Interspecific Competition between Two Exotic Parasitoids (Hymenoptera: Aphelinidae) of an invasive Bemisia tabaci Species (Hemiptera: Aleyrodidae)

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Interspecific competition between two exotic parasitoids (Hymenoptera: Aphelinidae) of an invasive *Bemisia tabaci* species (Hemiptera: Aleyrodidae)

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Abstract

Classical biocontrol programs usually employ multiple species to control a single pest; however, the beneficial effects are not always additive due to competition between the introduced species. Knowledge of these potentially negative interactions is crucial when determining whether the introductions were successful and the extent to which they influence pest suppression. Here, we report the results of such competition between 2 exotic wasp species [*Encarsia bimaculata* Heraty & Polaszek and *Eretmocerus* sp. nr. *emiratus* (Hymenoptera: Aphelinidae)] introduced into Florida for control of the circumglobal super pest currently known as Middle East-Asia Minor 1, a cryptic species within the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae). Levels of parasitism, progeny production, and host feeding were evaluated in the laboratory under various parasitoid combinations on 2 host plant species (collard and eggplant) that differed in leaf pilosity (i.e., number of trichomes on the abaxial leaf surface). Significant differences in parasitism were observed by treatment, but not host plant. *Encarsia bimaculata* produced fewer progeny when introduced before *Er.* sp. nr. *emiratus* on collard; however, this trend was reversed on eggplant. *Eretmocerus* sp. nr. *emiratus* produced less progeny in all combinations involving *En. bimaculata* on collard and when introduced before *En. bimaculata* on eggplant. Mortality caused by host feeding was atypical for the whitefly-parasitizing aphelinids (<5% in all treatments). Based on differences in parasitism and progeny production, no additional pest suppression would be gained by releasing *En. bimaculata* into an environment already under control by *Er.* sp. nr. *emiratus*.

Key Words: biological control; *Encarsia*; *Eretmocerus*; hyperparasitism; parasitoid; progeny production

Resumen

Los programas de control biológico generalmente emplean varias especies para controlar a una especie; sin embargo, los efectos benéficos no son siempre aditivos debido a la competencia entre las especies introducidas. El conocimiento de estas interacciones potencialmente negativas es crucial cuando se trata de determinar si las introducciones fueron exitosas y la cantidad con las cuales ellas influyeron en la supresión de la plaga. Aquí, reportamos lo resultados de dicha competencia entre dos especies de avispas exóticas [*Encarsia bimaculata* Heraty & Polaszek and *Eretmocerus* sp. nr. *emiratus* (Hymenoptera: Aphelinidae)] introducida a Florida para cel control de la super plaga circumglobal, conocida actualmente como Medio Este-Asia Menor 1, una especie criptica dentro del complejo de *Bemisia tabaci* (Hemiptera: Aleyrodidae). Los niveles de parasitismo, producción de progenie y alimentación del hospedero fueron evaluados en el laboratorio bajo diferentes combinaciones de parasitoides en dos plantas hospederas (col o berza y berenjena) que difirieron en su pubescencia o pilosidad (i.e., número de tricomas en la superficie foliar abaxial). Se observaron diferencias significativas de parasitismo entre tratamientos, pero no para plantas hospederas. *Encarsia bimaculata* produjo un menor número de progenies cuando fue introducido antes de *Er.* sp. nr. *emiratus* en col. Sin embargo, esta tendencia fue al contrario en berenjena. *Eretmocerus* sp. nr. *emiratus* produjo menos progenie en todas las combinaciones involucrando a *En. bimaculata* sobre col y cuando se introdujo antes *En. bimaculata* sobre berenjena. La mortalidad causada al alimentar al hospedero fue atípica para los afelínidos parasitoidesan de mosca blanca (<5% en todos los tratamientos). Basados en las diferencias de parasitismo y producción de progenie, no se ganaría supresión adicional de la plaga al liberar *En. bimaculata* en un ambiente en el que esté ya bajo control de *Er*. sp. nr. *emiratus*.

Palabras Clave: control biológico; *Encarsia*; *Eretmocerus*; hiperparasitismo; parasitoide; producción de progenie

Classical biological control programs typically involve the introduction of multiple exotic species for control of a single target pest (Denoth et al. 2002; Gould et al. 2008). Such introductions undoubtedly lead to competition between the introduced organisms for the shared resources vital to their survival and perpetuation. Competition for resources is commonplace in parasitoid Hymenoptera, many of which develop on a single host and display a high degree of niche overlap. Knowledge of the dynamics between competing parasitoid species, in relation to use of their shared host(s), has direct relevance to their utilization in biological control programs.

Whiteflies and their parasitoids are a model host–parasite system to evaluate the outcomes of such competition. Many whiteflies become pests after escaping their natural boundaries and establishing populations in new areas (Martin 1987). Such was the case following the invasion of a member of the *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) species complex in Florida, and later many other parts of the

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world, in the latter decades of the last century (Stansly & Naranjo 2010). These novel environments may not have a natural enemy cohort capable of controlling this whitefly species below the economic threshold, resulting in the need for the introduction of exotic natural enemy species.

Parasitoid wasps in the family Aphelinidae (Hymenoptera: Chalcidoidea) are one of the most successful groups of insects used to control sternorrhynchous Hemiptera (Greathead 1986), and are regarded as the most important natural enemies of whiteflies, including *B. tabaci*, used in biological control (Lahey & Stansly 2014; Liu et al. 2015). Over the past century, numerous species have been introduced for control of adventitious pest whiteflies with many projects ending in complete success (e.g., Rose & DeBach 1992; van Lenteren et al. 1996; Pickett & Pitcairn 1999; Liu et al. 2015). A recent example was a program aimed at establishing parasitoids of *B. tabaci* Middle East-Asia Minor 1 (MEAM1; formerly biotype B or *Bemisia argentifolii* Bellows & Perring) in the United States (Goolsby et al. 2005; Gould et al. 2008). This whitefly is established worldwide and causes annual economic losses in the billions (US dollars). Florida was the site of the first reported MEAM1 invasion into the United States, where it remains a key pest of tomato, watermelon, and many other plants of agricultural and economic importance (Hamon & Salguero 1987).

An effort to establish exotic biological control agents was initiated in the early 1990s in an attempt to control MEAM1 in Florida through the mass rearing and release of parasitoid wasps (Nguyen & Bennett 1995). Seven parasitoid species were chosen as the best candidates for long-term control of MEAM1 and were released in numerous counties across the state. Recent recoveries of parasitoid species from the *B. tabaci* species complex in Florida (Lahey 2014) have confirmed the establishment of two species released during this program, namely, *Encarsia bimaculata* Heraty & Polaszek imported from Guatemala, India, and Sudan (Nguyen & Bennett 1995; Heraty & Polaszek 2000), and *Eretmocerus* sp. nr. *emiratus* imported from Sudan (= *Eretmocerus sudanensis* Zolnerowich & Rose in Castillo & Stansly 2011). Host records for both species appear to be relatively narrow, with *En. bimaculata* known from two whitefly species, *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Heraty & Polaszek 2000), and *Er.* sp. nr. *emiratus* only from *B. tabaci* (Castillo & Stansly 2011).

The parasitoid genera *Encarsia* and *Eretmocerus* have different reproductive biologies that may influence their ability to suppress whitefly populations within the same environment. In most *Encarsia* species, females develop as primary parasitoids, whereas males develop as hyperparasitoids on the larvae and pupae of conspecific or heterospecific individuals, including *Eretmocerus* (Walter 1983; Hunter & Woolley 2001). Eggs are deposited internally after the host is punctured by the hypodermic-like ovipositor (Gerling et al. 1998). By contrast, both sexes of *Eretmocerus* develop as primary parasitoids, and egg deposition by the spatulate ovipositor is always external to the host whitefly in the space between the leaf surface and nymphal venter. Both species kill additional nymphs by host feeding. One of the goals of the current study was to determine the competitive influence of each species on the other's survival early in the parasitization process (i.e., <48 h after each species parasitizes the same host), providing insight into interactions between similar development stages of these parasitoids.

Recent field observations indicated a clear difference in the host plant preferences for each species of parasitoid with *Er.* sp. nr. *emiratus* favoring hirsute plant species, whereas *En. bimaculata* was found more often on plants with fewer plant trichomes (Lahey 2014). Previous studies suggest that it may be easier for *Eretmocerus* to oviposit under whitefly nymphs on hairy plants, or those with uneven surface texture, due to larger gaps underneath the nymph than on plants with smooth leaves (e.g., McAuslane et al. 1995; Headrick et al. 1996; De Barro et al. 2000). Such gaps may facilitate insertion of the blunt-tipped, slightly curved ovipositor of *Eretmocerus* (Gerling et al. 1998), an action that may be hampered on smooth leaf surfaces (McAuslane & Nguyen 1996). *Encarsia* are less likely to be influenced by this factor, but may experience reduced walking speed and host finding capacity on hirsute plants (van Lenteren et al. 1996). Based on these observations and life history traits, all experimental treatments were performed on a glabrous (collard) and hirsute (eggplant) host plant in the hopes of determining the extent to which plant preference plays a role in mediating biological control efficiency and species coexistence in these introduced parasitoid wasps.

Materials and Methods

HOST PLANTS

Collard [*Brassica oleracea* L. 'Georgia' (Brassicales: Brassicaceae)] and eggplant [*Solanum melongena* L. 'Nadia' (Solanales: Solanaceae)] seeds were germinated in 200-unit seedling trays and transferred into 15.2 cm diameter pots filled with Fafard 2S or 4S Mix growing media (Conrad Fafard Inc., Agawam, Massachusetts). Plants were fertilized after the appearance of the first 2 true leaves with 20-20-20 (N-P-K) general purpose fertilizer. Cultivation occurred under conditions of natural temperature, humidity, and light in mesh cages (Live Monarch, Boca Raton, Florida) located outdoors (average conditions: 22 °C, 80% RH, 11:13 h L:D photoperiod) at the Southwest Florida Research and Education Center (SWFREC; 26.7667°N, 81.7167°W) in Immokalee, Florida. Plants with at least 3 fully expanded true leaves were used in experiments.

INSECTS

Whitefly and parasitoid cultures were maintained on collard. The whitefly *B. tabaci* MEAM1 served as the parasitoid host used in the experiments. The whitefly colony was established from collections made on *Sonchus asper* (L.) Hill (Asterales: Asteraceae) located at the SWFREC during Jun 2013. Laboratory cultures of *En. bimaculata* and *Er.* sp. nr. *emiratus* were established by rearing the parasitoids from MEAM1-infested collard and eggplant grown outdoors at the SWFREC. All insects were kept in separate climate-controlled rooms maintained at 26.5 ± 2 °C, 70 ± 10% RH, and a 14:10 h L:D photoperiod. The whitefly colony was housed in a wood-framed cage (60 × 60 × 60 cm) with walls made of transparent plexiglass and antithrips polyethylene mesh to allow for ventilation. Parasitoid colonies were reared in separate BugDorm (Megaview Science Co., Ltd., Taichung, Taiwan) cages (60 × 60 × 60 cm) with front and back panels made of clear plastic and sides of polyester netting for airflow. Overlapping generations of the whitefly and parasitoids were maintained in their respective cages with fresh plants being introduced as needed.

Parasitoids used in experiments were collected from each colony by removing collard leaves that contained wasp pupae, cutting the leaves into pieces, and sequestering them in separate clear plastic emergence containers (Tri State Plastics, Dixon, Kentucky; 18.0 × 12.5 × 10.5 cm). The top of each container was modified to allow removable plastic vials to be inserted; emerged parasitoids moved upwards into the vials, which were then removed to obtain even-aged individuals for experiments. Each container was provisioned with a moist, braided cotton dental roll (Richmond Dental, Charlotte, North Carolina) to provide moisture and a thin strip of paper towel impregnated with honey to nourish the emerged insects. Single pairs of newly emerged (i.e., <72 h) *En. bimaculata* and *Er.* sp. nr. *emiratus* females and males were

aspirated directly into 0.5 mL PCR tubes with tips removed and a small piece of cotton inserted in the opposite side for air exchange. The intake was sealed with Parafilm M (Bemis Company, Inc., Neenah, Wisconsin) to prevent escape. Wasps were held for <1 h in their respective colony rooms before being used in experiments. If a wasp of either sex died at any time during the parasitization period, the experimental unit was discarded and repeated.

TREATMENTS

The competitive influence of one parasitoid species on the other and the effect of competition on suppression of MEAM1 were determined by comparing 6 treatments: 3 heterospecific (Eb/Ee, Ee/Eb, Ee+Eb) and 2 conspecific combinations (Ee only, Eb only) and a whitefly only control (used to test for parasitoid contamination). The 3 heterospecific treatments were either synchronous (i.e., a single female and male of both parasitoid species released at the same time; Ee+Eb) or asynchronous [i.e., a single female and male of *En. bimaculata* released first followed by a single female and male of *Er.* sp. nr. *emiratus* (Eb/Ee) and vice versa (Ee/Eb)]. The ability of combinations of these parasitoids to suppress MEAM1 populations was compared with treatments consisting of each parasitoid species alone. Statistically significant increases in numbers of parasitized nymphs or nymphs killed by host feeding would indicate greater control potential compared with the single species introductions.

EXPERIMENTAL DESIGN

The experiment was set up as a factorial design with 2 host plants (collard and eggplant) and 6 treatments. Approximately 50 unsexed MEAM1 adults were confined to the abaxial leaf surface under a clip-on cage (Stansly & Liu 1997) for a 24 h oviposition period. Clip-on cages were made from plastic medicine cups (top and bottom diameters of 4 and 2.5 cm, respectively) attached to metal hair clips. The top of each cup was lined with yarn to prevent damage to leaf tissue, and the bottom of the cup was removed and replaced with 60 mesh nylon screen. Experimental plants contained at least 3 fully expanded leaves onto which a single clip-on cage was fastened at a maximum of 3 cages per plant. After 24 h, the whiteflies and clip-on cages were removed and the eggs were monitored daily for emergence. After all the insects had hatched and settled on the host plant, all but approximately 30 nymphs were removed with a size 0 insect pin affixed to a wooden applicator stick. When the 3rd instar was reached (i.e., the preferred stage for both parasitoid species) (Qiu et al. 2007; Castillo & Stansly 2011), the clip-on cages were replaced, and the parasitoids were released according to treatment. In the asynchronous treatments, both wasp species had 24 h access to the whitefly nymphs regardless of their order of release, making for a total access period of 48 h (i.e., 24 h for *Er.* sp. nr. *emiratus* and 24 h for *En. bimaculata*). Conversely, the synchronous treatment consisted of both wasp species released together and lasted for 24 h. In the single species introductions, each pair of *En. bimaculata* and *Er.* sp. nr. *emiratus* male and female had a 48 h exposure period to the nymphs within the clip-on cage.

After the final exposure period, the clip-on cages and wasps were removed, and the plants were kept in a room maintained at 26.5 ± 2.0 °C, 70 ± 10% RH, and 14:10 h L:D photoperiod for 10 d to allow for development and differentiation of the wasps by morphological differences in their larval and pupal forms. *Eretmocerus* sp. nr. *emiratus* larvae are circular in shape and take on a milky-white appearance within their host (Castillo & Stansly 2011). *Encarsia bimaculata* larvae are hymenopteriform, sickle shaped, and translucent with a visible ileo-labial gland (Antony et al. 2004). Pupae of *Er.* sp. nr. *emiratus* and *En. bimaculata* can be differentiated by the absence and presence of meconial pellets near the vasiform orifice of the whitefly, respectively. Pupae of *Er.* sp. nr. *emiratus* are completely yellow and have red eyes, whereas *En. bimaculata* pupae are covered in black cuticle.

After 10 d, the leaves were removed and examined under a stereomicroscope. Counts were made of the number of unparasitized (i.e., empty whitefly pupal cases), *En. bimaculata* parasitized, and *Er.* sp. nr. *emiratus* parasitized nymphs, and those killed by host feeding. In all treatments where *Er.* sp. nr. *emiratus* was released, nymphs were turned over to check for the presence of hatched and unhatched parasitoid eggs. Each treatment was replicated 5 times on each host plant. All treatments in a replicate were conducted simultaneously, although not all replications were performed at the same time.

STATISTICAL ANALYSES

Mean percentage of parasitism, numbers of progeny produced by *En. bimaculata* and *Er.* sp. nr. *emiratus*, and numbers of nymphs killed by host feeding on each plant relative to the treatment type (excluding the control), were subjected to a 2-way analysis of variance (ANOVA) using the package lme4 (R version 3.1.2; R Development Team 2014). Means were separated using the difflsmeans function within the lmerTest package. Results were considered significant at an alpha value of 0.05.

Results

TOTAL PARASITISM

The total level of parasitism varied significantly among treatments (F = 11.5; df = 4,40; P < 0.001) but not among plant species (F = 0.10; df = 1,40; P = 0.751) (Fig. 1). There was no interaction between treatments and host plants (F = 1.51; df = 4,40; P = 0.218). The percentage of nymphs parasitized was similar in all treatments on collard with the exception of the single introduction of *En. bimaculata*, where parasitism was significantly lower (16.0 ± 4.3%). On eggplant, percentage of parasitism was more variable, being greatest in the synchronous parasitoid release treatment (58.9 ± 6.8%) and lowest when *En. bimaculata* was released alone (14.5 ± 1.4%).

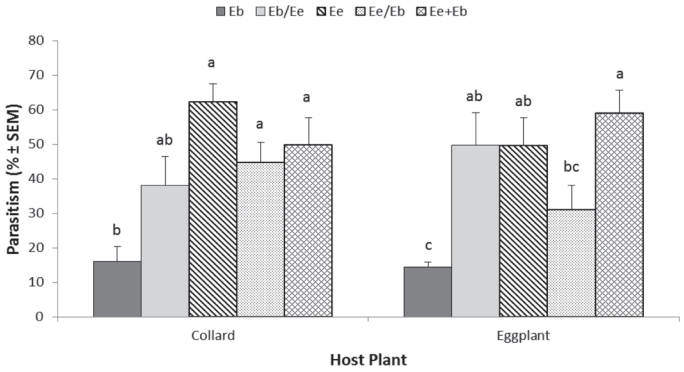

Fig. 1. Mean rates of parasitism (% ± SE) under all parasitoid release combinations and host plants. Treatment types are abbreviated as follows: Eb, *Encarsia bimaculata* only; Eb/Ee, *En. bimaculata* followed by *Eretmocerus* sp. nr. *emiratus*; Ee, *Er.* sp. nr. *emiratus* only; Ee/Eb, *Er.* sp. nr. *emiratus* followed by *En. bimaculata*; Ee+Eb, *Er.* sp. nr. *emiratus* and *En. bimaculata* together. Columns with the same lower case letter within each host plant are not significantly different (difflsmeans, P > 0.05).

PROGENY PRODUCTION OF EACH PARASITOID SPECIES

Significant differences in the number of progeny produced by *En. bimaculata* were found among parasitoid release treatments (F = 4.68; df = 3,32; P = 0.009) but not between plant species (F = 0.14, df = 1,32; P = 0.697) (Fig. 2). There was no treatment by host plant interaction (F = 1.20; df = 3,32; P = 0.274). *Encarsia bimaculata* produced significantly fewer progeny (1.2 ± 0.6) when introduced before *Er.* sp. nr. *emiratus* on collard; similar numbers of progeny were produced in all other treatments. On eggplant, *En. bimaculata* produced significantly fewer progeny (1.8 ± 0.7) when introduced after *Er.* sp. nr. *emiratus* compared with the single introduction of *En. bimaculata* (4.4 ± 0.2).

Significant differences in the number of progeny produced by *Er.* sp. nr. *emiratus* were found among treatments (F = 4.21; df = 3,32; P = 0.013) but not between plants (F = 0.01; df = 1,32; P = 0.920) (Fig. 3). There was no treatment by host plant interaction (F = 1.73; df = 3,32; P = 0.181). *Eretmocerus* sp. nr. *emiratus* produced significantly more progeny when introduced alone (21.8 ± 2.1) than in any combination with *En. bimaculata* on collard. On eggplant, *Er.* sp. nr. *emiratus* produced significantly fewer progeny (8.0 ± 1.3) when introduced before *En. bimaculata* compared with being introduced singly (17.2 ± 3.0) or in synchrony with *En. bimaculata* (17.2 ± 4.0).

HOST FEEDING

The number of nymphs killed by host feeding did not differ among treatments (F = 0.83; df = 4,40; P = 0.508) or between host plants (F = 1.53; df = 1,40; P = 0.220). There was no significant interaction between treatments and host plants (F = 2.55; df = 4,40; P = 0.053).

Across all treatments, the total number of nymphs killed by host feeding never exceeded more than 5% of those offered in a particular treatment. Host feeding was lowest in the single introduction of *Er.* sp. nr. *emiratus*, where 0.9% (n = 3) of nymphs were killed by host feeding. The introduction of *Er.* sp. nr. *emiratus* before *En. bimaculata* resulted in the greatest number of nymphs host fed upon at 2.8% (n = 9). When the host plant was taken into consideration, host feeding was both lowest and highest on collard, lowest when no host feeding was observed in the single species introduction of *Er.* sp. nr. *emiratus*, and highest when 4.4% (n = 7) of nymphs were killed in the synchronous treatment. On eggplant, 0.6% of nymphs were killed by host feeding when *En. bimaculata* was introduced before *Er.* sp. nr. *emiratus* (n = 1), as well as in the synchronous treatment (n = 1); host feeding was greatest in the single introduction of *En. bimaculata* at 2.6% (n = 4).

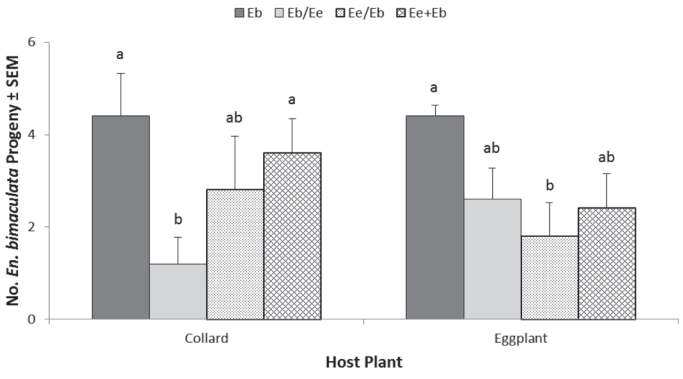

Fig. 2. Mean (± SE) number of progeny produced by *Encarsia bimaculata* under all parasitoid release combinations and host plants. Treatment types are abbreviated as follows: Eb, *En. bimaculata* only; Eb/Ee, *En. bimaculata* followed by *Eretmocerus* sp. nr. *emiratus*; Ee/Eb, *Er.* sp. nr. *emiratus* followed by *En. bimaculata*; Ee+Eb, *Er.* sp. nr. *emiratus* and *En. bimaculata* together. Columns with the same lower case letter within each host plant are not significantly different (difflsmeans, P > 0.05).

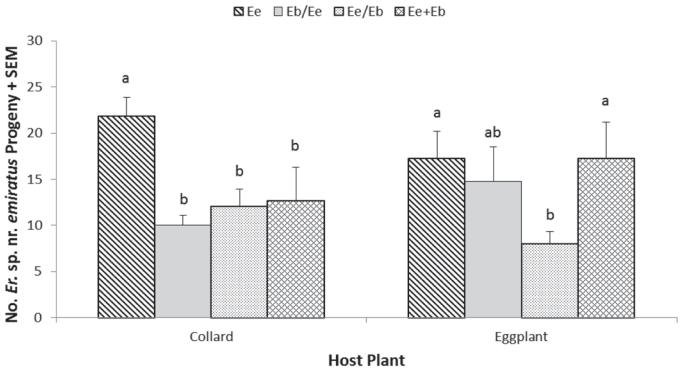

Fig. 3. Mean (± SE) number of progeny produced by *Eretmocerus* sp. nr. *emiratus* under all parasitoid release combinations and host plants. Treatment types are abbreviated as follows: Ee, *Er.* sp. nr. *emiratus* only; Eb/Ee, *Encarsia bimaculata* followed by *Er.* sp. nr. *emiratus*; Ee/Eb, *Er.* sp. nr. *emiratus* followed by *En. bimaculata*; Ee+Eb, *Er.* sp. nr. *emiratus* and *En. bimaculata* together. Columns with the same lower case letter within each host plant are not significantly different (difflsmeans, P > 0.05).

Discussion

We evaluated the ability of 2 parasitoid wasp species to contribute to the suppression of a shared host, and the impact each has on the other, on a glabrous (collard) and hirsute (eggplant) host plant. Our results suggest that in terms of parasitism, the order of release of the parasitoids did not affect the number of hosts parasitized on either host plant; however, differences in incidence of parasitized whiteflies were observed in previous studies relative to order of introduction. Pang et al. (2011) found significant decreases in the numbers of *B. tabaci* nymphs parasitized by *En. formosa* Gahan and *En. sophia* (Girault & Dodd) on tomato when the wasps were introduced simultaneously, or in sequence, compared with each species alone. In competition experiments under 2 host densities, Xu et al. (2013) found that the sequential introduction of *En. sophia*, followed by *Er. hayati* Zolnerowich & Rose, resulted in greater levels of parasitism than *En. sophia* alone when 30 *B. tabaci* nymphs were present on tomato. Conversely, sole access of *Er. hayati* to 10 nymphs contributed to greater levels of parasitism compared with allowing *Er. hayati* access first, followed by *En. sophia*.

The only differences we observed in parasitism levels were in the individual release treatments with *En. bimaculata* on both host plants, and when *En. bimaculata* was introduced after *Er.* sp. nr. *emiratus*, where the numbers of parasitized *B. tabaci* were significantly lower than in other treatments. This result is expected based on life history differences between the genera. *Encarsia* species mature more of their egg complement after emergence of the adult (i.e., synovigeny), whereas *Eretmocerus* emerge as adults with nearly their full egg complement (i.e., pro-ovigeny) (Jervis et al. 2001). As a result, *Eretmocerus* are able to oviposit in significantly more hosts than *Encarsia* earlier in their lifespan resulting in the observed differences in parasitism between treatments.

Progeny production of each parasitoid species was reduced in certain competition treatments relative to treatments in which each species was released individually. On collard, the number of progeny produced by *En. bimaculata* was significantly reduced (by 45.4% or 1 offspring) when introduced before *Er.* sp. nr. *emiratus*. The opposite combination, *Er.* sp. nr. *emiratus* followed by *En. bimaculata*, reduced

the progeny production of the latter on eggplant (by 7% or 0.6 offspring). All 3 competition treatments significantly reduced the number of *Er.* sp. nr. *emiratus* progeny produced on collard. Differences on eggplant were confined to the treatment in which *Er.* sp. nr. *emiratus* was followed by *En. bimaculata*. A reduction in the number of progeny is the expected result when parasitoids compete for hosts. Collier & Hunter (2001) found that *Er. eremicus* Rose & Zolnerowich reduced the progeny production of *En. sophia* by up to 50%, and *En. sophia* reduced the progeny production of *Er. eremicus* by up to 92%, when allowed access to 8 *B. tabaci* nymphs on cotton. Similarly, *Er. hayati* and *En. sophia* negatively affected the number of progeny produced by each other in sequential release combinations regardless of the number of available hosts (i.e., 10 or 30) (Xu et al. 2013).

The cause for such reductions appears to be due to 2 factors: multiparasitism and host feeding. A combination of these factors accounted for the negative influence *En. sophia* had on *Er. eremicus*, and multiparasitism was implicated in *Er. eremicus*' effect on *En. sophia* (Collier & Hunter 2001). In this study, we did not investigate the mechanism by which each species interfered with the other; however, host feeding across treatments occurred at an unusually low rate, decreasing the likelihood that this factor contributed to our results. It is possible that our method of mass rearing the parasitoids may have allowed early emerging parasitoids the opportunity to host feed, so that they became satiated prior to their use in experiments.

In both treatments in which each parasitoid species was introduced sequentially (i.e., Ee/Eb and Eb/Ee), we observed an empty *Eretmocerus* egg underneath a nymph containing an *En. bimaculata* pupa. In all cases (n = 4), circular entry scars were observed in the ventral integument of the whitefly where the *Eretmocerus* larva had penetrated. This observation indicates some capacity for *En. bimaculata* to successfully compete for the same host early (<24 h) in the parasitism process. In addition, this phenomenon was observed in both sequential treatments, suggesting that neither parasitoid has a capacity to discriminate between nymphs recently (<24 h) parasitized by the other.

The different host plants used in this study did not have any effect on total parasitism, progeny production, or the number of nymphs killed by host feeding by either parasitoid species. Previous studies indicated that *Eretmocerus* will parasitize more hosts on hirsute plants, or those with rough leaves (e.g., rockmelon), where whitefly nymphs do not lie flat against the leaf surface presumably making oviposition easier (Headrick et al. 1996; De Barro et al. 2000). Therefore, we expected that the single species treatment of *Er.* sp. nr. *emiratus* on eggplant would result in significantly greater parasitism than the same on collard; however, no differences in total parasitism were detected. Qiu et al. (2005) found *Er.* sp. nr. *furuhashii* to parasitize more hosts on glabrous collard leaves than on the increasingly pilose leaves of eggplant, cucumber, and tomato. In a similar study, *Er.* sp. nr. *furuhashii* and *En. bimaculata* parasitized significantly fewer whitefly nymphs on cucumber and tomato compared with less hairy eggplant and glabrous collard (Qiu et al. 2007). A potential caveat of these studies, and our own, is that they were run under no-choice conditions and may not reflect host plant choice by these parasitoids in the field. Clearly, more research is needed to elucidate the relationships between whiteflies, their parasitoids, and the host plant structures that may enhance whitefly biological control.

The results of this study suggest there would be no need to introduce *En. bimaculata*, or any other species of *Encarsia*, into an environment already under control by *Er.* sp. nr. *emiratus*. Although multigenerational studies should be conducted to say this definitively, the introduction of *En. bimaculata* would almost certainly reduce the positive effect of *Er.* sp. nr. *emiratus*, especially after the first generation when heteronomous hyperparasitism becomes possible (Walter 1983; Williams 1996).

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