Effect of Mosaic Viruses on Infection of Horseradish by *Spiroplasma citri*

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ABSTRACT

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Horseradish (*Armoracia rusticana*) crops in Illinois may be severely damaged by brittle root disease, caused by *Spiroplasma citri*. Horseradish, a vegetatively propagated crop, is virtually 100% infected with turnip mosaic virus, and cauliflower mosaic virus has also been found. Studies to determine the effect of these viruses on brittle root disease in horseradish showed that prior infection with mosaic viruses is neither a requirement nor a preventive for subsequent infection with *S. citri* or for development of brittle root symptoms.

Additional key words: *Circulifer tenellus*

Illinois produces about half of the 6 million kg of horseradish (*Armoracia rusticana* Gaertn., Mey. & Scherb.) grown in the United States each year (15). Brittle root disease has caused severe losses in the Illinois crop during several years since it was first reported in 1936 (8). Breeding for resistance to brittle root disease was hampered by lack of information on its cause and mode of transmission. In 1981, however, after *Spiroplasma citri* was cultivated from diseased horseradish (5,13), this spiroplasma was shown to be the causal agent of brittle root, with *Circulifer tenellus* as an experimental vector of the pathogen (5). Horseradish, a vegetatively propagated crop, is virtually 100% infected with turnip mosaic virus (TuMV) (6,7). Cauliflower mosaic virus (CaMV) has also been found in horseradish (2). Our study was conducted to determine whether mosaic viruses present in horseradish affect its infection by *S. citri* or play a secondary role in development of brittle root disease. If prior virus infection facilitates development of brittle root disease, then elimination of the virus from planting stock might be an effective means of control. A preliminary report of this work has been published (4).

MATERIALS AND METHODS

Source of test plants. In the first experiment, one group of horseradish plants (cv. Swiss) was produced from secondary roots ("sets") provided by a grower in Collinsville, IL, from his 1980 fall harvest. These sets, trimmed and planted in pots in the greenhouse, generated new plants that were assumed to be infected with TuMV and possibly CaMV; most showed veinclearing or chlorotic mottling typical of mosaic virus infection. The second group of plants was grown from seeds of the experimental line 1465a (provided by A. M. Rhodes, Department of Horticulture, University of Illinois, Urbana). Because *S. citri*, TuMV, and CaMV are not seed-transmissible, these seedlings were considered to be free from these pathogens. In the second experiment, plants were generated from sets collected near Collinsville, IL, in the fall of 1980 from field plants derived the previous spring by tissue culture of the cultivar Big Top Western (performed by Martin Meyer, Department of Horticulture, University of Illinois, Urbana, using techniques previously described [10]). During the growing season, many but not all of these plants had developed symptoms of virus infection.

Assay for virus infection. The presence or absence of mosaic viruses in all test plants was determined by repeated mechanical inoculations to turnip plants (*Brassica rapa* L. 'Purple Top White Globe'). Both TuMV and CaMV induce chlorotic and often necrotic lesions in turnip plants. Two young leaves from each horseradish plant were ground in a mortar with 1–2 ml of 0.2 M sodium phosphate buffer (pH 8), and this inoculum was rubbed with a cotton-tipped applicator onto Carborundum-dusted leaves of eight turnip seedlings 2–4 wk old. As controls, several turnip plants were inoculated with buffer only. Leaves were rinsed with distilled water and turnip plants were held in the greenhouse at 21–27 C for symptom development.

Transmission of *S. citri*. *C. tenellus* for transmitting the spiroplasma were selected from a colony initiated from insects originally collected in August 1979 in St. Clair County, IL, and maintained in greenhouse cages on sugar beet (*Beta vulgaris* L.). A sample of leafhoppers was tested for curly top virus transmission and was found free from the virus.

In the first experiment, a suspension of triply cloned *S. citri* isolate BR6 was prepared and injected into leafhoppers as previously described (5); a second group received equal volumes of PBS-sucrose alone. After injection, both groups of leafhoppers were caged with sugar beet plants for 19–20 days in a growth chamber as previously described (5). Then *S. citri*-injected leafhoppers were caged in groups of 10 on five set-derived (virus-infected) horseradish plants and five seedling (virus-free) horseradish plants for a 4-day inoculation access period. Leafhoppers injected with PBS-sucrose alone were caged on plants in the same manner. Five set-derived and five seedling horseradish plants not exposed to leafhoppers served as additional controls. Test plants were trimmed to one or two leaves and covered with cylindrical Mylar cages for confinement of leafhoppers. Insects were transferred subsequently to new test plants for another 4 days, with leafhoppers tested first on set-derived plants transferred to seedlings and vice versa. Inoculation access periods were conducted in an insectary with continuous illumination at a temperature of 26 C.

In the second experiment, transmission of *S. citri* was accomplished by allowing leafhoppers to feed on *S. citri*-infected plants and transferring them to test plants. Third- to fifth-instar nymphs were caged with several turnip plants that had been infected with *S. citri* isolate BR6 for about 45 days. Additional nymphs were caged with healthy turnip plants as controls. After an 8-day acquisition access period, the insects were held on sugar beet plants for 16 days. Then leafhoppers were caged in groups of 10 per plant on seven virus-infected and 10 virus-free horseradish plants derived from tissue culture for a 7-day inoculation access period. Control insects were treated in a similar manner. Throughout this test, the caged insects were held in an insectary under continuous light at 25–27 C.

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Table 1. Effect of prior virus infection on susceptibility of set-derived or seedling horseradish plants to *Spiroplasma citri*

Plant type[a]	Number per test	Number per test with prior virus infection	Number per test infected with *S. citri*		
			After *S. citri*-injected leafhopper access	After PBS-sucrose-injected leafhopper access	No leafhoppers
Set-derived	10	10	10	0	0
Seedling	10	0	7	0	0

[a]Set-derived plants were propagated from field-collected set roots and all were shown to be virus-infected by mechanical inoculation to turnip plants. Seedling plants were grown from seeds and all were shown to be virus-free by mechanical inoculation to turnip plants.

Table 2. Effect of prior virus infection on susceptibility of set-derived horseradish plants to *Spiroplasma citri*

Treatment of leafhoppers placed on test plants	Number of test plants[a]	Number with prior virus infection	Number infected after leafhopper access	
			With *S. citri*	With virus and *S. citri*
Fed on *S. citri*-infected turnip	7	7	5	5
	10	0	7	0
Fed on healthy turnip	7	7	0	0
	10	0	0	0

[a]Obtained from a field of plants originally derived by tissue culture. After one growing season in the field, some had become infected with viruses and others were virus-free, as shown by mechanical inoculation to turnip.

Confirmation of *S. citri* infection. All test plants were tested for the presence of *S. citri* by spiroplasma isolation attempts (5) from roots within 9 wk after initial exposure to leafhoppers. Presence of spiroplasmas in liquid medium was verified by dark-field microscopy.

RESULTS

***S. citri* transmission.** In the first experiment, all the set-derived horseradish test plants were infected by virus as determined by the development of mosaic symptoms on turnip plants. Whether this infection involved TuMV, CaMV, or both was not determined. All the seedlings were shown to be virus-free by this method.

All 10 set-derived plants and seven of 10 seedling plants exposed to spiroplasma-injected *C. tenellus* became infected with *S. citri* (Table 1). Test plants exposed to PBS-sucrose-injected leafhoppers or left without leafhoppers remained free from symptoms of spiroplasma infection. Attempts to isolate spiroplasmas were successful only from plants with symptoms of *S. citri*.

In the second experiment, the horseradish test plants derived from tissue culture were designated "virus-infected" or "virus-free" according to the reaction on inoculated turnip. *C. tenellus* previously fed on *S. citri*-infected turnip plants transmitted the spiroplasma to five of seven virus-infected plants and to seven of 10 virus-free plants (Table 2). Plants exposed to leafhoppers fed previously only on healthy turnip plants remained free from symptoms of spiroplasma infection, and isolation attempts from these plants were unsuccessful. In combined data from both experiments, 15 virus-infected test plants and 14 virus-free test plants subsequently became infected with *S. citri*.

Symptomatology. In the first experiment, both set-derived (virus-infected) and seedling (virus-free) horseradish plants showed typical symptoms of brittle root disease (5), except that seedling horseradish plants had a greater tendency toward collapse of the leaves. In addition, their diffuse, netlike root system made it impossible to evaluate the seedlings for the discolored phloem ring, considered the most diagnostic feature of brittle root in the large, fleshy root of set-derived plants. To avoid root system differences in the second experiment, the virus-infected and virus-free test plants were grown from field-collected sets produced on plants originally derived from tissue culture. Symptoms of *S. citri* infection in both groups were indistinguishable.

Symptoms of virus infection in horseradish were similar in all instances regardless of source of plant. These included mosaic of young leaf blades as they developed and occasional dark streaking of petioles. Virus-free plants were characterized by uniform, dark green leaves. In plants infected with both *S. citri* and a mosaic virus, symptoms of both diseases were distinguishable.

DISCUSSION

S. citri is known to occur in many plant hosts in mixed infections with other pathogens (3,9,11,14). Our results show that *S. citri* can induce brittle root symptoms in plants with or without mosaic virus. We cannot state categorically, however, that viruses or other infectious agents have no influence on the invasion of the spiroplasma or the development of disease. The effects of such mosaic viruses as TuMV and CaMV on growth and yield of horseradish have not been determined. Susceptibility to some diseases was increased by prior virus infection (1,12,16). Subtle effects, such as predisposition of the host plant to infection because of stress, possible attraction of inoculative leafhoppers to mottled plants, competition of two pathogens within the host plant, or increased or decreased host plant longevity owing to two or more pathogens, may be involved and deserve further attention.

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