Brittle Root of Horseradish in Illinois and the Distribution of *Spiroplasma citri* in the United States

Jacqueline Fletcher

Department of Plant Pathology, University of Illinois, Urbana 61801.
I gratefully acknowledge the contributions to the work described here by Cathy E. Eastman, Gerald A. Schultz, Robert M. Goodman, Karen O'Hayer, Michael McGuire, and Kathleen Franklin. I am also indebted to Robert E. Davis, USDA, Beltsville, MD, for help and advice.

Accepted for publication 29 July 1982.

ABSTRACT

Fletcher, J. 1983. Brittle root of horseradish in Illinois and the distribution of *Spiroplasma citri* in the United States. Phytopathology 73: 354-357.

The known distribution of *Spiroplasma citri* has been greatly expanded by the finding that this organism is the causal agent of brittle root disease of horseradish in Illinois and Maryland. Prior to the late 1970s, *S. citri* was known to occur in the United States only in the hot, dry regions of southern California and Arizona, where it causes citrus stubborn disease. One of the vectors of *S. citri*, *Circulifer tenellus*, has been collected from Illinois horseradish during most epidemic years. The wide host and geographical ranges of *S. citri* have prompted a reevaluation of the potential threat of this organism to agriculture in the midwestern U.S. and elsewhere and in crops other than citrus.

Additional key words: mycoplasmalike organism.

Fifteen years have passed since the 1967 findings of mycoplasmalike organisms (MLOs) associated with yellows diseases (11,16). Since that time, several spiroplasmas have been cultured from within or from the surfaces of plant tissue, and Koch's postulates have been completed for three plant diseases caused by spiroplasmas: citrus stubborn (9), corn stunt (7,32), and horseradish brittle root (BR) (13). The recent finding of the etiological role of *Spiroplasma citri* Saglio in BR disease of horseradish in Illinois (13) is only the second case in which this organism has been proven to be the causal agent of an agriculturally important plant disease. That this organism was found in the Midwest, over 1609 km (1,000 miles) from its previously known range in the western United States and in a climate thought by some to be unsuitable to the survival of both the organism and its vector, has implications that extend to reports in the late 1970s of the occurrence of *S. citri* in the eastern half of the U.S. Although offered without proof of pathogenicity, these reports take on new significance with the finding that the organism does occur and can cause disease in a field crop in the Midwest. In this article, hypotheses on the epidemiology of *S. citri* based on the expansion of the known geographical and host ranges of the organism and on reports of the activity and habits of certain insect vectors will be proposed.

BR of horseradish. Horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.) is a member of the Brassicaceae. First reported in 1936 (17), horseradish BR disease has occurred in epidemic proportions only sporadically in Illinois (1936, 1953, 1954, 1975, 1979) (13,27). Individual growers have lost up to 100% of their crop, while losses to the horseradish industry in Illinois have been as high as 80% (13). However, small numbers of BR-diseased plants are seen almost every year. Named for the typical loss of root flexibility, BR is characterized also by foliar chlorosis and curling, edge necrosis, stunting, reduction in number of branch roots, development of a darkened ring in the phloem region of the root, and sometimes wilting. Affected plants usually die within a few weeks of symptom onset (13).

Prior to 1980, all attempts to demonstrate the pathogen causing BR were unsuccessful. Some indirect evidence and similarities in symptomatology led to a suggestion of curly top virus involvement (17,30) but this was never substantiated.

***Spiroplasma citri*: Causal agent of horseradish BR.** In 1980, MLOs were seen in thin sections of phloem tissue of a BR-diseased horseradish plant from Illinois (25). Subsequently, a spiroplasma serologically indistinguishable from *S. citri* was found associated with BR horseradish (13,25). Illinois researchers completed Koch's postulates by introducing cultured horseradish spiroplasma into nonbrittle root (NBR) horseradish test plants via microinjected beet leafhoppers (*Circulifer tenellus* (Baker)), reisolating spiroplasma from symptomatic test plants, and showing the spiroplasma to be identical to the injected spiroplasma isolate by serology and polyacrylamide gel electrophoresis (13). Since then, in tests involving over 100 horseradish plants with and without BR symptoms tested in 1980–1981, *S. citri* was isolated from 100% of the BR-diseased plants tested, but from none of the NBR plants (J. Fletcher, *unpublished*).

Possible involvement of the beet leafhopper. Epidemics of BR seem to be associated with drought years in Illinois, although some BR may be seen in small patches every year. In nonepidemic years, the disease may be present due to survival of the pathogen in vegetatively propagated plants; secondary roots infected late in the growing season and symptomless at harvest may be used as planting stock the following spring, giving rise to the random occurrence of infected plants (17). Drought stress could cause malfunction of the roots and predispose the plant to disease. However, weather conditions may also affect movement of an insect vector. *C. tenellus*, known to be a natural vector of *S. citri* in California and an experimental one in Illinois, is a resident primarily of semiarid regions, but its range during a given season might be influenced by rainfall patterns outside of normally arid regions (2).

Drought conditions existed all over the southwestern United States in 1949–1954, and there was an eastern and northern spread of *C. tenellus* from breeding grounds in southern New Mexico and western Texas with the spreading drought (12). The beet leafhoppers established new breeding areas in Oklahoma in 1953–1954 (12). This wave of insect movement could have been a source of spread into Illinois in the 1953–1954 BR epidemics (Fig. 1). Beet leafhoppers were found in Illinois in large numbers in 1953 and 1954 (24). In those two years, curly top virus (CTV), for which the only known North American vector is *C. tenellus* (2), occurred in some states where the leafhoppers were found: Iowa, Minnesota, Kansas (12), and Illinois (12,27) (Fig. 1). CTV was also reported in tobacco and sugar beet on the East Coast in 1958 (15,28) although beet leafhoppers were not detected from sweep collections that year (28). Leafhoppers carrying CTV could also have carried *S. citri*. If so, *S. citri* could have been established in host plants of the regions

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

through which the leafhoppers passed; since the pathogen is not known to be passed transovarially in its insect vectors (20), subsequent generations of leafhoppers could have acquired it only from infected plants. Reservoirs of *S. citri* may still be present in these regions.

In 1976, *S. citri* was found in turnips in Washington (G. N. Oldfield, *personal communication*), and in 1980 the pathogen was isolated from plants and beet leafhoppers in Arizona (1). A spiroplasma presumed to be *S. citri* has recently been isolated from *C. tenellus* from southwestern Utah (G. N. Oldfield, *personal communication*) (Fig. 1). These observations are consistent with the hypothesis that inoculative *C. tenellus* may be migrating from the Southwest.

Other regions potentially threatened by citrus stubborn or horseradish BR disease. Citrus and horseradish are grown in several locations of the United States, yet the stubborn and BR diseases are confined to limited geographic regions. Both Florida and the Texas coast are important citrus-growing regions, and horseradish is grown on relatively large acreages in northern California and southwestern Wisconsin and to a limited extent in several eastern states. In Illinois, BR has been likened to a disease of California horseradish (13) supposedly caused by CTV (17); however, no proof was given that the two diseases were the same, and in later work, *S. citri* could not be isolated from California horseradish (25). The potential for damage to citrus and horseradish or other crucifers in other states is considerable if the pathogen should become established. It is possible that *S. citri* has not been introduced into southeastern Texas or into Florida; both areas are outside of the known range of the organism, although west Texas is a breeding ground for *C. tenellus* and the leafhopper was reported as breeding on a brassicaceous weed in Florida in 1925 (10). Beet leafhoppers have not been reported in Wisconsin, although CTV was found in tobacco in that state in 1954 (14). The horseradish-producing area of northern California is located midway between southern California and Washington, both of which have hosted populations of *C. tenellus* from which *S. citri* has been isolated. That Wisconsin and California horseradish crops have remained apparently free of BR may also be because the plants are cultivated differently in these two states than in Illinois. Illinois growers produce their crop each year from root pieces, which are usually lifted slightly with a hoe or knife to break off branch roots near the crown once or twice during the season. This practice, done to increase the size of the root, temporarily stresses the plant. In Wisconsin, where roots are not lifted, a more extensive root system develops. California horseradish is grown as a perennial from which the upper root only is harvested every 2 yr. In Illinois, BR symptoms are extremely rare in foliage of "volunteer" horseradish, which, regenerating from pieces of root left in the ground after harvest, has a much deeper and better developed root network than the newly planted crop. Thus, unstressed horseradish plants may harbor the pathogen without showing symptoms or may resist infection. However, the apparent absence of BR in California or Wisconsin is as yet unresolved. It may be that the pathogen is present in horseradish in these regions, but that BR symptoms are mild due to less water stress, or are masked by other disease(s).

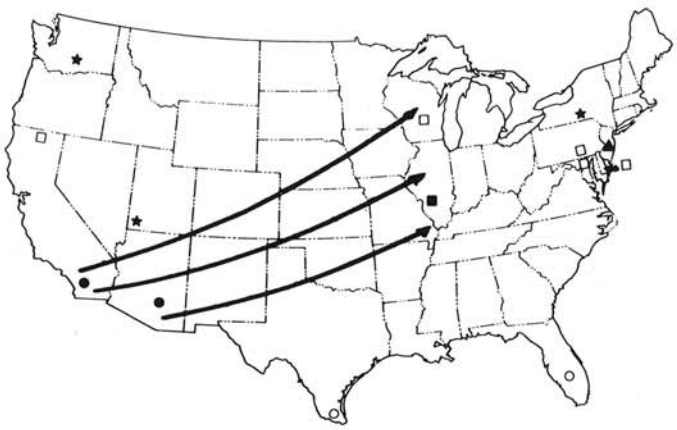

Fig. 1. Hypothetical migration routes (arrows) of the beet leafhopper, *Circulifer tenellus*, infectious with *Spiroplasma citri*, in the United States. Other symbols indicate isolation of *S. citri* from citrus trees (dark circles), citrus growing regions from which *S. citrii* has not been reported (light circles), isolation of *S. citri* from horseradish (dark squares), horseradish growing regions from which *S. citri* has not been reported (light squares), reports of isolation of *S. citri* from *C. tenellus* (dark stars), and a report of isolation of *S. citri* from lettuce (dark triangle).

BR disease in Maryland. In addition to the major horseradish-producing states of Illinois, Wisconsin, and California, small acreages are also grown for commercial sale in Oregon, Michigan, Pennsylvania, New Jersey, and, since 1980, Maryland. In late 1981, *S. citri* was isolated and identified from horseradish plants collected from a field in southern Maryland and showing symptoms characteristic of BR: yellowed foliage, death of older leaves, stunting of plants, brittleness of roots, and a darkened ring in the root phloem region (R. E. Davis, *personal communication*). No spiroplasmas were obtained from plants without symptoms from the same field. Isolation results from symptomatic and asymptomatic plants have been confirmed by our laboratory (J.

TABLE 1. Known hosts of Illinois horseradish isolates of *Spiroplasma citri*

Families and species	Common name	Infected in Illinois: Microinjection[a] (Vector)	Infected in Illinois: Feeding[b] (Vector)	Infected by California isolates
Apocynaceae				
Catharanthus roseus	Periwinkle	+ (BLH)	+ (BLH)	+
Asteraceae				
Callistephus chinensis	China aster	+ (ALH)	+ (ALH)	+
Brassicaceae				
Armoracea rusticana	Horseradish	+ (BLH, ALH)	+ (BLH, ALH)	ND
Capsella bursa-pastoris[c]	Shepherd's purse	+ (BLH)	+ (BLH)	ND
Barbarea vulgaris[c]	Yellow rocket	+ (BLH)	+ (BLH)	ND
Brassica kaber[c]	Wild mustard	+ (BLH)	+ (BLH)	ND
Brassica rapa	Turnip	+ (BLH)	+ (BLH, ALH)	+
Raphanus sativus	Radish	+ (BLH)	+ (BLH)	+
Polemoniaceae				
Phlox drummondi	Phlox	ND[d]	+ (BLH)	+

[a] Infection followed microinjection of *Circulifer tenellus* (BLH) or *Marosteles fascifrons* (ALH) with cultured spiroplasma, incubation time for the leafhoppers on sugarbeet or barley and inoculation access feeding on test plants.
[b] Infection by feeding was accomplished by allowing BLH or ALH acquisition access time on source plants, incubation time on sugar beet or barley, and inoculation access time on test plants.
[c] Experimental work by K. W. O'Hayer et al (*unpublished*).
[d] ND = not done.

Fletcher, *unpublished*). Further details will be published in a separate paper.

Expansion of known host and geographical ranges of *S. citri*. Horseradish is one of many brassicaceous plants, both wild and cultivated, reported to be natural or experimental hosts of *S. citri* (5,23). *S. citri* has a very wide host range with genera from about 20 plant families (23), including representatives of both herbaceous and woody plants, annuals and perennials, and dicots and monocots. Seven additional hosts were added in 1981 (1,29). The known hosts of the Illinois isolates of *S. citri* are shown in Table 1.

The finding of *S. citri* in Illinois and Maryland significantly expands its known geographical range in the United States. The pathogen has been recognized in hot and dry regions of the world where citrus is produced. Citrus stubborn was noted in California as early as 1915 (3). A similar disease ("little leaf") appeared in the eastern Mediterranean in the late 1920s; *S. citri* has since been isolated from affected citrus trees in several Mediterranean countries. *S. citri* may have originated in northern Africa; it occurs there today along with one of its California vectors, *C. tenellus* (4). In addition, one of the wild hosts of the pathogen in California is a mustard, *Brassica tournefortii*, which is native to northern Africa (4). It seems possible that the organism, carried within its plant host or insect vector, arrived in California and became established some time prior to 1915.

Other reports of *S. citri* east of the Mississippi River. Spiroplasmas later shown to be *S. citri* (8) were reported to occur in the northeastern United States prior to the finding in Maryland (6,19,22). In one case (19,22), a spiroplasma cultivated from field-collected lettuce plants affected with aster yellows disease in New Jersey was claimed to be the causal agent of aster yellows (19,21,22), a disease that has been presumed to be due to a noncultivable MLO. The report was accompanied by incomplete information and was not followed by a published report of details of symptomatology or proof of pathogenicity.

The geographical range believed supportive of the two organisms (31) has been noted as one point of discrepancy between the aster yellows agent and *S. citri*. However, proof of the natural occurrence of *S. citri* in horseradish in Illinois made it reasonable to consider survival of the organism on the East Coast. Such reasoning led to the search by Davis for BR-diseased horseradish in Maryland where the pathogen was subsequently found.

S. citri was also isolated and identified from a single pear twig from trees affected by pear decline in Connecticut (26). Although the relationship of the spiroplasma to the disease is unknown, the report of the organism in Connecticut should be pursued further.

Implications of *S. citri* in the East. *S. citri* occurs in Illinois and Maryland, where it causes a serious disease of horseradish (13, R. E. Davis and J. Fletcher, *unpublished*). The expansion of the known geographical range of the pathogen forces a reevaluation of the limitations of the organism. Average temperatures in these states are lower than in the Southwest, the humidity is greater, and rainfall patterns differ. The frequency of the occurrence of *C. tenellus* in Illinois is not known. It has not been collected in Maryland, although the presence of CTV in the state in 1958 (15,28) is indicative that the insect may occasionally be there.

The occurrence of *S. citri* in the Midwest and East may be linked to movement of the beet leafhopper, possibly from California and Arizona or from other breeding grounds in western Texas and New Mexico. More than one vector may be involved, either in bringing the pathogen into these areas or in spreading the organism once it is there. Insects known to naturally transmit *S. citri* are listed in Table 2. *Scaphytopius acutus acutus* and *Macrosteles fascifrons* (aster leafhopper) are both native to Illinois (Table 2) and the aster leafhopper is common on the East Coast as well. *S. acutus delongi*, a close relative of the Illinois *S. acutus acutus*, is a natural vector of *S. citri* in California. *M. fascifrons* was reported to transmit the microinjected spiroplasma from lettuce to healthy lettuce and aster (22). Kloepper et al (18) and our group in Illinois (K. W. O'Hayer et al, *unpublished*) have confirmed that microinjected aster leafhoppers can transmit *S. citri*, in the former case to *Plantago* sp. and in the latter to horseradish and to aster. O'Hayer et al (*unpublished*) have further demonstrated that the aster leafhopper can transmit *S. citri* after feeding on infected plants.

S. citri has already been reported on the East Coast (6,19,22,26, R. E. Davis and J. Fletcher, *unpublished*) and further investigation from this region should clarify some questions. It will be important to learn whether the pathogen has recently arrived in that area or has been there for a long period and was only recently detected. For example, although the known geographical range of *S. citri* has recently been expanded to include Illinois, this presumably is not a new location for the pathogen since horseradish brittle root was reported in the state as early as 1936. If *S. citri* has recently moved into the eastern states, its potential for damage is unknown. If, however, the organism has been endemic in these areas for some time, we may expect either to identify it as a causal agent in one or more diseases of unsolved etiology, or to find that it is not a dangerous pathogen in the region due to unsuitable conditions for the organism or its vectors. Similarly, the actual range of the organism may currently be much more extensive than the known locations.

TABLE 2. The occurrence in Illinois of insects known to be natural vectors of *Spiroplasma citri*

Circulifer tenellus	Not common; frequency unknown
Macrosteles fascifrons[a]	Common in horseradish and other plants every year
Scaphytopius acutus delongi	Not present[b]
Scaphytopius nitridus	Not present

[a] Work on transmission of Illinois isolates by *M. fascifrons* done by K. W. O'Hayer et al will be published in a separate paper.

[b] The close relative, *S. acutus acutus*, occurs routinely in Illinois. Tests are in progress to determine whether this leafhopper is capable of transmitting *S. citri*.

LITERATURE CITED

1. Allen, R. M., and Donndelinger, C. R. 1982. Transmission of *Spiroplasma citri* by two leafhopper vectors in Arizona. (Abstr.) Phytopathology 71:856.
2. Bennett, C. W. 1971. The curly top disease of sugarbeet and other plants. Phytopathological Monograph 7. American Phytopathological Society, St. Paul, MN. 81 pp.
3. Calavan, E. C., and Gumpf, D. J. 1974. Studies on citrus stubborn disease and its agent. INSERM (Inst. Nat. Sante Rech. Med.) Colloq. 33:181-186.
4. Calavan, E. C., Kaloostian, G. H., Oldfield, G. N., Nauer, E. M., and Gumpf, D. J. 1977. Natural spread of *Spiroplasma citri* by insect vectors and its implications for control of stubborn disease of citrus. Proc. Int. Soc. Citriculture 3:900-902.
5. Calavan, E. C., and Oldfield, G. N. 1979. Symptomatology of spiroplasmal plant disease. Pages 37–64 in: The Mycoplasmas: Plant and Insect Mycoplasmas. Vol. III. R. F. Whitcomb and J. G. Tully, eds. Academic Press, New York.
6. Charbonneau, D. L., Hawthorne, J. D., Ghiorse, W. C., and Vandemark, P. J. 1979. Isolation of a spiroplasma-like organism from aster yellows infective leafhoppers. Abstr. G21. Page 86 in: Proc. Am. Soc. Microbiol., 79th Annu. Meeting, 4–8 May 1979, Los Angeles, CA.
7. Chen, T. A., and Liao, C. H. 1975. Corn stunt spiroplasma: Isolation, cultivation, and proof of pathogenicity. Science 188:1015-1017.
8. Christiansen, C., Freundt, E. A., and Maramorosch, K. 1980. Identity of cactus and lettuce spiroplasmas with *Spiroplasma citri* as determined by DNA-DNA hybridization. Curr. Micro. 4:353-356.
9. Daniels, M. J., Markham, P. G., Meddins, B. M., Plaskitt, A. K., Townsend, R., and Bar-Joseph, M. 1973. Axenic culture of a plant pathogenic *Spiroplasma*. Nature 244:523-524.
10. DeLong, D. M. 1925. The occurrence of the beet leafhopper, *Eutettix tenella* Baker, in the Eastern U.S. J. Econ. Entomol. 8:637-638.
11. Doi, Y. M., Teranaka, M., Yora, T. K., and Asuyama, H. 1967. Mycoplasma PLT group-like microorganisms found in phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Ann. Phytopathol. Soc. Jpn. 33:259-266.
12. Douglass, J. R., Peay, W. E., and Cowger, J. I. 1956. Beet leafhopper and curly top conditions in the southern Great Plains and adjacent areas. J. Econ. Entomol. 49:95-99.

13. Fletcher, J., Schultz, G. A., Davis, R. E., Eastman, C. E., and Goodman, R. M. 1981. Brittle root disease of horseradish: Evidence for an etiological role of *Spiroplasma citri*. Phytopathology 71:1073-1080.
14. Fulton, R. W. 1955. Curly-top of tobacco in Wisconsin. Plant Dis. Rep. 39:799-800.
15. Heggestad, H. E., and Moore, E. L. 1959. Occurrence of curly top on tobacco in Maryland and North Carolina in 1958. Plant Dis. Rep. 43:682-684.
16. Ishiie, T., Doi, Y., Yora, K., and Asuyama, H. 1967. Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease. Ann. Phytopathol. Soc. Jpn. 33:267-275.
17. Kadow, K. J., and Anderson, H. W. 1940. A study of horseradish diseases and their control. Ill. Agric. Exp. Stn. Bull. 469:531-583.
18. Kloepper, J. W., and Garrott, D. G. 1983. Evidence for a mixed infection of spiroplasmas and nonhelical mycoplasmalike organisms in cherry with X-disease. Phytopathology 73:357-360.
19. Kondo, F., Maramorosch, K., McIntosh, A. H., and Varney, E. H. 1977. Aster yellows spiroplasma: Isolation and cultivation *in vitro*. (Abstr.) Proc. Am. Phytopathol. Soc. 4:190-191.
20. Liu, H.-Y. 1981. The transmission, multiplication, and electron microscopic examination of *Spiroplasma citri* in its vector, *Circulifer tenellus*. Ph.D. thesis, University of California, Riverside.
21. Maramorosch, K. 1981. Spiroplasmas: Agents of animal and plant diseases. Bioscience 31:374-380.
22. Maramorosch, K., and Kondo, F. 1978. Aster yellows spiroplasma: Infectivity and association with a rod-shaped virus. (Abstr.) Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. I Orig., Reihe A 241:196.
23. Oldfield, G. N., and Calavan, E. C. 1982. Stubborn disease in non-rutaceous plants. In: Description and Illustration of Virus and Virus-like Diseases of Citrus: A Collection of Color Slides (rev. ed.). J. Bové and R. Vogel, eds. Institut Français de Recherches Fruitieres Outre-Mer. Paris.
24. Petty, H. B. 1955. The insect pests of horseradish in southwestern Illinois. Ph.D. thesis, Univ. of Illinois, Urbana.
25. Raju, B. C., Nyland, G., Backus, E. A., and McLean, D. L. 1981. Association of a spiroplasma with brittle root of horseradish. Phytopathology 71:1067-1072.
26. Raju, B. C., Nyland, G., and Purcell, A. H. 1983. Current status of the etiology of pear decline. Phytopathology 73:350-353.
27. Rhodes, A. M. 1977. Horseradish—Problems and research in Illinois. Pages 137–148 in: Crop Resources. D. Seigler, ed. Academic Press, New York.
28. Schneider, C. L. 1959. Occurrence of curly top of sugar beets in Maryland in 1958. Plant Dis. Rep. 43:681.
29. Schultz, G., Eastman, C., Fletcher, J., O'Hayer, K., McGuire, M., and Goodman, R. M. 1981. Factors affecting infection of horseradish with *Spiroplasma citri* in brittleroot disease. (Abstr.) Phytopathology 71:903.
30. Severin, H. H. P. 1929. Additional host plants of curly top. Hilgardia 3:595-636.
31. Whitcomb, R. F. 1981. The biology of spiroplasmas. Annu. Rev. Entomol. 26:397-425.
32. Williamson, D. L., and Whitcomb, R. F. 1975. Plant mycoplasmas: A cultivable spiroplasma causes corn stunt disease. Science 188:1018-1020.