Notes brèves

A RE-EXAMINATION OF *NEOAPLECTANA RARA* DOUCET, 1986 (STEINERNEMATIDAE : RHABDITIDA)

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In 1986, Doucet published a description of *Neoaplectana rara*, originally found parasitizing *Heliothis* larvae (Lepidoptera : Noctuidae) in Córdoba, Argentina. Doucet commented that the species was distinguished from other members of the genus by the absence of cephalic papillae and the possession of only nineteen genital papillae in the male. This was very surprising since all previously examined members of the genus *Neoaplectana* are known to possess four cephalic papillae and a complement of 23 genital papillae (Poinar, 1979; Mráček, Weizer & Gerdin, 1981). Thus a re-examination of these characters was made in order to determine the distinctness of *N. rara* in comparison to other species in the genus.

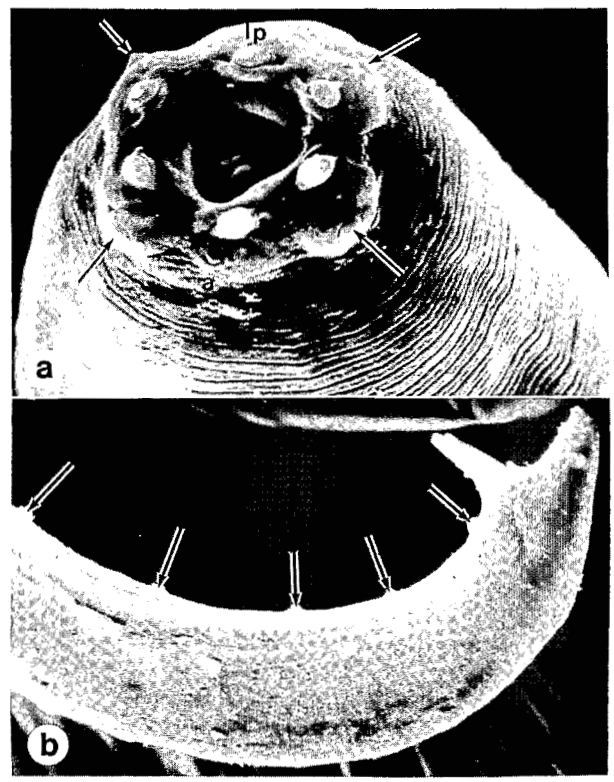

Fig. 1. *Neoaplectana rara* Doucet, 1986. Scanning electron micrographs. a : « En face » view of female showing inner row of labial papillae (l p), an outer row of cephalic papillae (arrows) and an amphid (a); b : Male tail showing a row of five pre anal papillae (arrows).

Materials and methods

Cultures of *Neoaplectana rara* Doucet were obtained from stored infective juveniles that had been reared on larvae of the wax moth, *Galleria mellonella.* For light microscope observations, first generation males were removed from the body cavity of wax moth larvae seven days after initial infection, killed by placing them in hot Ringer (70°), fixed in TAF and processed to glycerin.

Nematodes prepared for SEM were removed from larvae of the wax moth, washed three times in 0.05 % NaCl for 2 h intervals and then fixed in 2.5 % glutaraldehyde in cacodylate buffer at 4° for 2 h and postfixed in 1 % osmium tetroxide for 1.5-2 h. They were then dehydrated in an alcohol series and carried over to acetone where they were dried at critical point. After mounting them on stubs, they were gold-coated at 300 A in a Jeol Sputter JFC-1100 and examined in a Tesla BS 300 SEM.

Results

CEPHALIC PAPILLAE

Both light microscope and scanning electron microscope studies (Fig. 1 *a*) showed the presence of four cephalic papillae, along with an inner ring of six labial papillae and paired amphids on the head of *N. rara.* The cephalic papillae are not as distinct as on some of the other neoaplectanid species but they are clearly present.

GENITAL PAPILLAE

Both light microscope and scanning electron microscope studies (Fig. 2 *b*) demonstrated the presence of two rows of five precloacal genital papillae starting just anterior to the cloacal opening and running ventrolaterally anteriorly up the body. In addition, there were two pairs of papillae at the level of the cloaca, one pair adjacent to the cloaca, and one pair lateral and three pairs of papillae on the tail tip (Fig. 2 *a*, 2 *b*). Together with the single, ventral adanal papilla, it provides a total of 21 genital papillae.

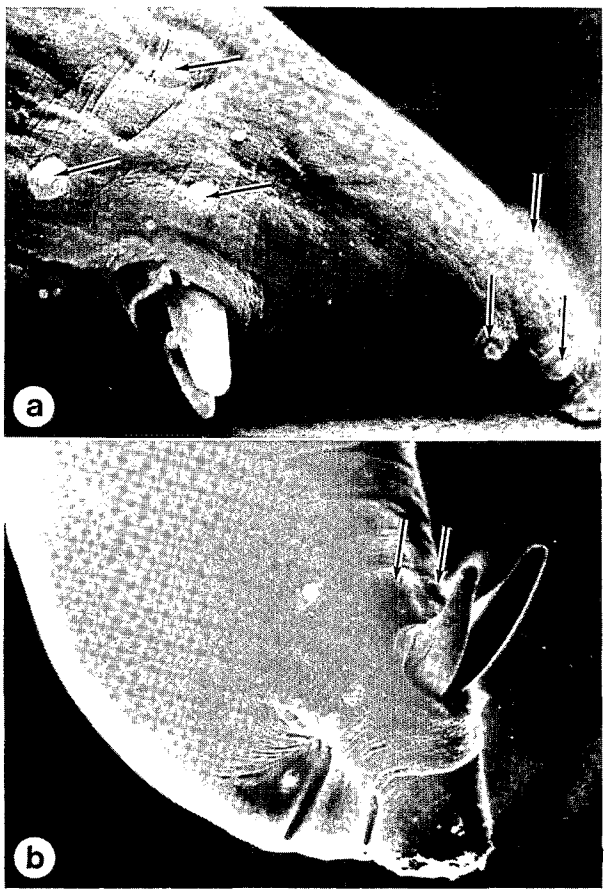

Fig. 2. *Neoaplectana rara* Doucet, 1986. Scanning electron micrographs. a : Tip of male tail showing three papillae on the tail tip and three papillae in the area of the cloacal opening (arrows); b : Tip of male tail showing the unusual pairing condition of the normally single adanal papillae (arrows).

Discussion

The present study clearly shows the presence of four cephalic papillae, along with six labial papillae, in *N. rara*. Such a condition is normal for members of the genus *Neoaplectana*. A total of 21 genital papillae were observed on the tail of the male. This is two more than was reported in the original description of Doucet (1986) but is still two less than what is normally found in members of this genus. This deviation from the norm in male genital papillae is interesting since with *N. rara* the same pair of papillae were always « missing », namely a pair that would normally occur in the region posterior to the cloaca. It should be noted that some variation also occurs in relation to the single preanal papilla. This papilla, which is normally single in members of the genus *Neoaplectana*, frequently is paired in *N. rara*. This pairing can consist of two papillae lying side by side (Fig. 2 *b*) or one above the other. In rare cases, this papilla may occur as a ventral precloacal series of three or four papillae extending up the tail or be absent altogether. Considering the amount of variation present regarding this and occasionally other papillae, there is a possibility that the " missing " pair is present in a relatively small proportion of the population which was not included in our sample. An examination of other field collected populations should help to resolve this point.

Aside from the variation of the genital papillae in *N. rara*, there is another character which is distinctive in this first described neoaplectanid from South America. This is the occurrence of a red color associated with the multiplication of the symbiotic bacterium during the first two days after insect mortality. As the bacteria, which we determined to be in the genus *Xenorhabdus*, multiply in the insect's hemolymph, a large number of variably sized red crystals are formed in the insect. These crystals are produced in such density that they impart a reddish color to the infected insect, similar but not identical to the reddish color produced in insects infected with nematodes of the genus *Heterorhabditis*. In the latter case, the pigment is a soluble dye produced by the symbiotic bacteria, *Xenorhabdus luminescens*. In the case of *N. rara*, the color is due to crystals which diminish in number approximately three to four days after infection. The crystals also appear in bacterial colonies of the bacterium grown on nutrient agar plates. This is the first time such red crystals have been found associated with the growth phase of a *Xenorhabdus* species, especially one associated with a species of *Neoaplectana*.

Since Doucet (1986) used the absence of cephalic papillae in *N. rara* as a diagnostic character for the species, and this is no longer valid, we would like to point out other characters which can be used since we feel that *N. rara* is distinct from previously described species of *Neoaplectana*. Aside from the presence of only 21 genital papillae, the male tail mucron of *N. rara* separates this species from *N. glaseri*, *N. anomali* and *N. intermedia* which lack it completely. The lemon yellow color of the spicules as well as their degree of curvature separate *N. rara* from *N. carpocapsae* and *N. bibionis*. The small size of the infective stages (443-563 µm) separate *N. rara*

from all other described species except *carpocapsae*. From this latter species, *N. rara* can be distinguished by a smaller average distance from the head to the pharynx base (102 (89-120) µm in *N. rara vs* 120 (103-190) µm in *N. carpocapsae*) and differences in ratio D (distance from the head to the excretory pore divided by distance from head to base of pharynx) which range from 0.30-0.39 in *N. rara* and 0.23-0.28 in *N. carpocapsae* (in infective juveniles).

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IN VITRO RESPONSE OF *CRICONEMELLA XENOPLAX* TO NONFUMIGANT NEMATICIDES

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Greenhouse tests have been conducted with populations of *Criconemella xenoplax* (Raski) Luc & Raski, stressed monthly with low doses of nonfumigant nematicides (NFN) (Yamashita, Viglierchio & Kuo, 1988). All stressed populations appeared to respond differently to one or more NFN treatments than did a wild population with no previous history of NFN stressing.

An *in vitro* bioassay was used previously to characterize various populations of *Xiphinema index* (Yamashita & Viglierchio, 1987 *a*), *Meloidogyne incognita* and *Pratylenchus vulnus* (Yamashita & Viglierchio, 1986 *b*) and *Heterodera Schachtii* (Viglierchio & Brown, 1989) for demonstration of consonant behaviors. A 24-hour exposure of nematodes to relatively high concentrations of NFN served to express other characteristics not evident in results from field and greenhouse trials. Differences between stressed and wild populations were often consistent with those expressed under greenhouse testing.

Subsequent studies implicated at least two general modes of increased tolerance to NFN (Yamashita & Viglierchio, 1987 *b, c*). One involved apparent genetic characteristics unique to specific populations, suggesting true resistance. A second mode appeared in all populations following a pulse with low NFN doses, suggesting a transient phenomenon much like enzyme production (Yamashita & Viglierchio, 1978 *b*). The following studies seek additional characterization of wild and various NFN-stressed populations of *C. xenoplax* for comparison to other nematode species and an improved understanding of NFN activity.

Materials and methods

Populations of *C. xenoplax* utilized in these experiments included the following : A wild population (W-P, with no previous history of nematicide treatments) and four nematicide-stressed populations with over a year history of continuous monthly subnematicidal stressing. One population was stressed with carbofuran (C-S-P), a second with oxamyl (Ox-S-P), a third with phenamiphos (P-S-P) and a fourth with aldicarb (A-S-P). The stressing procedures were as previously described (Yamashita, Viglierchio & Kuo, 1988).

The techniques utilized for the *in vitro* testing were essentially those previously described (Yamashita & Viglierchio, 1986 *b*, 1987 *a*; Viglierchio & Brown, 1988). The concentrations of nematicides employed are indicated in Table 1.

Experience revealed a tendency for slight variations between different stock culture pots within a population. Therefore the experiment was repeated three times, each time with nematodes from a different stock culture pot of the population being evaluated. Because of slight variations of nematode numbers in the aliquant, data were evaluated following a logit transformation [ln (number of active plus 0.5 divided by the number of inactive plus 0.5)]. Mean column comparisons were conducted using a Duncan's multiple range test with an upper significance level of 5 %.

Results

The observed touch responses are summarized in Tab. 1. The stressed population controls were significantly more responsive than W-P to touch stimulus. The A-S-P was the most responsive. Subjected to a 1.0 mM carbofuran treatment, the C-S-P was significantly more responsive in the bioassay than P-S-P and A-S-P which in turn were significantly more responsive than Ox-S-P and W-P. After an 0.5 mM oxamyl treatment, the C-S-P

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