Biological Control 50 (2009) 172–178

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus*, to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci*

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ARTICLE INFO

Article history:
Received 15 December 2008
Accepted 24 March 2009
Available online 31 March 2009

Keywords:
Entomopathogenic fungi
Predatory bug
Plant-sucking insects
Greenhouse pests
Lecanicillium spp.
Dissemination

ABSTRACT

The simultaneous use of two biocontrol agents for the concurrent control of three pest species was investigated. Leaf disc bioassays were conducted to establish a suitable method (surface dosing) for the dissemination of an entomopathogenic fungus (*Lecanicillium longisporum* or *L. muscarium*) by the predatory bug *Orius laevigatus*. Predatory bugs surface dosed with fungal conidia successfully disseminated conidia onto sweet pepper leaf discs. Most (98%) of the peach-potato aphids (*Myzus persicae*) that were subsequently maintained on the leaf discs became infected with the pathogen and died within 5 days. However, fungal conidia disseminated by surface dosed predatory bugs did not infect and kill the western flower thrips (*Frankliniella occidentalis*) or the sweetpotato whitefly (*Bemisia tabaci*). Plant trials were performed to assess the efficacy of using predatory bugs surface dosed with *L. longisporum* as an effective means of controlling *M. persicae* and *F. occidentalis* populations. The results indicated that the number of aphids and thrips were significantly lower (66% and 95%, respectively) on the plants where surface dosed predatory bugs were used as a control measure compared with plants where the fungal pathogen alone was used and were statistically comparable to the numbers on plants where the predatory bug alone was used. The potential for using this dual approach is discussed in the context of improved biological control of glasshouse pests.

1. Introduction

Thrips, aphids and whiteflies are major pests of horticultural industry crops. Due to the detrimental consequences for the environment caused by the application of chemical insecticides (Boatman et al., 2007), alternative means of pest control such as biocontrol agents are needed. However, biocontrol agents have limitations. For example, pest populations may have to be present in relatively high numbers before predatory bugs become effective, which can be unacceptable to retailers because of the damage caused (DeBach and Rosen, 1991). Therefore biocontrol agents are most effective when used as part of an integrated pest management (IPM) system.

Numerous studies have investigated the potential for insect predatory bugs to disseminate entomopathogenic microsporidia (Down et al., 2004) and viruses (Biever et al., 1982; Abbas and Boucias, 1984; Young and Yearian, 1987; Lee and Fuxa, 2000; Down et al., 2004). Other studies have shown that carabid beetles (Capinera and Barbosa, 1975) and sarcophagid flies (Lee and Fuxa, 2000) can also disseminate insect viruses. More recent studies have concentrated on the potential for (1) an additive effect when predatory insects and entomopathogenic fungi are used together to control insect pest species (Alma et al., 2007), and (2) the dissemination of fungal entomopathogens by non-pest insect species on the improved efficacy of the pathogen (Pell et al., 1997; Butt et al., 1998; Roy et al., 2001; Bruck and Lewis, 2002; Pell and Vandenberg, 2002; Bird et al., 2004; Meyling et al., 2006; Carreck et al., 2007). The dispersal of entomopathogens by non-target insects often provides a targeted way of dispersing the fungal pathogen directly into the pest population.

Entomopathogenic fungi belonging to the genus *Lecanicillium* (formerly *Verticillium*) (Zare and Gams, 2001) are antagonistic to insects (Askary et al., 1998; Gindin et al., 2000; Wang et al., 2004; Cuthbertson and Walters, 2005; Cuthbertson et al., 2005a,b; Lee et al., 2006), plant pathogens (Whipps, 1993; Verhaar et al., 1997; Askary et al., 1998; Benhamou and Brodeur, 2000, 2001; Miller et al., 2004) and plant-parasitic nematodes (Meyer et al., 1990). There is potential for *Lecanicillium* spp. to be used in IPM in glasshouse horticultural systems (Hall, 1981; Verhaar et al., 1996) in combination with other beneficial organisms to simultaneously control a number of plant pests and pathogens, including the dual control of aphids and cucumber powdery mildew fungus (Kim et al., 2007, 2008). Both *L. longisporum* (Petch)

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doi:10.1016/j.biocontrol.2009.03.010

Zare and Gams and *L. muscarium* (Petch) Zare and Gams have been commercially developed as mycopesticides and are used in several countries, including the United Kingdom, to control aphids (Vertalec®) and whitefly (Mycotal®).

This study investigates the efficacy of an approach for the concurrent control of aphids, thrips and whiteflies based on the simultaneous use of two biocontrol agents. The potential for the predatory bug, *Orius laevigatus* (Fieber) to be used to prey upon and control the western flower thrips, *Frankliniella occidentalis* (Pergande), and whilst dosed with an entomopathogenic fungus (*L. longisporum* or *L. muscarium*), to serve a dual function by disseminating the fungal pathogen to improve the control of other pest species [peach-potato aphid, *Myzus persicae* (Sulzer); sweetpotato whitefly, *Bemisia tabaci* (Gennadius)] was assessed.

2. Materials and methods

2.1. Biological materials

Myzus persicae were reared on oilseed rape (*Brassica napus* cv. Apex) at 20 °C, 60% RH under a 16:8 h L:D regime. *Bemisia tabaci* were reared under quarantine conditions in perspex cages (60 cm × 60 cm × 80 cm) on poinsettia (*Euphorbia pulcherrima* cv. Lilo Pink) at 23 ± 1 °C, 65% RH under a 16:8 h L:D regime and an artificial dawn and dusk (Cuthbertson et al., 2005a). Cultures of *F. occidentalis* were maintained within half liter glass Kilner jars on green beans (*Phaseolus vulgaris* L.) and pollen. The jars had ventilated lids covered with nylon mesh (75 μm pore) to allow airflow. The colonies were maintained at 24 °C, 65% RH and 16:8 h L:D according to Smith et al. (2005).

Orius laevigatus (Thripor-L®; Koppert Biological Systems Ltd., Haverhill, UK) were maintained in 1 L glass Kilner jars with ventilated lids covered with muslin, at 25 °C, 70% RH and 16:8 h L:D following the method of Bakker et al. (2000). The jars contained tissue paper to minimize cannibalism. The predatory bugs were fed on *Ephestia kuehniella* (Zeller) eggs (Entofood®, Koppert Biological Systems Ltd.), and green beans were provided as a water source and oviposition site.

Commercial formulations of *L. longisporum* and *L. muscarium* were obtained from Koppert Biological Systems Ltd. (Vertalec® and Mycotal®, respectively) and diluted and sprayed according to the manufacturer's instructions unless otherwise stated. *Lecanicillium longisporum*, recommended by the manufacturer for use against aphid pests, was used to investigate the dissemination of conidia by *O. laevigatus* and subsequent infection of *Myzus persicae*. *Lecanicillium muscarium*, recommended by the manufacturer for use against whitefly pests with some effect on thrips, was used to investigate the dissemination of conidia by *O. laevigatus* and subsequent infection of *B. tabaci* and *F. occidentalis*.

2.2. Dissemination of L. longisporum and L. muscarium by surface dosed O. laevigatus

Lecanicillium longisporum (Vertalec®) was plated out onto malt extract agar (MEA) plates, which were incubated over a shallow layer of water in a plastic box covered in foil, in a controlled environment room set at 20 °C, 70% RH and 16:8 h L:D. After 7–10 days, when *L. longisporum* growth had covered the agar and was sporulating, six adult *O. laevigatus* predatory bugs were allowed to walk inside each plate for 30 min. Predatory bugs used for the control treatment were confined to clean MEA plates for 30 min. Six plates were used for the fungal and control treatments, totaling 36 predatory bugs (replicates) for each treatment. Twelve leaf discs (25 mm) from a sweet pepper plant (*Capsicum annuum* L. cv. Bell Boy) approximately 2–2.5 months old were cut from one pair of leaves, using six different plants for a total of 72 discs. These were divided comparably between the fungal and control treatments. The leaf discs were placed with the abaxial surface uppermost in the wells of 12-well tissue culture plates (diameter 22.2 mm). The wells had previously been coated with Fluon Fluoropolymer dispersion (Whitford Plastics Ltd., Runcorn, UK), to prevent the insects from climbing the walls, and partially filled with agar (Agar No. 3, Oxoid) to maintain high moisture levels. One predatory bug, either treated by confining to fungal lawn or control, was placed on each leaf disc. The lids were placed over the well-plates, which were then wrapped in Saran wrap and a weight placed on top. The well-plates were incubated at 20 °C, 70% RH and 16:8 h L:D. After 48 h the predatory bugs were removed from the leaf discs. The leaf discs were carefully transferred and placed abaxial surface uppermost into glass tubes (diameter 25 mm, height 50 mm), which had been partially filled with agar (Agar No. 3, Oxoid) to maintain high moisture levels. Ten newly mature, apterous *M. persicae*, acclimated to sweet pepper by placing on 2-month-old sweet pepper plants as neonate nymphs and allowing to develop to maturity, were placed on each leaf disc and the tube sealed with a cotton wool plug. The tubes were placed in a desiccator, with a humidity of 80%, maintained by KOH solutions (Solomon, 1951) at the appropriate specific gravity, and the desiccator placed at 20 °C, 70% RH and 16:8 h L:D. Aphid survival was recorded daily, and once all the aphids per replicate had died, the bodies were surface sterilized and placed in sealed humid chambers in order to confirm whether or not they were infected with the fungal pathogen. Surface sterilization was performed by submerging the cadavers in a 1% sodium hypochlorite solution for 1 min and then washing twice in sterile distilled water for 1 min. This method has previously been shown to kill fungal conidia on the external surfaces of insects (El-Hamalawi, 2008). The bodies (grouped in their original replicates) were placed on moistened filter paper in 35 mm diameter petri dishes. The dishes were sealed with parafilm and placed at 20 °C, 70% RH. The bodies were monitored for 2 weeks for signs of subsequent fungal growth, indicating that they were infected with *L. longisporum*.

The predatory bugs were maintained (no food provided) on fresh sweet pepper leaf discs, and their bodies were also surface sterilized after they died to see if they had become infected with the pathogen.

This experiment was repeated to investigate the dissemination of *L. muscarium* conidia (Mycotal®), by *O. laevigatus*, and the possible subsequent infection of *F. occidentalis* and *B. tabaci* adults. Once the adult *F. occidentalis* and *B. tabaci* had been placed on the leaf discs, they were maintained in a controlled environment cabinet at 22 °C, 80% humidity and 16:8 h L:D. Replicates were set up over two time points such that a total of 36 and 24 predatory bugs (replicates) were used for *F. occidentalis* and *B. tabaci*, respectively.

2.3. Whole plant assays for concurrent control of M. persicae and F. occidentalis

Sweet pepper plants, approximately 2–2.5 months old (ca. 20 cm in height) were treated with one of five pest control measures: (1) introduction of five *O. laevigatus* predatory bugs that had been surface dosed with *L. longisporum* conidia as described in Section 2.2; (2) introduction of five *O. laevigatus* predatory bugs that had not been dosed with fungal conidia; (3) sprayed with the commercial formulation of *L. longisporum* (Vertalec®) following the manufacturer's instructions (giving approximately 1.5×10^5 conidia/cm^2 leaf surface); (4) sprayed with the commercial formulation of *L. longisporum* and also introduced with five *O. laevigatus* predatory bugs that had not been dosed with fungal conidia; and (5) no pest control. A 0.02% solution of the non-ionic wetting agent Agral (Syngenta Crop Protection Ltd., Cambridge, UK; active ingredient: alkyl phenol ethylene oxide) was added to the conidial suspension