Seasonal Transmission of X-Disease Agent from Cherry by Leafhopper *Colladonus montanus*

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ABSTRACT

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The leafhopper *Colladonus montanus* acquired the agent of the Green Valley type of X-disease from cherry and transmitted it to celery and to healthy Mazzard cherry seedlings. Transmission rates to celery increased throughout the growing season from 0 in April to a high of 25% in August. Results suggest that cherry may be a source of inoculum for spread to cherry and other host plants. *C. montanus* did not transmit the agent of the peach yellow leaf roll type of X-disease from peach in field and greenhouse experiments.

Additional key words: cherry buckskin, epidemiology, western X-disease

X-disease (XD) of stone fruits, caused by a leafhopper-borne mollicute (9), has caused substantial losses in cherries and peaches (16,17). XD was first described in California as cherry buckskin disease (14). Three strains of the causal agent have been described in California based on symptom differences: peach yellow leaf roll (PYLR) (12), Napa Valley (7), and Green Valley (GV) (15). The number of cherry-producing areas in California has been greatly reduced over the past 50 yr because of this disease (16,21). A recent PYLR epidemic has severely affected the peach industry in northern California (11).

The leafhopper *Colladonus geminatus* (Van Duzee) was thought to be responsible for the early XD outbreaks in eastern Washington and Oregon because of its abundance (23) and relatively high efficiency in transmitting the X-disease agent (XDA) (20). We used the leafhopper *C. montanus* (Van Duzee) in our studies because of its relative abundance in and near cherry and peach orchards in central California (13,19) and its efficiency as a vector of XDA to celery (4,5). We evaluated the transmission of the GV type of XDA (GVX) by *C. montanus* from X-diseased cherry and peach. A preliminary report has been published (3).

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MATERIALS AND METHODS

Inoculum sources. Four 20- to 30-yr-old sweet cherry trees (*Prunus avium* L. cv. Bing) grafted on cv. Mazzard rootstocks that had fruit symptoms of GV throughout each tree in 1977 were used as inoculum sources in 1978. These four trees were in a commercial orchard near Lodi, CA. Peach trees used were cling stones 6, 12, or 17 yr old both with and without symptoms of PYLR. They were located in commercial orchards in Sutter and Yuba counties, CA.

Transmission procedures. *C. montanus* colonies of a biotype used by previous workers (2,5) were reared in a greenhouse in Berkeley, CA, on celery (*Apium graveolens* L. cv. Tall Utah 52-70). Noninfective fourth instar leafhoppers (600 per test period) were given 1-wk acquisition access feeding on the four GV-infected cherry trees during five periods in 1978: 8–15 April, 20–27 May, 1–8 July, 11–18 August, and 22–29 September. Minimum and maximum temperatures recorded in tree canopies were 3 and 31, 4 and 33, 7 and 41, 7 and 40, and 9 and 37 C, respectively.

Leafhoppers were transported to the orchard in cylindric, plastic containers measuring 5 × 10 cm fitted with nylon mesh and Parafilm (American Can Co., Greenwich, CT) at opposite ends. Each container was placed in an 80-cm nylon mesh sleeve cage sewn at one end. The open end of the bag was slipped over a branch exhibiting XD symptoms, and the base of the bag was secured with a hose clamp around a strip of foam rubber. The Parafilm was removed from the cage, and leafhoppers were shaken onto the foliage within the sealed sleeve cage. Two sleeve cages were placed on the northeast (shaded) side of each tree.

After 1 wk, branches were cut off below the base of the attached sleeve cage and returned to the greenhouse. The surviving insects were shaken off and placed on individual 3-mo-old celery plants for 1 wk. Thirty-five leafhoppers from each of the eight intermediate celery plants were removed, caged singly on one-leaf-stage celery test plants, and transferred at weekly intervals to fresh test plants for as long as the leafhoppers survived. Leafhoppers that died during the first week were replaced with those remaining on the intermediate celery. During the experiment, caged vectors were kept in a constant-light growth chamber at 25 C. Celery plants were kept in the greenhouse about 6–9 wk for symptom development.

In June 1979, 100 GV-infective leafhoppers (which had been given a 1-wk acquisition access period on GV-infected celery) were given a 1-wk inoculation access period on Mazzard cherry seedlings in the greenhouse, using 10 leafhoppers per plant. The same number of noninfective leafhoppers was confined on 10 healthy Mazzard cherry seedlings as controls.

In October 1979, in the same cherry orchard and using the procedures described above, 165 noninfective leafhoppers were caged on each of eight GV-infected trees (four of which had been injected [18] with 2 g of oxytetracycline in 1 L of water in April 1979) and on two symptomless trees for a 1-wk acquisition access period.

Transmission from peach. On 28 August 1979, 130 leafhoppers were bagged on branches of each of ten 12-yr-old peach trees in Yuba County. Three trees had symptoms of PYLR, four had had PYLR symptoms before being injected 11 mo earlier with oxytetracycline at 1½ g a.i., and three were apparently healthy. The same field and laboratory procedures were followed as previously described for cherry.

On 11 and 20 September 1979, the same procedures were repeated in Sutter County using six 6-yr-old and six 12-yr-old trees. In each age group, two trees were healthy, two had PYLR symptoms, and two had had PYLR symptoms in 1978 and subsequently had been injected with oxytetracycline 1 mo before leafhopper acquisition access feeding. These procedures were repeated in May and June 1980 with trees of the same age in a different orchard in Sutter County.

Comparison of GV and PYLR. A total of 140 second through fifth instar leafhoppers were given 1-wk acquisition

access feeding on GV-infected celery; 50 fourth instar leafhoppers were given 1-wk access on PYLR-infected celery. GV had been maintained in the greenhouse for 8 mo. PYLR had been maintained in celery for 20–25 yr (5). After the 1-week acquisition access feeding period and 2 wk on the intermediate plant, the leafhoppers were individually placed on 2-wk-old celery plants for 1-wk inoculation access periods, then transferred weekly for as long as the leafhoppers survived.

RESULTS

Transmission from cherry. Leafhoppers transmitted GVX from cherry to celery in four of the five access periods throughout the growing season. The percentage of transmission was highest following the August and September acquisition feeding periods (Fig. 1). No transmission occurred from the first acquisition period on 8–15 April; the percentage of transmission increased to 4.6% in May and 4.7% in early June (Table 1).

The average latent period (LP) for transmitting leafhoppers in each sampling group was calculated using the midpoints of the acquisition access period and the inoculation access periods during which transmission occurred. Figure 1 indicates the range of variability in transmission from each tree. Transmission rates from each tree generally increased until August, with one exception. Transmission from one tree was highest (17%) in May and lower (6%) thereafter; net transmission from this tree (5.3%) was the lowest of the four trees tested throughout the sampling period. XD symptoms were less severe in this tree, although the disease occurred throughout the tree the previous year. The highest transmission rate over the entire season (15.1%) was from a tree near total collapse throughout 1978. This tree died in 1979.

In October 1979, two groups of 150 *C. montanus* from each of four cherry trees with GV failed to transmit XDA to celery. However, two groups of 150 leafhoppers that fed on one of four trees that had been injected with oxytetracycline 7 mo earlier transmitted GVX to celery with an average LP of 37 days; 83% of the leafhoppers lived past this date, with 14% transmitting. This tree exhibited severe foliar and fruit symptoms of XD before and after antibiotic treatment, probably as a result of absorbing only about half the antibiotic (G. Nyland, *personal communication*). No transmission occurred from the other three treated, diseased trees or from the two symptomless trees.

Four of 10 seedlings exhibited leaf reddening and curling symptoms of XD 2½ mo after GV-infective *C. montanus* were given inoculation access to healthy Mazzard cherry seedlings. Ten noninfective *C. montanus* that were given a 1-wk acquisition access feeding on each cherry seedling exhibiting symptoms of XD transmitted the causal agent to celery, producing typical XD symptoms (4,6).

Transmission from peach. None of the 4,500 leafhoppers placed on PYLR-infected trees at various times during the 1979 and 1980 growing seasons transmitted the PYLR agent (PYLRX) to celery. Leafhoppers were transferred weekly to new plants until the insects died, a period of 3–4 mo. Survival ages of the leafhoppers that fed on PYLR-diseased trees, on uninfected trees, and on trees injected with oxytetracycline were not significantly different.

Comparison of GV and PYLR in celery. Using GVX in celery source plants, 13 of 40 (32%) fourth instars transmitted XDA to celery, whereas transmission rates were 3 of 40 (7.5%) for second instars, 5 of 40 (12.5%) for third instars, and 2 of 23 (9%) for fifth instars. Differences between transmission rates of second, third, and fifth instars were not significant (chi square = 0.70, d.f. = 2, $P < 0.5$). However, differences between fourth instars and all other instars were significant (chi square = 11.56, d.f. = 3, $P < 0.01$). All but one of 50 fourth instars that fed for 1 wk on PYLR-diseased celery transmitted this type of XDA to celery. Our results confirm those of Gold (2), who found that the fourth instar was the most efficient stadium for acquisition of XDA and that PYLRX was transmitted more efficiently to celery than was GVX.

GVX and PYLRX induced similar symptoms in celery. Petioles became brittle, then chlorotic. GV was more severe, causing a more rapid collapse of the plant than PYLR. Young celery plants infected with GVX collapsed approximately 5–10 days after initial symptom expression. Three-month-old celery plants infected with GVX exhibited the same symptoms but lived up to 3 wk before collapsing; growth was arrested in older plants. The age of inoculated plants did not affect survival time as greatly in PYLR-diseased celery. Young (<3 wk old) celery with PYLR seldom collapsed

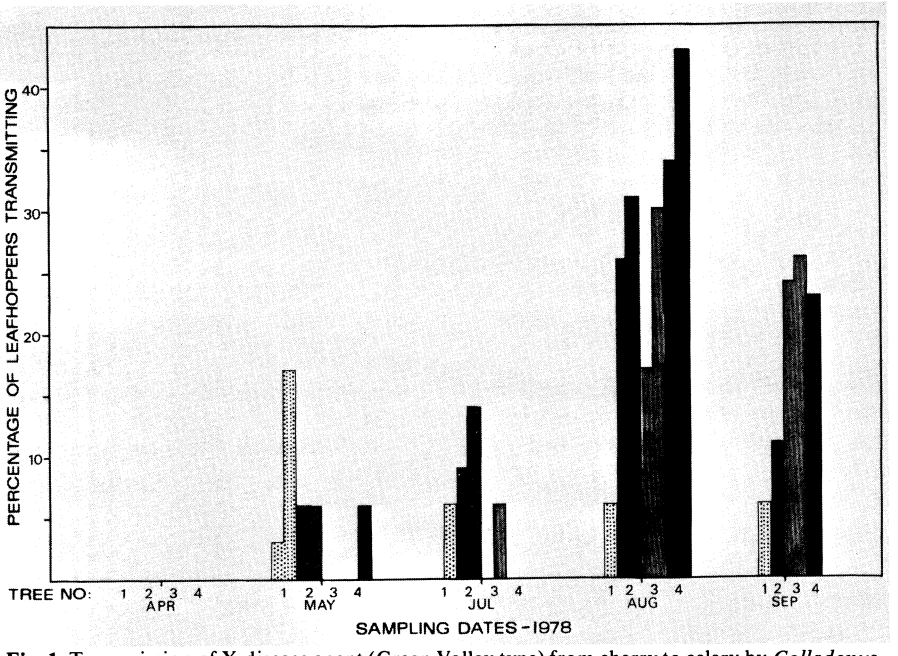

Fig. 1. Transmission of X-disease agent (Green Valley type) from cherry to celery by *Colladonus montanus*. The same four trees (1–4), denoted by different kinds of bars, were used throughout the study. Each bar represents a separate limb; the absence of a bar indicates no transmission. Only five limbs were tested in September.

Table 1. Seasonal transmission of the Green Valley type of the X-disease agent by *Colladonus montanus* from cherry to celery, Lodi, CA, 1978

Acquisition access period	Leafhoppers transmitting/ tested (no.)[x]	Transmission (%)	Average latent period (days)[y]
8–15 April	0/260 a	0	...
20–27 May	12/261 b	4.6	38
1–8 July	12/255 b	4.7	41
11–18 August	63/262 d	24.0	35
22–29 September	31/156[z] c	19.9	41

[x] Means followed by different letters are statistically different at the 1% level (Duncan's multiple range test).

[y] Determined from the midpoints of the acquisition access period and the inoculation access periods during which transmission occurred.

[z] Smaller sample size because of unexplained mortality in three sleeve cages.

before 5 wk after symptom expression; celery infected at a later age (>2 mo old) usually lived 9–12 wk after symptoms appeared.

DISCUSSION

Transmission from cherry. Results showed that cherry was a potential source of inoculum for spread of XDA within orchards. In 1954, Nielson and Jones (10) reported transmission of XDA from sweet cherry on Mahaleb rootstock to cherry with groups of *C. geminatus*. Their field results with nursery stock showed a 30% transmission efficiency when sweet cherry was used as the source and indicator host, as determined with groups of leafhoppers rather than individuals. Rosenberger and Jones (20) evaluated individual leafhopper transmission efficiencies of *Scaphytopius acutus* (Say), probably the most economically important vector of XDA in the East (1), and *Paraphlepsius irroratus* (Say), which may be an important vector of the agent in Michigan (20). They reported a 9% transmission efficiency with *S. acutus* and 24% with *P. irroratus* when chokecherry was used as the source plant and celery as the indicator host. In our experiments, the average transmission by *C. montanus* of GVX from cherry to celery was 12%. Cherry apparently is about as effective as a source of inoculum of GVX in California as is chokecherry in the East.

Herbaceous plants can be infected by XDA (1,6), but a perennial herbaceous host has not been reported. Although chokecherries occur naturally within California (8), they are not found near stone fruit orchards. Reeves et al (17) noted that under certain circumstances the roguing of XD cherry trees gave good results in western states, suggesting that cherry may be an effective source of inoculum. Our studies support this hypothesis.

Because the major differences among the leafhopper transmission experiments from cherry consisted of the dates and conditions of acquisition access, most differences in transmission rates were presumably the result of different acquisition rates from cherry. It is possible that lower acquisition rates in April and May were caused by a low titer of XDA in cherry during that period. The average LP for each group of leafhoppers on a single branch decreased as net transmission increased, which may reflect an increase in titer of XDA later in the summer. In the early fall, net transmission decreased as the average LP increased, reflecting a possible decrease in titer. Titer changes could be caused by local temperatures and other environmental factors or by a normal seasonal response of the pathogen in infected cherry.

In our 1979 field experiment designed to determine the duration of protection with an antibiotic, XDA was transmitted by *C. montanus* from one of four cherry trees treated with oxytetracycline. This was consistent with the trend of net transmission in late September 1978 for untreated diseased trees (Fig. 1).

C. montanus did not transmit PYLRX from infected peach trees to celery. Jensen (5) reported that *C. montanus* varies markedly in its ability to transmit XDA, depending upon the indicator plant used; peach is a poor source plant for transmission. Celery is a better source and indicator plant for PYLRX than peach (4).

Several lines of evidence suggest that peach is not a significant source of XDA inoculum for spread within orchards. Stoddard (22) found that removal of diseased peach trees does not give any measure of control but that removal of diseased chokecherries does. Studies in the East indicated that trees infected initially are usually located at or near the edge of an orchard, which leads to the conclusion that the source of infection is outside the peach orchard (22). Rosenberger and Jones (20) reported no transmission with *P. irroratus* and *S. acutus*, two important leafhopper vectors of XDA in the East, when X-diseased peach was used as the source plant and celery as the indicator host.

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