Mechanical Transmission, Susceptibility, and Host Response in Bing Sweet Cherry and Three Rootstocks by the Walnut Strain of Cherry Leafroll Virus

ADIB ROWHANI and S. M. MIRCETICH, Assistant Plant Pathology Specialist and Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, College of Agricultural and Environmental Sciences, University of California, Davis 95616

ABSTRACT

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A purified preparation of the walnut isolate of cherry leafroll virus (CLRV) was successfully transmitted to cherry (*Prunus avium* cv. Bing), to seedling Mazzard rootstock (*P. avium*), and to Mahaleb rootstock (*P. mahaleb*) but not to rooted cuttings of Colt rootstock (*P. avium* × *P. pseudocerasus*). CLRV-W infection produced a characteristic pitting symptom in the woody cylinder of susceptible cultivars. Its presence was confirmed by enzyme-linked immunosorbent assay in extracts prepared from symptomatic leaves or bark tissues. Extracts of Colt were uniformly negative, as were tests from buffer-inoculated control tissues.

Cherry leafroll virus (CLRV) is a member of the nepovirus (6) group. It naturally infects many wild and cultivated woody plant species (6). In the United States, CLRV has been isolated from walnut (10,12), elm (7), golden elderberry (5), and dogwood (16). More than 30 different English walnut (*Juglans regia* L.) cultivars can be infected by walnut isolates of CLRV (CLRV-W), but they do not exhibit recognizable leaf symptoms (12). However, CLRV-W infection of grafted walnuts incites a blackline at the scion-rootstock graft union resulting in decline and death of English walnut propagated on Northern California black (*J. hindsii* (Jeps.) Jeps. ex R. E. Smith) or hybrid paradox (*J. hindsii* × *J. regia*) rootstocks (10,12). CLRV is pollen-borne, spreading to healthy English walnut during the cross-pollination process (10,11). In cherry, CLRV has been reported to be transmitted by species of *Xiphinema* nematodes (3,4).

English walnut cultivars have been experimentally infected with CLRV-W but not by cherry or golden elderberry isolates of the virus (14). In California, commercial walnut orchards containing blackline-diseased trees are commonly adjacent to sweet cherry orchards, and although these cherry trees are apparently free of CLRV, we sought to determine their susceptibility and response to the virus. In this report, we have characterized the response of sweet cherry (*Prunus avium* (L.) L., cv. Bing), Mazzard (*P. avium*), Mahaleb (*P. mahaleb* L.), and Colt cherry (*P. avium* × *P. pseudocerasus*) to mechanically inoculated CLRV.

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MATERIALS AND METHODS

Virus and antiserum sources and ELISA test. A walnut strain of cherry leafroll virus (CLRV-W8) was obtained from naturally infected English walnut (12). Virus was increased and purified from *Chenopodium quinoa* Willd. and used for tree inoculation at a concentration of 0.5 mg/ml. The indirect enzyme-linked immunosorbent assay (ELISA) was performed as described previously (15). All ELISA tests were performed in flat-bottom, polystyrene plates, and the color reactions were determined by scanning the plates in a microplate reader (Emax). Absorbance was measured at 450 nm for the peroxidase enzyme-conjugate/O-phenylenediamine substrate reaction 30 min after the addition of substrate. For these tests, the plates were coated with pure anti-CLRV-IgG from rabbit (1 μg/ml) and incubated for 1.5 hr at 37 C. After washing, the plates were loaded with samples (200 μl per well) to be tested for the presence of the virus and incubated at 4 C overnight. The next day the plates were washed, egg yolk anti-CLRV antibody (1 μl/ml) was added, and the plates were incubated at 37 C for 1.5 hr. The plates were then washed and peroxidase conjugated rabbit anti-chicken antibody (0.6 μg/ml) was added to the plates and incubated for an additional 1.5 hr at 37 C. After the final wash, O-phenylenediamine at a concentration of 0.7 mg/ml was added to the plates. The plates were incubated in the dark at room temperature for 30 min before the color reaction was measured. In all tests an ELISA color intensity at least 2.5-fold greater than the average of the healthy (buffer inoculated) control was considered as positive for the virus. Each ELISA value is the representation of the average of 2 replicates tested in the same plate.

Field inoculation experiment. Two-year-old field-grown Bing sweet cherry trees on Colt or Mahaleb rootstocks were inoculated beneath the bark at two sites about 5 cm above the graft union. The inoculum was applied with a glass rod dipped into the virus preparation and rubbed on the cambial surface of the bark and woody cylinder (10). The same number of control trees were inoculated with 0.05 M phosphate buffer, pH 6.5.

All trees were analyzed by ELISA 3 mo after inoculation and then annually for three consecutive years. Bark cores, removed with a No. 8 cork borer (1.2 cm in diameter) below or above the inoculation sites, provided tissue for the analysis. The inner bark and cambium tissues were homogenized in ELISA extraction buffer at maximum speed in a polytron homogenizer for 10 sec. Leaf tissues were similarly extracted for ELISA.

To measure movement of the virus in Bing 3 yr after inoculation, inner bark and cambium tissue samples were col-

lected from positions in 10-cm increments above each of the inoculation sites and tested by ELISA. The average rate of movement per year was calculated from the total movement in each tree during the 3-yr experimental period. At the end of the experiment, the trees were sacrificed by removing the union (trees cut off 25 cm above and 10 cm below the scion-rootstock union). The trunk sections were autoclaved, debarked, and the woody cylinder examined for symptoms (Fig. 1).

Lathhouse inoculation experiments. One-year-old Mahaleb and Mazzard seedlings and rooted cuttings of Colt grown in 3.75-L plastic containers were inoculated and maintained in the greenhouse. Each rootstock was inoculated with virus and buffer controls in June as described above, held in the greenhouse until November, then moved to a lathhouse. Extracts from bark and leaf (5 randomly selected leaves per tree in May) tissue samples were tested by ELISA, and after a 2-yr incubation, the inoculated stems were autoclaved and the bark was removed and examined.

RESULTS

Transmission of CLRV-W in the field experiments. In the field, CLRV was detected in the scions of 8 out of 10 inoculated Bing-scion/Colt-rootstock (Bing/Colt) trees and all 5 trees of Bing/Mahaleb (Table 1). All ELISA-positive and ELISA-negative trees were identified 1 yr after inoculation and reconfirmed after 3 yr. Only the Mahaleb extracts of bark patches taken 3 cm below the union (but not the Colt extracts) were ELISA-positive for CLRV. These root bark assays were done in the third year after inoculation. Infected trees produced apparently normal vegetative growth. Bark extracts of Colt rootstocks supporting infected Bing scions were ELISA-negative, as were all buffer-inoculated control trees. Average absorbance values for CLRV-infected Bing extracts was 0.76 and for infected Mahaleb was 0.74. In contrast, absorbance values for the Colt extract prepared from infected Bing/Colt trees and buffer-inoculated trees produced values that averaged 0.06 and 0.07, respectively. Also, based on ELISA results, the rate of virus movement in the Bing scions averaged 15 cm/yr, independent of the rootstock.

Stem pitting symptoms developed on the woody cylinders of all ELISA-positive Bing scions. This symptom was extensively exhibited throughout the Mahaleb rootstock. On Colt, the stem pitting was restricted to the union area of six trees or extended 3–5 cm on 4 others (Fig. 1). On infected Bing/Colt trees, the woody cylinder at the scion/rootstock unions was invaginated, showing a brownline symptom similar to that developed in prune brownline disease on grafted French prune (9). Some trees developed small areas of bark canker on the Colt rootstock (e.g., Fig. 1A). In contrast, the controls displayed woody cylinders with smooth surfaces (Fig. 1A and B, right specimens).

Lathhouse experiments. Among the containerized plants, the leaves on three of five inoculated Mazzard trees were stunted and exhibited chlorotic ringspot symptoms. In contrast, the vegetative growth on Mahaleb or Colt plants appeared normal. Extracts prepared from symptomatic leaves and of the bark tissues from the inoculated Mazzard and Mahaleb plants were positive by CLRV-ELISA and all other extracts were negative. Only the inoculated Mahaleb and Mazzard plants developed stem pitting symptoms (Table 2 and Fig. 2A and B). No stem pitting occurred on virus-inoculated Colt (Fig. 2C) or on buffer-inoculated controls (Fig. 2A–C, left specimens).

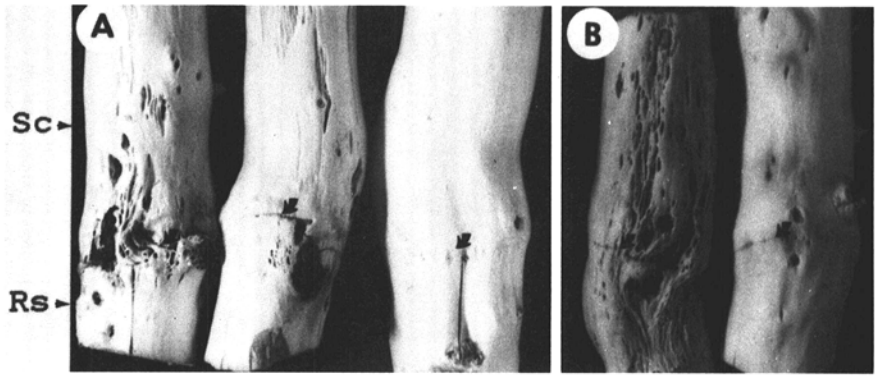

Fig. 1. Union trunk areas of (**A**) Bing/Colt and (**B**) Bing/Mahaleb sweet cherry trees grown for 3 yr in the field. A purified preparation of cherry leafroll virus-walnut strain or buffer controls were used to mechanically inoculate the inner bark surface of Bing scions. Note the necrotic canker in the Colt rootstock portion (A-Rs, middle specimen) invaginations and brownline at the graft union (arrows). Extensive stem pitting symptoms are evident in all inoculated Bing scions and Mahaleb rootstocks where bark extracts were positive by ELISA. Pitting and canker symptoms were also developed on Colt, however bark extracts were negative for CLRV by ELISA. All controls were free of symptoms and negative by ELISA (right specimens).

Table 1. Transmission of CLRV-W8 to sweet cherry cv. Bing grafted on two rootstocks and grown in the field. Three years after inoculation, bark samples were collected from 10 cm above and 3 cm below the graft union and tested by ELISA

Scion/rootstock	Inoculum	Scion	Rootstock
Bing/Colt	CLRV-W8	8/10[a]	0/10
	Buffer	0/10	0/10
Bing/Mahaleb	CLRV-W8	5/5	5/5
	Buffer	0/5	0/5

[a] Number of trees infected with CLRV-W8 and with stem pitting/number of trees inoculated. Average absorbance values for CLRV-infected Bing extracts was 0.76 and for Mahaleb and Colt rootstock extracts of CLRV-infected Bing scion were 0.74 and 0.06, respectively. The average absorbance values for buffer-inoculated Bing trees and their rootstocks Mahaleb and Colt were 0.07, 0.05, and 0.07, respectively.

Table 2. Transmission of CLRV-W8 to Mazzard, Mahaleb, and Colt cherry rootstocks

Rootstocks[a]	Inoculum	CLRV-positive[b]	Stem pitting[c]
Mazzard	CLRV-W8	5/5	5/5
	Buffer	0/3	0/3
Mahaleb	CLRV-W8	5/5	5/5
	Buffer	0/3	0/3
Colt	CLRV-W8	0/5	0/5
	Buffer	0/3	0/3

[a] One-year-old potted plants were inoculated by rubbing the cambial surfaces beneath the bark flap with a purified virus preparation.

[b] Infection determined by ELISA using bark tissue extracts; number of trees positive/number tested. Average absorbance values for CLRV-infected Mazzard, Mahaleb, and Colt were 0.30, 0.37, and 0.07 and for buffer-inoculated trees were 0.08, 0.09, and 0.06, respectively.

[c] Stem pitting symptoms observed in wood cylinders 2 yr after inoculation; number of trees with stem pitting/number inoculated.

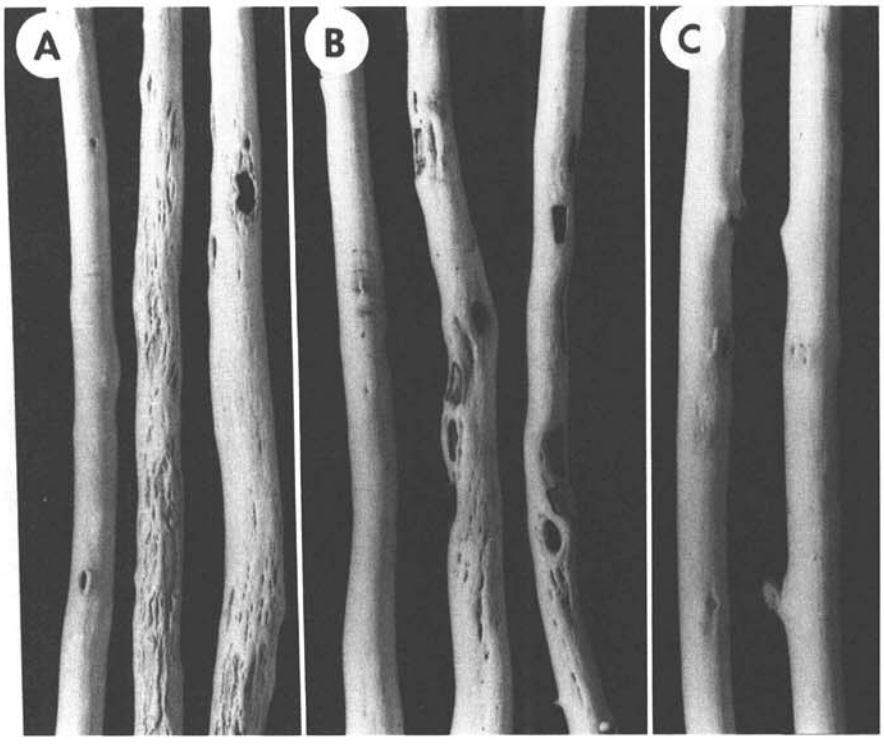

Fig. 2. Stems of 3-yr-old Mazzard (**A**) Mahaleb (**B**) and Colt (**C**) trees grown in pots. A purified preparation of cherry leafroll virus-walnut strain or buffer controls were used to mechanically inoculate the inner bark surfaces. Stem pitting symptoms occurred in Mazzard and Mahaleb. All virus-inoculated Colt and buffer-inoculated controls (left specimens) were symptomless, and they were negative by CLRV-ELISA. All symptomatic plants were positive by CLRV-ELISA.

DISCUSSION

The walnut strain of cherry leafroll virus (CLRV-W8) was found to be infectious in cherry, inducing symptoms on infected cv. Bing scion and on Mahaleb and Mazzard rootstocks. The stem pitting symptom developed on cv. Bing and on Mahaleb and Mazzard was similar to the stem pitting induced by tomato ringspot virus and by diseases of unknown etiology (8). Stem pitting in scions of Bing/Colt trees was less severe than that observed in scion or rootstocks of Bing/Mahaleb trees. Previously, CLRV had been reported to remain as a localized (necrotic) infection when Colt rootstocks were directly graft-inoculated (2). In this study we did not observe necrotic tissue around the inoculation sites on Colt trees and did not detect CLRV-W8 in either directly inoculated Colt trees or in the Colt rootstocks of CLRV-infected Bing scions. Even so, 4 of 10 Bing/Colt trees developed stem pitting in the rootstock. The pitting extended only 2–4 cm below the scion-rootstock union, and ELISA tests of Colt bark extracts from the pitted areas were negative. These results indicate that the limited stem pitting observed on Colt rootstock adjacent to the graft union was attributable possibly to the stress exerted on the rootstock by the pitted scion and/or a response to the invagination or brownline at the graft union. Although Mazzard (F12/1) rootstock that was graft-inoculated with the cherry strain of CLRV developed symptoms of interveinal necrosis, defoliation, and dieback (13), in this study only chlorotic ringspots, stunting, and severe stem pitting were noted in Mazzard plants artificially infected with CLRV-W8. These observations, in addition to the fact that two strains of CLRV (golden elderberry and cherry) did not infect English walnut trees (14), suggest that strains of CLRV originating in various perennial hosts may have differing pathogenicities or may induce differing symptoms on different perennial hosts.

The spread of CLRV-W and the resulting blackline disease of English walnut trees on black or paradox rootstocks has directed nurseries to propagate walnut scions on English walnut seedlings in establishing new orchards. The use of English walnut seedlings as a rootstock creates a potential problem, because the virus will infect the rootstock and may be spread by species of *Xiphinema* to other fruit tree species (1,6). In particular, California's walnut and cherry orchards are grown adjacent or in near proximity to each other; thus, it is conceivable that this virus could spread from walnut trees to cherry trees. However, use of the CLRV-immune cherry rootstock Colt should prevent the spread of this virus from walnut source trees.

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