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Potential for use of garlic oil to control *Lycoriella ingenua* (Diptera: Sciaridae) and *Megaselia halterata* (Diptera: Phoridae) in commercial mushroom production

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The repellent effects of garlic oil to *Lycoriella ingenua* and *Megaselia halterata* were examined in static olfactometers. In a choice between treated and control glass beads, adult female *M. halterata* were repelled by garlic solutions at concentration levels as low as 0.1% (v/v). When provided with a choice between compost treated with a range of garlic concentrations (0.1–20%) adult female *M. halterata* demonstrated a clear preference for untreated compost. In contrast, olfactometer studies involving *L. ingenua* females were inconsistent. For L. *ingenua*, the highest concentration of garlic oil (20%) significantly reduced subsequent adult emergence from treated spawned compost. With increasing concentrations of garlic oil, *M. halterata* emergence was progressively reduced. For both pest species, application of garlic oil post-oviposition had no significant effect on subsequent survival of immature life stages and surprisingly enhanced final emergence.

1. Introduction

Mushroom (*Agaricus bisporus* (Lange) Imbach) production is a significant component of the horticultural industry in the United Kingdom and Ireland. With an estimated output value of £152 M, of which Northern Ireland accounted for £37 M, mushroom production represents 10% of the total value of horticultural production in the UK in 2014. In addition, mushroom production in the Republic of Ireland is valued at £115 M (Anon, 2015 Bord Bia).

The major insect pests of mushroom cultivation are *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) and *Megaselia halterata* (Wood) (Diptera: Phoridae). Descriptions of their lifecycles, together with the current problems associated with their control, are reported elsewhere (Fletcher et al., 1989; Jess and Kilpatrick, 2000; Jess and Bingham, 2004; Jess, 2010). Differences in their life cycles affect their occurrence in the mushroom cultivation process and consequently, their relative importance as pests. In brief, both species are multivoltine throughout the mushroom production cycle (Fig. 1). However, as sciarid larvae are not obligate mycelium feeders, development occurs in unspawned compost and adult *L. ingenua* will readily lay eggs in this substrate. By contrast, oviposition by *M. halterata* is stimulated by mycelium development and is optimal during spawn-run. Under normal mushroom cultivation conditions, the total development time for *M. halterata* is 30 d, compared to 21 d for *L. ingenua*.

Expenditure on control measures for these pests represents an estimated 2% of the annual output value (White, 1995). Despite this, a further 4% is lost in yield reduction due to pest infestation leading to a 6% overall cost burden to growers. Resistance to different insecticides has been long known within sciarid populations (Binns, 1976; White and Gribben, 1989) and is now considered widespread in Great Britain (Smith and White, 1996). Also in this region, Smith (2002) recorded resistance in *Lycoriella* spp. populations to the benzoylurea insecticide, diflubenzuron, one of the few remaining insecticides approved for control of this pest in mushrooms. Further development of insecticide resistance among invertebrate pests of mushroom cultivation will probably exacerbate future problems associated with insect pest control in the mushroom production industry. Additionally, mushrooms are a perishable commodity with limited processing requirement and interval between harvest and consumption. Consequently, reliance on a chemical control strategy raises concerns by consumers about the potential introduction of pesticide residues into the food chain. Environmental considerations and increased public awareness of

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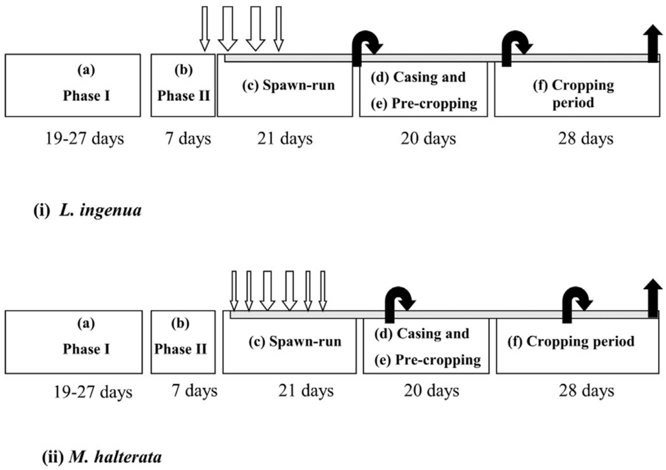

Fig. 1. Diagrammatic representation of the process of commercial mushroom production and the development of (i) *Lycoriella ingenua* and (ii) *Megaselia halterata* under experimental conditions. (a) Phase 1, initial composting of raw ingredients; (b) Phase 2, a pasteurisation process to produce an *Agaricus bisporus*-selective compost; (c) spawn-run, colonisation of compost by *A. bisporus* mycelium; (d) casing, addition of moist 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; initial oviposition; immature stages; adult emergence and re-infestation (adapted from White, 1995).

pesticide related problems has imposed increasingly restrictive legislation regarding insecticides. Consequently, pesticide registration costs have increased and the rate of novel active ingredients being developed for this industry has declined.

Whilst a survey of annual pesticide usage within the Northern Ireland mushroom production industry indicated a significant reduction in the use of chemical insecticides, problems remained with regard to the control of phorids and sciarids (Lavery et al., 2011). As the mushroom industry progresses toward the production of full-grown compost (Phase III), the pest significance of phorids in particular is likely to increase at sites for both compost production and mushroom cultivation. There is an urgent need to develop a sustainable alternative strategy to chemical pest control for the mushroom industry. This strategy should be widely applicable but may be of particular interest to the organic sector, where there is an opportunity for added value.

Plant extracts and other natural compounds can deter phytophagous insects, particularly in the protected environment (Bouda et al., 2001). The use of plant extracts to control pests and diseases in mushroom cultivation was investigated by Baars et al. (2008), in which, an unspecified (for reasons of commercial confidentiality) plant extract reduced phorid emergence by 60% and 90% when applied as preventative and curative treatments, respectively. Garlic (*Allium sativum* L.) extracts comprise a number of commercially available pest control products, but the efficacy and mode of action of these is variable (Flint et al., 1995; Liu and Stansly, 1995; Park et al., 2006; Prowse et al., 2006; Erler et al., 2008; Bell et al., 2016). The aim of this study was to evaluate garlic extract as a repellent to the two main fly pest species, *L. ingenua* and *M. halterata*.

2. Materials and methods

2.1. Cultures

Adult flies used in all experiments were taken from wild-collected cultures, reared on mushroom cultivation substrates, under controlled environmental conditions. Flies were housed in breeding and emergence cages (62.0 × 42.0 × 44.0 cm) constructed from wooden frames with perspex walls, ceilings and with mesh sleeves for access. *Lycoriella ingenua* cultures were maintained at 20 ± 1 °C and 70 ± 5% RH. A mixture of pasteurised, unspawned compost with compost inoculated with 1% *A. bisporus* spawn (strain A15, Sylvan UK – as used throughout), was exposed to adult sciarids in four plastic containers (22.5 × 11.5 × 7.5 cm) for one week. Subsequently, the containers were relocated to another emergence cage for three weeks to allow emergence. The culture technique for phorids was similar, except that these were maintained at 22 ± 1 °C and 70 ± 5% RH and the substrate used was compost inoculated with 1% *A. bisporus* spawn, which had been incubated for 1 weekat 22 ± 1 °C and 70 ± 5% RH in darkness. Twenty-four hours before adult flies were collected for use in experiments, the emergence cages were vacuum-cleaned, to ensure that all flies were at a similar stage of development.

2.2. Olfactometer experiments

A static-air olfactometer (Fig. 2) similar to that described by Tibbles et al. (2005) was used to investigate the behavioural responses by females of both *L. ingenua* and *M. halterata* to a range of garlic oil concentrations. The olfactometer comprised a 27.5 × 15.5 × 10.0 cm release arena with two 2.0 cm diameter ventilation holes, covered with fine mesh. Adult flies were released from a 4.5 cm diameter centrally mounted Petri dish with a lid attached to string threaded through a small hole in the top of the release arena. The choice-test pots were 300 ml sterile plastic honey jars with lids (Medfor HJL0300E) attached to the floor of the release arena and connected to it by glass tubes (4.5 × 0.5 cm internal diameter). Four tubes to each choice-test pot were positioned equidistantly from the centre of the lid and flush to the floor of the arena creating a pitfall trap for flies. The test pots were positioned equidistantly in the centre of arena floor. For each experiment, olfactometers were arranged in a Latin square inside a controlled environment chamber and replicated on five occasions to ensure that each treatment appeared once in each olfactometer.

At the beginning of each experiment, treatments and olfactometers were randomly assigned. On each trial, 20 adult female flies were placed into the Petri dish within the olfactometer and subsequently released. During experiments, olfactometers were maintained in darkness for 16 h at 20 ± 1 °C and 70 ± 5% RH for sciarids and 22 ± 1 °C and 70 ± 5% RH for phorids. Treatments were garlic oil solutions (1.0 ml) made from 99.9% food grade garlic concentrate (Agariguard® Ecospray Ltd) applied to pitfall test pots containing either 2.5 g glass beads (5.0 mm diameter) or 2.5 g of compost inoculated with 1% *A. bisporus*. Water (1.0 ml) was used for the control pots. The garlic oil solution concentrations were: 0.1%, 1.0%, 10.0%, 20.0% v/v (garlic oil/water). At each concentration, three scenarios were investigated: treated glass beads versus untreated glass beads, treated compost versus untreated glass beads, treated compost versus untreated compost. Each Latin square experiment was repeated on five occasions meaning each treatment was replicated 25 times.

At the end of each occasion (24 h), the numbers of flies within each choice-test pot and release chamber were counted. Relative proportions of flies within pots were analysed using Generalized Linear Mixed Models (GLMMs) fitted to a binomial distribution with a logit link function, and included overdispersion, using GenStat 16th Edition.

2.3. Effects of garlic application on *L. ingenua* and *M. halterata* oviposition and development of immature stages

Eight experiments examined the effects of garlic oil application, before and after oviposition, for both species, at two population