Some observations on a Tylenchid nematode

*Howardula* sp. parasitizing the mushroom phorid *Megasalia halterata* (Phoridae, Diptera)

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SUMMARY

*Howardula* sp. parasitizes the Mushroom phorid *Megasalia halterata* destroying the fat body and follicular membrane of the fly, decreasing copulation and egg output. With increasing hyperinfestation the fly is rendered sterile. *Howardula* sp. is host density dependent and reaches a maximum incidence in September and October. Under laboratory conditions fly populations were eliminated in three to five generations. The implications for possible biological control of *M. halterata* are discussed.

INTRODUCTION

*Megasalia halterata* Wood is a small black fly frequently found on mushroom farms and it may reach epidemic proportions as it did in West Sussex in 1953 (Moreton & John, 1955) and in 1961. *M. halterata* can reduce the yield of mushrooms as well as transmitting virus, bacterial and fungal pathogens: the fly also irritates pickers. Chemical control of phorids is effective but costly. Also, the problems of toxic residues and the possibility of fly resistance to the insecticide are good reasons for seeking alternative methods of control.

A Tylenchid nematode parasitizing the phorid was discovered by Hussey (1959) when dissecting flies. He referred to the nematode as a species of *Bradynema* in that and subsequent papers, but Riding (1971) has now described the nematode in detail under the generic name *Howardula*. The potentiality of this nematode parasite as a biological control agent was immediately apparent and this paper describes the life cycle of the nematode and various effects of the nematode on the development of the fly.

MATERIALS AND METHODS

The initial population of infested phorids was obtained from Dr N. W. Hussey, Glasshouse Crops Research Institute, Littlehampton, and this material was used to investigate the life cycle of *Howardula* sp., flies being dissected and examined all through the phorid life cycle.

For studies on the incidence of parasitism in commercial practice, phorids were
collected from nine mushroom farms in Surrey, Berkshire, Hampshire and West Sussex at intervals from January 1968 to August 1970: 100 flies from each sample were dissected.

Phorids were reared in a sterile inoculation cabinet with the two glove entrances replaced by removable framed gauze disks. Jars of spawned compost were added or removed after unscrewing the disks, the flies being attracted to the opposite end of the chamber by a bright light, so that they did not escape during this operation. The jars of spawned compost were placed on the floor of the chamber on top of slightly wetted absorbent paper which maintained a suitable level of humidity. The cages were kept in a constant temperature room at 25 °C, the gauze disks allowing a limited amount of air to circulate within the cage, but not enough to cause drying out.

The life cycle of *M. halterata* took 21 days at 25–27 °C. The population was sampled every 3–4 weeks to estimate the incidence of *Howardula* sp. and when the level of parasitism reached 50% unparasitized flies were added to the breeding cage together with eighteen jars of mushroom compost. Dilution of the parasite in this way enabled the population to continue breeding for longer, for when not manipulated in this way flies were eliminated in four to six generations.

To study the effect of *Howardula* on the internal anatomy of the phorid, legs and wings of anaesthetized flies were removed and the tough cuticle pierced by finely pointed tungsten wire. Flies were fixed in Carnoy solutions, processed and embedded in wax. Sections 5–7 μm thick were cut on a hot plate where they were dried. Sections were coated with 1% celloidin in an alcohol-ether mixture before the wax was removed by xylene prior to staining. Mayer's haemalum/eosin, Mann's methyl blue eosin or Mallory's triple stain were used to differentiate the sections before they were finally dehydrated in absolute alcohol, cleared in xylene and mounted with Canada balsam.

**RESULTS**

*Life cycle of Howardula* sp.

The incidence of parasitism in the flies sampled from the commercial mushroom farms (Text-fig. 1) showed an annual fluctuation in the level of parasitism from a minimum in the spring to a maximum in early autumn. The change in parasite density coincided with a change in fly density in the mushroom houses: although numbers of *M. halterata* were not estimated, flies were very rare from March to May and abundant in September to November.

The mature male *Howardula* is free living but the female is a parasite in the abdominal haemocoelomic cavity of *M. halterata*. The female nematode lays eggs during the development of the fly pupae and first stage larvae hatch about the time of the emergence of the adult fly. The first stage larva moults a few hours after hatching and the second stage larvae rapidly accumulate fat globules, indicating that they absorb food from the host; no further development of larvae occurs inside the host.

It was noted in male flies that nematode development did not go beyond the early second stage larva and usually not beyond the first larval stage: when removed by dissection these larvae did not develop outside the host, in contrast to the development of first stage larvae from female flies placed in isotonic Ringer.
A nematode parasitizing Megasalia halterata

Female flies release second stage nematode larvae via the genital tracts during abortive attempts at oviposition after the few viable eggs present have been laid. Some larvae may escape through the gut and anus, but are unable to get out by any other route: nematodes still inside the fly die about 24 h after the death of the host: all larvae within male flies die as there is no mode of exit.

After liberation into the mushroom compost, second-stage larvae develop into fourth-stage females or mature males in 1–2 days at optimum temperatures. Female nematodes moult twice during this period and males three times, but each stage remains ensheathed within the previous cuticle: both sexes escape from the moulted cuticles at one exsheathment. Copulation was never observed and fertilized female nematodes were never recovered from compost but sperm was always found in females recovered from flies.

The method of entry by fourth stage infective females into the host was not established. First-instar maggots of M. halterata were never found to be parasitized but fourth-stage infective nematodes were frequently present in second and third instar maggots and even in young pupae.

The fourth-stage infective female undergoes a final moult within 1–2 days of entering the haemocoel of the host. Very rapid growth occurs before the parasite becomes sexually mature, usually mid-way through the host's pupation period and eggs are laid soon after the fly emerges. The adult female Howardula sp. is polymorphic: the two commonly occurring forms are described by Riding (1971).

**Effect of parasite on host**

(a) On the internal anatomy of Megasalia halterata

Longitudinal sections through unparasitized and parasitized flies are shown in Plate 1. The most obvious effect of Howardula sp. on the host is the damage done to
the fat body. In uninfected flies it completely fills the abdominal cavity as a mass of tightly packed polyhedral cells or trophocytes: individual trophocytes are large, frequently vacuolated with granular inclusions and they ramify between the internal organs. In contrast, parasitized flies have a depleted fat body which is often disintegrated and sparse, particularly in the dorsal and posterior regions where most larval nematodes usually aggregate. In many infected flies the fat body is completely destroyed, probably due to mechanical damage caused by the constant locomotory activity of nematode larvae and also by their feeding, indicated by the occurrence of parasites in nests of eroded trophocytes.

The nematode larvae are usually oriented along the longitudinal axis of the host in the antero-lateral regions, but posteriorly they mainly lie transversely. A few larvae are found in the thoracic region where they cause dispersion of the flight muscles: in legs the nematodes also damage muscles and they have also been found in haemo-coelomic cavities of the insect's head.

Adult parasites occur in the abdomen and are often surrounded by trophocytes in such close proximity that it is difficult to determine the host-nematode interface. The host’s gonads are reduced and in parasitized female flies the membranes of the polytrophic ovarioles are thin and frequently perforated, so that larval nematodes can pass freely between the oocytes from the abdominal cavity. In parasitized flies the ovary is never packed with oocytes and eggs and there appears to be less sperm in parasitized male flies.

(b) Effect of parasite on copulation of Megasalia halterata

At a commercial mushroom house infested with *M. halterata*, all the phorids which alighted in 30 min on a 1 yd² of the external surface of a mushroom shed door were collected. Flies in copulation were collected separately from those that were not: flies were then dissected to find out whether they were parasitized. Thirty-eight of the ninety-three copulating flies were parasitized compared with fifty-four parasitized in seventy-three non-copulating individuals, and it was thus concluded that *Howardula* sp. markedly affects the vitality of *M. halterata* and reduces its ability to copulate. (Using $x^2$, $P < 0.1\%$.)

(c) On egg production of Megasalia halterata

Mushroom mycelium was grown on sterile 2% malt agar in Petri dishes and a Perspex container was inverted over the exposed mycelium. Female flies were mated in a large flight chamber and individual females were introduced into the Perspex containers through a small aperture in the top and allowed to oviposit on the mycelium: the total number of eggs produced by each fly was recorded. After natural death each fly was dissected to find out if the fly was parasitized or not. The presence of *Howardula* had a very marked effect on the number of eggs laid by *M. halterata*; eighty parasitized flies laid an average of 42 eggs each whereas forty-eight unparasitized flies laid an average of 148 eggs each.

Seven-day-old flies, which had been allowed sufficient time for copulation and egg maturation but which had not been exposed to mushroom compost, were dissected and the number of fly eggs and the number of adult parasites were counted in each
A nematode parasitizing Megasalia halterata specimen, the results being summarized in Text-fig. 2. One to two adult nematodes per fly were found most frequently but three to five parasites commonly occurred, and as many as thirteen Howardula females per fly were found. It is clear that Howardula has a very marked effect on egg production; when ten or more parasites were present the fly was unable to reproduce.

Text-fig. 2. Percentage reduction of eggs laid by flies subjected to multiple parasitism.

Text-fig. 3. Incidence of parasitism of flies maintained in breeding cages over several generations. ■, Farm A; □, farm B; □, farm C.
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(d) Effect of Howardula sp. on populations of Megasalia halterata

The female *M. halterata* lays eggs in mushroom compost and larvae hatch in 2–7 days (Hussey 1961) and feed on the mycelium: if mushroom beds are attacked soon after spawning, mycelial growth can be entirely inhibited (Hussey 1960) with resulting crop failure. Depending on temperature the three larval instars take 4–33 days and pupation 1–9 weeks before flies emerge (Hussey, 1961). Mating occurs within three days of emergence and a flight period is thought necessary to induce copulation, because flies normally leave the breeding site (Hussey, 1965). The flies do not feed and females return to the mushroom mycelium to lay eggs for about ten days (Hussey & Gurney, 1964).

In the populations of phorids sampled from three commercial mushroom farms the percentage parasitism varied from 3.7 to 8.5% initially. Experimentally about 1000 flies were used to establish populations in breeding cages and although large fly populations were produced the incidence of parasitism exceeded 90% by the third generation (Text-fig. 3). In two of the colonies fewer than a dozen flies were produced in the fourth generation and these were unparasitized. In the third colony a high level of parasitism was maintained until the sixth generation, when the population of both host and parasite was also eliminated.

DISCUSSION

Assessing the role of nematodes in the regulation of insect populations is fraught with problems (Sweetman, 1963), but from the observations on breeding of infected phorids there can be little doubt that *Howardula* sp. was directly responsible for the elimination of the phorid population in three to six generations under experimental conditions.

Hussey (1968) reported preliminary experiments on the efficacy of *Howardula* sp. as a parasite of *M. halterata*: the proportion of parasitized phorid larvae decreased as their density increased and he therefore inferred that some mechanism other than random contact enables nematodes to reach the maggots, resulting in hyperparasitism of some hosts while others remained uninfected.

Tylenchid (Sphaerularid) parasites rarely kill their hosts and for this reason they are considered to have less biological control potential than mermithids and steiner-nematids and Rühm (1956) concluded that they were of little importance in controlling scolytid beetles. In general this may be true but there are examples of sphaerularids which may be significant in regulating pests, e.g. *Tripium sciarae* in *Bradysia paupera* (Poinar, 1965) and *Contortylenchus elongatus* in *Ips confusus* (Nickle, 1963).

The seasonal fluctuation of *Howardula* sp. populations (Text-fig. 1) is similar to that with other parasites, e.g. both *Heterotylenchus pavlovskii* and *Parasitylenchus diplogenus* (Welch, 1965) also have a host density dependent autumn peak. Hussey (1959) also showed that *Howardula* sp. was most abundant at this time, reaching an average parasitism of 80% and he reported that the incidence of the nematode in mushroom sheds tended to rise at the end of the cropping period when phorid numbers were decreasing. This divergence from the usual host density dependent relationship
A nematode parasitizing Megasalia halterata indicates that the parasite was effectively reducing the fly population at that time of year.

Hussey (1959) suggested that if the autumn and late cropping situation could be shifted to boost the proportion of parasitized flies earlier in the year, then it might be possible to prevent the annual outbreaks which are so troublesome in September and October. The present findings support this suggestion but, 'early in the season' is judged to be June–July when sufficient phorids are naturally present to avoid the usual biological control procedure of having to introduce the pest before the parasite. If Howardula sp. could be released in such quantities that a high incidence of hyper-parasitized flies was produced in one generation, the phorids could be eliminated and consequently wild populations would be decreased.

The main problem of manipulating Howardula sp. as a biological control agent is concerned with the method of release. In the free-living stage (second-stage larvae to infective female) there are problems of the viability of the second-stage larvae and other stages which could not easily be stored prior to release. The release of parasitized flies would be less tedious and more efficient because nematode larvae would be deposited near phorid eggs. More research is required before definite conclusions can be drawn about the feasibility of using Howardula to control M. halterata but there is sufficient evidence from this study to suggest that further investigations are warranted.

REFERENCES


EXPLANATION OF PLATE

Plate 1. Longitudinal sections through (a) parasitized and (b) unparasitized Megasalia halterata, showing larvae in abdominal cavity in parasitized flies and depleted fat body, and well-formed fat body in unparasitized flies.