

Effect of *Hirschmanniella caudacrena* on the Submersed Aquatic Plants *Ceratophyllum demersum* and *Hydrilla verticillata*¹

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Abstract: In vitro pathogenicity tests demonstrated that *Hirschmanniella caudacrena* is pathogenic to *Ceratophyllum demersum* (coontail). Symptoms were chlorotic tissue, deformed stems, and, finally, death of the plant. Inoculum densities of 500 nematodes per 5-cm-long cutting in a test tube containing 50 ml of water resulted in death and decay of some of the cuttings within 8 weeks; 100 nematodes killed the plants in 12 weeks, and 50 and 25 nematodes killed them in 16 weeks. The lowest inoculum level of 10 nematodes did not seriously affect the plants at 16 weeks when the experiment was terminated. A second test conducted outdoors in glass jars containing 3 liters of water and two cuttings weighing a total of 15 g fresh weight showed damage, but results were not statistically significant. *Hydrilla verticillata* inoculated with *H. caudacrena* was not affected seriously.

Key words: aquatic plant, biological control, *Ceratophyllum demersum*, coontail, *Hirschmanniella caudacrena*, hydrilla, *Hydrilla verticillata*, pathogenicity test, rice root nematode.

Nematodes parasitizing aquatic plants have received little attention, although several reports indicate that they can cause serious plant damage (2-4,8-10,13,16,17,20,23,24,27,28). Between June 1983 and June 1985, we conducted a comprehensive survey of plant-parasitic and free-living nematodes associated with aquatic plants in Florida. High populations of *Hirschmanniella caudacrena* (Sher, 1968) were found within the tissues of the submersed plant *Ceratophyllum demersum* L. at Newnan's Lake (Alachua County) and at Rodman Reservoir (Putnam County). We detected up to 58 nematodes per gram of fresh plant tissue (8). Plants from these locations usually exhibited chlorosis. *Hirschmanniella caudacrena* was found also in plants growing in Lochloosa Lake, Orange Lake (Alachua County), and Lake Rousseau (Citrus County), but at much lower population densities.

Hirschmanniella caudacrena was first re-

ported from soil planted to paddy rice (*Oryza sativa* L.) in Louisiana (21), and its pathogenicity to rice was demonstrated (13). The species is known to be associated also with several, mostly aquatic, plants: *Cabomba* sp. (15,23), *Cabomba caroliniana* Gray (7), *Cordia* sp. (22), *Hydrilla verticillata* L. f. Royle (6,7,10), *Ludwigia repens* Forst. (6,7), *Najas* sp. (24,25), *Oryza sativa* L. (21), *Pistia stratiotes* L. (6,7), *Sagittaria subulata* L. Buch. (6,7), *Sagittaria* sp. (6,7,21), *Sabal palmetto* (5), *Vallisneria americana* Michx. (6,7,21), and *Vallisneria* sp. (21).

Pathogenicity tests with nematodes infecting submersed plants have not been conducted previously. Our objective was to determine the pathogenicity of *H. caudacrena* to the submersed aquatic plants *C. demersum* and *H. verticillata* and to assess the potential of the nematode to control these plant species.

Ceratophyllum demersum, commonly called coontail or hornwort, is a perennial submersed plant with slender, elongated stems and whorled leaves (1,9). Unlike most submersed aquatics, coontail is a rootless plant; however, early in the growing season modified branches may be buried in the substrate (14). The plants may subsequently detach from the substrate, continue to grow, and form dense mats floating beneath the surface. Coontail is a cosmopolitan species found in quiet ponds and lakes as well as in slowly flowing rivers and canals

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(14). In New Zealand, coontail is a serious weed problem in lakes supplying water to hydro-electric power plants (18); in the United States, it is considered a substantial problem in Connecticut, Indiana, Iowa, Minnesota, Ohio, Virginia, Wisconsin (26), and Alabama (11).

Hydrilla verticillata, commonly called hydrilla, is a perennial submersed plant with long, branching stems which form large mats. Hydrilla, which is native to the old world, was introduced into Florida probably around 1960 (12). Presently hydrilla is the most important noxious aquatic weed in Florida, covering 45,502 acres of waterways (19).

MATERIALS AND METHODS

Ceratophyllum demersum was obtained from Cross Creek (Alachua County, Florida) and was kept in tapwater in outdoor pools. Plants from Cross Creek have never been found infected with *Hirschmanniella caudacrena*. New apical shoots 5 cm in length and bearing 5–7 nodes were cut and used for the test. The shoots were washed with tap water under high pressure to remove attached debris, algae, and small aquatic animals and then rinsed in five changes of deionized water (Experiment 1) or tap water (Experiment 2). Surface sterilizing the plants with 3% Chlorox (sodium hypochlorite) was phytotoxic.

Hydrilla verticillata was obtained from Manatee Springs (Levy County, Florida). Apical shoots 10 cm long were prepared as described in the previous paragraph.

Hirschmanniella caudacrena was extracted from infected coontail plants from Newnan's Lake (Alachua County) with a Baermann funnel method as modified by Oostenbrink (15). The plants were washed, cut into small pieces with scissors, and spread evenly on a non-gauze milk filter (Kendall Company, Agricultural Products, Boston) which was supported by a wire mesh in an aluminum pan containing tap water. After 48 hours, nematodes that migrated through the filters were hand picked or collected with a pipet randomly and concentrated in a small drop of deionized

water. The nematode suspensions were added with a pipet to the water in which the plants were growing.

At the end of each of the three experiments that follow, nematodes were isolated from plant tissues and soil by the milk filter method described in the previous paragraph.

Experiment 1 (Ceratophyllum demersum): Plants from apical shoots were grown in test tubes (25 × 150 mm) containing 5 cm³ autoclaved, fine builders sand as a substrate and 50 ml deionized water. The stem base of each plant was buried in the sand to facilitate nematode infection by preventing the plant from floating. The water level in the test tubes was maintained at 50 ml, and a slight algal film that formed was removed occasionally with a pipet.

The cuttings were inoculated the day after planting. Treatments were 0, 10, 25, 50, 100, and 500 *H. caudacrena*, with each treatment replicated 10 times. The plants were kept in an incubator at 25 C with a 12-hour day length at approximately 155 $\mu\text{moles photons m}^{-2} \text{sec}^{-1}$ for 16 weeks. At 4-week intervals, the plants were rated visually for damage by estimating the coloration or decay of the stems and leaves (see footnote, Table 1).

Experiment 2 (Ceratophyllum demersum): Forty glass jars containing 100 cm³ autoclaved, fine builders sand and 3 liters tap water were placed in two empty outdoor pools. Two apical cuttings (5–7 nodes and 15 g fresh weight) with numerous side shoots were planted in each jar and anchored in the sand by fastening two rubber stoppers (total weight 8 g) with a rubber band to the stem base of each plant. This was done to prevent the plant from floating and interfering with penetration through the stem base by the nematodes which sink to the bottom of the jar and are unable to swim upward. The plants were inoculated 1–2 weeks after planting with 0, 2,000, 4,000, and 8,000 nematodes per jar (equivalent to 0, 133, 267, and 533 nematodes per gram of fresh plant tissue). Each treatment was replicated 10 times. Each jar was covered with perforated parafilm to pre-

vent possible disturbance by rainfall or animals. The pools containing the jars were covered with shadecloth (approximately 75% shade) to prevent excessive algal growth. Water temperature in the jars ranged from 23 C to 28 C during the experiment, which began at the end of May 1985 and was terminated 3 months later at the end of August. The plants were blotted dry with paper towels before determining their fresh weights at the beginning and end of the experiment.

Experiment 3 (Hydrilla verticillata): Apical shoots 10 cm long were used for the test. The experimental design and procedures were as for Experiment 1, except that larger test tubes (25 × 200 mm) were used.

RESULTS

Experiment 1 (Ceratophyllum demersum): Symptoms of feeding were observed after 4 weeks in all treatments containing nematodes (Table 1). After 8 weeks, plant damage at inoculum levels 100 and 500 was manifested as brownish stems and leaves (Fig. 1A–D) and after 12 weeks the plants were almost totally decayed; a 2-cm layer of decayed plant material was present in most test tubes at inoculum level 500. By 16 weeks plants at inoculum levels 25 and 50 were almost totally decayed. A closer look at the leaves revealed destruction of chloroplasts (Fig. 1F, G). Plants did not die at the 10 nematode inoculum level, but the leaves and stems were slightly chlorotic. Some of the control plants also exhibited slight yellowing at 12 and 16 weeks.

Both inoculated and uninoculated plants grew during the first 8 weeks, as determined by the production of side shoots and by an increase in length of 1–2 nodes in some of the plants. Those producing side shoots (Fig. 1D) did not increase much in length. At the termination of the experiment, no nematodes were recovered from the controls, the 10 or the 500 inoculum level treatments and those recovered from the 25, 50, and 100 inoculum levels represented 12%, 34%, and 47%, respectively, of the original inoculum.

Experiment 2 (Ceratophyllum demersum):

TABLE 1. Damage to stems and leaves of *Ceratophyllum demersum* caused by *Hirschmanniella caudacrena* and final nematode populations.

Inoculum level per 50 ml water	Damage rating												Final no. nematodes recovered
	4 weeks			8 weeks			12 weeks			16 weeks			
	Stem	Leaves		Stem	Leaves		Stem	Leaves		Stem	Leaves		
0	0.0	0.0		0.0	0.0		0.0	0.4		0.0	0.4		0
10	0.1	0.0		1.1	0.3		1.9	1.0		2.1	1.3		0
25	0.3	0.1		1.8	1.1		3.9	3.1		5.0	5.0		3
50	1.0	0.3		2.5	1.5		4.5	4.2		5.0	4.6		17
100	1.8	1.3		4.3	4.1		5.0	4.7		5.0	5.0		47
500	1.5	0.3		4.7	4.1		5.0	5.0		5.0	5.0		0
F value	10.79	9.93		48.54	24.51		39.48	35.09		70.16	63.07		
P	0.0001	0.0001		0.0001	0.0001		0.0001	0.0001		0.0001	0.0001		

Data are means of 10 replicates per treatment.

Damage rating scale: stems (from base), 0 = no damage, 1 = yellowish between base and third node, 2 = brownish between base and third node, 3 = whole stem yellowish, 4 = all brownish, 5 = whole stem decayed; leaves (from base), 0 = no damage, 1 = yellowish between base and third node, 2 = brownish between base and third node, 3 = all leaves yellowish, 4 = all leaves brownish, 5 = all leaves decayed.

TABLE 2. Effect on plant weight of *Ceratophyllum demersum* caused by *Hirschmanniella caudacrena* after 3 months and final nematode numbers in plant tissue and substrate.

Inoculum level per 3 liters water	Plant weight (nearest gram)			Final no. nematodes recovered		
	Initial	Final	Change	Plant tissue	Substrate	Total
0	15	12 ± 5	-3	0	0	0 ± 0
2,000	15	11 ± 5	-4	330	7	337 ± 468
4,000	15	10 ± 4	-5	263	22	285 ± 486
8,000	15	8 ± 5	-7	570	21	591 ± 976

Data are means (± standard deviation) of 10 replicates per treatment.

The effect of nematode feeding on cuttings of *C. demersum* was assessed by observing changes in plant weights. After 3 months, all plants had decreased in weight (Table 2). The decrease was greater as the inoculum level increased, but the differences were not statistically significant ($P = 0.05$). Only 17%, 7%, and 7% of the inoculum was recovered at the 2,000, 4,000, and 8,000 inoculum levels, respectively, at the end of the experiment.

Experiment 3 (Hydrilla verticillata): None of the inoculum levels tested had an effect on the hydrilla plants, except for a slight discoloration of the stem bases at the 50, 100, and 500 inoculum levels at 8 weeks. All plants grew well, producing side shoots and roots. The nematodes penetrated the stem bases and migrated along the first internode (5–8 mm). At the end of the experiment, only a few dead nematodes were observed in the stem bases and in the substrate in some of the replicates.

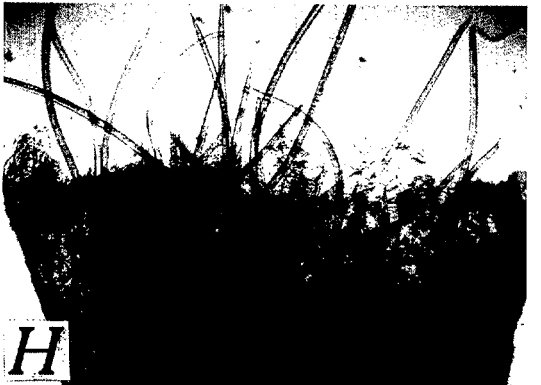
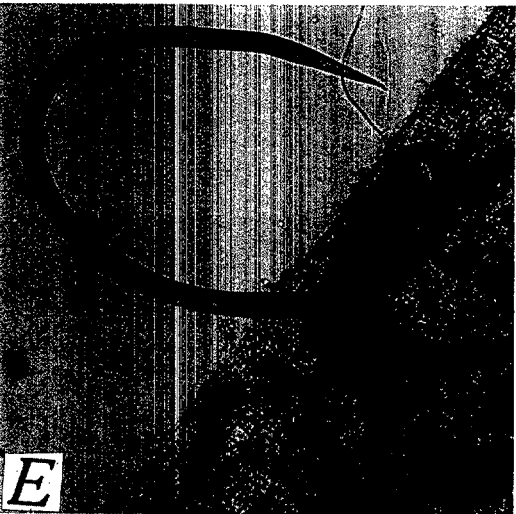
DISCUSSION

From field observations, it was suspected that *H. caudacrena* was pathogenic to *C. demersum*. Experiment 1 confirmed this hypothesis. Plant response to nematode infection was chlorotic tissue, deformed stems, and finally death of the plant. The highest inoculum densities caused the greatest damage to the plant cuttings in the shortest period of time. The lowest in-

oculum density did not seriously affect the plants.

The number of nematodes present at the termination of the experiment did not exceed the inoculum level in any of the treatments. That implies that no reproduction occurred. That is not the case under other conditions, however, because we obtained populations of the nematode for research purposes from coontail plants in a nearby lake. Also, in preliminary experiments on the life cycle of the nematode, several cuttings were inoculated each with a single gravid female; young adults were detected after 26 days. Thus reproduction occurred with the life cycle taking about 26–30 days. It is possible, of course, that reproduction occurred and that many of the nematodes died. That is almost certainly the case at the 500 inoculum level in which the plants died and were almost totally decayed after 12 weeks. Four weeks later when the experiment was terminated, all of the nematodes may have died and decayed so there was no way to determine whether reproduction occurred. This may have been the case to a lesser degree at the other inoculum levels with some nematodes surviving. That cannot explain why no nematodes were recovered at the 10 inoculum level, however. It is highly probable that many nematodes did not penetrate through the milk filter and hence were not recovered.

FIG. 1. Effect of *Hirschmanniella caudacrena* on *Ceratophyllum demersum*. A) Cutting 8 weeks after inoculation with 100 nematodes. B) Uninoculated control plant. C) Cutting 8 weeks after inoculation with 500 nematodes. D) Uninoculated control plant. E) Nematodes penetrating a stem of *C. demersum*. F) Leaf cells of *C. demersum* almost devoid of chlorophyll after having been fed upon by *H. caudacrena*. G) Healthy cells. H) Nematodes inside broken stem of *C. demersum*.



While the measure of pathogenicity (i.e., weight loss) showed no statistical differences in the second test, numerical differences did occur. This test was conducted outdoors during our hottest weather. Even though the jars were covered with shade cloth, it is possible that the water temperatures up to 28 C may have affected both damage to the plants and the number of nematodes that survived.

Ceratophyllum demersum is a rootless plant, and the nematodes occur in the leaves and stems (Fig. 1E, H). A comparison of nematode populations in single branches of the plant showed that the stems always support higher nematode densities than do the leaves. One-third of the plant consists of a well-developed aerenchyma in the form of canals. The nematodes occur and move freely within these airspaces. Nematodes were observed to penetrate through the outside of stems to reach the aerenchyma (Fig. 1E). Nematode feeding destroys the chloroplasts of the epidermal cells of stems and leaves (Fig. 1F), resulting in discoloration and finally death of the affected plant part.

Since coontail propagates itself mainly vegetatively in Florida, nematode dissemination most likely is by infected new plant fragments. However, infection could take place early in the growing season when modified branches of the plant are partially buried in the sediment. Coontail usually floats beneath the water surface, and a nematode attempting to migrate from one plant to another might sink to the bottom of the lake and be unable to swim up to the floating plants. Replicated sediment samples taken monthly over a period of 10 months from under coontail in Newnan's Lake yielded no more than three nematodes per 250 ml soil, which suggests that the nematode completes its entire life cycle in the plant and is disseminated in plant fragments.

Nematode population densities were high inside plants collected from lakes but plant abundance did not appear to be affected adversely. We assume that the

growth rate of the plant exceeds the damage caused by the nematode.

The other plant tested, *H. verticillata*, was not affected by *H. caudacrena* even at high inoculum levels, in spite of the fact that the nematodes penetrated the stems and migrated in the first internodal area. Hydrilla was often present together with *C. demersum* in many lakes, and nematodes infecting the latter plant may well have migrated to hydrilla. Hydrilla samples received from near Bangalore, Karnataka State, India, were infested with another species, *Hirschmanniella oryzae* (v. Breda de Haan, 1902) Sher, 1968. Those plants were growing in a lake closely bordering a paddy rice field, which suggests that hydrilla may serve as an alternate host for the nematode.

Our experiments indicate that due to the high inoculum levels needed to stress *Ceratophyllum demersum* it is currently impractical to use *Hirschmanniella caudacrena* alone as a biological control agent.

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