Herbaceous Host Plants of Western X-Disease Agent

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Supported by Research Grant AI-03490 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Accepted for publication 13 July 1971.

ABSTRACT

Western X-disease (WX) is an important disorder of stone fruits, apparently caused by mycoplasma. The agent of WX was shown experimentally to cause disease symptoms in the following herbaceous plants: periwinkle, chrysanthemum, China aster, radish, turnip, cauliflower, filaree, strawberry, carrot, coriander, celery, and *Gomphrena globosa*. Parsley has also been reported to be susceptible to infection. All transmissions of the WX agent were accomplished by means of the leafhopper vector, *Colladonus montanus*. WX agent-free leafhoppers recovered the WX agent from all symptom-bearing plants that were tested, and transmitted it to celery indicator plants. The incubation period of WX symptom development in susceptible hosts was ca. 4-6 weeks. Susceptibility to WX infection varied among the herbaceous hosts; carrot was the most resistant, and several species, such as celery and turnip, were very susceptible. Periwinkle plants, after developing symptoms of WX, remained susceptible to the aster yellows agent and produced symptoms distinctive of aster yellows disease. Phytopathology 61: 1465-1470.

Additional key word: virus.

Western X-disease (WX) is an important disorder of stone fruits in western North America. It was described originally as a virus disease of sweet cherry under the name "cherry buckskin" (11), and was subsequently shown to involve many strains of an agent which differ in their activity and in the symptoms they induce in peach and cherry (12, 16). The name "Western X-disease" was first applied to the disease as expressed in peach (14) to distinguish it from a similar disease reported on peach in Connecticut in 1933 under the name "X-disease" (13, 15).

Western X-disease was, until very recently, thought to be caused by a virus. It now appears probable that the disorder results from infection by mycoplasma-like organisms (1, 5, 9).

Within the genus *Prunus*, the Western X-disease agent (WXA) is transmissible to peach (*Prunus persica*), nectarine (*P. persica* var. *nectarina*), sweet cherry (*P. avium*), sour cherry (*P. cerasus*), apricot (*P. armeniaca*), plum hybrids (probably *P. munsoniana* x *P. salicina*), western chokecherry (*P. virginiana* var. *demissa*), eastern chokecherry (*P. virginiana*), Bessey cherry (*P. besseyi*), and Manchu cherry (*P. tomentosa*) (16).

Evidence that the host range of the X-disease agent includes herbaceous plants was first provided in 1944 when Kunkel (8) reported dodder transmission of a disease agent from infected peach trees to carrot, parsley, periwinkle, and tomato. More recently, an infectious agent was transmitted by dodder from X-diseased chokecherry to milkweeds (*Asclepias syriaca*), periwinkle, and tomato (3). In both the above investigations, attempts to transmit the infectious agent from these herbaceous plants to peach by means of dodder were unsuccessful. However, a disease of milkweeds, the agent of which could be transmitted by dodder to milkweed, periwinkle, and tomato (3), had the same symptoms as those produced by the dodder-transmitted agent from X-diseased chokecherry. Dodder transmission of an infectious agent from WX-affected peach to carrot, periwinkle, and parsley has also been reported (17).

Experimental transmission of WXA from peach to celery and from celery back to peach was accomplished by means of the leafhopper vector, *Colladonus geminatus* Van Duzee (6, 7). Subsequently, the eastern X-disease agent was transmitted from chokecherry to periwinkle and back to chokecherry by means of the vector, *Scaphytopius acutus* Say (4).

Experimental transmission of a disease agent from WX-affected celery to strawberry, by means of the leafhopper *Colladonus montanus*, was also recently reported (2). The disease produced in strawberry was lethal, and appeared sufficiently similar to the lethal decline disease of strawberry to suggest that lethal decline might be caused by a strain of WXA.

The present paper discusses the host range of WXA among herbaceous plants, with special reference to several newly reported hosts. The transmission studies were carried out by means of the leafhopper vector, *Colladonus montanus*, and involved the yellow leaf roll strain of WXA.

MATERIALS AND METHODS.—WXA inoculum used in these studies was obtained originally from a peach with symptoms of the yellow leaf roll strain of WXA, near Marysville, Calif. (10). Since 1956, WXA has been maintained most of the time in celery, and has been transmitted to new celery plants at intervals of ca. 2 months.

The test insects were reared in the greenhouse on celery (*Apium graveolens* L. 'Utah Green') grown from seed. The other plants used also were grown from seed in the greenhouse unless otherwise noted.

Plants tested as possible hosts of WXA were exposed for 7-14 days to infective leafhoppers previously fed on WX-affected celery for 4-6 weeks. The test plants were then held in the greenhouse for symptom development. Representative inoculated plants, with or without symptoms, were subsequently used in infectivity recovery tests. Noninfective leafhoppers were caged on such plants for an

acquisition feeding period varying from a few days to 2 weeks. These leafhoppers were then caged on healthy celery test plants for transmission feedings; usually groups of 10-40 insects/plant were tested. The test insects, after a 30-day period for incubation of WXA in the insects, were transferred to new celery plants at weekly intervals for 3-4 weeks.

Leafhoppers used in these WXA recovery tests were taken from WXA-free colonies whose non-infectivity was confirmed by caging them on healthy celery plants. All such control plants remained healthy. Groups of WXA-free leafhoppers also were allowed to feed on apparently healthy plants of some of the plant species tested as possible hosts of WXA. These plants also remained free of disease symptoms.

RESULTS.—The herbaceous plants infected with WXA in these studies are listed in Table 1. Symptoms were evident in 4-6 weeks in most hosts, but carrot required 6-8 weeks.

Plants inoculated with WXA which did not develop evidence of infection are listed in Table 2.

Periwinkle.—The older leaves of infected periwinkle became yellow, and the young leaves developed light-green or chlorotic tissue which was evident first along the veins and subsequently in the interveinal areas. Chlorosis sometimes appeared on only one-half the leaf or in restricted areas such as the leaf margin. Some young leaves also were observed to curl, pucker, or develop downward-cupping.

Periwinkle usually was invaded systemically by WXA; such plants had sparse foliage, reduced plant size, and small, chlorotic leaves (Fig. 1-f). Occasionally, one portion of a plant remained normal in appearance for months after the rest of the plant was obviously diseased. Some diseased plants remained alive for ca. 1 year after becoming infected, but their vigor declined progressively.

Flower symptoms included reduced size, deletion of color pigment along the margins of the petals, and modification of the petal form to more acutely pointed tips (Fig. 1-e). The flowers developing on infected plants were smaller in size and number; after 3 months, few (if any) flowers were produced. These small flowers were partly white in contrast to the large, rose-colored petals of control plants (Fig. 1-c).

Periwinkle simultaneously affected by aster yellows and Western X-disease.—Aster yellows and Western X-disease are characterized by different symptoms though they have some insect vectors in common. The causal agents of these diseases do not protect against each other in periwinkle. Plants which had developed typical symptoms of WX were secondarily inoculated with the aster yellows agent by means of the aster leafhopper. These plants subsequently developed axillary shoots and witches'-broom typical of aster yellows.

TABLE 1. Infection of herbaceous plants with Western X-disease agent (WXA) by means of the vector, *Colladonus montanus*, and recovery of WXA from these hosts

Host plant	Transmissions	Recovery of WXA from herbaceous hosts	
		No. source plants tested	No. transmissions to celery
APOCYNACEAE			
Vinca rosea L. (periwinkle)	45/61[a]	6	14/14[a]
AMARANTHACEAE			
Gomphrena globosa L.	4/6	1	1/2
COMPOSITAE			
Chrysanthemum carinatum L. (annual chrysanthemum)	7/7	2	15/16
Callistephus chinensis Nees (China aster)	5/6	2	16/16
CRUCIFERAE			
Raphanus sativus L. (radish)	27/48	7	17/24
Brassica oleracea var. *botrytis* L. (cauliflower)	7/15		
Brassica rapa L. (turnip)	38/41	2	30/39
GERANIACEAE			
Erodium moschatum L'Her. (filaree)	5/5	2	22/22
ROSACEAE			
Fragaria vesca L. (strawberry)	3/6		
UMBELLIFERAE			
Coriandrum sativum L. (coriander)	7/11	5	57/58
Daucus carota L. var. *sativa* DC. (carrot)	9/73	4	59/71

[a] Numerator = number of plants infected; denominator = number of plants inoculated.

TABLE 2. Plants inoculated with WXA which did not develop evidence of infection[a]

Species	Plants inoculated	Insects /plant	Infectivity controls[b]	
			Peach	Celery
Medicago sativa L. (alfalfa)	3	20	3/3[c]	
Plantago major L. (common plantain)	12	31	3/3	21/21
Sonchus oleraceus L. (common sow-thistle)	6	21		2/2
Lycopersicum esculentum Mill. (tomato)	6	35		4/4
Nicotiana tabacum L. (Turkish tobacco)	5	35		3/3
Nicotiana rustica L. var. *humilis* (rustic tobacco)	11	40		15/15
Salix sp. (willow)	4	20	8/8	

[a] Unsuccessful attempts were made to recover infectivity from the following inoculated but symptomless test plants by means of noninfective vectors: three alfalfa; three plantago; one tomato; two willows. The test insects used in the Western X-disease agent (WXA) recovery trials were fed on 42 peach seedlings and 7 celery plants. All plants remained healthy.

[b] Insects used to inoculate experimental plants were tested for infectivity on peach and/or celery. *Colladonus geminatus* leafhoppers were used in tests with alfalfa, plantain, willow, and rustica tobacco; *C. montanus* leafhoppers were used in tests with sow-thistle, tomato, turkish tobacco, and rustica tobacco.

[c] Numerator = number of plants infected; denominator = number inoculated.

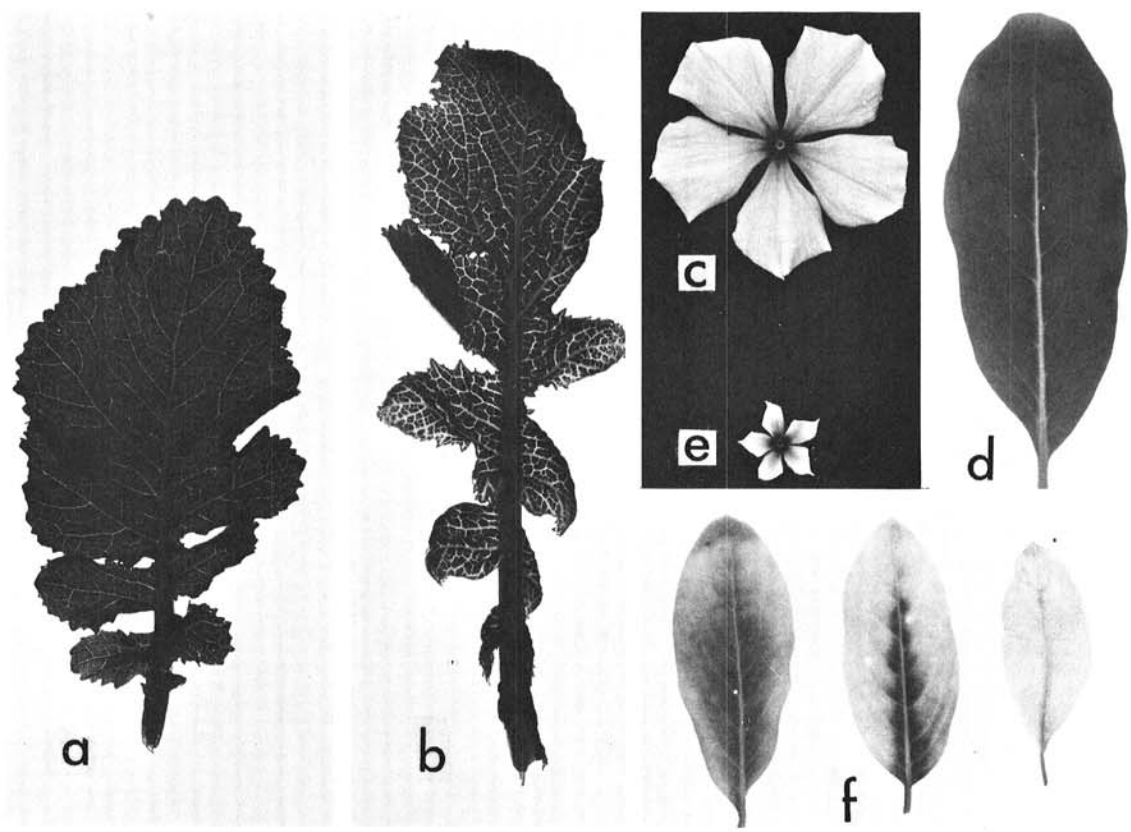

Fig. 1. Symptoms of Western X-disease in turnip and periwinkle. **a, b)** Young healthy and diseased leaves, respectively, of turnip. **c, d)** Healthy flower and leaf of periwinkle. **e, f)** Diseased flower and leaves of periwinkle.

Gomphrena globosa.—Infective leafhoppers were placed on *Gomphrena* plants at the six-leaf stage. Insect survival on *Gomphrena* was poor. Each of six plants received 25 leafhoppers, but nearly all died within 4 days. An additional 25 leafhoppers were added to each plant, and they also died rapidly. After 4 days, only 10 of 150 leafhoppers were alive. These, however, effected transmission of WXA when transferred to healthy celery.

Chlorosis and stunting were evident in the youngest leaves of four of the six test plants. These plants remained small, and died within 4 months of inoculation. WXA-free leafhoppers recovered WXA from the single *Gomphrena* plant assayed for WXA infection.

Chrysanthemum.—Twenty-five infective leafhoppers were caged on each of seven healthy young chrysanthemum plants for 8 days. All seven test plants developed disease symptoms. The leaves and leaflets were smaller than normal, and the color of the younger foliage was lemon yellow rather than dark green. Diseased chrysanthemum plants grew to only half the height of healthy controls in 50 days. WXA-free leafhoppers recovered WXA from chrysanthemums and transmitted it to 15 of 16 celery plants.

Aster.—Six china aster seedlings, in the four-leaf stage, were inoculated with WXA by means of infective *C. montanus* leafhoppers. Each plant was fed on by 25 insects for 7 days. Approximately 1 month later, the younger leaves on two of the inoculated plants were smaller and paler green than normal. Within 4 months after inoculation, five of six test plants had developed symptoms of retarded growth, and had died. The sixth inoculated plant and the controls remained alive. Noninfective leafhoppers recovered WXA from both of two aster plants assayed.

Radish.—Large radish plants (cultivar White Icicle) and small, newly transplanted seedlings were susceptible to WXA. WXA was transmitted to 27 of 48 radish plants exposed to infective leafhoppers, and was recovered from the seven diseased plants tested by WXA-free leafhoppers. In an experiment to compare the relative susceptibility of radish and turnip seedlings to infection by WXA, 15 leafhoppers from an infective colony were caged on each of 10 small radish and 10 small turnip seedlings for 8 days. Disease symptoms developed in all 10 turnip but in only 2 of 10 radish seedlings.

On older diseased radish plants, the youngest leaves became light green to chlorotic and were stunted. Older leaves were golden yellow in color, had stiff petioles, upward curving of entire leaves, and some upward-cupping of leaflets. Three months after inoculation, diseased plants had lost all but a few of the youngest leaves which were small and dark green. Root size was only half that of healthy control plants, which also had considerable foliage and flower development.

In radishes infected as small seedlings, the oldest leaves became chlorotic and the leaflets developed upward-cupping. The youngest leaves later became dwarfed, and were darker green than normal. Three months after inoculation, the youngest four leaves were dwarfed, dark green, and curled downward and inward at the tips and along the lateral margins. Puckering occurred between the veins and veinlets. The oldest leaves had a mosaic pattern of light-green to chlorotic patches, and the petioles were stiffer than normal. The distance between the leaflets was shortened, causing the leaflets to overlap.

Cauliflower.—Fifteen young cauliflower seedlings were inoculated with WXA by caging 15 infective leafhoppers on each plant for 10 days. WX symptoms were evident as reddish-purple color in the leaves of 7 of 15 plants. On medium-aged leaves, the veins and veinlets became purple first. This color later appeared in interveinal tissue. The petioles also became purple.

Turnip.—Turnip was one of those herbaceous plants most susceptible to infection by WXA. Of 41 turnip plants exposed to infective *C. montanus*, 38 (93%) became diseased. Noninfective leafhoppers recovered WXA from two plants and transmitted it to 30 of 39 celery plants.

Primary symptoms consisted of puckering of the tissue between the veins of the youngest leaves, followed within a few days by the development of purple color in the older leaves. The younger leaves showed conspicuous veinlet clearing (Fig. 1-b). The plants later acquired a flattened growth habit in contrast to the upright and green growth of normal plants. Within 2 months of inoculation, the oldest leaves of some diseased plants had died; in a few cases, the entire plants died. Healthy control plants remained green for months except that the main veins became purple on some of the oldest leaves.

Filaree.—Filaree plants, grown from seed, were fed upon by 25 infective *C. montanus* leafhoppers for 7 days when the plants were in the six- to seven-leaf stage. Four weeks later, all five test plants showed disease symptoms. The younger leaves were pale green, especially toward the margin of the leaves, and the oldest leaves were rose-colored along the margins. Infected plants made little growth thereafter. The young and middle-aged leaves became increasingly chlorotic, and the older leaves became more red. Infected plants deteriorated progressively; all died during the 3rd or 4th month after inoculation. Control plants grew vigorously and lived until they were discarded.

Five weeks after the filaree plants had been inoculated, WXA-free leafhoppers were caged on two of them for 8 days. WXA was recovered by the leafhoppers from both plants and transmitted to 22 of 22 celery plants.

Coriander.—Infective leafhoppers from WX-affected celery were caged on 11 young coriander plants in the six-leaf stage for 7 days. Five plants received 10 leafhoppers each, and six plants received 25 leafhoppers each. Seven of 11 coriander plants developed disease symptoms.

Symptoms were evident first as light chlorosis on the margins of the youngest leaves.Within a few days, necrosis as well as chlorosis developed in these leaves. Leaves of medium age remained smaller than normal, and developed chlorotic margins and some curling.

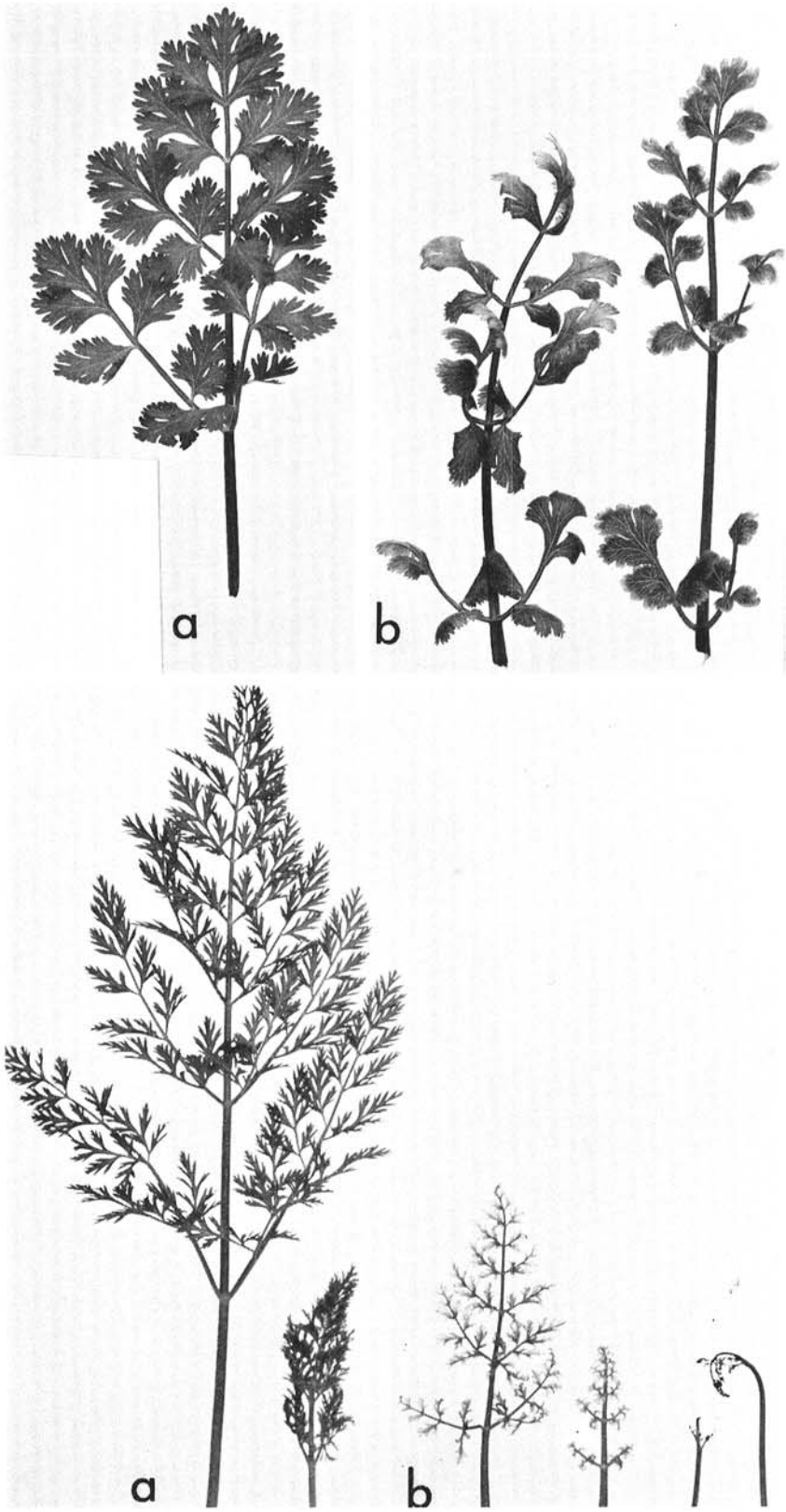

Fig. 2. (Above) Symptoms of Western X-disease in coriander. **a)** Healthy leaf. **b)** Diseased leaves. (Below) Symptoms of WX in carrot. **a)** Medium-aged and youngest leaf of healthy control. **b)** Medium-aged and youngest leaves of infected plant.

Two months after inoculation, all younger leaves were dwarfed and chlorotic or necrotic (Fig. 2, above). The oldest leaves were drooping or horizontal in position, and some were bronze or purple. Infectivity was recovered from five of five diseased coriander plants by means of WXA-free *C. montanus*.

Carrot.—Of the herbaceous plants listed as hosts for WX, carrot has proved to be the least susceptible. Only nine of 73 carrots, exposed to infective leafhoppers, developed disease symptoms. In several of the tests, the infective leafhoppers fed alternately on carrot and celery plants for several weeks. In other experiments, groups of 10-15 vectors, taken from the same infective colony, were caged on equal numbers of carrot or celery plants. In both types of experiments, a high percentage of the celery plants but only an occasional carrot became diseased.

Recovery of infectivity by WXA-free leafhoppers was accomplished from the four diseased carrots assayed. Transmission occurred to 59 of 71 indicator celery plants. Recovery was also attempted from 11 carrot plants which, though exposed to infective leafhoppers, failed to develop disease symptoms. No disease developed in any of the 338 celery plants fed on by leafhoppers transferred from inoculated but symptomless test carrots.

Symptoms of WX in red-cored Chantenay carrot were: severe stunting and some distortion of the new growth; small leaves and leaflets; shortened internodes; and petioles which were stiff and thickened. Some older leaves developed irregular chlorosis, and younger leaves became brown or "burned" on their margins or over the entire leaves (Fig. 2, below).

Within 3 months after inoculation, the foliage of some diseased carrots wilted due to collapse and death of the root system.

DISCUSSION.—In the present work, WX occurred in 12 species of herbaceous plants in seven plant families. The agent that causes eastern X-disease has been transmitted by means of dodder to milkweed and tomato (3). Eastern X and WX appear to be closely related diseases, and may even be identical. It would be surprising, therefore, if milkweed and tomato are immune to WX, although in the present study, attempts to infect tomato with WXA by means of leafhoppers appear to be unsuccessful. However, it is possible that tomato can be infected more readily by means of dodder than by insect vectors.

Although WX occurs in nature in several wild species of *Prunus*, the disease has not yet been shown to occur in nature in herbaceous plants. The results of this study, however, suggest that WXA has potentially a wide host range among herbaceous plants, and may be infecting annual and perennial weeds in orchards. Such plants would serve as reservoirs of WXA, and might be better sources of inoculum and hosts of some species of leafhoppers than are peach and cherry trees.

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