Virescence of Horseradish in Illinois

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ABSTRACT

Eastman, C. E., Schultz, G. A., Fletcher, J., Hemmati, K., and Oldfield, G. N. 1984. Virescence of horseradish in Illinois. Plant Disease 68:968-971.

A virescence-inducing agent, transmitted experimentally by *Circulifer tenellus*, was found in field-grown horseradish in Illinois. Symptoms in flowering horseradish, Madagascar periwinkle, radish, and wild mustard included virescence, phyllody, shortening of internodes, and bunching of leaves with occasional axial proliferation. Mycoplasmalike organisms of typical polymorphic morphology were found in mature sieve elements from virescent periwinkle test plants. The virescence agent was found periodically in mixed infections with *Spiroplasma citri* in brittle root-diseased horseradish.

Additional key words: *Armoracia rusticana*

Oldfield et al (7) described virescence and rosetting in Madagascar periwinkle test plants (*Catharanthus roseus* (L.) G. Don, cited as *Vinca rosea*) exposed to beet leafhoppers (*Circulifer tenellus* (Baker)) that had been collected from several locations in California. Mycoplasmalike organisms (MLOs) were found in sieve elements from these plants during electron microscopic examination. Virescent Madagascar periwinkle, broccoli (*Brassica oleracea* L., Botrytis group), *Phlox* sp., and the brassicaceous weeds *B. geniculata* (Desf.), *Raphanus sativus* L., and *Sisymbrium irio* L.—all collected in southern California—were used successfully as sources for transmission of a virescence agent to periwinkle by laboratory-reared *C. tenellus* (6). We report the presence in Illinois of a virescence-inducing agent in horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.). The agent is transmitted experimentally by *C. tenellus* and appears to be associated with the presence of an MLO. Our findings further indicate that although found occasionally in mixed infections with *Spiroplasma citri* Saglio et al in brittle root-diseased horseradish, the virescence agent is not directly involved in this disease. A preliminary report of this work has been published (2).

Accepted for publication 30 April 1984.

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MATERIALS AND METHODS

The history of the *C. tenellus* colony and the methods for caging these leafhoppers on source and test plants have been described (5). In addition, saran screen cages 22 cm high and 5.5 cm in diameter were used to confine leafhoppers to individual plants in some tests. Test plants used were Madagascar periwinkle (Mixed Color and White), horseradish (Swiss) grown from sets obtained from a grower in Collinsville, IL, radish (Sparkler), and wild mustard (*Brassica kaber* DC. (L. C. Wheeler)) (11) grown from seed (F. and J. Seed Service, Woodstock, IL).

Three experiments, originally designed to obtain transmission of *S. citri* from brittle root-diseased horseradish, were conducted with field-grown vegetative horseradish collected near Collinsville, IL, in July (experiment 1) or October (experiments 2 and 3) 1980. In experiment 1, adults and nymphs of *C. tenellus* were caged for 16 days with nine horseradish plants with or without symptoms of brittle root disease. The leafhoppers were then placed in groups of two to 25 on horseradish or periwinkle test plants and transferred serially at 4- to 7-day intervals to new test plants, alternating between horseradish and periwinkle, until all insects were dead. In experiments 2 and 3, nymphs were caged with nine horseradish plants with or without symptoms of brittle root disease for 16–18 days, then held on sugar beet plants (*Beta vulgaris* L. 'Giant Western') for another 5–10 days. An equal number of leafhoppers were caged only on sugar beet plants during this period. Then the leafhoppers were placed in groups of five to 50 on individually caged horseradish or periwinkle test plants or held in groups of 200–400 in cages containing six horseradish and three periwinkle plants for 8–25 days.

Two attempts were made to obtain transmission from virescent flowering horseradish collected from Madison County in 1981. In the first test, cut flower stalks were used as sources. In the second test, roots from virescent and nonvirescent plants were dug from the field, potted individually in a greenhouse soil mixture, and allowed to regenerate foliage before use as sources. In both tests, *C. tenellus* nymphs were caged with virescent horseradish and a healthy sugar beet plant (as a supplemental feeding host) for 21–24 days. Equal numbers of nymphs were caged with nonvirescent horseradish and a sugar beet plant as a control. Then leafhoppers were placed in groups of five to 10 on radish or wild mustard plants or held in groups of 75–300 in cages containing three to six periwinkle plants for 3–16 days.

Terminal shoots of two virescent periwinkle test plants were selected for electron microscopic examination. Midribs, petioles, and fine stems were cut into 2-mm^2 pieces in cold 6% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.2, and immediately immersed in fresh fixative on ice for 1 hr. After rinsing in cold potassium phosphate buffer for another hour, the plant tissues were postfixed in 2% osmium tetroxide in the same buffer on ice for 2 hr. Specimens were dehydrated in an acetone series for 3 hr, then embedded in Spurr's resin "standard medium" (10) with propylene oxide used to improve infiltration. Ultrathin and thick sections were cut with glass knives from 20 fixed specimens, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-600 transmission electron microscope.

RESULTS

Detection in Illinois. A virescence-inducing agent was detected in 1980 during experimental attempts to transmit *S. citri*—the causal agent of horseradish brittle root (3,8)—by *C. tenellus* from brittle root-diseased plants collected from southwestern Illinois. In experiment 1, brittle root symptoms (3,9) developed only in horseradish plants exposed to leafhoppers caged previously with brittle root-diseased horseradish (Table 1). Likewise, extensive chlorosis and stunting (1) developed only in

periwinkle plants exposed to leafhoppers fed on brittle root-diseased horseradish. Attempts to isolate spiroplasmas by methods described previously (3) were successful only from plants with these symptoms. All test plants with symptoms of *S. citri* infection were dead or dying within 4 mo.

A second syndrome developed in other periwinkle plants, including those caged with leafhoppers fed previously on brittle root-free horseradish (Table 1). These symptoms included partial or complete virescence of the flowers (Fig. 1), phyllody, shortening of internodes with bunching of leaves, axial proliferation, and occasionally, sinuous midveins and yellow spots on leaves. These plants were still alive when discarded after 4 mo. Virescence symptoms were not observed in horseradish test plants, all of which remained vegetative. Attempts to isolate spiroplasmas from virescent periwinkle were unsuccessful. Periwinkle plants not exposed to leafhoppers showed no symptoms. These findings indicated that a virescence-inducing agent could be transmitted by *C. tenellus* from field-grown horseradish with or without brittle root disease.

In experiments 2 and 3, virescence developed in 11 of 24 periwinkle plants but only when brittle root-diseased horseradish plants were used as pathogen sources. Horseradish test plants remained vegetative and could not be evaluated for virescence by symptomatology. None of the test plants developed symptoms of *S. citri* infection, possibly because the horseradish plants used as sources were collected late in the season (October).

Transmission from virescent flowering horseradish. Vegetatively propagated horseradish, grown as an annual in Illinois, rarely flowers before harvest; however, a field of an experimental line of horseradish (Illinois 984a) with a high percentage of flowering plants was encountered in Madison County in August 1981. Of the 920 flowering plants examined, 4.3% expressed virescence (Fig. 2). Foliar symptoms of brittle root disease were noted in 0.8% of 1,231 flowering and vegetative plants examined. Spiroplasmas were isolated from brittle root-diseased plants but not from virescent horseradish. Virescent horseradish plants were found in two additional fields, one containing several rows of Illinois 984a and the other planted with mixed stock. In the latter field, six *C. tenellus* were collected in sweep-net samples.

On the basis of symptoms expressed in two experiments in 1981, *C. tenellus* caged previously on virescent horseradish transmitted the virescence agent to 11 of 20 radish, 4 of 5 wild mustard, and 9 of 20 periwinkle test plants. Virescence did not develop in plants exposed to leafhoppers caged previously with nonvirescent horseradish. Symptoms in periwinkle resembled those observed in the tests performed in 1980, although the virescence was less pronounced and often affected the flowers on only one branch. Virescence and occasional phyllody were noted in affected radish and wild mustard plants. All virescent plants continued to grow and remained free of symptoms other than those noted; some of these

Table 1. Transmission of *Spiroplasma citri* or a virescence-inducing agent from brittle root-diseased or brittle root-free horseradish by *Circulifer tenellus* (1980)

Agent source	Test plant	No. of plants infected with[a]	
		S. citri[b]	Virescence agent
Brittle root horseradish	Periwinkle	2/47	11/47
	Horseradish	4/15	...[c]
Non-brittle root horseradish	Periwinkle	0/47	9/47
	Horseradish	0/15	...
None[d]	Periwinkle	0/38	0/38
	Horseradish	0/15	...

[a] No. of plants infected/no. of plants tested, based on symptomatology.
[b] Presence of *S. citri* was confirmed by isolation.
[c] None of the horseradish plants flowered, so the presence of a virescence-inducing agent could not be determined by visual examination of plants for symptoms.
[d] Test plants in this group were not exposed to leafhoppers.

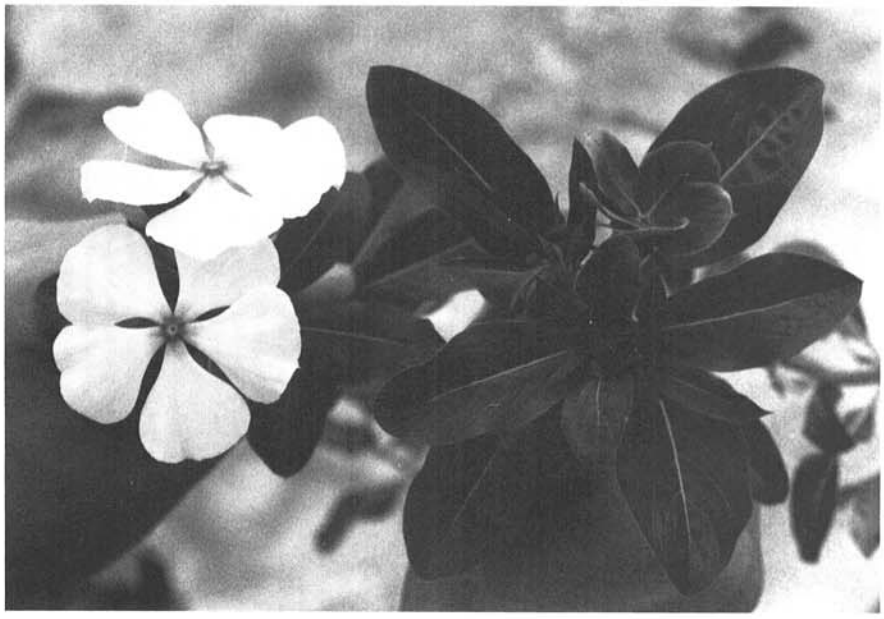
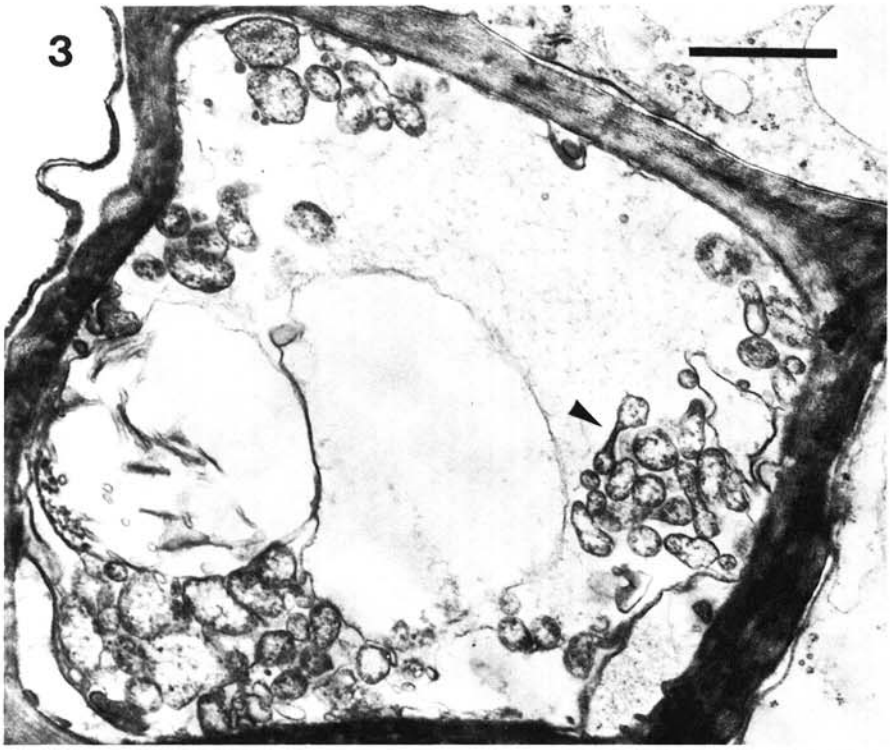
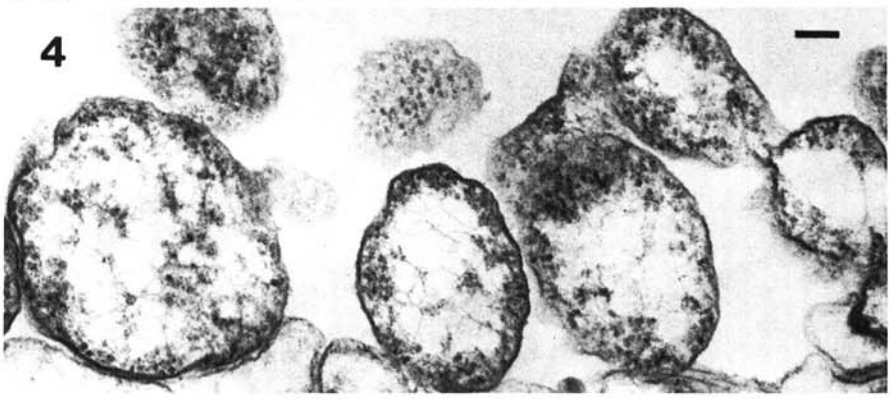
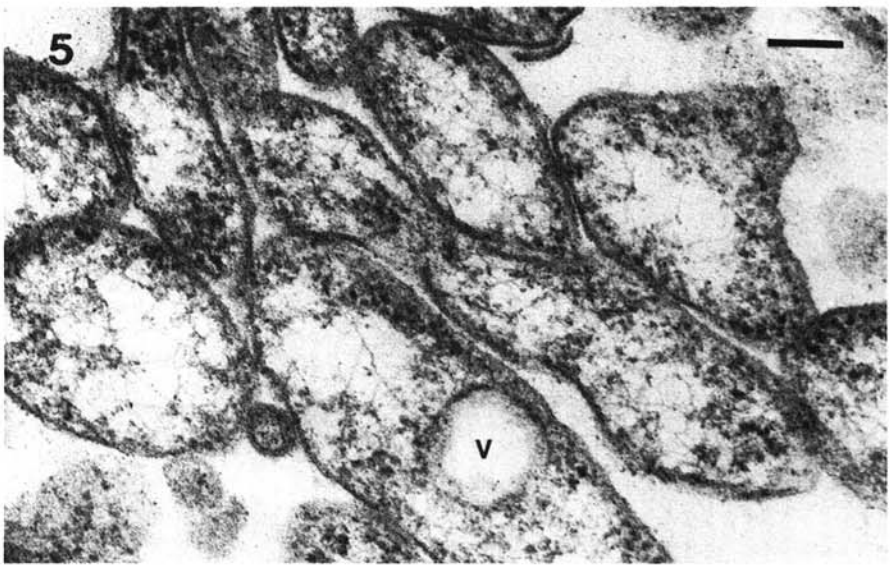


Fig. 1. (Right) Periwinkle infected with a virescence agent transmitted from horseradish by *Circulifer tenellus*. (Left) Healthy periwinkle.

Fig. 2. (A) (Left) Normal horseradish flowers compared with (right) virescent horseradish flowers. **(B)** Virescent flower stalks from horseradish.







Figs. 3–5. Mycoplasmalike organisms (MLOs) within sieve element cells of virescent periwinkle plants. **(3)** MLOs in a single sieve element cell; note elongated forms and possible binary fission (arrow). Bar = 1 μm. **(4)** Ovoid MLOs containing ribosomes and DNA-like strands. Bar = 0.1 μm. **(5)** Ovoid and elongate MLOs; note vacuole (v). Bar = 0.1 μm.

virescent periwinkle are still being maintained in a greenhouse 3 yr after inoculation.

Electron microscopy. Typical polymorphic MLOs were noted in 40% of the several hundred examined sections of mature sieve elements from two periwinkle plants showing virescence symptoms (Figs. 3–5). Spherical or ovoid MLO bodies ranged from 110 to 750 nm in diameter in cross section. Small electron-dense spherules were observed occasionally between the larger bodies (Fig. 3). Morphological features of MLO bodies indicative of binary fission (4) were often seen (Fig. 3). Filamentous bodies or helical forms were not detected. Vacuoles were observed within the cytoplasm of larger MLO bodies (Fig. 5) and were more frequent in large bodies (up to 950 nm in diameter) with scattered contents, presumably undergoing natural degeneration. Although these examinations were done on a small number of virescent plants, the results support the possible involvement of an MLO in the virescence condition of horseradish in Illinois.

DISCUSSION

Symptoms induced by a virescence agent in periwinkle and brassicaceous plants in our experiments were quite similar to those reported previously for a virescence agent in California. This similarity and the transmission of both agents by *C. tenellus* indicate that the virescence-inducing agents found in California and Illinois may be closely related. Although MLOs were found in virescent test plants in both states, their role in the induction of the virescence conditions described here has not been established.

No obvious adverse effects of the virescence condition on foliage or roots of vegetatively propagated horseradish were observed. However, the length of time the virescence agent has been present in field-grown horseradish in Illinois, the extent of crop infection, and any effects it has on yield are not known.

The nature of the relationship between the Illinois virescence agent and *S. citri* has not been fully elucidated. Both agents can be transmitted by *C. tenellus* and have occurred together in mixed infections in horseradish. Each agent induces distinctly different symptoms, but only symptoms associated with *S. citri* infection characterize brittle root disease of horseradish.

ACKNOWLEDGMENTS

We thank M. R. McGuire, K. W. O'Hayer, K. Franklin, and J. Kitasako for technical assistance and C. J. D'Arcy, H. Jedlinski, and M. C. Shurtleff for suggestions. We also thank R. E. Davis (USDA Plant Virology Laboratory, Beltsville, MD) for first calling to our attention the similarity in symptoms induced by the Illinois and California virescence agents. This publication is a contribution of the Illinois Natural History Survey and the Illinois Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana-Champaign, supported in part by Hatch grants 30-15-12-351 and 30-15-68-398.

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