Arabidopsis thaliana as an Experimental Host of the Mollicute *Spiroplasma citri*

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ABSTRACT

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Beet leafhoppers (*Circulifer tenellus*) that had been confined on *Spiroplasma citri* BR3-infected turnip plants were caged with seedlings of *Arabidopsis thaliana* (ecotypes Lansburg Erecta, Columbia, and Niedersans) for 4–7 days. Symptoms of *S. citri* infection in *Arabidopsis* included stunting of the basal rosette, curled and deformed cauline leaves, floral stunting and necrosis, reduced silique size and seed set, and reduced internode length on the floral stalk with terminal bunching of flowers and siliques. Infection of test plants occurred with as few as one leafhopper per plant. Spiroplasmas were cultured from 34 of 42 exposed plants, and the protein profiles of reisolated spiroplasmas in polyacrylamide gels were indistinguishable from those of cultured *S. citri* BR3. *S. citri* was also detected in infected *Arabidopsis* samples by ELISA. These findings extend the potential of *Arabidopsis* as a host plant for genetic studies of host-pathogen interactions.

Arabidopsis thaliana (L.) Heynh. (family Brassicaceae) has rapidly gained prominence as a model plant for the investigation of plant genetics and molecular genetic approaches to questions in plant physiology, biochemistry, and development (10). Because of its small size, rapid generation time, and small, simple genome, this plant has many advantages for genetic analysis. Recently, plant pathologists have found that *Arabidopsis* may also be useful in the study of host-pathogen interactions. Viruses (9,11,13), fungi (8), bacteria (1,2, 12,14,15), and mycoplasmalike organisms (5) have been shown to infect this mustard. Genes conferring resistance to the bacterial phytopathogens *Xanthomonas campestris* pv. *campestris* (14) and *Pseudomonas syringae* strains (2) have been reported. An urgent need for additional information about pathogens of *Arabidopsis* has been expressed (8).

The wall-less prokaryote *Spiroplasma citri* Saglio et al, a member of the class Mollicutes, causes a serious disease in several brassicaceous crop and weed species, including cultivated horseradish (3,4), and is the causal agent of stubborn disease of citrus (6). Phloem-feeding leafhoppers, including the beet leafhopper (*Circulifer tenellus* (Baker)) are the natural vectors of *S. citri* (7). Demonstration that *Arabidopsis* is susceptible to *S. citri* would provide a useful system for study of the nature of host-spiroplasma interactions, or of the plant's response to infection. In this paper we report the reaction of several ecotypes to infection by *S. citri*.

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MATERIALS AND METHODS

Spiroplasma strain and culture conditions. *S. citri* strain BR3, originally cultivated from Illinois horseradish affected by brittle root disease (4), has been maintained in leafhopper-inoculated turnip (*Brassica rapa* L.) plants in a growth chamber (16 hr of light at 27 C, 8 hr of dark at 22 C). Spiroplasmas were cultured in LD8 broth at 31 C (4).

Leafhopper vector. A colony of healthy *C. tenellus* was established with insects collected in 1979 and 1984 from horseradish fields in southwest Illinois. Leafhoppers from this colony were used to transmit *S. citri* to turnip plants used as sources in the present study and to inoculate *Arabidopsis* test plants. Mid-size nymphs were given a 7-day acquisition access period on infected turnip, held 14 days on healthy sugar beet plants (a nonhost of *S. citri*) in the growth chamber (16 hr of light at 27 C, 8 hr of dark at 22 C), and then confined as adults on test plants.

Spiroplasma transmission studies. *Arabidopsis* ecotypes Lansburg Erecta, Columbia, and Niedersans (seeds obtained from David Meinke, Oklahoma State University) were greenhouse-grown in a soil-peat mixture in clay pots (7.6 cm diameter) placed on trays containing charcoal. One to several leafhoppers, fed previously on *S. citri*-infected turnip, were caged on seedlings or young flowering plants (Fig. 1) in the growth chamber for an inoculation access period of 4–7 days. Cylindrical cages of polyester film (Mylar) with polyester mesh tops were used to confine leafhoppers to individual pots containing one or more test plants. Plants caged without leafhoppers or with leafhoppers not exposed to *S. citri* source plants served as controls. Following the inoculation access period, the leafhoppers were removed and the plants were returned to the growth chamber for symptom development.

Spiroplasma detection methods. Spiroplasmas from infected plant samples were isolated in LD8 broth (4). For polyacrylamide gel electrophoresis (PAGE), cells were pelleted and proteins

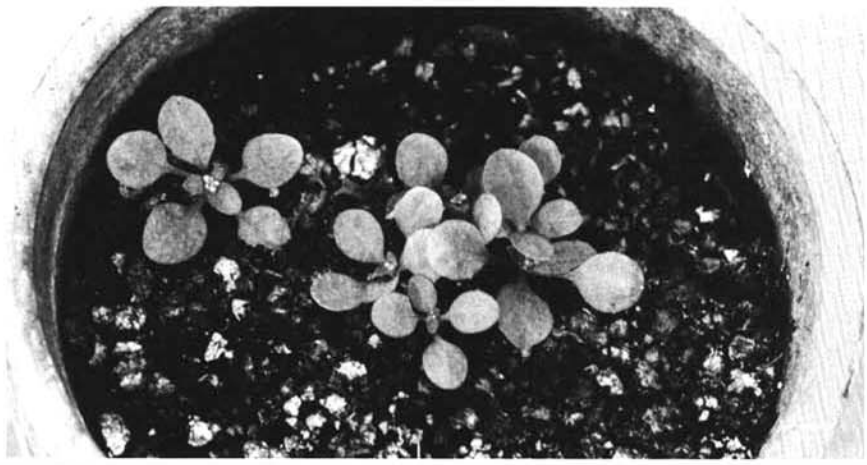

Fig. 1. *Arabidopsis thaliana* plants, in a 7.6-cm-diameter pot, at the stage when caged with leafhoppers for inoculation access.

were solubilized and electrophoresed (4) in 1.5-mm sodium dodecyl sulfate slab gels of 10% acrylamide. Foliage and/or flowering stalks of *Arabidopsis* were ground in PBS-Tween at a ratio of 0.3:0.4 g/ml for double antibody sandwich ELISA (3). In one case, a 1:10 dilution of this extract was also tested.

RESULTS

***S. citri* infection in test plants.** Plants became infected after exposure to as few as one leafhopper per plant. Transmission success increased with additional leafhoppers, but prolonged feeding by many insects, healthy or infected, on individual young seedlings was deleterious. The overall infection rate of test plants with *S. citri* was 43 of 51 exposed plants (84%). This included 26 of 31 exposed Lansburg Erecta plants (84%), eight of 10 exposed Columbia plants (80%), and nine of 10 exposed Niedersans plants (90%).

Symptoms of *S. citri* infection, which began to appear 14 days after the first day of leafhopper exposure, were similar among the three ecotypes. Infected plants often had small or absent rosettes, stunted floral stalks (Fig. 2), and curled (C-shaped) or deformed cauline leaves (Fig. 3A). Internodes on flower stalks were shortened, with terminal bunching of flowers and siliques (Fig. 3B). Flowers and siliques were sometimes reduced or necrotic, although the pedicel remained green. Seed set was often drastically reduced. The disease caused progressive decline and premature death of the host plant. These symptoms are similar to those described for *S. citri* in other brassicaceous plants (4). No virescence, phyllody, or asymmetry of floral structures was observed.

Reisolation and characterization of spiroplasmas. Spiroplasmas were isolated from 34 of 42 exposed *Arabidopsis* plants, with the positives including all symptomatic plants and occasionally symptomless plants that had been exposed to spiroplasma-infected leafhoppers. Spiroplasmas were never cultured from control plants. PAGE protein patterns of spiroplasmas cultured from *Arabidopsis* were indistinguishable from those of cultured BR3 but differed from those of *S. kunkelii* or *S. melliferum*.

ELISA. A_{405} readings for 12 healthy *Arabidopsis* plants ranged from 0.007 to 0.026 and were indistinguishable from wells containing buffer alone. Ten infected plant samples (five Niedersans, three Columbia, and two Lansburg Erecta) had A_{405} readings of 0.408–1.750; a 1:10 dilution of one sample read 0.705.

DISCUSSION

Our findings demonstrate that the brassicaceous weed *Arabidopsis* can serve as a host to both the phytopathogen *S. citri* and its leafhopper vector *C. tenellus*, which we observed to reproduce on *Arabidopsis* (*unpublished*). Infection of *Arabidopsis* is detectable by symptomatology, isolation of the pathogen in culture media, or ELISA. To our knowledge this is the first report of the infection of *Arabidopsis* by a spiroplasma. Another mollicute, the beet leafhopper-transmitted virescence agent (BLTVA), has been shown to infect *Arabidopsis* (5). Symptoms of *S. citri* infection appear in *Arabidopsis* in as few as 14 days, compared with 5 wk for symptoms of BLTVA infection. The symptomatology of these two mollicutes in *Arabidopsis* is very different and would provide an interesting comparative study.

All three *Arabidopsis* ecotypes examined were susceptible to the spiroplasma. If a resistant ecotype could be found, as with the *X. c. campestris* system (11,14), perhaps the genes for resistance in the host could be identified. Whether or not that occurs, the *Arabidopsis-S. citri* interaction may be a valuable model for

Fig. 2. Compared with healthy *Arabidopsis thaliana* plant (23.5 cm long), plant (7.2 cm long) infected with *Spiroplasma citri* has stunted floral stalk, small siliques, short internodes, and terminal bunching of siliques and flowers.

Fig. 3. *Arabidopsis thaliana* plants infected with *Spiroplasma citri* showing **(A)** curled (C-shaped) cauline leaves and normal flowers and **(B)** necrotic siliques and terminal bunching of floral organs.

investigations of molecular aspects of spiroplasma infection in plants.

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