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Biological control of sciarid and phorid pests of mushroom with predatory mites from the genus *Hypoaspis* (Acari: Hypoaspidae) and the entomopathogenic nematode *Steinernema feltiae*

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Abstract

In small-scale experiments, the predatory mites, *Hypoaspis aculeifer* (Canestrini) and *H. miles* Berlese, applied at 700 mites m^{-2}, and the entomopathogenic nematode, *Steinernema feltiae* (Filipjev) applied at 3×10^{-6} nematodes m^{-2} controlled sciarids and phorids in mushroom compost and casing substrates. For both mite species, earliest application to the growing substrate following sciarid infestation reduced sciarid emergence. In contrast, later application of each biological control agent provided more effective control of phorid emergence. The behaviour of adult mites suggested that *H. aculeifer* were more positively geotactic than *H. miles* although both species could penetrate compost and casing substrates to a depth of 2–12 cm. A majority of *S. feltiae* nematodes resided at a depth of 2–4 cm in both substrate types. Independent application of *H. aculeifer* provided more comprehensive control of sciarids and phorids than the other biological agents studied, owing to its better dispersal within compost and casing, and ability to attack larvae of differing ages.

Introduction

Mushroom cultivation is a significant component of the horticultural industry in the United Kingdom and is an economically important method of food production. With an estimated output value of £149M (Anon., 2001a), mushroom production represented 8% and 1% of the total value of horticultural and agricultural production, respectively in the UK in 2000. Commercial mushroom production in Northern Ireland comprises 375 growers annually producing *c*. 21,000 tonnes of mushrooms with a market value of £26M (Anon., 2001b).

The protected environment used for mushroom cultivation provides optimum environmental conditions for infestation by a diverse invertebrate fauna (Fletcher *et al*., 1989). The principal invertebrate pests associated with mushroom cultivation are Diptera from the families Sciaridae, Phoridae and Cecidomyiidae. Other arthropod pests are mushroom mites (order Acarina) from the families Tyroglyphidae, Anoetidae, Eupodidae and Tarsonemidae. The remaining invertebrate pests of mushroom cultivation are nematodes comprising saprophagous and mycophagous species from the orders Rhabditida and Tylenchida,

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respectively. Previously, application of pesticides to mushroom crops was relatively uncomplicated, involving the incorporation of relatively inexpensive, organochlorine or organophosphorus or, latterly, benzoylurea insecticides into the compost or casing substrates (Jess & Kilpatrick, 2000). In addition to mycotoxic effects due to insecticide application, there are other problems with chemical control strategies, including pest resistance and general environmental issues. Resistance to different organophosphorus insecticides has been reported within sciarid populations (Binns, 1976; White & Gribben, 1989) and is now considered widespread in Great Britain (Smith & White, 1996). A survey of annual pesticide usage within the Northern Ireland mushroom production industry estimated that 1.8 t of insecticide active ingredients were applied to 129 ha of mushroom crops, primarily for the control of mushroom flies (Diptera: Sciaridae, Phoridae) (Kidd *et al*., 1998).

In addition to constraints associated with chemical control strategies, increasing consumer demand for reduced pesticide inputs is a further consideration in the development of novel pest-control programmes in mushroom production, accentuating the significance of biological control. The protected environment of mushroom production is ideally adapted for the development and use of this control strategy (Richardson, 1987; White, 1995). The limited number of invertebrate pest species associated with mushroom cultivation increases the potential for adoption of a biological control strategy within this industry. However, mushroom crops are also susceptible to pathogens, including weed fungi, and it is unlikely that biological control agents will be used in isolation (Jess & Kilpatrick, 2000). It is envisaged that they will be used in conjunction with other control measures within an integrated crop management system.

Hitherto, research investigations of biological control of invertebrate pest species of mushroom cultivation have been largely confined to the use of *Bacillus thuringiensis* (Berliner) (Bacillaceae) (Cantwell & Cantelo, 1984; White & Jarret, 1990) and entomopathogenic nematodes (Richardson, 1987; Epsky *et al*., 1988; Nickle & Cantelo, 1991; Grewal *et al*., 1992; Grewal & Richardson, 1993). While a number of predatory mite species have been found within substrates used in mushroom cultivation, their potential to control associated insect pests has been overlooked (Binns, 1973). More recently, owing to constraints imposed on chemical control strategies, particularly within the horticultural industry, the significance of predatory mites as biological control agents is being re-examined (Chambers *et al*., 1993; Lind, 1993; Wright & Chambers, 1994; Ali *et al*., 1997, 1999; Enkegaard *et al*., 1997).

Mites from the genus *Hypoaspis* (Acari: Hypoaspidae) occur naturally in glasshouses in Europe (Enkegaard *et al*., 1997). Knowledge of the biology of *Hypoaspis* mites is limited and originates from investigations of *H. aculeifer* (Canestrini) (Lobbes & Schotten, 1980; Ragusa & Zedan, 1988; Murphy & Sardar, 1991; Glockemann, 1992). Relatively few studies concern *Hypoaspis miles* Berlese (Shereef *et al*., 1980; Rasmy *et al*., 1987; Glockemann, 1992; Chambers *et al*., 1993; Wright & Chambers, 1994; Ali, 1996). *Hypoaspis* spp. prey on soil-dwelling life-stages of many invertebrates including the larvae of sciarids and shore flies, bulb mites, Collembola, nematodes and pupae of thrips, leafminers and gallmidges (Ragusa *et al*., 1986; Epsky *et al*., 1988; Gillespie & Quiring, 1990; Enkegaard *et al*., 1995; Brodsgaard *et al*., 1996; Lesna *et al*., 1995, 1996, 2000). Both species have the potential to control infestations of sciarid flies and thrips in glasshouse ornamental plants (Gillespie & Quiring, 1990; Glockemann, 1992; Chambers *et al*., 1993). In addition, these species are polyphagous (Karg, 1961; Enkegaard *et al*., 1997) and can reproduce by arrhenotokous parthenogenesis (de Jong *et al*., 1981; Usher & Davis, 1983), attributes which increase their potential as biological control agents. However, the biology of these mites may be significantly affected by the type of prey consumed (Lobbes & Schotten, 1980; Murphy & Sardar, 1991; Enkegaard *et al*., 1997).

Hypoaspis aculeifer is an effective predator of the bulb mites *Rhizoglyphus echinopus* Famouze & Robin (Acari: Acaridae) (Ragusa & Zedan, 1988) and *Rhizoglyphus robini* Claparède (Acari: Acaridae) (Lesna *et al*., 1996, 2000). The latter workers emphasized the efficiency of this mite when preying on cryptic invertebrates and related this to its relatively small size with female somal lengths of *c*. 700 μm. Bulb mite populations were decimated or completely eradicated from lily bulb scales within 4–6 weeks of introduction of *H. aculeifer* mites. *Hypoaspis aculeifer* is a generalist predator and its foraging strategy relies on fungal stimuli, which attract it to an area where the probability of encountering fungivorous prey is high (Hall & Hedlund, 1999). This strategy suggests that *H. aculeifer* may have some potential as a predator of invertebrate pests during mushroom cultivation.

Adult *H. miles* mites are larger than *H. aculeifer*, with female somal lengths of 810 μm. *Hypoaspis miles* provided persistent control of the glasshouse fly *Bradysia difformis* Frey (Diptera: Sciaridae) on glasshouse ornamental plants throughout an 11-week period (Chambers *et al*., 1993). Mushroom cultivation is normally completed within a period of 10 to 11 weeks. Therefore, if mites from the genus *Hypoaspis* could provide a similar control of the dipteran pests of mushrooms, the entire period of mushroom cultivation could be protected by a single introduction of this mite. Lind (1993) reported that introduction of *H. miles* at a population level equivalent to 600 mites m^{-2} reduced the emergence of adult *Lycoriella castanescens* (Lengersdorf) (Diptera: Sciaridae) from spawned mushroom compost by 96%. More recently, Ali *et al*. (1999) recorded 60% reduction in the emergence of adult *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) following introduction of *H. miles* at a rate of *c*. 900 mites m^{-2} at the casing stage of mushroom cultivation.

To provide a sustainable alternative strategy to chemical pest control, a diversity of control methods must be integrated into the pest control programme. Therefore, it is essential that the effects of these control systems on specific biological control agents are evaluated. This study investigates the influences of the predatory mites, *H. miles* and *H. aculeifer* and the entomopathogenic nematode, *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) on populations of *L. ingenua* and *Megaselia halterata* (Wood) (Diptera: Phoridae) within different mushroom cultivation substrates.

Materials and methods

Mushroom culture

A description of the salient features and terminology associated with commercial mushroom production and the relationships with life-cycles of *L. ingenua* and *M. halterata* are outlined in fig. 1.

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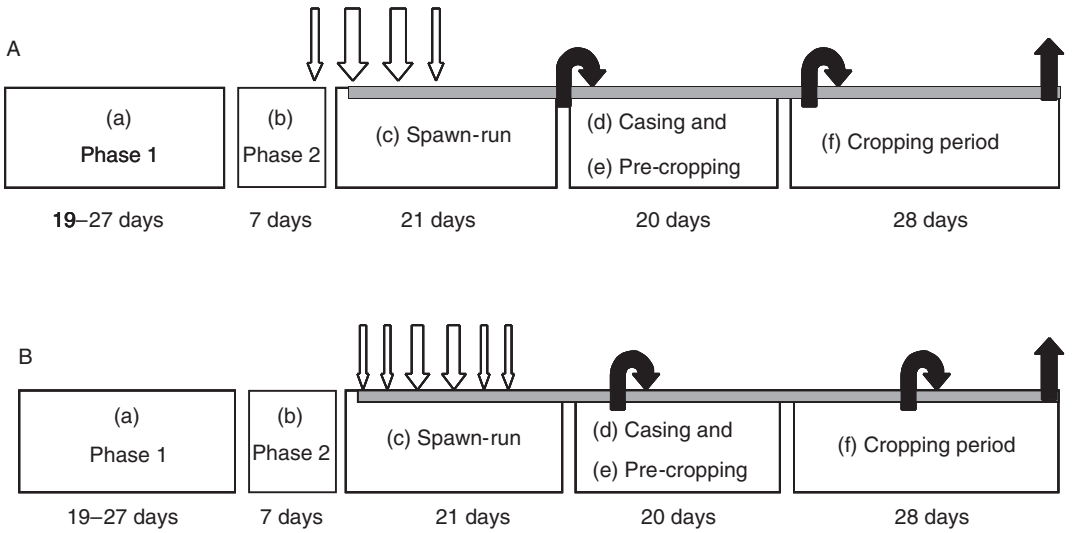

Fig. 1. Diagrammatic representation of the process of commercial mushroom production and the development of (A) *Lycoriella ingenua* and (B) *Megaselia helterata* under experimental conditions. (a) Phase 1, initial composting of raw ingredients; (b) Phase 2, a pasteurization process to produce an *Agaricus bisporus*-selective compost; (c) spawn-run, colonization of compost by *A. bisporus* mycelium; (d) casing, addition of moise peat-chalk layer required to promote fruiting; (e) pre-cropping, mycelial growth through compost and casing layer culminating in production of primordia; (f) cropping period, production of mushrooms; oviposition ⇒; immature stages ▭; adult emergence ▬ (adapted from White, 1997).

Experimental

Experiments were conducted in environmental chambers maintained at a temperature of 20°C (± 1°C), 75% humidity (± 5%) and 16 h daylength. Adult *L. ingenua* and *M. halterata* were removed from laboratory cultures using insect pooters and immediately anaesthetized using carbon dioxide. Adult flies were placed into experimental units at sex ratios specified for each experiment. Unless stated otherwise, experimental units comprised 400 cm^3 polystyrene food containers with lids ventilated by a single 1 cm diameter hole plugged with non-absorbent cotton wool.

Commercially produced Phase 2 compost (McGeary Mushroom Compost Limited, UK) was inoculated with mushroom spawn (Sylvan Spawn 130®) at a rate of 5 g kg^{-1}. A mixture of ground limestone and sphagnum peat (15 kg limestone to 300 l of peat) provided the casing substrate. *Hypoaspis* mites were obtained from the plant protection product Hyposure® (quality controlled by Biological Crop Protection Limited, UK) and supplied in a substrate comprising a mixture of peat and vermiculite. Immediately before application to experimental units, the substrate containing the mites was spread onto a white, butchers' tray. Negative geotactic behaviour of *H. miles* adult mites encouraged their migration to the vertical sides of the tray, from which they were removed using a fine moistened paintbrush. *Hypoaspis aculeifer* adults were also removed by this method but it was noted that adults of this species tended to remain within the substrate, a behaviour suggesting positive geotaxis. In all experiments, the application rate for mites was calculated as the number of mites related to the surface area of the experimental unit. Introductions of both predatory mite species in the following experiments were equivalent to 700 mites m^{-2}.

The nematode *S. feltiae*, was obtained commercially (Nemasys M®, quality-controlled by MicroBio, UK). Dormant, third stage, infective nematodes were prepared and applied according to label instructions. Application rates for the nematode were calculated by adding a measured quantity of Nemasys M® to a measured volume of water to produce a suspension of active nematodes. This solution was agitated for 15 min at room temperature, following which, a 1 ml aliquot was removed and placed on a McMaster microscope counting slide. The slide was examined at a magnification of ×100 under a binocular microscope and the number of nematodes in the 1 ml suspension was counted. This process was replicated three-fold and a mean number of nematodes in 1 ml was calculated. Application rates could be adjusted by further dilution.

Nineteen days after the initial inoculation of sciarid or phorid adults, 5.0 cm^2 discs cut from yellow insect-monitoring traps (Agralan UK) were attached to the inner surface of each container lid to trap emerging adults. Following an overall incubation period of 35 days, these traps were examined at ×100 magnification using a binocular microscope. The total numbers and gender of adult sciarids and phorids in each experimental plot were recorded.

Count data were subjected to analysis of variance, using Genstat 5.0 (Release 4.2) (Lawes Agricultural Trust, IACR, Rothamsted, UK).

Experiment 1. Predation rates of two species of Hypoaspis *mites on* Lycoriella ingenua *following their introduction to spawned mushroom compost at different time intervals*

The potential effectiveness of biological control agents may be affected by the development stage of the prey at time of introduction. Therefore, each species of *Hypoaspis* was introduced on a number of occasions, coinciding with different life stages of *L. ingenua*, to determine the optimum time for introduction of each predatory mite species.

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The experimental design utilized a randomized-block design with eight treatments spatially arrayed in five replicates. Forty 400 cm^3 labelled polystyrene food containers were uniformly filled with 200 cm^3 of spawned mushroom compost. One male and two gravid female anaesthetized sciarid adults were introduced to each container. After three days, 15 g of soya meal was evenly distributed on the surface of the compost in each container to sustain sciarid larvae. Five adult *H. aculeifer* mites were introduced to each of five containers at 2, 9 and 16 days after sciarid inoculation. This process was repeated for adult *H. miles* mites whilst the remaining ten untreated containers provided the controls.

Experiment 2. Effects of introduction of H. miles, H. aculeifer *and* S. feltiae *to mushroom cultivation substrates on emergence of adult* Lycoriella ingenua

To be effective biological control agents in mushroom cultivation, the selected organisms must be able to control insect pest populations within the different growing substrates used during this process. This experiment examined the potential of three biological control agents to limit adult sciarid emergence within unspawned compost, spawned compost and casing.

The experimental design comprised 12 treatments replicated four-fold and spatially arrayed within a randomized-block design. Each of the three substrates was put into sixteen 400 cm^3 polystyrene containers. Owing to the different densities of the substrates, the required quantities were measured volumetrically. For each substrate, polystyrene containers were uniformly half-filled. To determine moisture content, sub-samples of each substrate were weighed and then dried at 80°C for 16 h before re-weighing. Distilled water was added to substrates, as required, to standardize moisture content of the substrates. Anaesthetized sciarid adults, two gravid females and one male, were introduced to each container. Two days after sciarid inoculation, five adult *H. aculeifer* mites were introduced into each of four containers of the three substrates. This process was repeated for adult *H. miles*, which was equivalent to 700 mites m^{-2} for each mite species. *Steinernema feltiae* nematodes were also applied to each of four containers of the three substrates at a rate equivalent to 3×10^6 nematodes m^{-2}. The remaining 12 untreated containers provided the controls.

Experiment 3. Effects of introduction of H. miles, H. aculeifer *and* S. feltiae *into mushroom compost on emergence of* Megaselia halterata

Hypoaspis mites are polyphagous but there are no previous references to their predation on phorids *Megaselia* spp. Results from previous studies on the effect of *S. feltiae* on phorids have been variable (Cantelo *et al.*, 1977; Richardson, 1987; Scheepmaker 1998a,b). Therefore, the objective of this experiment was to examine the effectiveness of these biological agents to control *M. halterata* within spawned mushroom compost. Varying the time for introduction of these biological agents could provide some indication of the pest life stage most susceptible to predation by mites and parasitism by nematodes.

The experimental design comprised three replicates of 16 treatment combinations, randomly allocated to three discrete blocks. Forty-eight 400 cm^3 labelled polystyrene food containers were uniformly filled with 200 cm^3 of spawned mushroom compost and three anaesthetized phorid adults were introduced to each container at a ratio of 2 gravid females to 1 male. Each of the three biological control agents was applied to an appropriate container on four occasions throughout the experiment, namely: one day before phorid inoculation (-1 day), on the same day of phorid inoculation (0-day) and two (2-day) and four day (4-day) after phorid inoculation. At each occasion, five adult mites from each species and 2.37×10^4 nematodes were introduced to the appropriate containers with one set of containers left blank as a control.

Experiment 4. Migration of the biological control agents H. miles, H. aculeifer *and* S. feltiae *within the profile of mushroom compost substrates*

The resident position and the ability of biological control agents to migrate within the profile of cultivation substrates may affect their potential to control invertebrate pests. The aim of this experiment was to determine the ability of three biological control agents, *H. miles, H. aculeifer* and *S. feltiae*, to migrate within the profile of casing and compost. In addition, the resident position for each biological control agent, in the absence of invertebrate pests, may be established.

Experimental units were prepared from plastic drainpipe tubing (10.0 cm diameter and 0.25 cm thick wall). Tubing was cut into transverse sections of 2.0 cm and a disc of plastic-coated wire mesh (mesh size 1 cm^2) was glued to the underside of each section, which was then filled with appropriate compost substrate. Six identical sections were prepared and stacked to form a 12.0 cm column. The sections were joined together using insulating tape to seal the joints between sections on the outer wall. Either *H. aculeifer*, *H. miles* or *S. feltiae* were introduced onto the top surface of an experimental unit containing either spawned compost or casing, providing six treatments. Fifty mites from each species and 1.0×10^5 nematodes were applied to appropriate units. Each column was placed in a tissue culture bag, which was sealed using an electric heat-sealer. Treatments were replicated six-fold and the 36 units were randomly allocated to six discrete blocks.

After a period of five days, the columns were carefully dismantled and each section was examined for the presence of the biological control agents. *Hypoaspis* mites were extracted by placing the sections into Tullgren funnels (Burkard, UK) equipped with 100 W tungsten bulbs providing the source of light and heat for an extraction period of 24 h. Mites were collected in 7.5 × 2.5 cm glass vials containing a solution of 75% lactic acid in distilled water. The numbers of mites of each species from the genus *Hypoaspis* were counted using a binocular light microscope at ×100 magnification.

Nematode extraction involved the use of a modified Baermann funnel extraction for a period of 48 h before nematodes were drawn off and counted in an annulated counting dish using a binocular light microscope at × 100 magnification (Hooper, 1986).

To test the null hypothesis that the distribution of each species was equal across all substrate depths, count data for all biological control agents were subjected to Chi-squared analysis (maximum likelihood method) using Genstat 5.0 (Release 4.2) (Lawes Agricultural Trust, IACR, Rothamsted, UK).

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Results

Experiment 1

Earliest introduction of either mite species at two days after inoculation with *L. ingenua* reduced adult sciarid emergence compared to the controls ($F = 45.8$, df = 3, 28, $P < 0.001$) (fig. 2). Mean adult sciarid emergence from experimental units to which *H. aculeifer* mites were applied, was lower than those receiving applications of *H. miles* mites on all introduction occasions ($F = 25.3$, df = 1, 28, $P < 0.001$). No significant differences were recorded for mean adult sciarid emergence between times of introduction with *H. aculeifer* mites. However, earliest introduction of *H. miles* at two days after sciarid inoculation resulted in the lowest sciarid emergence for this mite species ($F = 3.6$, df = 3, 28, $P < 0.05$). Introduction of this mite species at nine days after sciarid inoculation produced lower mean adult sciarid emergence than the final introduction of mites at 16 days after sciarid inoculation ($F = 3.6$, df = 3, 28, $P < 0.05$).

Experiment 2

The greatest mean sciarid emergence was recorded from spawned compost and the least recorded from casing substrate, irrespective of effects of biological control agents ($F = 20.5$, df = 2, 33, $P < 0.001$) (fig. 3). Considering all substrate types, the mean sciarid emergence from those which received biological control agents, was significantly lower than that recorded from controls ($F = 49.0$, df = 3, 33, $P < 0.001$). All biological control agents introduced to spawned compost produced lower sciarid emergence than the control ($F = 8.8$, df = 6, 33, $P < 0.001$). However, the introductions of *H. aculeifer* and *S. feltiae* to this substrate resulted in lower mean sciarid emergence than that observed following the introduction of *H. miles* ($F = 8.8$, df = 6, 33, $P < 0.05$). Introduction of each of the three biological control agents to unspawned compost decreased mean sciarid emergence when compared to the control ($F = 8.8$, df = 6, 33, $P < 0.001$). Introductions of biological control agents to casing substrate did not significantly reduce emergence of adult *L. ingenua*. However, a trend for lower emergence from experimental units treated with these agents, particularly *H. aculeifer*, was observed when compared to controls (fig. 3).

Experiment 3

The mean adult phorid emergence from compost, into which biological control agents had been introduced, was lower than untreated controls. ($F = 19.0$, df = 3, 30, $P < 0.001$) (fig. 4). Phorid emergence from experimental units, which received introductions of *S. feltiae*, was lower than those receiving introductions of *H. aculeifer* ($F = 19.0$, df = 3, 30, $P < 0.001$). Introductions of biological control agents at two and four days post-inoculation with phorid adults resulted in lower mean adult phorid emergence compared to introductions coinciding with inoculation, and one day pre-inoculation with phorid adults ($F = 3.1$, df = 3, 30, $P < 0.05$).

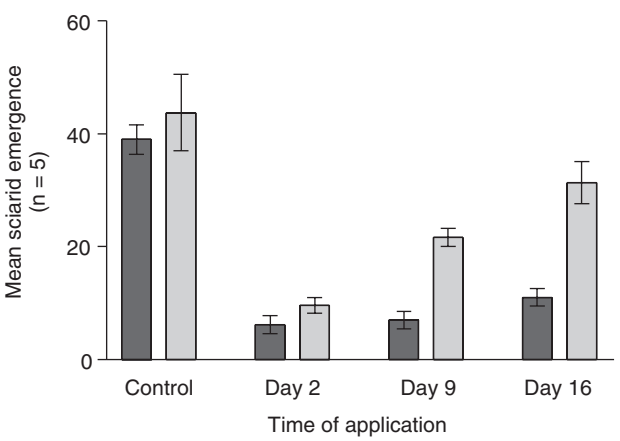

Fig. 2. Emergence of adult *Lycoriella ingenua* from spawned compost, following introduction of *Hypoaspis aculeifer* (■) and *H. miles* (▒) at different time intervals. Bars represent ±S.E.

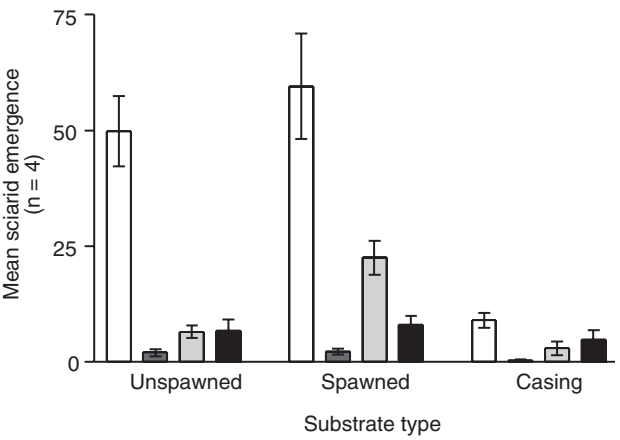

Fig. 3. Emergence of adult *Lycoriella ingenua* from three mushroom cultivation substrates, following introduction of *Hypoaspis aculeifer* (■), *H. miles* (▒) and *Steinernema feltiae* (■). Bars represent ±S.E. □, Control.

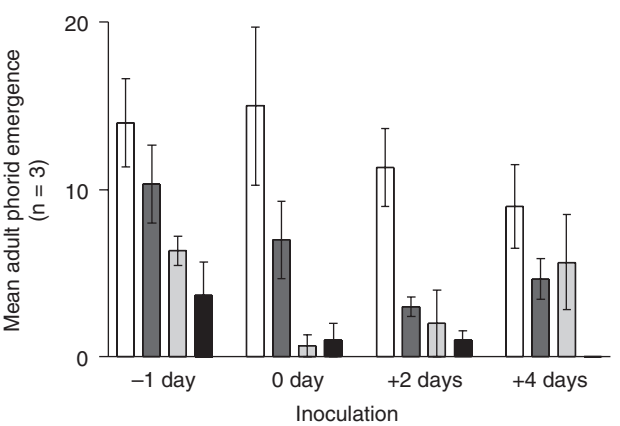

Fig. 4. Emergence of adult *Megaselia halterata* from spawned compost, following introduction of *Hypoaspis aculeifer* (■), *H. miles* (▒) and *Steinernema feltiae* (■) at different time intervals relative to *M. halterata* oviposition. Bars represent ±S.E. □, Control.

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Experiment 4

The vertical distribution of *H. miles*, *H. aculeifer* and *S. feltiae* in the two substrates is shown in fig. 5. All species were found at each substrate depth. *Hypoaspis miles* were relatively evenly distributed throughout the casing (fig. 5a). However, within the compost, a majority of *H. miles* were found in the lower two layers (10–12 cm) ($\chi^2 = 45.4$, df = 25, $P < 0.01$) (table 1, fig. 5a). Within casing and compost, most *H. aculeifer* were found in the final three layers (casing: $\chi^2 = 68.1$, df = 25, $P < 0.001$; compost: $\chi^2 = 74.1$, df = 25, $P < 0.001$) (table 1, fig. 5b). In contrast, a significantly greater proportion of *S. feltiae* were found in the upper layer (2 cm) of both casing ($\chi^2 = 4648.5$, df = 25, $P < 0.001$) and compost ($\chi^2 = 13380.0$, df = 20, $P < 0.001$), and 92% of nematodes were recovered from the two upper layers of both substrates (table 1, fig. 5c).

Discussion

The control of insect pests during mushroom production must occur at an early stage in the cropping cycle to prevent significant damage and consequent yield losses. An additional consideration is that the principal insect pests of mushroom are multivoltine and therefore, effective control of preceding generations will limit population levels in subsequent generations. Therefore, it is during the early phase of spawn-run (fig. 1) that control of invertebrate pests is most beneficial in reducing yield losses and further pest population increases. Currently, there are no effective biological or chemical controls recommended for application during spawning phase. The benzoylurea chitin inhibitor diflubenzuron or the entomopathogenic nematode *S. feltiae* are normally applied at the casing stage. Considering that sciarid infestation may occur at the conclusion of phase l composting, almost a complete generation may have developed before control measures are introduced. Additionally, the early spawn-running phase is most susceptible to oviposition by adult female phorids and considerable development of immature stages will occur before casing and subsequent application of chemical insecticide. It is imperative within an integrated pest management system that controlling pressures on pest populations are introduced early and maintained throughout the cropping cycle.

For both predatory mite species, in experiment 1, the earliest application into the compost provided significant reduction in subsequent adult emergence of *L. ingenua*, which is of considerable practical importance. It was also notable that *H. aculeifer* mites were more effective than *H. miles* mites at controlling sciarid emergences at all application times. Effects of prey maturity and condition on feeding behaviour of *H. miles* have been observed previously (Shereef *et al*., 1980; Ali, 1996). Both studies noted that this predatory mite did not feed on sciarid eggs. Ali (1996) also observed a preference of *H. miles* for early larval instars of *L. ingenua* prey. Large individuals of second and third instar sciarid larvae were observed actively repelling adult female *H. miles*, which were considered the most efficient predatory life-form of this species. However, it was also considered that the reduced speed of movement of first instar larvae and their inability to penetrate the substrate may have influenced predation. Wright & Chambers (1994) also reported that *H. miles* consumed a greater number of smaller rather than larger larvae of the glasshouse sciarid *B. difformis*.

Knowledge of the predation of *H. aculeifer* on sciarid larvae is limited to a study which observed its effect on populations of *B. difformis* (Gillespie & Quiring, 1990). In this current study, *H. aculeifer* mites were more effective predators of *L. ingenua* than *H. miles* when introduced at later stages. This suggests that mites of *H. aculeifer* have a greater ability to attack the more mature larval stages of its prey. Alternatively, its smaller size relative to adult *H. miles* may facilitate better penetration into the substrate, increasing its ability to locate its prey. The advantages of the smaller size of this species and its ability to locate cryptic prey, has been discussed in relation to its role as a predator of the bulb mite *R. robini* (Lesna *et al*., 1996). While adult *H. aculeifer* mites are smaller than adult *H. miles* mites, the mouthparts of each species are similar in size and structure. The greater ability of *H. aculeifer* to penetrate both types of cultivation substrate suggests that this mite species may have an advantage in predation within inaccessible areas. In addition, the apparent preference of this species to remain in the lower regions of the substrate profile suggest that it may be more effective at controlling insect pest species within compost and casing layers.

In the second experiment, most adult sciarids emerged from spawned compost. This confirms previous observations on *L. castanescens* (Hussey & Gurney, 1968; Binns, 1975; Scheepmaker *et al*., 1996) and *L. ingenua* (Ali, 1996). Al Amidi (1993) observed that *L. ingenua* females laid equal numbers of eggs on spawned and unspawned compost. The complex relationship between mycelium growth and larval development has been described by Binns (1975, 1980). Therefore, it may be concluded that larval development rather than oviposition preferences between cultivation substrates is the principal factor affecting adult sciarid emergence in experiment 2. The retardation of sciarid larval development in unspawned compost is likely to have increased the vulnerability of sciarid larvae to predation by

Table 1. The mean numbers of *Hypoaspis miles*, *H. aculeifer* and *Steinernema feltiae* recovered at different depths of casing and compost substrates.

Substrate depth (cm)	*H. miles*		*H. aculeifer*		*S. feltiae*	
	Casing	Compost	Casing	Compost	Casing	Compost
2.0	1.3	0.5	3.7	1.7	15400	21100
4.0	1.3	1.0	2.2	3.0	4770	5630
6.0	2.0	2.7	3.0	4.0	582	2120
8.0	1.8	2.7	4.5	5.2	109	715
10.0	1.7	4.8	3.5	9.3	52	471
12.0	2.7	1.7	7.7	9.0	24	330

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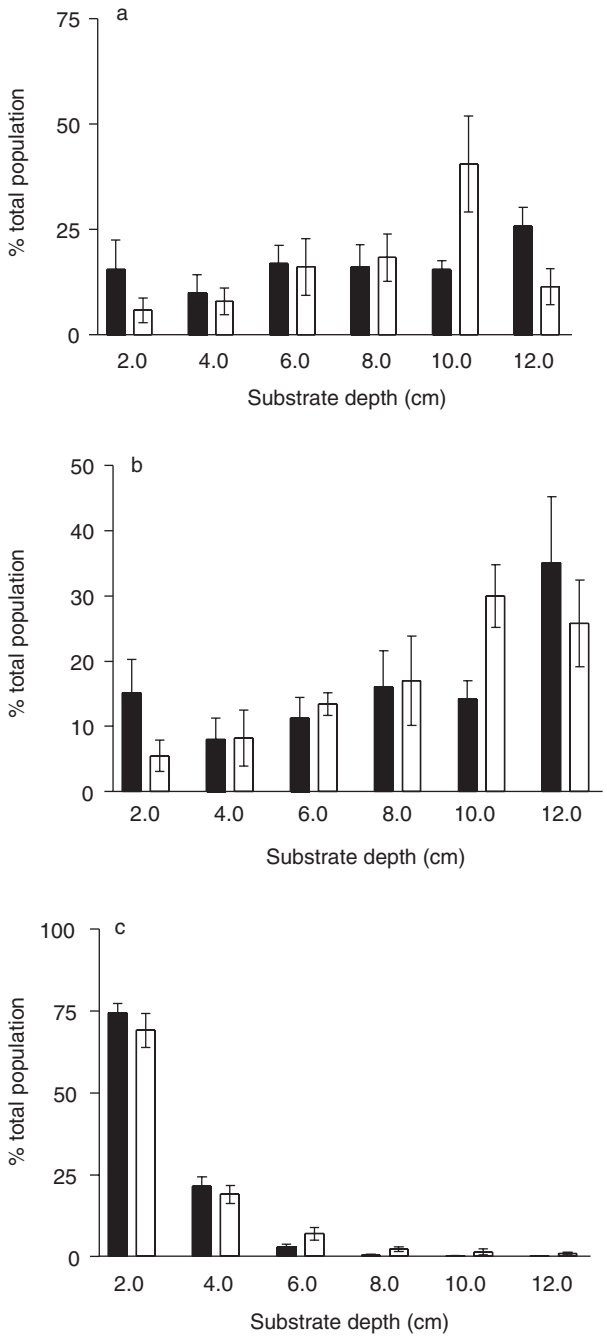

Fig. 5. Vertical distribution of three biological control agents (a) *Hypoaspis miles*, (b) *H. aculeifer* and (c) *Steinernema feltiae* in two mushroom cultivation substrates, casing (■) and compost (□). Bars represent ±S.E.

adult *H. miles* mites and, consequently, the differences between predation rates of the biological control agents within this substrate are less significant.

Fewer adult sciarids emerged from casing than from spawned or unspawned compost. In the absence of *Agaricus bisporus* mycelium, mushroom casing is a less suitable substrate for development of sciarid larvae than either of the latter substrates. Therefore, determination of the relative effectiveness of the biological control agents within this substrate was inconclusive. However, an observed trend indicated lower adult sciarid emergence from casing substrate, which received biological control agents when compared with controls (fig. 3). The trend also implied lower sciarid adult emergence from casing substrate, which received introduction of *H. aculeifer*, compared with *H. miles* or *S. feltiae*.

Introduction of the entomopathogenic nematode *S. feltiae* provided considerable control of both insect pest species across all substrate types. The susceptibility of insect pests of mushrooms to parasitism by *S. feltiae* has been previously investigated with variable results, particularly with regard to phorids (Cantelo *et al*., 1977; Richardson, 1987; Scheepmaker *et al*., 1998a,b). Initial attempts to parasitize phorids and sciarids with *S. feltiae* in compost-filled Petri dishes were unsuccessful (Cantelo *et al*., 1977). However, in larger-scale experiments, Richardson (1987) demonstrated the potential of *S. feltiae* and another rhabditid nematode *Heterorhabditis heliothidis* Khan, Brooks & Hirschmann to control *L. castanescens*, *M. halterata* and *Heteropeza pygmaea* Winnertz (Diptera: Cecidomyiidae). Because infective larvae of *H. heliothidis* can penetrate insect cuticle in addition to natural body openings, this nematode was considered to be a more effective parasite (Richardson, 1987). Infective larvae of *S. feltiae* penetrate the insect host through natural body openings and this is considered a limitation, particularly in relation to the requisite size and related maturity of its host (Richardson, 1987; Scheepmaker, 1998a,b). This limitation was considered to be the principal reason for the inefficiency of control of phorid larvae, which are much smaller than sciarid larvae at comparative development stages. Scheepmaker (1998a,b) made similar observations and proposed later nematode applications to coincide with increased maturity of phorid larvae. In the current study, *S. feltiae* provided considerable control of *M. halterata* in spawned mushroom compost and there was a notable trend for increased effect of later application of nematodes.

Observations on the vertical distribution of the biological control agents within substrate profiles, in the absence of prey, may be considered of limited value. Lesna *et al*. (1996) concluded that the arrestment of *H. aculeifer* to a particular region was in response to the presence of the prey bulb mites *R. robini*. Lind (1993) observed that movement of *H. miles* depended on its physiological state. Starved mites tended to move more rapidly with increased negative geotaxis and hence greater dispersal. Mites which were well-fed had slower and more deliberate movements, which limited their dispersal range. Both mite species used in profile study were well fed on *Acarus siro* Linnaeus (Acari: Acaridae) before introduction to experimental units. Nevertheless, adult *H. miles* mites exhibited a greater degree of negative geotaxis to that displayed by adult *H. aculeifer* during experimental preparations. However, experimental results indicated that both species could penetrate either substrate within the range of 2.0 and 12.0 cm, with a greater proportion of each species residing at depths between 10.0 and 12.0 cm. In contrast, a majority of *S. feltiae* nematodes were recovered from both substrates at depths between 2.0 and 4.0 cm. This may be considered a severe limitation, particularly during early phase 3 of mushroom growth when the immature stages of the pest species may reside at the compost and casing interface at a depth of 5.0 cm. Whilst further pest infestation will occur at the casing surface, a latent period

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will occur before pest larvae reach a level of maturity, which is susceptible to parasitism by nematodes of *S. feltiae*. Therefore, it may be concluded that the mite species of the genus *Hypoaspis* investigated in this study are potentially more suitable candidates for the biological control of *L. ingenua* and *M. halterata*.

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