J.M. NEVES[1)], N. SIMÕES[1)] & M. MOTA[2)]: *Evidence for a sex pheromone in* Steinernema carpocapsae.

There are many references to the existence of sex attraction in nematodes (Bone, 1982), but no information on sexual attraction in steinernematids which are excellent candidates for biological control (Klein, 1990; Klein & Georgis, 1994). The aim of this study was to investigate sexual communication between adults of *Steinernema carpocapsae* Weiser.

S. carpocapsae Az20 and *S. carpocapsae* Breton were grown on 9 cm diameter Petri plates containing 10 ml of fortified lipid agar medium (FLA) prepared with 1.6% nutrient broth, 1% sunflower oil and 1.2% bacteriological agar. Each plate was inoculated with 1 ml of the strain specific symbiotic bacterium *Xenorhabdus nematophilus* (Poinar and Thomas) Thomas and Poinar from exponential growth cultures, swirled to ensure uniform bacterial dispersion onto the agar surface and incubated at 30°C for 24 h. 1000 infective juveniles, previously surface-sterilized in 10% hypochlorite solution for 10 min, were then added to each FLA plate. After three days incubation at 23°C, young virgin males and females were obtained. These young adults were collected by washing plates with 5 ml Tyrode's solution for 5 min, allowing the adults to settle in the washings and the supernatant was discarded. Male and female nematodes were separated in a discontinuous sucrose gradient (Neves *et al.*, 1996). Solutions of potential attractants were prepared by incubating 6000 females or males in 6 ml Tyrode's solution for 3 h at 23°C in the dark. Nematode-free solution was removed by aspiration, to give a stock solution equivalent to the incubation of 3 females (male) · hour · μl. Subsequent solutions were prepared by dilution with Tyrode's solution, which was used as a control solution in all experiments.

Females were shown to be virgin by examination of samples microscopically and by maintaining some separated to confirm that no progeny was produced.

Single young males were placed in the wells of 48-cell culture plates in 10 μl of Tyrode's solution and maintained at 23°C. Ten minutes later, 40 μl of undiluted female incubation solution were added to each well. Males were observed under stereomicroscope and the number becoming active was recorded at 2 min intervals for 20 min. One hundred replicates were done for each assay.

Sexual attraction was studied in (6 × 2 × 2 cm) migration chambers (Bonner & Etges, 1967) made of plexiglass and divided into two compartments, A (1 ×

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2 × 2 cm) and B (5 × 2 × 2 cm), separated by a strip (2 × 2 cm) of Whatman nº 1 filter paper. Compartment A received incubation solution and compartment B, the nematodes. This compartment was subdivided into a 1 cm middle zero zone (0) and two 1 cm wide zones towards compartment A (+2, +1) and two 1 cm away from compartment A (−1, −2). Each chamber received 3 ml Tyrode's solution, and 1 ml of an incubation solution was added to compartment A and allowed to diffuse for 2 hr at 23°C. Then 100 males or females were placed in zone 0 of compartment B. After two hours, the number of nematodes in each zone of compartment B was recorded.

Cross-attraction experiments tested the responses of males and females of both strains to incubation solution from the same and opposite sexes of each strain, at 23°C under uniform illumination with four replicates of each combination.

Dosage dependency responses of male *S. carpocapsae* Az20 were examined using incubation solutions equivalent to 0, 0.75, 1.5 and 3 *S. carpocapsae* Az20 female · hour.

Data from migration and cross-attraction experiments were submitted to the ANOVA test, distributions were compared using a chi-square test and dosage responses analyzed by linear regression.

Males of *S. carpocapsae* Az20 exposed to an incubation solution of virgin females of the same strain had a particular behaviour characterized by brusque and rapid undulatory movements of the whole body. In a solution equivalent to 3 female·hour, male responses began after 2 min exposure and, at 4 min, 77% of males were excited. After this time, the percentage of excited males decreased and no activity was observed 10 min after application.

When males were placed in the migration chamber 2 hr after the female incubation solution, they became excited within minutes and began to move towards the source. Two hours later the male distribution in the migration chamber was different from control ($\chi^2 = 292$, $P \leqslant 0.05$). In controls, 38% of males remained in zone 0, and others migrated randomly. However, in the presence of the female incubation solution, 23% of males remained at the inoculation point, significantly fewer (9%) migrated to the −1 and −2 zones and significantly more (68%) migrated to +1 and +2 zones. In the presence of female incubation solution, 27% of males reached the +2 zone demonstrating the orientated movement of the males to the female incubation solution (Fig. 1).

Female incubation solution neither excited nor stimulated migration of females placed in zone 0. The percentage of females migrating towards the female source (32%) was not significantly different from 30% females migrating in the opposite direction ($\chi^2 = 1.5$, $P > 0.05$). *S. carpocapsae* Az20 male incubation solution

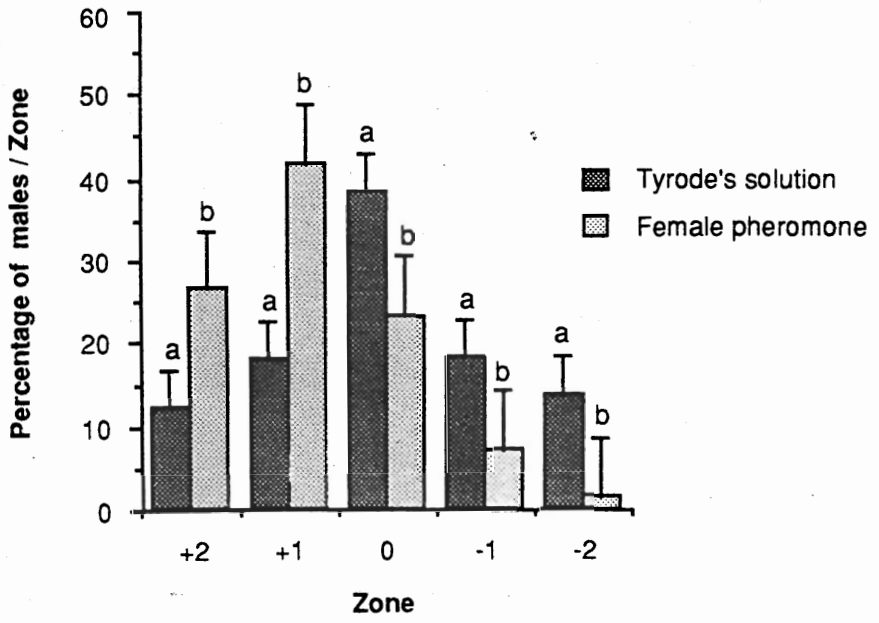

Fig. 1. Percentage of *S. carpocapsae* males migrating to zones of a migration chamber in the presence of Tyrode's solution (■) and female pheromone (▣) (3 female · hour) as attractant. Means of 100 replicates for each zone are significantly different ($P \leqslant 0.005$).

did not cause any modifications of the behaviour and migration of males and females of *S. carpocapsae* Az20 ($\chi^2 = 0.75$ and 2.72 respectively, $P > 0.05$).

Incubation solutions from females of both *S. carpocapsae* Az20 and *S. carpocapsae* Breton caused a significant and similar migration of males of both strains, respectively 59-68% for Az20 strain and 61-64% for Breton strain. The percentage of *S. carpocapsae* Az20 males migrating towards the Az20 female source dosage dependent, increasing linearly over the range 0 to 3 female · hour solutions ($Y = 31.980 + 11.139x$; $R^2 = 0.98$, $P \leqslant 0.05$).

These data provide an additional perspective in the reproductive biology of *S. carpocapsae* and are a basis for future investigation. They show that virgin *S. carpocapsae* females produce an attractant which appears to be a sex pheromone since it affected only males, although it was not strain specific. Similar pheromones have been reported from *Pelodera teres* Schneider (Dougherty) Jones, 1966) and *Heterodera* species (Green, 1966). Dosage dependency reponses have been reported for several nematodes examined for pheromones and optimum chemical concentrations for attractions have been reported for other nematodes (Green, 1966; Green *et al.*, 1968; Balakanich & Samoiloff, 1974). Similar cross attraction between females and males of other strains were demon-

strated for the sex pheromones of females of *Radopholus similis* (Cobb) Thorne citrus strain and males of the banana strain (Huettel *et al.*, 1982).

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