9% in 1988 to 1989 (Table 3). If orchard 8, which did not follow the abatement program, is excluded from the mean, then mean percent WXD-infected trees remaining in the orchards decreased from 1.7% in 1986 to 1987 to 0.3% in 1988 to 1989. Also, growers increased their use of long-residual insecticides from a mean residual longevity of 31.7 days in 1986 to 75.0 days in 1989 (Table 2). If orchard 8 is again excluded, then the mean residual longevity increased from 31.7 days in 1986 to 84.4 days in 1989. The mean number ± SEM of healthy trees per orchard increased from 837 ± 216 in 1986 to 941 ± 176 in 1990. This increase in the number of healthy trees was due to an in-

crease in the number of replanted trees attaining the fourth year of growth, a decrease in the number of new WXD-infected trees, and corresponding decrease in the number of trees removed from the orchards.

The stepwise linear regression of percentage of new WXD infections against insecticide residual longevity and the percentage of WXD-infected trees remaining in the orchards from one year to the next (N = 25) indicated that percentage of WXD-infected trees remaining in orchards was the best predictor of new infections (Table 4). The regression analysis suggests that the WXD-infected tree removal component of the abatement program is primarily responsible for the reduction of new WXD infections and explains 75% of the variation, whereas the insecticide residual longevity contributed only a minor amount to the decrease of new infections. Inclusion of both insecticide residual longevity and percent WXD-infected trees remaining in the orchards explained 81% of the variation.

Because leafhopper populations were not monitored in 1986, leafhopper populations were not included in the stepwise linear regression analysis. However, the correlation of the mean number of *C. montanus* per trap per year for 1987 and 1988 with insecticide residual longevity for 1987 and 1988 (N = 18) was not significant (F = 2.94; df = 1,17; P = 0.104; r^2 = 0.14). No *F. florii* were captured during the study. Also, Spearman's rank correlation coefficients of the mean number of *C. montanus* per trap per year for 1987 and 1988 with insecticide use pattern and insecticide residual longevity for 1987 and 1988 (N = 18) was not significant (–0.05 and 0.027, respectively, $P > 0.1$). The lack of a significant correlation between *C. montanus* populations and insecticide residual longevity was expected because *C. montanus* is highly dispersive (9,17) and would be captured in yellow sticky traps in spite of lethal insecticide residues. In addition, because no *F. florii* that can cause rapid tree-to-tree spread of WXD were captured during the study, the low percentage (0.0 to 6.4%) of new WXD-infected trees (Table 3) was expected and new infections probably resulted from *C. montanus* transmission.

Orchards 7 and 9 were adjacent to cherry orchards that had large numbers of WXD-infected trees. These adjacent orchards practiced no WXD abatement procedures, neither WXD-infected tree removal nor applications of long-residual insecticides to reduce the leafhopper populations. Orchards 7 and 9 practiced thorough WXD-infected tree removal (Table 3) and insecticide programs (Table 2) but had 2.7 and 2.2% new WXD-infected trees (respectively) in 1988 to 1989 evaluations and 1.1 and 0.2% new WXD-infected trees (respectively) in 1989 to 1990 evaluations (Table 3). In 1987, orchards 7 and 9 had the highest *C. montanus* population and in 1988 orchard 9 had the highest *C. montanus* population (Table 2). We speculate that the *C. montanus* transmitted WXD from the adjacent orchard into orchards 7 and 9. The data were re-analyzed without orchards 7 and 9 by stepwise linear regression. The percentage of new WXD infections was regressed against the percentage of WXD-infected trees remaining in the orchards from one year to the next and insecticide residual longevity (N = 20). The regression analysis again indicated that the percentage of diseased trees remaining in the orchards was the best predictor of new infections (Table 4). The WXD-infected tree removal component of the abatement program was primarily responsible for the reduction in new WXD infections and explained 84% of the variation, while insecticide residual longevity contributed only a minor amount of the suppression of new infections. Inclusion of both insecticide residual activity and percent WXD-infected trees remaining in the orchards explained 91% of the variation. The increase in explained variation from 75%, when the percentage of new WXD infections was regressed against the percentage of WXD-infected trees remaining in the orchards from one year to the next with orchards 7 and 9 included, to 84%, with orchards 7 and 9 excluded from the regression analysis, indicates that a large immigration of WXD-infected *C. montanus* was probably the cause of new infections in orchards 7 and 9. The high rate of new infections in orchards 7 and 9, which practiced thorough abatement procedures but were adjacent to a large source of diseased trees, suggests that WXD abatement procedures should be practiced on an area-wide basis to be most effective. Orchards containing WXD-infected trees and not practicing WXD abatement procedures can serve as sources of both inoculum and leafhoppers. Dispersal of WXD-infected leafhoppers from these orchards can cause new WXD infections in adjacent orchards and elimination of dispersing WXD-infected leafhoppers cannot be achieved by repeated insecticide applications.

This study found that abatement of WXD in cherry can be achieved by the rigorous removal of WXD-infected trees and repeated applications of long-residual insecticides. WXD-infected tree removal is primarily responsible for the reduction of new WXD infections while insecticide residual longevity contributes only a minor amount in suppression of new infections. WXD abatement procedures should be most effective when practiced on an area-wide basis.

Table 3. Percentage of trees infected with western X-disease (WXD) remaining in the orchards from one year to the next and percentage of trees newly infected with WXD in nine sweet cherry orchards near Stockton, California

	Percent WXD-infected trees remaining			Percent new WXD-infected trees		
Orchards	**1986 to 1987**	**1987 to 1988**	**1988 to 1989**	**1987 to 1988**	**1988 to 1989**	**1989 to 1990**
1	0.5	0.0	0.0	0.7	0.5	0.7
2	1.5	1.6	0.8	1.9	2.5	0.9
3	0.7	1.2	0.9	0.9	0.2	0.2
4	0.4	0.0	0.0	0.7	0.3	0.8
5	0.0	0.0	0.0	0.6	0.5	0.4
6	9.1	4.6	0.6	5.3	2.0	1.7
7	0.0	0.2	0.0	0.0	2.7	1.1
8	...	3.9	5.6	...	3.5	6.4
9	...	0.0	0.0	...	2.2	0.2
Mean	1.7	1.3	0.9	1.4	1.6	1.4
+ SEM	3.0	1.7	1.7	1.7	1.2	1.8

[a] Number of WXD-infected trees remaining in the orchards from one year to the next year divided by the number of WXD-susceptible trees.

[b] Number of healthy trees that became infected with WXD the following year divided by the number of WXD-susceptible trees.

Table 4. Coefficients and corresponding statistics from the stepwise multivariate linear regression of new western X-disease (WXD) infections against WXD-infected trees remaining in the orchards from one year to the next and insecticide residual longevity in orchards near Stockton, California

Coefficients		Statistics			
WXD-infected trees remaining	**Insecticide residual longevity**	***F***	**df**	r^2	***P***
Nine sweet cherry orchards					
0.74	...	68.1	1,24	0.75	0.000
0.56	2.1	48.6	2,23	0.81	0.014
Seven sweet cherry orchards[a]					
0.74	...	92.4	1,19	0.84	0.000
0.55	2.2	88.2	2,18	0.91	0.002

[a] Without orchards 7 and 9.

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