

## Newly Discovered Plant Hosts of *Spiroplasma citri*

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### ABSTRACT

O'Hayer, K. W., Schultz, G. A., Eastman, C. E., and Fletcher, J. 1984. Newly discovered plant hosts of *Spiroplasma citri*. Plant Disease 68:336-338.

*Circulifer tenellus*, injected with an Illinois isolate of *Spiroplasma citri* obtained from brittle root-diseased horseradish (*Armoracia rusticana*), transmitted the spiroplasma to yellow rocket (*Barbarea vulgaris*), wild mustard (*Brassica kaber*), and shepherd's purse (*Capsella bursa-pastoris*). *Circulifer tenellus* that had fed on experimentally infected yellow rocket, wild mustard, or shepherd's purse transmitted *S. citri* to plants of the same species and to horseradish. These weeds, new additions to the host list of *S. citri*, are the first wild plant species common in the Midwest that have been shown capable of serving as sources for transmission of this spiroplasma.

Brittle root disease of horseradish (*Armoracia rusticana* Gaertn., Mey., & Sherb.) causes root discoloration, chlorosis of leaves, stunting, and death of plants (5,12). Epidemics of this disease accompanied by severe crop losses occurred in Illinois in 1936, 1953-1954, 1975 (12), and 1979. In addition to these large but sporadic outbreaks, brittle root disease occurs at a low incidence in Illinois horseradish almost every year (12) and has also been reported in Maryland (4). The association (11) and causal role (5) of the mollicute *Spiroplasma citri* in brittle root were recently reported, and the beet leafhopper (*Circulifer tenellus* (Baker)) was shown to transmit the pathogen from brittle root-diseased to brittle root-free horseradish (5).

The brittle root epidemic of 1979, preceded by 3 yr of negligible incidence of the disease, was characterized by a sudden widespread occurrence of diseased plants, which indicated that the vectors were probably inoculative when they entered the horseradish fields. One possible explanation was that the vectors had acquired the pathogen from local weeds. In addition, weedy horseradish fields were among those with the highest incidence of brittle root disease. These observations led us to hypothesize that local weed species might serve as

reservoirs from which *S. citri* could be disseminated by vectors to horseradish.

Wild plants have been shown to play an important role in the spread of many plant diseases (2,13). Several wild brassicaceous species have been infected experimentally with *S. citri* (9,10), and field-collected samples of many of these and of other weed species have been found to harbor spiroplasmas in California (9,10) and Arizona (1). However, no similar transmission tests with wild plant species had been done with Midwestern isolates of *S. citri*. Therefore, we conducted a study to determine if three wild brassicaceous species common in the Midwest (14)—yellow rocket (*Barbarea vulgaris* R. Br.), wild mustard (*Brassica kaber* (DC.) L. C. Wheeler), and shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.)—could serve as sources for transmission of an Illinois isolate of *S. citri* to horseradish by *Circulifer tenellus*.

### MATERIALS AND METHODS

The source of *C. tenellus* colonies, rearing methods, and the manner in which leafhoppers were caged during transmission tests have been described (8). Yellow rocket, wild mustard, and shepherd's purse were grown from seed (F. and J. Seed Service, Woodstock, IL), whereas horseradish plants (cultivar Swiss) were grown from secondary roots supplied by a grower in Collinsville, IL. Cloned horseradish test plants (5) were used, with one member of each clone exposed to leafhoppers fed on spiroplasma-infected plants and the other to leafhoppers fed on healthy plants. Test plants were 21-35 days old when exposed

to insects. They were sprayed with carbaryl immediately after leafhoppers were removed and again 7 days later to kill any emerging nymphs.

To determine whether the three weed species were susceptible to infection by *S. citri* and to generate infected plants that could be used as pathogen sources in subsequent transmission tests, *C. tenellus* injected with cultured *S. citri* were used to inoculate yellow rocket, wild mustard, and shepherd's purse test plants. Methods used to isolate, cultivate, and characterize horseradish *S. citri* isolate BR3 (5) and the procedures for injection of leafhoppers (8) have been described previously. For injection, the isolate was subcultured to a final dilution of  $10^{-25}$ . Each insect received about  $2 \times 10^5$  colony-forming units of *S. citri* in 0.2-0.3  $\mu$ l phosphate-buffered saline containing 10% sucrose. After injection, leafhoppers were held on sugar beet plants for 22 days under 16 hr light (28 C) and 8 hr dark (24 C), then transferred in groups of 10 to each of five yellow rocket, wild mustard, and shepherd's purse test plants. As controls, groups of 10 uninjected leafhoppers were caged on equal numbers of test plants of each species. After 7 days, the surviving insects from each group were transferred to a fresh plant of the same species for an additional 7 days. Insects were held on test plants under constant fluorescent illumination at 27 C. Plants were held for symptom expression in a growth chamber under 14 hr light (28 C) and 10 hr dark (22 C).

To determine if *C. tenellus* could acquire and subsequently transmit *S. citri* from infected yellow rocket, wild mustard, and shepherd's purse, nymphs were confined in separate cages with four source plants of each weed species, which had been inoculated with *S. citri* 35-42 days before. Leafhoppers had access to source plants for 7 days and were held on sugar beet plants for an additional 26 days. Insects confined with each source plant species were transferred in groups of 10 per plant to five test plants of the same species and to five horseradish test plants. As a control, groups of 10 *C. tenellus* that had fed on healthy yellow rocket, wild mustard, or shepherd's purse plants were transferred to equal numbers

Accepted for publication 14 December 1983.

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of test plants of each species in the same manner. After 7 days, surviving insects from each group were transferred to a fresh test plant of the same species for an additional 7 days. This experiment was conducted in a naturally illuminated greenhouse room with average 24-hr maximum and minimum temperatures of 35 and 21 C, respectively. Test plants were held for symptom expression in a separate naturally illuminated greenhouse room with average 24-hr maximum and minimum temperatures of 28 and 19 C, respectively.

Infections in test plants were identified by disease symptoms and confirmed by either enzyme-linked immunosorbent assay (ELISA) for yellow rocket, wild mustard, and shepherd's purse or by isolation of spiroplasmas (5) for horseradish. ELISA techniques were similar to those of Clark and Adams (3) and were found reliable for detection of *S. citri* in the three weed species on the basis of agreement between results of isolation attempts and ELISA (J. Fletcher, unpublished).

## RESULTS

Injected *C. tenellus* transmitted *S. citri* to all three weed species tested (Table 1). *C. tenellus* fed on yellow rocket, wild mustard, and shepherd's purse source plants transmitted *S. citri* to test plants of the same species and to horseradish (Table 1). All control plants in both experiments remained free of symptoms. All test plants, including controls, were tested for the presence of *S. citri*; positive results were obtained only with plants showing symptoms. In these experiments, *C. tenellus* survived well on wild mustard, yellow rocket, and shepherd's purse. In addition, we observed that *C. tenellus* reproduced on all three weed species.

Symptoms in test plants first appeared 2-3 wk after initial exposure to inoculative leafhoppers. Infected horseradish plants showed symptoms typical of brittle root disease. Infected shepherd's purse, yellow rocket, and wild mustard all showed chlorosis and stunting of young leaves, with older leaves often developing chlorosis and sometimes downward curling of the margins 1-2 wk after initial symptom onset (Fig. 1). None of the yellow rocket plants, including controls, flowered. Several shepherd's purse control plants, but only one infected plant, developed flowers. This infected plant produced apparently normal flowers on a stunted, chlorotic flower stalk. Many control and infected wild mustard plants flowered and developed fruits. Infected plants produced small, pale flowers on stunted flower stalks (Fig. 1C). Fruits of infected wild mustard plants were small, shrunken, and contained small, poorly developed seeds. Most infected test plants of all three weed species were still alive although severely stunted and chlorotic when discarded 6-7 wk after inoculation.

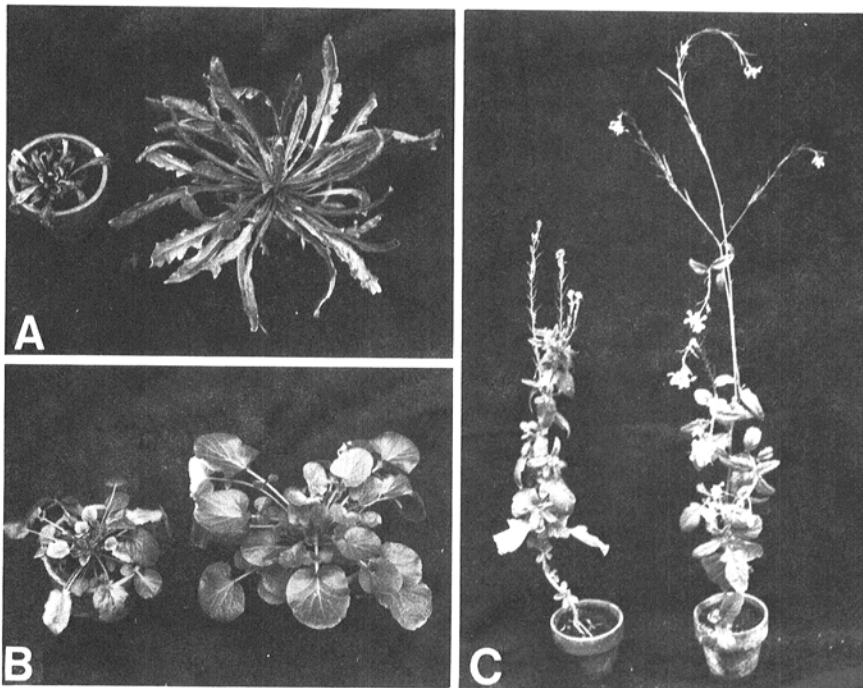
**Table 1.** Transmission of *Spiroplasma citri* to yellow rocket, wild mustard, shepherd's purse, and horseradish by *Circulifer tenellus*

Leafhopper treatment	Source plant	Test plant	Insect groups transmitting <sup>a</sup>	Plants infected <sup>b</sup>
Injected	None	Yellow rocket	3/5	4/10
		Wild mustard	4/5	6/9 <sup>c</sup>
		Shepherd's purse	4/5	4/10
Fed	Yellow rocket	Yellow rocket	5/5	10/10
		Horseradish	5/5	10/10
Fed	Wild mustard	Wild mustard	5/5	10/10
		Horseradish	5/5	9/10
Fed	Shepherd's purse	Shepherd's purse	5/5	10/10
		Horseradish	5/5	10/10

<sup>a</sup> Number of insect groups transmitting/total number of groups tested.

<sup>b</sup> Number of plants infected/total number of plants tested. Infections were confirmed by isolation of spiroplasmas (horseradish) or by ELISA (weed species).

<sup>c</sup> One additional test plant showed symptoms but died before infection could be confirmed.



**Fig. 1.** Foliar chlorosis and stunting resulting from *S. citri* infection in (A) shepherd's purse, (B) yellow rocket, and (C) wild mustard. Note stunting of flower stalks and flowers in (C). In A-C, the plant on the left was exposed to inoculative *C. tenellus* and the plant on the right to control leafhoppers.

## DISCUSSION

*S. citri* induces stubborn disease of citrus (7), and considerable work has been done to identify hosts of this spiroplasma in California and Arizona. *S. citri* was found in a number of field-collected wild plant species in these states (1,9,10). This led to speculation that weeds and other noncitrus hosts may be involved in the epidemiology of citrus stubborn disease (6).

This discovery that *Barbarea vulgaris*, *Brassica kaber*, and *Capsella bursa-pastoris* are susceptible to infection by *S. citri* expands the known host range of this pathogen and is the first such study to use a Midwestern isolate of the spiroplasma. In a preliminary search, we did not isolate spiroplasmas from wild plants collected near horseradish fields, but intensive studies of this type have yet to be conducted. Our demonstration that these

three species can serve as sources for transmission of *S. citri* to horseradish is the foundation for long-term research now in progress to determine the possible involvement of local or regional wild plant populations in the epidemiology of brittle root disease in Illinois.

## ACKNOWLEDGMENTS

We thank K. Franklin and M. R. McGuire for technical assistance and M. E. Irwin, C. J. D'Arcy, and J. V. Maddox for reviewing this manuscript. Research support was provided by the Illinois Agricultural Experiment Station Projects 68-0398 and 12-0351 and by the Illinois Natural History Survey.

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