The Mediterranean Cereal Cyst Nematode, *Heterodera latipons*: a Menace to Cool Season Cereals of the United States[1]

N. Greco[2], N. Vovlas[2], A. Troccoli[2] and R.N. Inserra[3]

INTRODUCTION: Cool season cereals, such as hard and bread wheat, oats and barley, are among the major staple crops of economic importance worldwide. These monocots are parasitized by many pathogens and pests including plant parasitic nematodes. Among nematodes, cyst-forming nematodes (*Heterodera* spp.) are considered to be very damaging because of crop losses they induce and their worldwide distribution. The most economically important cereal cyst nematode species damaging winter cereals are: *Heterodera avenae* Wollenweber, which occurs in the United States and is the most widespread and damaging on a world basis; *H. filipjevi* (Madzhidov) Stelter, found in Europe and Mediterranean areas and most often confused with *H. avenae*; and *H. hordecalis* Andersson, which seems to be confined to central and north European countries. In the 1950s and early 1960s, a cyst nematode was detected in the Mediterranean region (Israel and Libya) on the roots of stunted wheat plants (Fig. 1 A,B). It was described as a new species and named *H. latipons* based on morphological characteristics of the Israel population (Franklin 1969). Subsequently, damage by *H. latipons* was reported on cereals in other Mediterranean countries (Fig. 1).

MORPHOLOGICAL CHARACTERISTICS AND DIAGNOSIS: *Heterodera latipons* cysts are typically ovoid to lemon-shaped as those of *H. avenae*. They belong to the *H. avenae* group because they have short vulva slits (< 16 µm) (Figs. 2A; 3A) (Baldwin and Mundo-Ocampo 1991). The fenestration of *H. latipons* cysts shows two distinct semi-fenestrae, which are more than a semifenestral width apart (Figs. 2A,B; 3A). The underbridge is strong and shows a pronounced thickening in the middle (Figs. 2C; 4A,B) from which the name of the species is derived. The extremities of the underbridge are bi-trifurcate (Figs. 2C; 4A,B). Bullae are few to absent.

Morphometrics (from Franklin 1969):
Cysts: Fenestral length 58-76 µm; fenestral width 15-27 µm; semi-fenestral length 13-19 µm; vulval slit length 6-9 µm; vulval bridge length 18-39 µm; underbridge length 80-125 µm; underbridge width 7-14 µm; sub-crystalline layer present.
Females: Body length (excluding neck) 348-645 µm; body width 277-510 µm; neck length 58-103 µm; stylet length 21-28 µm.
Embryonated eggs: length 100-124 µm; width 44-56 µm.
Males: Body length 960-1406 µm; body width 25-32.5 µm; stylet length 22-29 µm; spicule length 32-36 µm; lateral field with four longitudinal incisures.
Second stage juveniles (J2s): Body length 401-478 µm; body width 19-22 µm; tail length 42-54 µm; length of the hyaline tail tip 20-31 µm; stylet length 23-25 µm; lateral field with four incisures.

Fig. 1. Damaging effect of *Heterodera latipons* on cereals. A) Uneven growth of durum wheat in a nematode infested field in Syria. B) Chlorotic and stunted barley stand in experimental plots infested by the nematode in Cyprus.

[1]Contribution No. 483, Bureau of Entomology, Nematology and Plant Pathology – Nematology Section.
[2]Nematologists, C.N.R., Istituto per la Protezione delle Piante, Sezione di Bari, 70126 BBari, Italy.
[3]Nematologist, Division of Plant Industry, FDACS, P.O. Box 147100, Gainesville, Florida 32614-7100.

Cyst, J2, male and female morphology provides diagnostic information for the identification of this nematode species (Handoo 2002). The cysts of *H. latipons* closely resemble those of *H. hordecalis* and *H. turcomanica*. These three cyst-forming nematodes share similar morphological characters such as circular semi-fenestrae separated by a distance longer than semi-fenestra diameter, rather typical underbridge having in the middle a pronounced enlargement facing the vulval slit (Figs. 2C; 4A-C). These morphological similarities make the separation of these three cyst nematodes difficult.

Cysts of *H. hordecalis* when compared with those of *H. latipons* appear paler and more transparent with a less pointed cone tip. The most important differentiating character between these two species is the vulval slit which in *H. latipons* is much shorter than in *H. hordecalis* (6-9 µm *vs.* 17-25.5 µm) (Figs. 3A,B). It is worthwhile to mention that *H. hordecalis* cysts have length values of vulval slit exceeding those (<16 µm) considered standard for the species included in the *H. avenae* group (Baldwin and Mundo-Ocampo 1991). The underbridge enlargement is more sclerotized in *H. latipons* than in *H. hordecalis* (Fig. 4A-C). Moreover, the J2 stylet knobs are more anteriorly concave in *H. hordecalis* than in *H. latipons*, while tail and hyaline portion of the tail in *H. latipons* are slightly shorter than in *H. hordecalis*, 42-54 µm *vs.* 44-64 µm and 20-31 µm *vs.* 29-46 µm, respectively (Fig. 5). The tail tip is less pointed in *H. latipons* than in *H. hordecalis*.

Cysts of *H. latipons* differ from those of *H. turcomanica* by having a shorter vulval slit (6-9 µm *vs.* 9-14 µm) and lacking the small gland-like sacs beneath the cuticle, which are reported in *H. turcomanica*, and for the absence of bullae. Moreover, eggs of *H. latipons* are larger (48x112 µm) than those of *H. turcomanica* (40x77 µm). No J2s, males and host plants were found for *H. turcomanica* (Franklin 1969).

Heterodera latipons cysts are similar to those of *H. avenae*, which also have circular semi-fenestrae, but they lack an underbridge and the semifenestrae are much closer than in the three species mentioned above (Fig. 3C). Morphological characters of J2s are very useful for the separation of *H. latipons* from *H. avenae.* Second-stage juveniles of *H. latipons* are shorter than those of *H. avenae* (454 µm *vs.* 575 µm) and also have a shorter hyaline portion of the tail (20-31 µm *vs.* 35-45 µm) *Heterodera latipons* cysts differ from those of *H. filipjevi*, a cyst nematode occurring on cereals in Europe, because they lack bullae which are present in those of *H. filipjevi*. J2s of *H. latipons* differ from those of *H. filipjevi* for the shorter hyaline portion of the tail (20-31 µm *vs.* 31-39 µm).

Bekal *et al*. (1997) were able to identify by molecular methods individual cysts and females of *H. avenae*, *H. filipjevi*, *H. latipons* and *H. mani*, based on restriction fragment length polymorphisms (RFLPs) of amplified nuclear ribosomal DNA. These species were also differentiated by electrophoresis on cellulose acetate plates using the enzymes esterase and malate dehydrogenase and aliquots of 25 females (Mokably *et al*. 2001). No attempt has been made to identify *H. hordecalis* and *H. turcomanica* by molecular methods. The separation of these two species based on morphological differentiation is not reliable when only a few specimens are available for the diagnosis.

GEOGRAPHICAL DISTRIBUTION: *Heterodera latipons* has been reported in Israel, Libya, Bulgaria (Franklin 1969; Stoyanov 1982), Greece, Poland, Scotland, USSR (Mulvey and Golden, 1983), Italy (Tacconi 1976), Spain (Romero 1980), Jordan, Lebanon, Syria (Greco, unpublished), Cyprus (Philis 1988), Turkey (Rumpenhorst *et al*. 1996), Japan (Momota 1979) and Prince Edward Island, Canada (Mulvey and Golden 1983).

HOSTS: According to Franklin (1969), *Heterodera latipons* parasitizes barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rye (*Secale cerale* L.) and wheat (*Trititicum aestivum* L.). Other hosts include canary grass (*Phalaris* sp.) (Mor *et al*. 1992). It seems that hard and bread wheat (*T. durum* Desf.) are poor hosts of this nematode (Scholz 2001).

SYMPTOMS, BIOLOGY AND DAMAGE: Slight to severe yellowing of cereal stands can be observed at an early stage of nematode infestation. Later, infested fields show patchy plant growth (Fig. 1) associated with poor tillering and shorter spikes. These symptoms are similar to those caused by other biotic and abiotic soil stresses. Before plant flowering, white lemon-shaped females can be observed on the roots by the naked eye or under a dissecting microscope after gently shaking or washing the roots to remove adhering soil.

The biology of *H. latipons* is similar to that of *H. avenae*. *Heterodera latipons* mature females produce a gelatinous matrix without eggs, as the eggs are retained inside the nematode body. At the completion of the life cycle, the female dies and becomes a cyst containing eggs. Cysts are the survival stage of the nematode. Laboratory experiments (Scholz 2001) demonstrated that eggs hatch in greater numbers from older (4-5 months) than from younger (1-2 months) cysts after exposing the cysts at temperatures of 5°C or 10°C. Temperatures in the range 5-15°C appear to be the most suitable for egg hatch, while at 20-25°C egg hatch seems to be suppressed. Hatch in water appears similar to that in barley root diffusates (Scholz 2001). Field studies under Mediterranean conditions indicate that nematode J2s occur in the soil from November to February when soil temperatures

do not exceed 18°C (Mor *et al*. 1992; Philis 1999; Scholz 2001). Usually J2s in soil peak at plant emergence (Philis 1999). Juveniles invade roots behind the root apex (at root apex in *H. avenae*) (Mor *et al*. 1992) at the beginning of plant emergence in November-December. Females and males develop by the end of January, and females lay eggs by February. Embryonated eggs can be observed by early March, when well-developed white females can be easily observed on the roots. From April onwards white females turn to brown cysts coated by a sub-crystalline layer. In cooler areas, as in inland Syria, the development of the nematode can be delayed (Scholz 2001). Accumulated day degrees, above 7°C, for the development of white females and cysts with coiled embryos were 215 and 386, respectively (Philis 1999). Only one generation per growing season is completed. Roots infested by *H. latipons* do not show the typical branching of *H. avenae*-infested roots (Mor *et al*. 1992) and symptoms on above-ground plant parts can be observed as soon as severe root infestation occurs.

Although severe damage has been observed, no investigation has been undertaken to relate nematode population densities with yield of the host crops. Therefore, little information is available on the extent of yield losses caused by *H. latipons*. Philis (1988) reported up to 50% yield loss of barley in Cyprus. In Syria, Scholz (2001) reported barley yield loss of 24% in a field infested with 28 eggs and juveniles/g soil located in an area with 279 mm annual rainfall, but no significant yield reduction was observed in soil infested with up to 10 eggs/g soil in areas with 411 mm annual rainfall. These findings would confirm field observations that the nematode is more damaging under water-stress conditions. Moreover, damage is more severe in fields infested concomitantly by *H. latipons* and the fungus *Bipolaris sorokiniana* (Sacc.) Shoemaker, the causal agent of the common root rot and seedling blight of barley, as the nematode increases the aggressiveness of the fungus (Scholz 2001).

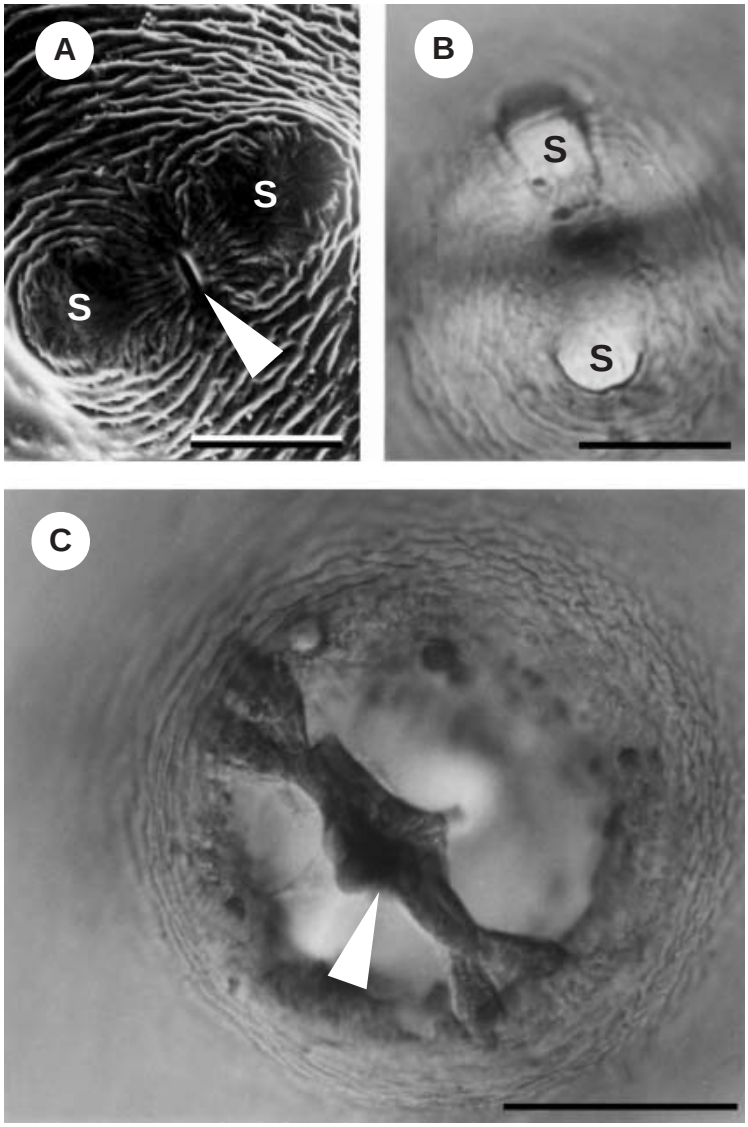

Fig. 2. Micrographs and photographs showing the anatomical characteristics of *Heterodera latipons* cysts. A) Scanning electron microscopy (SEM) micrograph of the vulval area of a newly formed cyst. Note the short vulval slit (arrow) and the area in which fenestration (S) will occur at a late stage of cyst formation. B) Light microscopy photograph of a perineal pattern of a nematode cyst showing well-separated and circular semi-fenestrae (S). C) Perineal patterns observed with a light microscope showing a well-developed and a rather long underbridge, bifurcated at both ends and with a sclerotized enlargement (arrow) in the middle. Scale bars = 20 μm.

SURVEY AND DETECTION: As for other cyst-forming nematodes, in the absence of a host crop, soil samples must be collected and processed to extract cysts. Perineal patterns of cysts must then be prepared, observed under a microscope and compared with those of the original description (Franklin 1969). In the presence of a host crop (winter cereals), close examination of roots from February (Mediterranean coastal area) onwards will reveal the presence of white lemon-shaped females. However, at a later stage of development, females become easily detached from the roots making the detection of the nematode very difficult even in cases of heavy infestation, especially in non-sandy soils. According to Mor *et al*. (1992), but contrary to what is stated by Philis (1997), roots of cereals infested by *H. latipons* do not show the typical branching of those infested by *H. avenae*. Moreover, females of *H. latipons* are rather isolated while in *H.avenae* they tend to be grouped.

Considering the large variety of climatic conditions under which *H. latipons* has been reported and its temperature requirements for development (Mor *et al*.1992; Philis 1997 1999, Scholz 2001), there is no doubt that this cyst-forming nematode would become a very noxious pest for winter cereals, especially in temperate states in the United States. About 10,000 acres in the

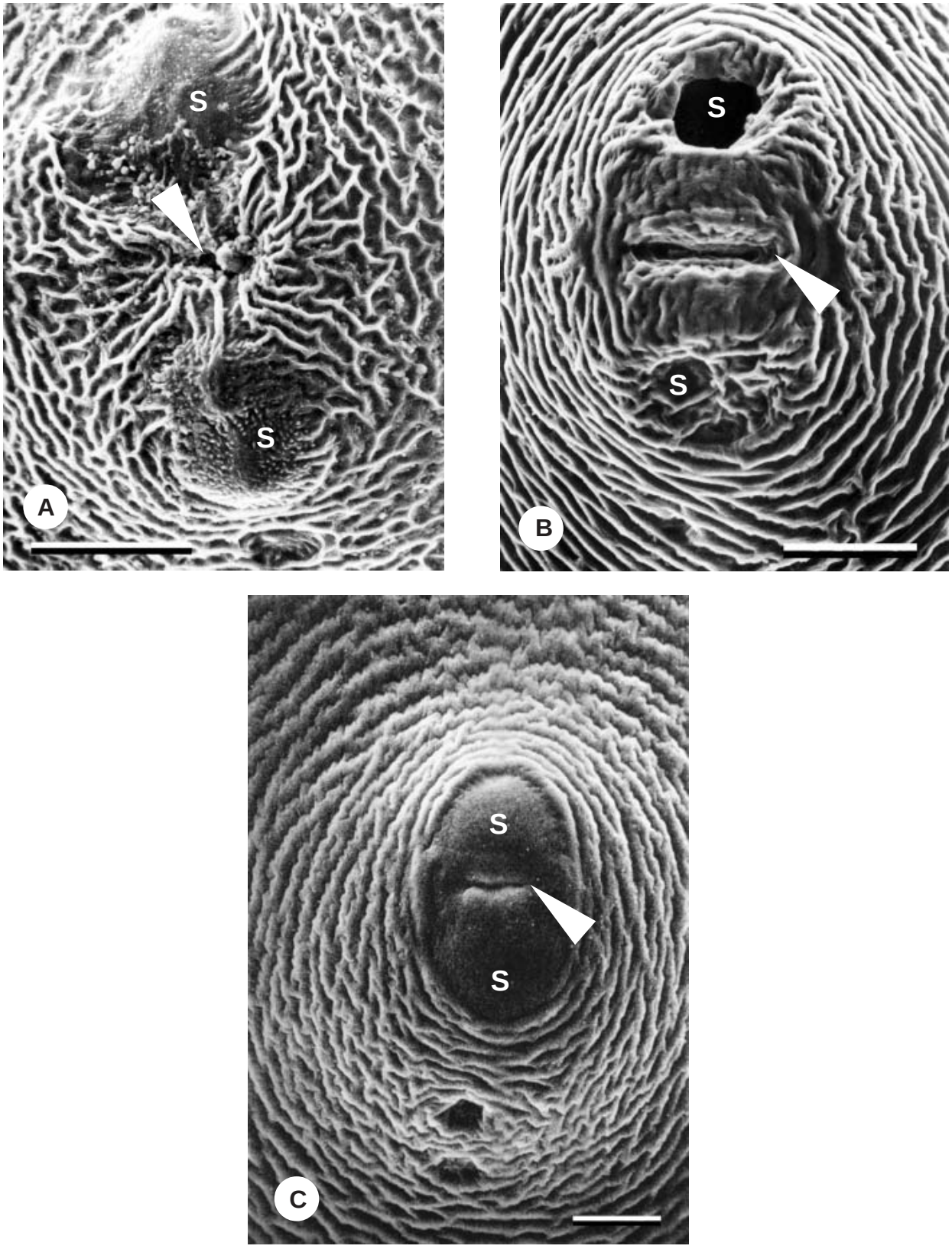

Fig. 3. SEM micrographs of the vulval area of (A) *H. latipons*, (B) *H. hordecalis*, and (C) *H. avenae*. Note that semi-fenestrae (S) are well separated in *H. latipons* and *H. hordecalis* and close to each other in *H. avenae*. Also note differences in the length of the vulval slit (arrow) of the three species. Scale bars = 20 μm.

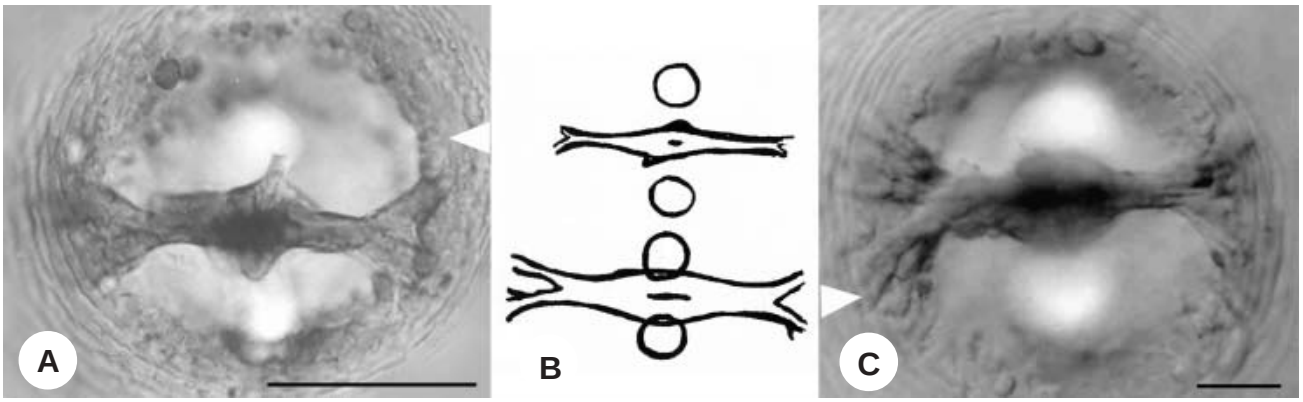

Fig. 4. Light microscopy photographs of the perineal area of cysts of (A) *H. latipons* and (C) *H. hordecalis*. Note the underbridge with bifurcated ends in both species and with an enlargement in the middle, which is more sclerotized in (A) *H. latipons*. Diagram of the underbridge (B): *H. latipons* (upper) and *H. hordecalis* (lower). Scale bars = 20 µm.

Florida Panhandle are cultivated to wheat (Florida Department of Agriculture and Consumer Services 2002). These wheat-growing areas need to be monitored for the introduction of this pest.

CONTROL METHODS: *Heterodera latipons* has a rather narrow host range and the best way to control the nematode is by crop rotation. Information on the decline of the nematode soil population in the absence of a host is lacking, but because the nematode behaves similarly to *H. avenae*, a 3-5 year crop rotation would be satisfactory. Scholz (2001) tested the reaction to *H. latipons* on several cultivars and lines of barley, wheat and oats, including those suggested to identify the pathotypes of *H. avenae*. He found that several barley cultivars of the international differential set for pathotyping *H. avenae*, the cv Rihane and the line M64-76/Bon/Jo/York, were resistant. Several wheat cultivars of the international set and all the hard and bread wheat tested were resistant to *H. latipons.* No resistance was observed in oat cvs of the international testing set. Because in tests conducted by Scholz (2001) the reproduction rate of *H. latipons* was rather low on susceptible cultivars, the observed resistance needs to be confirmed.

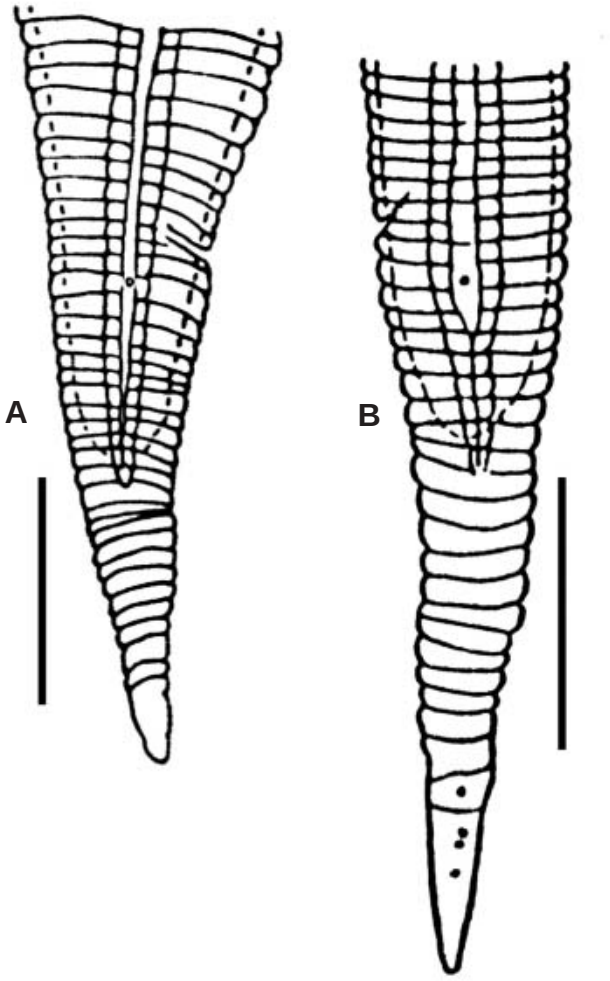

Fig 5. Tails of (A) *H. latipons* and (B) *H. hordecalis*. Note that tail and tail hyaline portion lengths are shorter in *H. latipons*. Scale bars = 20 µm.

LITERATURE CITED

Andersson, S. 1974. *Heterodera hordecalis* n.sp. (Nematoda: Heteroderidae) a cyst nematode of cereals and grasses in southern Sweden. Nematologica 20: 445-454.

Bekal, S., J.P. Gauthier and R. Rivoal. 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. Genome 40: 479-486.

Florida Department of Agriculture and Consumer Services. 2002. The Florida Agricultural Facts Directory. Division of Marketing and Development, Tallahassee, Florida. 168 pp.

Franklin, M.T. 1969. *Heterodera latipons* n.sp., a cereal cyst nematode from the Mediterranean region. Nematologica 15: 535-542.

Handoo, Z.A. 2002. A key and compendium to species of the *Heterodera avenae* group (Nematoda: Heteroderidae). Journal of Nematology 34: 250-262.

Mokably, A., S. Valette and R. Rivoal. 2001. Différenciation de quelques espèces de nématodes à kystes des céréales ed des graminées par èlectrophorèse sur gel d'acétate de cellulose. Nematologia Mediterranea 29: 103-108.

Momota, Y. 1979. The first report of *Heterodera latipons* Franklin, 1969 in Japan. Japanese Journal of Nematology 9: 73-74.

Mor, M., E. Cohn and Y. Spiegel. 1992. Phenology, pathogenicity and pathotypes of cereal cyst nematodes, *Heterodera avenae* and *H. latipons* (Nematoda: Heteroderidae) in Israel. Nematologica 38: 494-501.

Mulvey, R.H. and A. M. Golden. 1983. An illustrated key to the cyst-forming genera and species of Heteroderidae in the western hemisphere with species morphometrics and distribution. Journal of Nematology 15: 1-59.

Philis, I. 1988. Occurrence of *Heterodera latipons* on barley in Cyprus. Nematologia Mediterranea 16: 223.

Philis, I. 1997. *Heterodera latipons* and *Pratylenchus thornei* attacking barley in Cyprus. Nematologia Mediterranea 25: 305-309.

Philis, I. 1999. The life cycle of the Mediterranean cereal cyst nematode *Heterodera latipons* in Cyprus. Nematologia Mediterranea 27: 43-46.

Romero, M.D. 1980. *Heterodera latipons* especie nueva para España. Nematologia Mediterranea 8: 95-98.

Rumpenhorst, H.J., I.H. Elekçioglu, D. Sturhan, G. Oztürk and S. Enneli. 1996. The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. Nematologia Mediterranea 24: 135-138.

Scholz, U. 2001. Biology, pathogenicity and control of the cereal cyst nematode *Heterodera latipons* Franklin on wheat and barley under semiarid conditions, and interactions with common root rot *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph: *Cochliobolus sativus* (Ito *et* Kurib.) Drechs. Ex Dastur.]. Ph.D. Thesis Dissertation, University of Bonn, Germany. 161 pp.

Stoyanov, D. 1982. Cyst-forming nematodes on cereals in Bulgaria. EPPO Bulletin 12: 341-344.

Tacconi, R. 1976. Sulla presenza di *Heterodera carotae* Jones, 1950 e *Heterodera latipons* Franklin, 1969 nel Veneto. Redia 59: 305-311.

PI-03T-03