Biological Control 80 (2015) 70–76

Contents lists available at ScienceDirect

Biological Control

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

A simple method of rearing insect natural enemies of spider mites

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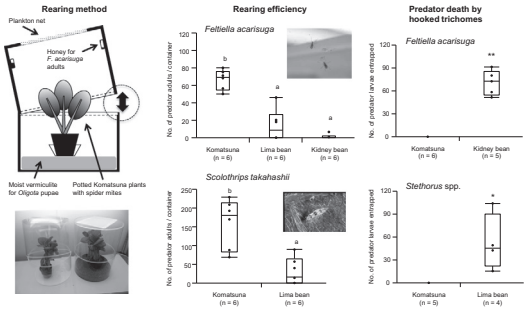
HIGHLIGHTS

- We have four important insect predator groups of pest spider mites.
- Rearing these insect predators is difficult due to the lack of suitable host plants.
- Prey spider mites can be efficiently maintained on komatsuna plants (*Brassica rapa*).
- The insect predators can be efficiently reared on prey-infested komatsuna plants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 April 2014
Accepted 24 September 2014
Available online 7 October 2014

Keywords:
Rearing method
Scolothrips takahashii
Feltiella acarisuga
Stethorus spp.
Oligota spp.
Spider mite

ABSTRACT

Establishing effective methods of rearing natural enemies can facilitate basic and applied studies on their use in biological pest control. Insect predators of four genera (*Oligota*, *Stethorus*, *Scolothrips* and *Feltiella*) and predatory mites are important natural enemies of pest spider mites in agricultural crops. However, fewer laboratory studies have focused on insect predators due to the difficulty of their rearing. To establish a simple rearing method, we compared the rearing efficiency of four insect predator groups (*Oligota* spp., *Stethorus* spp., *Scolothrips takahashii* and *Feltiella acarisuga*) on potted komatsuna plants (*Brassica rapa*) infested with the two-spotted spider mite *Tetranychus urticae*, a new plant-prey combination for predator rearing in large containers, with that on potted bean plants infested with *T. urticae*. Significantly more *Feltiella acarisuga* and *Stethorus* spp. adults were obtained when offered *T. urticae*-infested komatsuna plants than infested lima bean or kidney bean plants (*Feltiella*: 70, 9 and 0 adults, respectively; *Stethorus*: 72, 3 and 0 adults, respectively). More adults of *S. takahashii* and *Oligota* spp. were obtained on *T. urticae*-infested komatsuna than on infested lima bean (*Scolothrips*: 181 and 27 adults, respectively; *Oligota*: 152 and 16 adults, respectively). Many individuals of all four predator groups such as larvae of *F. acarisuga* and *Stethorus* spp. were entrapped by the hooked trichomes on lima and kidney bean leaves, whereas no entrapped predators were observed on the komatsuna plants. These results show potted komatsuna plants infested with *T. urticae* to be suitable for efficient rearing of all four predator groups.

1. Introduction

Establishing effective methods of rearing natural enemies is a prerequisite for facilitating basic and applied studies on their use in biological pest control (Van Lenteren and Woets, 1988). For example, large numbers of predators or parasitoids must be provided to evaluate their susceptibility to various pesticides in the laboratory; for this purpose, information on (mass-) rearing

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http://dx.doi.org/10.1016/j.biocontrol.2014.09.015
1049-9644/

methods of some beneficial insects and mites has been reported (Sterk et al., 1999). We need more information about efficient rearing methods for other natural enemies to clarify their pesticide susceptibility, biological characteristics and ecological interactions with pest arthropods.

Spider mites are an important pest of crops, fruit and ornamental plants (Helle and Sabelis, 1985a; Huffaker et al., 1970). Chemical control of spider mites is challenging, so their natural enemies, which can be roughly classed into predatory mites and insect predators, have received attention in past decades (Helle and Sabelis, 1985b; McMurtry and Croft, 1997). Predatory mites can depress spider mite populations to low densities (Amano, 2001; Chazeau, 1985), while voracious insect predators may prevent outbreaks of spider mites (Gagné, 1995; Kishimoto and Adachi, 2008; Takahashi et al., 2001). Because of the ease of rearing predatory mites, their biological characteristics have been studied extensively (Dicke and Baldwin, 2010; Ellner et al., 2001; Kappers et al., 2005; McMurtry and Croft, 1997; Sabelis and Van De Baan, 1983), and some of them are commercially available for biological pest control (Escudero and Ferragut, 2005; Fournier et al., 1985; Van Lenteren et al., 1997).

In contrast, fewer laboratory studies have focused on insect predators, due to the difficulty of rearing them (but see e.g., Gotoh et al., 2004; Kishimoto and Adachi, 2008; Shimoda, 2004). Four genera of insect predators are important natural enemies of spider mites: *Oligota* (Coleoptera: Staphylinidae), *Stethorus* (Coleoptera: Coccinellidae), *Scolothrips* (Thysanoptera, Thripidae), and *Feltiella* (Diptera: Cecidomyiidae) (Abe et al., 2011; Biddinger et al., 2009; Shimoda and Takabayashi, 2001; Takahashi et al., 2001). Low densities of these insect predators can be reared on detached bean leaves placed on moist cotton wool, called "leaf discs," infested with the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) in small containers (Gotoh et al., 2004; Kishimoto and Adachi, 2008). However, this rearing system tends to be arduous and inefficient, because prey-infested leaf discs must be supplied frequently (often every few days) for voracious predators (Chazeau, 1985; Gotoh et al., 2004); and the hooked trichomes on bean leaves may affect predator survival (Riddick and Wu, 2011; Sengonca and Gerlach, 1984).

The aim of this paper was to establish a simple and improved method for rearing high densities of insect predators of the four genera on whole plants cultivated in a pot, hereafter called "potted plants", that support abundant prey. We have focused on a brassica vegetable, komatsuna (*Brassica rapa* L., var. perviridis), as a candidate for the host plant, since: (1) it can be easily cultivated in the laboratory or greenhouse; (2) many individuals of *T. urticae* can be maintained on potted komatsuna plants for several weeks; and (3) it has no hooked trichomes to interfere with insect predators. In this study, we compared the efficiency of rearing the chief natural enemies of the four insect groups in Japan [*Oligota* spp., *Stethorus* spp., *Scolothrips takahashii* Priesner and *Feltiella acarisuga* (Vallot)] on *T. urticae*-infested komatsuna plants in large containers with the same process on infested lima bean and kidney bean plants. We show that potted komatsuna plants infested with *T. urticae* are suitable for efficient rearing of all four predator groups.

2. Materials and methods

2.1. Plants and insects

Three lima bean plants (*Phaseolus lunatus* L., cv. Sieva) or five kidney bean plants (*Phaseolus vulgaris* L., cv. Nagauzuramame) were cultivated in a plastic pot (diameter: 9 cm, depth: 7 cm) in a climate-controlled room (23 ± 1 °C, 60 ± 10% RH and 16 L: 8 D). Three komatsuna plants (*B. rapa* L., var. perviridis, cv. Rakuten) were cultivated in a plastic pot in a greenhouse (23 ± 3 °C and 16 L: 8 D). *T. urticae* was reared on potted kidney bean plants in a climate-controlled room (23 ± 1 °C, 60 ± 10% RH and 16 L: 8 D). Several pieces of *T. urticae*-infested kidney bean leaves were placed on potted lima bean (2–3 weeks after germination), kidney bean (2–3 weeks after germination) or komatsuna plants (4–5 weeks after germination) for 1 day; each potted plant had approximately 4000 mites at all stages (ca. 400 were adult females) and was used for the rearing experiments.

Oligota spp. [*O. kashmirica benefica* Naomi and *O. yasumatsui* Kistner; which may be involved in the genus *Holobus* (Hoy, 2011; Löbl and Smetana, 2004)], *Stethorus* spp. [*Stethorus japonicus* H. Kamiya and *Stethorus pusillus* (Herbst)], *S. takahashii*, and *F. acarisuga* were collected from kudzu vine plants (*Pueraria lobata* [Wild] Ohwi) infested with the red spider mite *T. puerariicola* Ehara & Gotoh in Tsukuba, Ibaraki Prefecture, Japan, in 2009, 2010 and 2011 (Fig. 1). *S. takahashii* adult females, discriminated morphologically from males, were used in the rearing experiments. Adults of *Oligota* spp. and *Stethorus* spp. were individually introduced into a plastic container (diameter: 9 cm, height: 4.5 cm), containing a *T. puerariicola*-infested kudzu vine leaf disc, for 24 h in a laboratory (25 ± 1 °C, 30 ± 10% RH and 16 L: 8 D); female predators laying eggs were selected for the experiments. Because of the difficulty in discriminating the sexes in *F. acarisuga*, both adult females and males emerging from the pupae on kudzu vine leaves in a netted plastic cage (25 × 33 × 30 cm) were used. Because the taxonomy of the genus *Feltiella* is ambiguous (Abe et al., 2011; Ganaha-Kikumura et al., 2012), identification of *F. acarisuga* was confirmed by sending the specimens to Dr. Junichiro Abe at NARO, Western Region Agricultural Research Center, Fukuyama, Hiroshima, Japan.

2.2. Rearing experiments

Three *Stethorus* spp. females were introduced, using a fine paintbrush, onto potted komatsuna, lima bean or kidney bean plants infested with *T. urticae* in a columnar acrylic container (diameter: 30 cm, height: 40 cm: Fig. 2) in a climate-controlled room (25 ± 1 °C, 30 ± 10% RH and 16 L: 8 D). To supply additional prey, a new pot of each plant species carrying the mites was placed in the container every 5–10 days (Fig 2, Fig. S1). Thirty days after predator introduction, the numbers of adult and other stages (egg, larva and pupa) in the container were counted under a binocular microscope (Leica MZ16; Leica, Tokyo, Japan). Six replicates were made for each plant species.

Five adult females of *S. takahashii* were introduced into a container in which a potted komatsuna or lima bean plant, infested with *T. urticae*, had been placed. The numbers of adult and other stages (larva and pupa) were investigated 30 days later. Other conditions and procedures were the same as those for *Stethorus* species. Six replicates were made for each plant species.

Thirty adults (including females and males) of *F. acarisuga* in the netted cage were collected using a glass tube (diameter: 1 cm, length: 7.5 cm) and then transferred into a container with a pot of komatsuna, lima bean or kidney bean plants infested with *T. urticae*. Small amounts of honey were applied to the wall of the container: *F. acarisuga* adults feed on sugary foods and do not attack spider mites (see Figs. 1d, 2). A new pot of each plant species with the mites was supplied every 5–7 days. Two weeks later, the number of adult stages was investigated. The other conditions and procedures were the same as those for *Stethorus* species. Six replicates were made of each plant species.

Fully-developed *Oligota* spp. larvae on prey-infested leaves will die within a few days if they cannot burrow into moist soil to pupate (Shimoda, 2004). Thus, four female predators were introduced onto a pot of prey-infested komatsuna or lima bean plants