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Biological Control 33 (2005) 74–80

Biological Control

www.elsevier.com/locate/ybcon

Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp.

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Received 4 October 2004; accepted 6 January 2005

Abstract

Bemisia tabaci (Gennadius) is a serious pest of vegetable, ornamental, and agronomic crops throughout the world. To control *B. tabaci*, *Eretmocerus eremicus* Rose & Zolnerowich, and *Eretmocerus mundus* Mercet are considered the most effective parasitoids in dry tropical regions. In parasitoids, choosing the 'right' hosts has direct consequences for their reproductive success and efficiency as biocontrol agent. Therefore, being able to discriminate a parasitized host from an unparasitized one would be important to prevent wasting time, eggs, and to reduce the mortality risk for their offspring. We evaluated intra- and interspecific host discrimination and the chance of super-parasitism or multi-parasitism in two populations of *E. mundus* (sexual and asexual) and *E. eremicus.* Different combinations and sequences of female introduction were carried out for the various populations and species. Experienced females avoided super-parasitism. However, naïve females did lay eggs under hosts that were previously parasitized by conspecific females. *E. eremicus* females avoided to multi-parasitize hosts parasitized by *E. mundus.* However, *E. mundus* females did multi-parasitize the hosts that had been parasitized earlier by *E. eremicus.* In the case of super-parasitism, the outcome showed that neither of the *E. mundus* populations was stronger, whereas in the case of multi-parasitism *E. mundus* appeared stronger than *E. eremicus.* Since those populations and species are morphologically similar a molecular method had to be developed to identify the outcome of super- or multi-parasitism, which is presented in Appendix A.

Keywords: *Bemisia tabaci*; Competition; *Eretmocerus eremicus*; *E. mundus*; ITS1 and ITS2 marker; Multi-parasitism; "Nru I" enzyme; Super-parasitism

1. Introduction

Currently, *Bemisia tabaci* (Gennadius) is a serious pest of vegetable, ornamental, and agronomic crops throughout the world. It has caused enormous damage to many crops during the past three decades (Gerling, 1990; Gerling and Mayer, 1996). So far, several biological control strategies have been evaluated for management of *B. tabaci*, e.g., the use of hymenopteran parasitoids, either native or exotic (for a review see Goolsby et al., 1998). Currently, two species of *Eretmocerus* are commercially available: *E. eremicus* Rose & Zolnerowich and *E. mundus* Mercet. *E. eremicus* is indigenous to the United States (Rose and Zolnerowich, 1997). It seems to be effective for control of *B. tabaci* on poinsettia (Hoddle and van Driesche, 1999). *E. mundus* is recorded from many parts of the Mediterranean basin (Mound and Halsey, 1978). It is considered the most important controlling agent for *B. tabaci* in the plastic greenhouses in southern Spain (Rodriguez et al., 1994). These two *Eretmocerus* species now used are arrhenotokous, but another population of *E. mundus*, which has been found in Australia, is thelytokous (de Barro et al., 2000). Because a thelytokous population only produces female offspring, it is

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1049-9644/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2005.01.006

considered the best candidate for biological control of *B. tabaci* (de Barro et al., 2000).

In the evaluation of parasitoids for biological control, one aims to select the most effective species. One aspect that may have an important effect on the parasitoid's efficiency is its foraging behavior (Godfray, 1994). During foraging behavior, a female parasitoid must make a number of decisions that are relevant to its reproductive success, namely: how long to stay in a patch to search for hosts, and whether to accept a host for oviposition. Part of this last decision is based on whether the host is healthy or already parasitized (see review in Hoffmeister and Roitberg, 1997). Emerging parasitoid larvae should be able to defeat the host defenses (e.g., encapsulation), which are induced by oviposition (Tuda and Bonsall, 1999). If more than one oviposition occurs by females of the same parasitoid species (a phenomenon called super-parasitism), the larvae face competition with other (related or unrelated conspecific) parasitoid larvae. A host can be parasitized more than once by females of the same species (super-parasitism) or by females of a different species (a phenomenon called multi-parasitism) of parasitoid (van Dijken and Waage, 1987). Superparasitism and multi-parasitism can delay the development of the progeny, increases larval mortality, and results in smaller offspring, particularly in solitary parasitoids (e.g. Potting et al., 1997; Vet et al., 1994). Therefore, an important element of host selection is the capability to distinguish between parasitized and unparasitized hosts, so-called "host discrimination."

Host discrimination confers an advantage to parasitoid females by reducing the wasting of time and eggs, and by minimizing the mortality risk for the offspring (van Lenteren, 1976, 1981). Host discrimination is perhaps particularly important in solitary parasitoids because only one larva is expected to complete its development (e.g., Hofsvang, 1990; van Alphen and Visser, 1990; van Lenteren, 1981). Therefore, to avoid competition among its own progeny, intraspecific host discrimination is frequently found in solitary parasitoids but interspecific host discrimination is rare (Agboka et al., 2002; Royer et al., 1999; van Baaren et al., 1994; van Lenteren, 1981).

Several mechanisms for host discrimination have been described in parasitoids to detect a parasitized host (external, internal or a combination; see, e.g., reviews by Gauthier and Monge, 1999; Potting et al., 1997; van Lenteren, 1976, 1981). In many cases "marking pheromones," have been implicated in mediating host discrimination (review in Nufio and Papaj, 2001). Host discrimination can also be mediated by chemical and/or physical changes in hosts induced by the presence of eggs or larvae (review in Nufio and Papaj, 2001). For instance, a hatching larva of an earlier oviposition may change the physiology of the host, enabling discrimination by conspecific parasitoids (Bai, 1991). However, in most parasitoids, the expression of host discrimination is influenced by internal factors of the adult parasitoid as well, e.g., egg load (Islam and Copland, 2000), different oviposition time intervals (Outreman et al., 2001; Ueno, 1999), and experience of the females (van Alphen and Visser, 1990; van Lenteren and Bakker, 1975, 1981).

To date, elements of host searching and oviposition behavior have been studied for *E. eremicus* and *E. mundus* (Foltyn and Gerling, 1985; Gerling et al., 1990; Headrick et al., 1995; Greenberg et al., 2002). However, super-parasitism, multi-parasitism, and host discrimination of whitefly parasitoids has been studied only to a limited degree and interspecific discrimination has not been studied at all in these species. Therefore, we embarked upon a study describing host discrimination and competition among *Eretmocerus* species and populations. In this research, we evaluate intra- and interspecific host discrimination of the two populations of *E. mundus* (sexual and asexual) and of a sexual population of *E. eremicus.* To obtain better insight in host discrimination among these populations and species, we distinguish different types of discrimination: "self" (where the host has been parasitized by the same female), "intra population" (parasitized by a conspecific female from the same population), "interpopulation" (parasitized by a conspecific female from another population), and "interspecific" (parasitized by a female from the other species).

2. Materials and methods

2.1. Maintenance of the insects

We used three populations of *Eretmocerus: E. eremicus* that is commercially available (ErCal, Koppert Biological Systems, The Netherlands), and non-commercial populations of *E. mundus* from Spain (sexual) and Australia (asexual). All three populations were maintained on *B. tabaci* and poinsettia (*Euphorbia pulcherima* Willd. ex Klotzsch) plants. A culture of *B. tabaci* was maintained on poinsettia plants in a greenhouse (25 °C and 75% RH).

2.2. Host discrimination

For the experimental work, leaf parts (3 × 4 cm) were cut from poinsettia plants infested with *B. tabaci* nymphs. Plant parts were fixed on moist pieces of cotton wool in a Petri dish to prevent desiccation. Subsequently, a map of the nymphal distribution was drawn for each leaf part and a 1-day-old naïve female parasitoid was introduced. Oviposition events were marked on the map using a stereo microscope; we called this phase the 'initial foraging period.' When the "first females" had achieved some ovipositions, the female was removed and