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The Clover Cyst Nematode, *Heterodera trifolii*, a Potential Pest of Clover and Vegetable Crops in Florida.[1]

R. N. Inserra[2], N. Vovlas[3], S. Walker[4], and R. A. Dunn[5]†

INTRODUCTION: The loss of inexpensive nematicides because of environmental and health concerns has increased the interest of farmers and agricultural specialists in cropping systems to better managing nematode populations in infested soils. Leguminous cover crops, such as cowpea (*Vigna unguiculata* L.), joint-vetch (*Aeschynomene americana* L.), lespedeza (*Lespedeza* spp.) and velvet bean (*Mucuna deeringiana* (Bort) Merril), have received special attention for their resistance to some root-knot nematodes (*Meloidogyne* spp.) species. Also, these legumes fix nitrogen through their symbiotic relationship with the bacteria *Rhizobium* and *Bradyrhizobium* spp. (Rodriguez-Kabana et al. 1988).

Clovers (*Trifolium* spp.) are other cover crops of potential value for production of forage and green manure. Red clover (*T. pratense* L.) lines adapted to the environmental conditions of Florida have been selected recently. Red clover cultivar 'Cherokee' is particularly promising for spring forage production and resistance to root-knot nematodes in north-central Florida (Chambliss and Quesenberry 1991). An expansion of clover in north-central Florida could favor attacks by the clover cyst nematode, (*Heterodera trifolii* Goffart) (Fig. 1 A,B), in mild winter and early spring months. The distribution of the clover cyst nematode *H. trifolii* in Florida is limited and confined to a few areas in the central western part of the state (Hardee, Hillsborough, and Lake counties) where no evidence of crop damage has been observed.

Recently, an infestation of *H. trifolii* was detected in Pinellas Co. on white clover (*T. repens* L.) grown continuously in a screenhouse (records of the Division of Plant Industry). Plants were severely stunted and adversely affected also by nutritional disorders. In a section of the site, clover plants were concomitantly infected by *H. trifolii* and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. This circular provides Florida growers with information about the potential damage that the clover cyst nematode can cause under conditions conducive to its attacks.

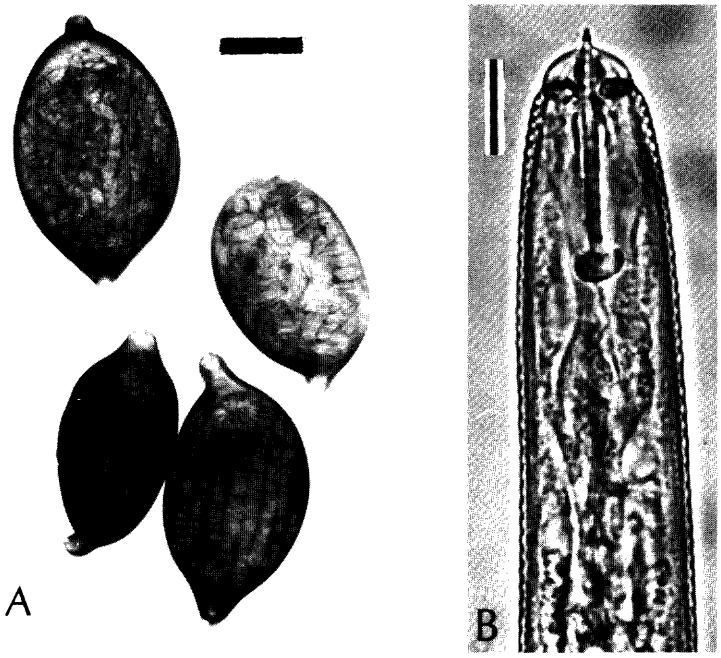

Fig. 1. Selected life stages of *Heterodera trifolii*. Scale bars = 200 µm in A and 10 µm in B. A) Cysts. Note eggs in the specimens with transparent cuticle. B) Anterior body portion of a second-stage juvenile.

[1] Contribution No. 450, Bureau of Entomology, Nematology & Plant Pathology - Nematology Section.

[2,4] Nematologist and Biological Scientist, respectively, Division of Plant Industry, P.O. Box 147100, Gainesville, FL 32614-7100.
[3] Nematologist, Istituto Nematologia Agraria, CNR, 70126 Bari, Italy.
[5] Professor, Entomology and Nematology Department, IFAS, University of Florida, Gainesville, FL 32611.

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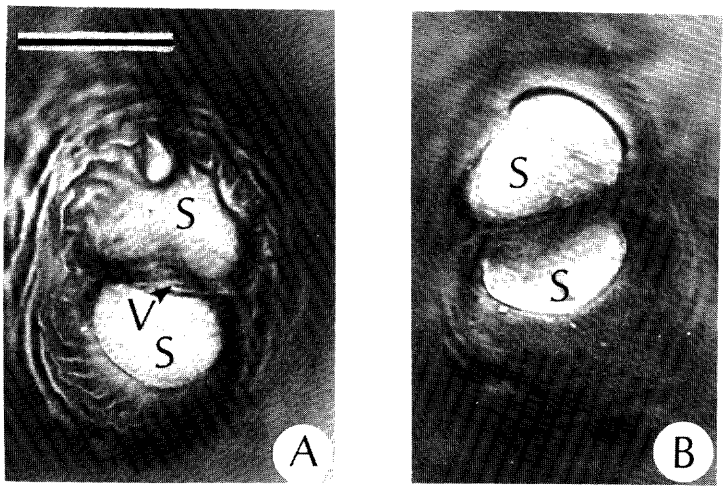

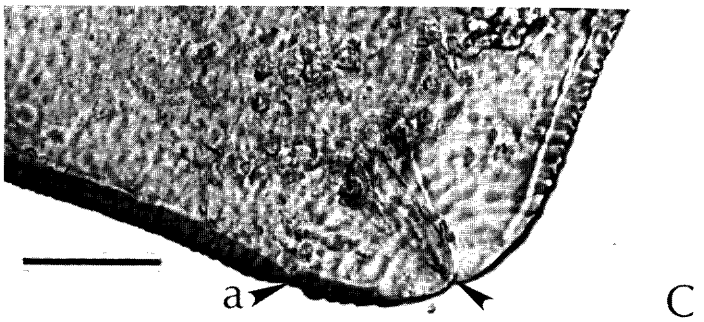

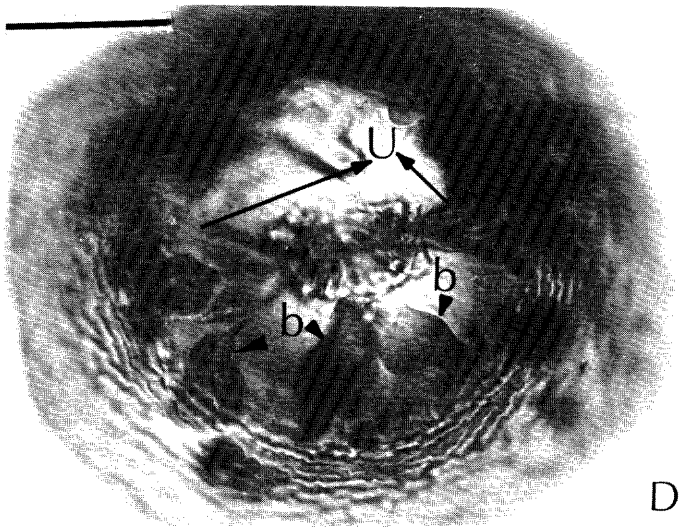

Fig. 2. Vulval cones of *Heterodera trifolii* cysts. Scale bars = 42 µm in A and B, and 45 µm in C and D. A,B) End views of vulval cone showing semifenestrae (S) and vulval slit (V). C) Lateral view of vulval cone showing anus (a) and vulva (V). D) Underbridge (U) and prominent bullae (b).

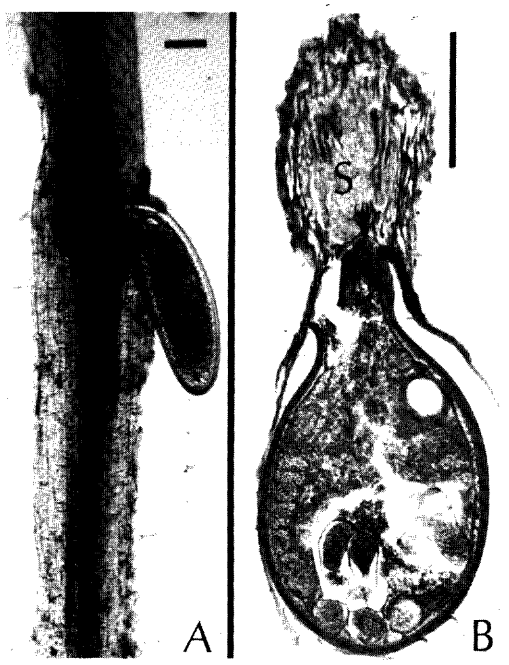

Fig. 3. White clover roots infected with swollen juveniles of *Heterodera trifolii*. Scale bars = 75 µm in A and 90 µm in B. A) Swollen juvenile protruding from the surface of a feeder root. B) Root cross section infected by a swollen juvenile feeding on a syncytium (S) in the stele.

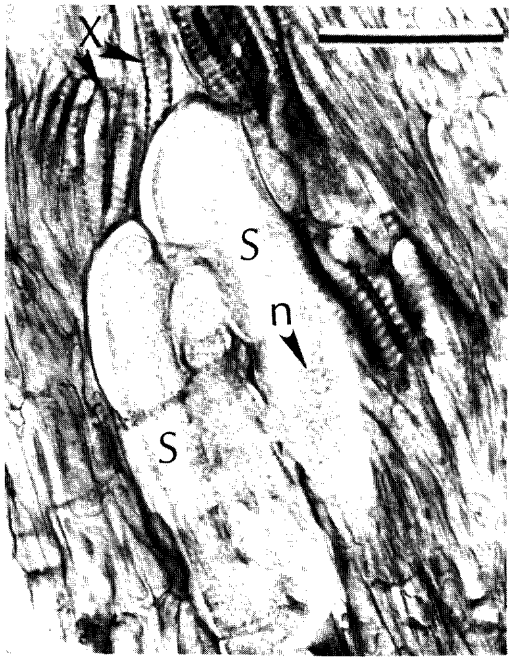

Fig. 4. Longitudinal section of a white clover root showing a syncytium (S) induced by *Heterodera trifolii* in the stele. n = nucleus, x = xylem elements. Scale bar = 50 µm.

COMPARATIVE MORPHOLOGICAL CHARACTERISTICS OF CYST-FORMING NEMATODES FOUND IN FLORIDA: *H. trifolii* belongs to the large group of cyst forming nematodes with morphological characters similar to those of the sugarbeet cyst nematode, *H. schachtii* Schmidt. *H. trifolii* has lemon-shaped bullate cysts with a vulval slit > 35 µm long (Figs. 1A,2A-D) (Mulvey and Anderson 1974). *H. trifolii* differs from other *Heterodera* species reported in Florida (Donaldson 1964; Esser and Langdon 1967; Inserra *et al.* 1989; MacGowan 1983; Vovlas *et al.* 1989) as follows:

H. trifolii cysts have irregular globose bodies (bullae) below the fenestra (transparent or open region of the vulval cone) (Fig. 2D), whereas cysts of *H. cyperi* Golden *et al.* and *H. graminophila* Golden and Birchfield do not. *H. trifolii* cysts have longer underbridge (vaginal bifurcation extending across the vulval cone) (Fig. 2D) than that of *H. fici* Kirjanova cysts (80-105 vs. 75 µm), longer fenestra, and a shorter vulva-anus distance than those of *H. schachtii* cysts (40-60 vs. 24-38 µm and 54-68 vs. 65-111 µm, respectively). *H. trifolii* cysts are undistinguishable from those of *H. glycines* Ichinoe (Hirschmann 1956). Juvenile stages emerging from eggs (second-stage juveniles = J2) of *H. trifolii* (Fig. 1B), however, have stylets longer than those of *H. glycines* J2 (25.5-29 vs. 22-24 µm). The upper values of J2 body length of *H. trifolii* (> 500 µm) are greater than those of *H. fici, H. glycines* and *H. schachtii* (< 500 µm). Lateral field of *H. trifolii* J2 has four lines while that of *H. leuceilyma* J2 has three. Body, stylet and tail length of 40 *H. trifolii* J2 from Pinellas county ranged 444-531, 27-28, and 54-64.5 µm, respectively.

GEOGRAPHICAL DISTRIBUTION: *H. trifolii* is a cosmopolitan species present in North America, western and eastern Europe, Israel, India, Australia, and New Zealand (Mulvey and Anderson 1974). In the United States, this nematode has been reported in 30 states including California and Hawaii (Norton and Isely 1967).

HOST RANGE: Forage legume hosts of *H. trifolii* include *Trifolium repens* L., which is the most susceptible host, and *T. fragiferum* L. (strawberry clover), *T. isthmocarpum* Brot., *T. pratense* L., *T. resupinatum* L. (Persian clover), and *T. retusum* L. (teasel clover). Other *Trifolium* spp. allow slight nematode reproduction (Eriksson 1972; Norton and Isley 1967). Host preference of *H. trifolii* varies with nematode race or isolate. For example, white and red clover are not suitable hosts of the sugarbeet race reported from The Netherlands (Maas *et al.* 1982; Maas and Heijbroek 1982), but are infected by the clover race present in the USA. Other forage legume hosts are *Lotus oroboides* (Humb., Bonpl. and Kunth) Ottley, *Melilotus* spp., *Medicago pironae* Vis. and *Vicia villosa* Roth. Non-forage hosts include *Phaseolus vulgaris* L. (bean), *Dianthus caryophyllus* L. (carnation), *Pisum sativum* L. (garden pea), *D. chinensis* L. 'Heddewigii' (pink), *Spinacia oleracea* L. (spinach) as well as some weeds *Galeopsis* X *tetrahit* L., *Rumex crispus* L., and *Spergula arvensis* L., and *Hebe* X *andersonii* (Lindl. and Paxt.) Cockayne, (an ornamental shrub). The weeds and the ornamental mentioned above are used as differential hosts to characterize nematode physiological races (Maas *et al.* 1982). Some curcurbits, *Cucumis melo* L., *Curcurbita maxima* L., and *C. pepo* L., allow slight nematode reproduction. The sugarbeet race of *H. trifolii*, which is not present in the USA, infects mainly *Beta vulgaris* L. (sugarbeet) and cruciferous crops including *Brassica oleracea* L. Capitata Group (cabbage), *B. oleracea* L. Botrytis Group (cauliflower), *Raphanus sativus* L. (radish), and *Rheum rhabarbarum* L. (rhubarb) (Maas *et al.* 1982).

BIOLOGY: *H. trifolii* J2 emerge from eggs inside the cysts, migrate through the soil and penetrate host roots. Inside root tissues, J2 become sedentary and swollen (Fig. 3 A,B) and complete their development by feeding on specialized stelar cells (Fig. 4). Swollen females rupture root tissues with the posterior portion of their bodies which protrude from the root surface. In some instances, swollen juveniles of the Florida population were observed with their posterior bodies protruding from the root surface (Fig. 3A,B). Females retain the majority of eggs inside their bodies and the remaining in egg sacs. Gravid females die at the end of their life cycle and become cysts (Fig. 1B) which contain 200-500 eggs in the Florida population. At 21-27° C (70-80° F), nematode development from J2 invasion to the appearance of females with egg masses requires 22-28 days. At these temperatures brown cysts appear 33 days after J2 root penetration (Mulvey and Anderson 1974). At lower temperatures, ranging from 10 to 20° C (50-68° F), brown cysts appear 60 days after J2 penetration in the roots (Mulvey and Anderson 1974). Under field conditions and on a susceptible host such as white clover, *H. trifolii* can have from three to eight generations per year (Mulvey and Anderson 1974). Egg hatch and root penetration by J2 can occur at temperatures as low as 10° C (50° F). The optimal temperatures for nematode development are 25-26° C (77-79° F) (Mulvey and Anderson 1974). Temperatures above 30° C (86° F) prevent J2 emergence from eggs (Maas and Heijbroek 1982).

SYMPTOMS AND YIELD LOSSES: Aboveground symptoms induced by *H. trifolii* infections are not specific. Infected pastures show vacant patches where plants are dead or chlorotic and poorly developed (Eriksson 1972; Maas and Heijbroek 1982). Concomitant infection of pathogenic fungi (*Rhizoctonia* spp. and *Fusarium* spp.), mild temperatures and low soil moisture increase plant damage caused by this nematode. Yield losses vary with nematode physiological races. Damage induced by the sugarbeet race is more severe to sugarbeet than clover (Maas and Heijbroek 1982). White clovers are considered to be more susceptible to the clover race of *H. trifolii* than red clovers (Eriksson 1972). However, yield losses depend more on the clover cultivar than on the clover species used.

Although *H. trifolii* has been known in Florida since 1955, this nematode has not become a major economic pest of pastures in the state. One major reason is the limited use in the past of white and red clovers as forage legumes. The absence in Florida of the aggressive sugarbeet race of *H. trifolii*, which attacks crops more commonly grown in Florida (such as cabbage, radish, spinach, sugarbeet, and turnip) has also limited the impact of this nematode on Florida agriculture. High soil temperatures of late spring, summer and early fall above 30° C (86° F), which inhibit J2 emergence, may also

play a role in suppressing nematode densities in infested soil. Frequent use of clover as a cover crop in north-central Florida may increase the incidence of nematode infestations in the state.

NEMATODE MANAGEMENT: *H. trifolii* cysts can survive for many years in the absence of host plants. However, the number of viable cysts declines with time (80% in one year) because of the adverse effects of biological antagonists such as nematophagous fungi (Maas and Heijbroek 1982). Crop rotations with non-host crops (e.g., *Zea mays* L. (corn) and other cereals) or non-host forage legumes (e.g., as cowpea and joint-vetch) are effective in suppressing the population densities of this pest. More costly management practices, including soil solarization or chemical methods, are not economically feasible for pastures. White clover cultivars resistant to *H. trifolii* have been selected in northern Europe (Mulvey and Anderson 1974). Substitution of white clovers with certain red clover cultivars could be effective in nematode-infested fields. Host preference of nematode populations in the field should be known before adopting new red clover cultivars.

SURVEY AND DETECTION: The sugarbeet race of *H. trifolii* is not present in Florida, but its accidental introduction can dramatically change the economic importance of this pest in the state. Cruciferous crops (cabbage, cauliflower, radish and turnip) in addition to sugarbeet and spinach, are potential hosts of the nematode. These crops should be checked for patches of stunted plants with chlorotic leaves and other nutrient deficiency symptoms. Soil and roots from these suspected plants should be collected and roots examined with the aid of a stereomicroscope for the presence of swollen females and cysts of this nematode race.

Pastures with the new red clover cultivars, such as 'Cherokee,' should also be monitored for patches of stunted plants infected by new isolates of *H. trifolii* clover race which, so far, has been detected only on white clover in Florida.

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