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STUDIES ON *HETERODERA DAVERTI* ON EGYPTIAN CLOVER *TRIFOLIUM ALEXANDRINUM*

by

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Summary. Egyptian clover in Giza was severely infected with *Heterodera daverti* Wouts *et* Sturhan 1978. The nematodes were identified on the basis of the morphology of second stage juveniles, characters of the cyst vulval cone and host range.

Egyptian clover, *Trifolium alexandrinum* L. has been reported as a good host for the soybean cyst nematode *Heterodera glycines* Ichinohe in Giza (Elmiligy, 1968; Ghorab, 1972) but the species was later re-identified as *H. cajani* Koshy (Aboul-Eid and Ghorab, 1974). Egyptian clover has also been reported as susceptible to *H. trifolii* Goffart (Massoud, 1980).

In the investigations reported here, the species of *Heterodera* severely infecting Egyptian clover was examined by light and electron scanning microscopy and identified as *H. daverti* Wouts *et* Sturhan, 1978 on the basis of morphometrics, morphology and host range.

Materials and methods

Roots of Egyptian clover and soil samples were collected in the Giza Province during the growing season. White females were still attached to the roots but brown cysts were collected from the soil samples. A population of the nematodes was maintained on Egyptian clover plants in the greenhouse for further studies.

Second stage juveniles, males and white females were fixed in F.A., 4:1 (Seinhorst, 1966) and examined by light microscopy. Cone tops (posterior end of the cyst) were prepared by the technique described by Mulvey (1972); the posterior ends from dry cysts were soaked in water for 24 hrs before dissection and any adhering body contents were cleaned out; the vulval cones were then bleached for a few minutes in hydrogen peroxide, washed in distilled water and passed through a graded series of ethyl alcohol, cleaned in clove oil and mounted on glass slides in Canada balsam.

Females and cysts prepared for scanning electron microscopy (SEM) were dehydrated to absolute alcohol in a diffusion chamber before transfer to a graded series of amyl acetate in absolute alcohol and finally to absolute amyl acetate. Specimens were ultrasonicated for one minute, dried to their critical point, mounted on stubs with double sided Scotch tape and gold coated. The prepared specimens were viewed in a ISI 130 dual-stage SEM at 10 KV accelerating voltage.

Nematode reproduction was studied on 20 cultivars of leguminous crops (Table I). Plants were grown singly in 15 cm diameter pots containing steam-sterilized soil. Two thousand eggs and juveniles obtained from 20 crushed cysts of *H. daverti* maintained on Egyptian clover were added to each pot. There were three replicates for each host, with and without nematodes. All the pots were kept in a greenhouse at 20-35°C. Eight weeks later the plants were uprooted and the soil was screened on a pair of collecting sieves (20 meshes/in over 60 meshes/in). The soil samples suspended in water then carefully poured into a glass cylinder (20 cm in height and 10 cm in diameter) lined with a strip of filter paper. After adding several drops of soapy solution, the paper strip was removed from the container and cysts and females were counted.

Results

The morphology and morphometrics of *H. daverti* found on Egyptian clover are as follows:
Second stage juveniles (Fig. 1, C, D) n=20: body length 471 (386-498) μm ; stylet length 25 (21-26) μm ; tail length 54 (40-61) μm ; hyaline tail terminus 30 (19-34) μm .

TABLE I - Reproduction of *H. daverti* from Egypt on selected host plants.

Host	Cultivar	No. of cyst/pot	Cyst Index % *
1. <i>Trifolium alexandrinum</i> L.	Meskawi	2500	100
2. <i>T. alexandrinum</i> L.	Khadrawy	1800	72.0
3. <i>T. alexandrinum</i> L.	Fahel	1180	47.2
4. <i>T. alexandrinum</i> L.	Seidy	250	10.0
5. <i>T. pratense</i> L.	Chlean	1400	56.0
6. <i>T. repens</i> L.	Tamar	1330	53.2
7. <i>Phaseolus vulgaris</i> L.	Contender	750	30.0
8. <i>P. aureus</i> Roxb.	Berbon	330	13.2
9. <i>Pisum sativum</i> L.	Little marvel	660	26.4
10. <i>Trigonella foenum graecum</i> L.	Giza 2	345	13.8
11. <i>Vicia faba</i> L.	Giza 2	310	12.4
12. <i>Vigna sinensis</i> L. (Savi)	Black eye	300	12.0
13. <i>Lupinus digitatus</i> L.	Giza 2	60	2.4
14. <i>Lens culinaris</i> Medic.	Giza 9	25	1.0
15. <i>Dolichos lablab</i> L.	—	65	2.6
16. <i>Cicer arietinum</i> L.	Giza 2	20	0.8
17. <i>Cajanus cajan</i> L.	—	30	1.2
18. <i>Arachis hypogaea</i> L.	Giza 4	40	1.6
19. <i>Glycine max</i> (L.) Mer.	Williams	0	0.0
20. <i>Medicago sativa</i> L.	Atlantica	0	0.0

$$* \text{ Cyst Index} = \frac{\text{No. cysts on cv.}}{\text{No. cysts on cv. Meskawi}} \times 100$$

The short body length, short sturdy stylet, short tail and hyaline tail portion are characteristic of the species.

White adult females (Fig. 2A-D): these pass through a distinctive pale yellow phase before the brown cyst stage develops; a subcrystalline layer is present in young females; a gelatinous matrix was found attached to the vulval end of the developing cyst.

Cysts (Fig. 1, E, F, Fig. 3A-D) n=20: body length 655 (530-760) μm ; body width 381 (380-430) μm ; vulval slit 48 (45-52) μm ; fenestral length 42 (36-45) μm ; fenestral width 37 (33-41) μm . Cysts typically lemon-shaped, with a coarse zig-zag cuticular pattern. Newly formed cysts are characterized by the strongly developed bullae and heavy underbridge of the cone tops.

Males (Fig. 1, A, B) n=10: body length 1150 (1100-1200) μm ; body width 35 (28-40) μm ; stylet length 29 (28-31) μm . The presence of abundant males in the populations indicated that *H. daverti* is amphimictic.

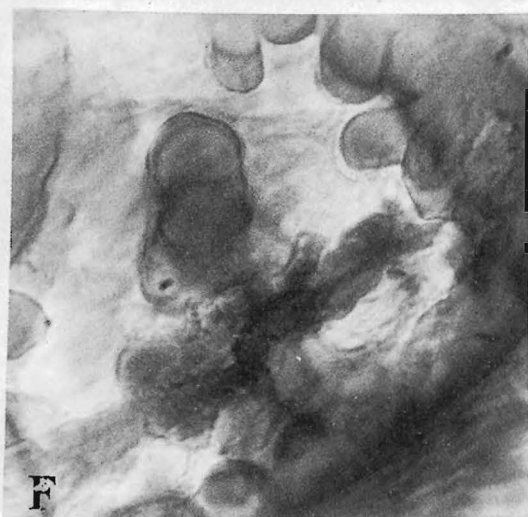
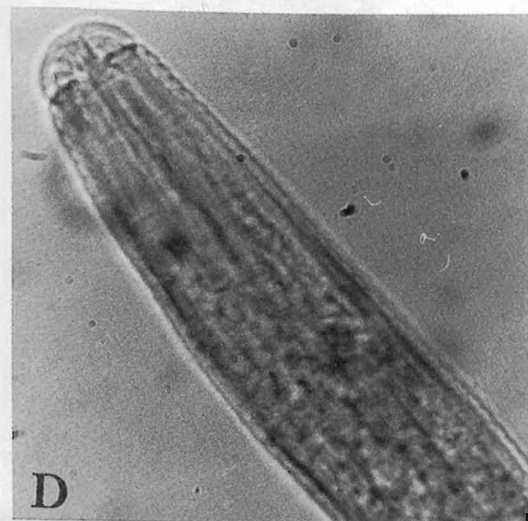
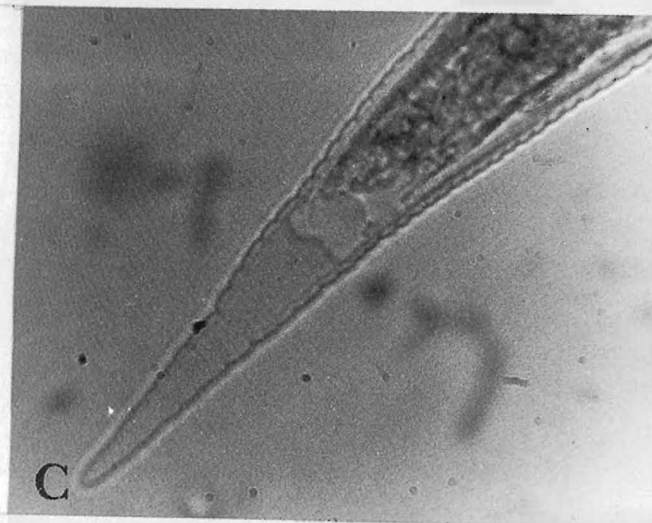
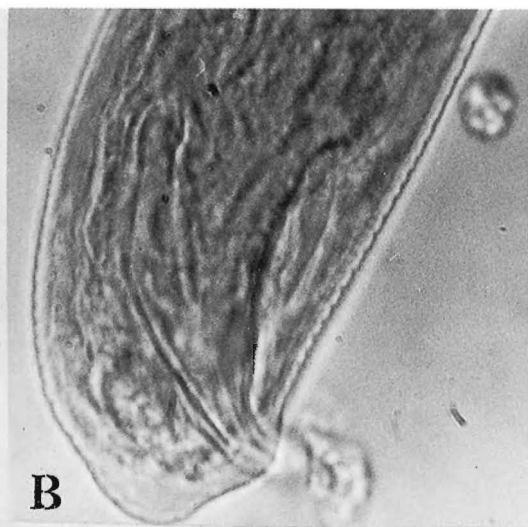
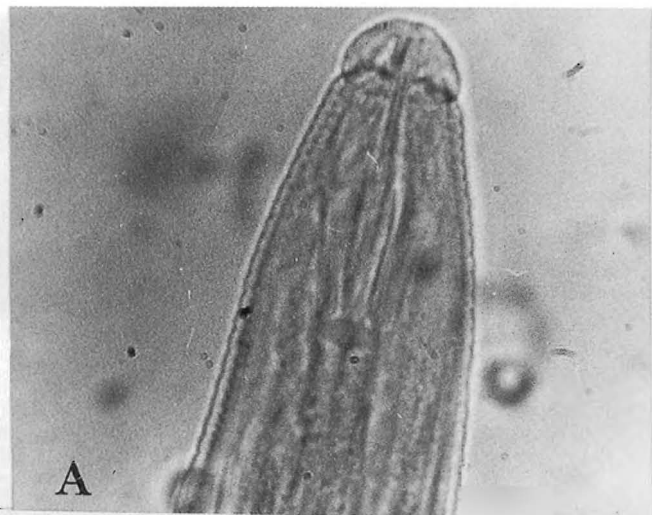
H. daverti reproduced on all of the tested leguminous cultivars, except those of soybean and alfalfa (Table I). The largest number of cysts per pot were

recovered from *T. alexandrinum* cv. Meskawi. *T. alexandrinum* cvs Meskawi and Khadrawy, *T. pratense* cv. Chlean and *T. repens* cv. Tamar were the most suitable hosts for *H. daverti*.

Discussion

H. daverti belongs to the group of *Heterodera* with lemon-shaped ambifenestrate cysts, long vulval slit and well-developed bullae and underbridge. It is distinguished from species with similar cyst features by characters of the second stage juvenile. *H. daverti* and *H. trifolii* cannot be distinguished by vulval cone characteristics but the juvenile morphology differs. Second stage juveniles of *H. trifolii* are longer and have longer and more robust stylets, and longer tails than those of *H. daverti*. *H. cajani* is differentiated from *H. daverti* by its shorter juvenile body length, shorter juvenile stylet and shorter tail and hyaline tail portion. *H. daverti* is most similar to *H. glycines* from which it can be separated by the longer juvenile stylet, slightly longer total length and relatively long hyaline portion of the tail.

Fig. 1 (front page) - An Egyptian population of *Heterodera daverti*: A, anterior body region; B, posterior body region of male; C, tail and D, head of second stage juvenile; E, side view (dorso - ventral) of cone top and underbridge and F, anterior view showing developing underbridge and bullae of cysts.



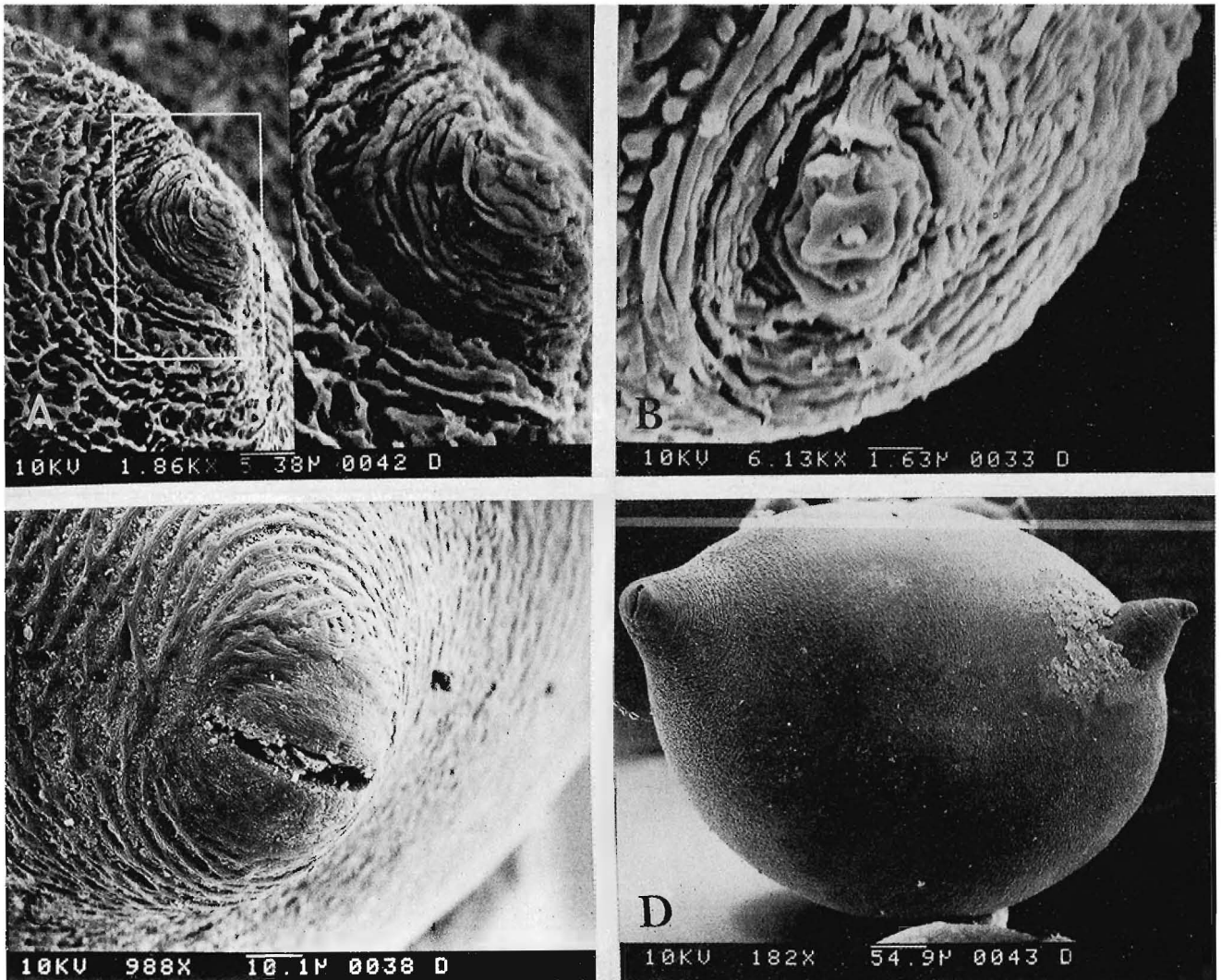


Fig. 2 - SEM photomicrographs of an Egyptian population of *H. daverti*: A, anterior body region (lateral); B, face view of the lip patterns; C, vulval aperture at top level; D, whole body of mature white females.

The measurements of vulval cones of the Egyptian population of *H. daverti* agree with those of *H. daverti* and are similar to those of *H. trifolii*, so that it can be placed in Mulvey's (1974) group No. 4 or in the Schachtii sub-group according to Stone's (1975) terminology.

The presence of males is an important factor in the diagnosis as *H. daverti* is characterized by abundant males, being an amphimictic species, whereas males in *H. trifolii* are rare.

The results of the host range test showed that *H. daverti* can infect and reproduce on at least 10 leguminous crops, but the most suitable host was the Egyptian clover cv. Meskawi. The type host for *H. daverti* is *T. repens*.

The Eastern Mediterranean is a centre of origin for the genus *Trifolium*, and the presence of *H. daverti* indicates that perhaps the nematode co-evolved in that region with that host group.

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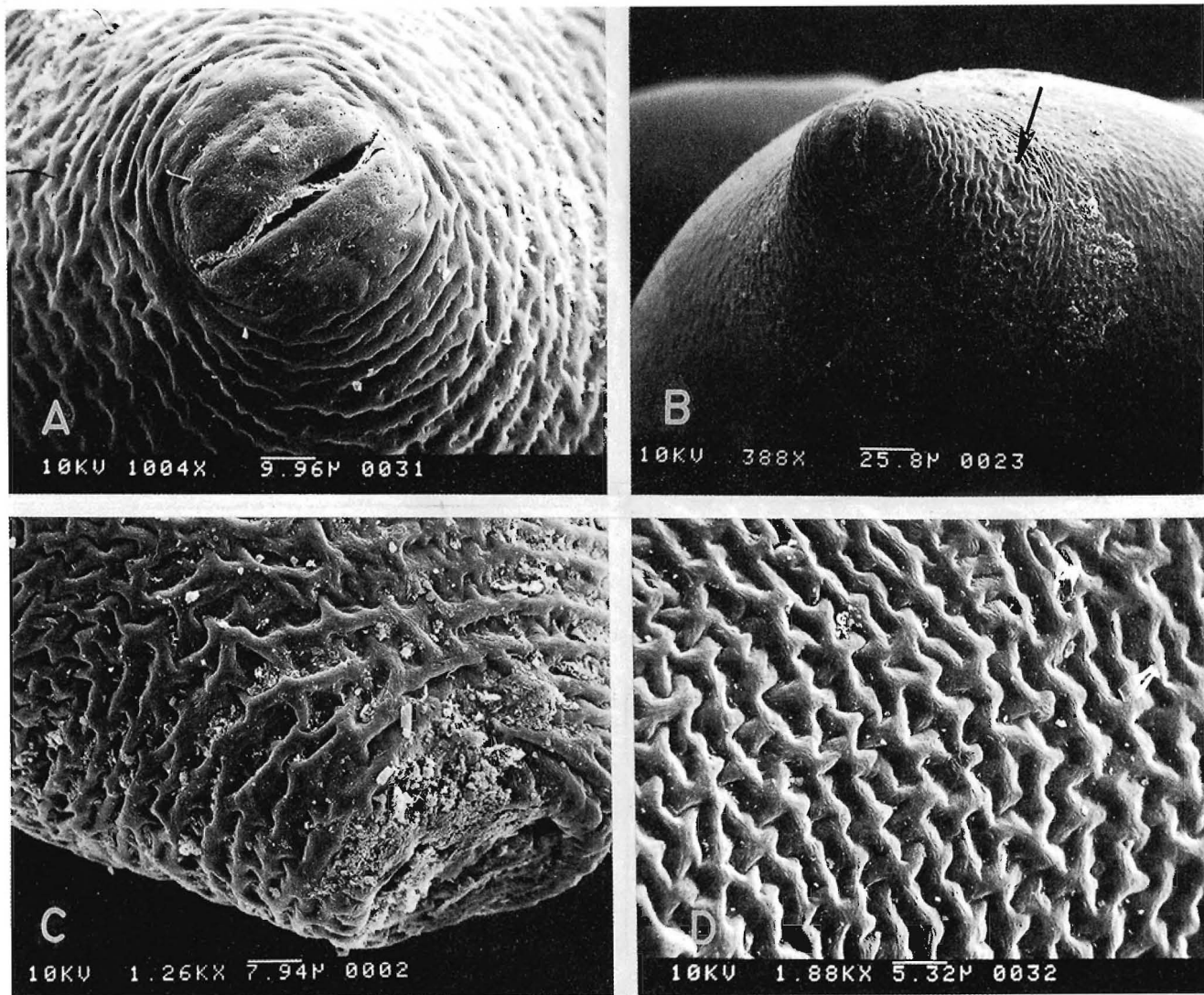


Fig. 3 - SEM photomicrographs of cyst cones of an Egyptian population of *H. daverti*: A, top view of unfenestrated vulval area showing long vulval slit; B approximate side view of unfenestrated perineal area showing vulval slit, zig-zag perineal ridges, and anal opening (arrow) and C, side view of fenestrated perineal area of cyst cones; D, coarse zig-zag cuticular pattern at the mid body cyst.

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