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HOST SPECIFICITY AND RISK ASSESSMENT OF *TRICHOGRAMMA FUENTESI* (HYMENOPTERA: TRICHOGRAMMATIDAE), A POTENTIAL BIOLOGICAL AGENT OF *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) is a non-native moth attacking prickly pear cactus, *Opuntia* spp., in southeastern U.S. The insect is also an important threat to ecological systems and to native and endangered *Opuntia* spp. in southwestern USA. The egg parasitoid *Trichogramma fuentesi* Torre (Hymenoptera: Trichogrammatidae) was discovered attacking wild *C. cactorum* in Florida. To evaluate the potential effect of inundative releases of *T. fuentesi* against *C. cactorum*, the host searching behavior of *T. fuentesi* on *C. cactorum* eggs and host suitability of selected lepidopteran eggs were studied in the laboratory. Host suitability was studied on the native blue cactus moth, *Melitara prodenialis* Walker, and 6 selected species of butterfly eggs [*Danaus plexippus* (L.), *Dryas iulia* (Hübner), *Junonia coenia* (Hübner), *Papilio glaucus* (L.), *Papilio polyxenes* (F.), and *Vanessa cardui* (L.)] to assess the potential for non-target effects from *T. fuentesi*. The proportion of parasitism of the native cactus moth (*M. prodenialis*) was 98%; significantly higher than the non-native cactus moth, *C. cactorum* (11% average parasitism rate). The high proportion of parasitism for all native non-target species tested and the lowest proportion of parasitism for the exotic target species suggested that *T. fuentesi* not be considered for inundative releases in a biological control approach against *C. cactorum.*

Key Words: egg parasitoids, *Cactoblastis cactorum*, cactus moth, host range testing, non-target effects

RESUMEN

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) es una especies exótica que ataca a el nopal, *Opuntia* spp., en el suroriente de los Estados Unidos de Norte América. Este insecto representa una amenaza para ecosistemas con especies nativas de *Opuntia* spp. en el suroriente de USA. El parasitoide de huevos *Trichogramma fuentesi* Torre (Hymenoptera: Trichogrammatidae) fue descubierto atacando a *C. cactorum* en Florida. Para evaluar el posible impacto de liberaciones inundativas de *T. fuentesi* contra *C. cactorum*, se realizaron estudios de laboratorio sobre el comportamineto de busqueda de *T. fuentesi* sobre huevos de *C. cactorum,* y la el potencial de otros huevos de lepidópteros como hospederos para los parasitoides. Se evaluaron como posibles hospederos, la polilla azul de los cactus, *Melitara prodenialis* Walker, y huevos de 6 especies de mariposas [*Danaus plexippus* (L.), *Dryas iulia* (Hübner), *Junonia coenia* (Hübner), *Papilio glaucus* (L.), *Papilio polyxenes* (F.), y *Vanessa cardui* (L.)] para determinar los posibles efectos de *T. fuentesi* en organismos no blanco. El porcentaje de parasitismo en la especie nativa, *M. prodenialis*, fue del 98%; el cual fue significativamente mayor que en la especies exótica, *C. cactorum* (11%). Los altos niveles de parasitismo en todas las especies nativas no-blanco y los niveles de parasitismo bajos sobre la especie blanco sugieren que *T. fuentesi* no debe ser considerada para liberaciones inundativas en programas de control biológico contra *C. cactorum.*

Palabras Clave: parasitoides de huevos, *Cactoblastis cactorum*, polilla del cactus, biología, especificidad de hospederos

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Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) is an invasive pest of *Opuntia* spp. in southeastern U.S. (Hight et al. 2002; Zimmermann et al. 2001). This moth is often found in the same habitat as the native blue cactus moth, *Melitara prodenialis* Walker (Lepidoptera: Pyralidae). Given that *C. cactorum* is rapidly expanding its geographical range in the U.S., there is considerable interest to explore control measures against this insect, including the application of inundative biological control. Bennett & Habeck (1992) were the first to suggest the use of biological control as a management option against *C. cactorum*. Pemberton & Cordo (2001) evaluated various biological control approaches for *C. cactorum* and discussed their associated level of risk to non-target species. The release of non-host specific parasitoids native to South America had the highest level of risk whereas the release of parasitoids specific to the cactus moth had the least level of risk. Pemberton & Cordo (2001) considered inundative releases of parasitoids present in Florida that were attacking *C. cactorum* to have a moderate level of risk due to potential non-target effects to native species.

In Florida, an unidentified egg parasitoid of the family Trichogrammatidae had been found attacking *C. cactorum* but at a low level (Bennett & Habeck 1992). In addition, 2 larval parasitoids of the family Chalcidae associated with the exotic cactus moth were identified. However, these chalcidoid pupal parasitoids have a broad host range and can potentially cause negative effects on native butterflies and moths (Peck 1963). Recent field surveys conducted in northern Florida to identify natural enemies of *C. cactorum* led to the discovery of 2 naturally occurring egg parasitoids, *Trichogramma pretiosum* Riley and *T. fuentesi* Torre (Hymenoptera: Trichogrammatidae) (Paraiso et al. 2011). *Trichogramma fuentesi* was the most commonly encountered species, and thus was suggested as a possible inundative biological control agent against *C. cactorum* (Paraiso et al. 2011).

Because *Trichogramma* species are generally polyphagous, concerns exist about possible detrimental effects of inundative releases on non-target hosts (Oatman & Platner 1986; Andow et al. 1995; Orr et al. 2000). *Trichogramma fuentesi* has been observed in South and North America attacking eggs of agricultural pests primarily in the Noctuidae and Pyralidae families (Fry 1989; Wilson & Durant 1991; Pinto 1999; Querino & Zucchi 2003). However, little information is known about this parasitoid host range in non-agricultural habitats or on native species in northern Florida. Therefore, it is important to assess this parasitoid's host range before it is considered for use in a biological control program. Kuhlmann et al. (2006) published recommendations for host range testing of parasitoids being evaluated in classical biological control programs, based on a comprehensive review of existing methods for the selection of host species. In the approach proposed by Kuhlmann et al. (2006), an initial list of hosts, developed from a literature review, was divided into several categories based on ecological similarities and phylogenetic/taxonomic affinities. The number of potential non-target species was reduced by eliminating those species with different spatial, temporal and morphological attributes, and species that cannot be tested because they are difficult to obtain from field collection or commercial insectaries (Kuhlmann et al. 2006). We used a similar approach for selection of non-target host species for evaluating the host specificity of *T. fuentesi*.

Two types of experimental designs are generally used to assess host specificity; no-choice and choice tests (McEvoy 1996; Mansfield & Mills 2004). No-choice tests assess the physiological host range, which in the case of parasitoids, determines the host species suitable for completion of development. Choice tests indicate the preference of egg parasitoids for various suitable hosts and are generally expected to better estimate the ecological host range (McEvoy 1996; Mansfield & Mills 2004). Our study assessed host suitability of non-target hosts for the development of *T. fuentesi* by using no-choice tests.

MATERIALS AND METHODS

Trichogramma Rearing

Experiments were conducted at the facilities of the USDA-Agricultural Research Service and Florida Agricultural and Mechanical University-Center for Biological Control in Tallahassee, Florida, USA. *Trichogramma fuentesi* females used in this study were isolated from a laboratory-reared colony, which had originated from field-collected parasitized *C. cactorum* eggs in northern Florida. Parasitoid identity was confirmed by R. Stouthamer (Department of Entomology, University of California, Riverside, California) by analyzing ribosomal DNA Internal Transcribed Spacer 2 (ITS-2) sequences. Eggs of *C. cactorum* from a mass-rearing colony maintained on artificial diet were used as hosts for rearing *T. fuentesi* and also as the source of experimental eggs. Although it has been demonstrated that rearing diets might affect parasitoids' acceptance of host eggs (Song et al. 1997; Moreau et al. 2009), preliminary observations did not show any difference in acceptance and level of parasitism by *T. fuentesi* for *C. cactorum* eggs of wild females or of diet-fed laboratory females. To culture *T. fuentesi*, host eggsticks were glued onto note card strips (4 × 2 cm) with non-toxic Elmer's® glue (Elmer's Products Inc., Columbus, Ohio). The note card strips were then placed into Petri dishes (9 × 2 cm) lined with filter

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paper (Ahlstrom®, Mt. Holly Springs, Pennsylvania). A fresh raisin dipped in honey was glued to a 1 × 1 cm note card in the center of the Petri dish to provide an energy source for emerging wasps. Newly emerged parasitoids were maintained without access to hosts for 3 days to allow mating to occur before conducting the experiments (Paraiso et al. 2012). Based on previous studies, *T. fuentesi* reared on *C. cactoblastis* eggs achieved their optimal oviposition activity 2 to 3 days after emergence (Paraiso et al. 2012). Petri dishes were sealed with Parafilm® (Pechiney-Plastic Packaging, Menasha, Wisconsin), and arranged on plastic trays lined with moist paper wipes to increase relative humidity up to 60–80%. The colonies were maintained in a growth chamber at 28 ± 1 °C and 16:8 h L:D.

Host Specificity Tests

Non-Target Host Species Selection.

A list of non-target host species was developed based on recommendations made by Kuhlmann et al. (2006) and consultation with entomologists, biological control university researchers, and local butterfly producers. The species used were selected based on ecological, habitat, and temporal similarities to the target host. An initial list was developed that included all lepidopteran hosts likely to occur in the same habitat where *Opuntia* spp. were found in northern Florida. The list was then divided into 5 groups based on the following traits: taxonomic/phylogenetic similarity to *C. cactorum*, ecological affinities of other lepidopteran hosts present on *Opuntia* spp., endangered/threatened butterflies, known natural enemies of weeds, and other beneficial lepidopterans of economic value present in the area surveyed. The final list contained 22 species from 7 lepidopteran families. Because egg availability was limited, not all host species from this list could be tested, but at least one host representative of each group was selected. Eggs of non-target species were either purchased from a local commercial butterfly rearing facility (Old Oak Butterfly Farm, Brooker, Florida) or collected from our laboratory colony of *M. prodenialis*.

No-Choice Tests

Host acceptance and suitability were assessed by exposing a single mated and honey-fed female *T. fuentesi* to 10, single, less than 1 day old eggs of each host species at the center of a Petri dish (30 × 10 mm) lined with filter paper. The containers were incubated under laboratory conditions (25 ± 1 °C, 16:8 h L:D, and 60–70% RH) until parasitoid emergence. The number of replications for each host depended on the availability of the eggs, but at least 5 individuals per species were used. Because of egg supplier limitations, the host eggs were tested over a period of 2 days, but experimental and rearing conditions remained the same for both trials. The proportion of eggs parasitized (as indicated by black coloration) per female, the number of emerged parasitoids per parasitized egg, and the sex ratio of parasitoid progeny were recorded.

Data Analysis

A complete randomized experimental design blocked by day was applied to analyze the data. Analysis of variance was used to compare the proportion of eggs parasitized by *T. fuentesi* per host species, the number of adult parasitoid progeny per parasitized egg, and the proportion of female wasps per host species for eight lepidopteran hosts (PROC GLIMMIX). The relationship between the different variables and host species was characterized using a logistic regression analysis (PROC LOGISTIC). The proportion of eggs parasitized per host and the proportion of emerged adult progeny was scored as a binary response (occurring, not occurring) and fit to a model using a binary distribution. Comparisons of the proportions were made using Least Square Mean Comparison test. The SAS Statistical Software Version 9.2 (SAS Institute, Cary, North Carolina) was used to perform all the statistical analyses.

Results

Trichogramma fuentesi attacked non-target hosts in all groups tested but the proportion of eggs parasitized varied significantly ($F = 8.61$, df = 6, 142, $P < 0.0001$). The percent parasitism by *T. fuentesi* on the non-target host eggs ranged from 28 to 98% (Table 1). The highest level of parasitism was observed on *M. prodenialis* and *Dryas iulia* (Hübner) (Lepidoptera: Nymphalidae) (98 ± 2% and 86 ± 4%). The lowest level of parasitism (11 ± 4%) was recorded for the target pest insect, *C. cactorum*. The level of parasitism for the native cactus moth, *M. prodenialis*, was nearly 9 times higher than the non-native cactus moth, *C. cactorum*. About 65% of *C. cactorum* eggs that were drilled by *T. fuentesi* became parasitized. Therefore, the egg mortality could be higher than the percent of parasitism observed if the act of drilling without egg laying injured the moth egg. The number of parasitoid progeny per parasitized host egg was also statistically different among the lepidopteran hosts ($F = 5.74$, df = 7, 102, $P < 0.0001$) (Table 1). *Trichogramma fuentesi* exhibited gregarious behavior for all of the non-target hosts with at least two parasitoids emerging per parasitized host egg (Table 1). The proportion of females was not influenced by host species ($\chi^2 = 12.97$, df = 7, $P = 0.073$). Female–biased progeny were recovered from all host species tested (Table 1).

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TABLE 1. PROPORTION OF EGGS PARASITIZED, NUMBER OF PARASITOIDS PER PARASITIZED EGGS, AND PROPORTION OF FEMALES (MEAN ± S.E.) OF THE EGG PARASITOID, *TRICHOGRAMMA FUENTESI*, ON POTENTIAL LEPIDOPTERAN HOST SPECIES.

Host species (10 eggs)	Family	Number of replicates	Proportion of eggs parasitized	Number of parasitoid progeny/parasitized egg	Proportion of females
Cactoblastis cactorum (Berg)	Pyralidae	20	0.11 ± 0.04 d	2.7 ± 0.25 c	0.73 ± 0.10
Melitara prodenialis Walker	Pyralidae	13	0.98 ± 0.02 a	5.1 ± 0.67 a	0.59 ± 0.08
Danaus plexippus (L.)	Danaidae	9	0.64 ± 0.09 b	4.1 ± 0.69 a	0.68 ± 0.09
Dryas iulia (Hübner)	Nymphalidae	26	0.86 ± 0.04 a	4.8 ± 0.46 a	0.85 ± 0.04
Junonia coenia (Hübner)	Nymphalidae	45	0.33 ± 0.04 c	3.6 ± 0.34 b	0.82 ± 0.05
Vanessa cardui (L.)	Nymphalidae	48	0.62 ± 0.04 b	2.6 ± 0.25 c	0.71 ± 0.05
Papilio glaucus (L.)	Papilionidae	5	0.28 ± 0.12 cd	1.9 ± 0.64 c	0.92 ± 0.12
Papilio polyxenes (F.)	Papilionidae	5	0.57 ± 0.15 cb	6.8 ± 1.60 a	0.67 ± 0.14

Means with different letters are significantly different according to Least Square Mean Comparison test at $P \le 0.05$.

DISCUSSION

The genus *Trichogramma* comprises more than 200 species (UCD 2012) that differ greatly in their searching behaviors and host preferences (Hassan 1989). When a *Trichogramma* female finds a patch of "preferred" host eggs, she will usually stay on the eggs until all or most of them are parasitized (Hassan 1989). Less preferable host eggs may be totally rejected or the parasitoid may lay fewer eggs (Hassan 1989). Host rejection is based on both chemical and physical characteristics, and generally happens quickly after initiation of host examination (De Jong & Pak 1984). In this study, the number of eggs parasitized was measured and not total host mortality, which would have also included host eggs killed by the act of drilling without receiving a parasitoid egg. However, the frequencies of drilling events and egg laying events were not significantly different, suggesting that each drilling event was followed by an egg laying event (Paraiso, unpublished data). Therefore, in this study, host mortality on eggs of *C. cactorum* was directly estimated from the number of eggs parasitized (drilling followed by egg laying). Because of the 1:1 frequency of drilling:egg laying behavior observed for *T. fuentesi* (Paraiso, unpublished data), when she drilled into a *C. cactorum* egg then she also oviposited into that egg. Although the number of parasitoids/parasitized *C. cactorum* egg was not recorded in the behavioral observation part of this study, nearly 3 parasitoids/parasitized *C. cactorum* egg were found during the host range trials of this study (Table 1), suggesting that *T. fuentesi* females had a gregarious tendency and often laid more than 1 egg into a host egg during a single drilling event.

Significant differences were identified among the 8 host species for the proportion of eggs parasitized and the number of parasitoid progeny per parasitized egg (Table 1). Overall, female *T. fuentesi* parasitized a lower proportion of *C. cactorum* host eggs and deposited fewer eggs into this host as compared to most of the tested non-target hosts (Table 1). Based on the high proportion of non-target host eggs parasitized in this study, the host range of *T. fuentesi* appears to be fairly broad. The high level of parasitism of the native cactus moth (*M. prodenialis*) demonstrated that it was a suitable host for *T. fuentesi*. In fact, *M. prodenialis* eggsticks had been collected in Florida that had been parasitized by *T. fuentesi*. Parasitism of *C. cactorum* by *T. fuentesi* may be considered a host shift from the native moth to the non-native moth.

Pemberton & Cordo (2001) ranked the release of generalist parasitoids known to attack *C. cactorum* in Florida, such as *T. fuentesi*, as a moderately high risk biological control approach. Criteria used to rank the risk of biological control

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approaches included the negative impact on rare species and non-target hosts in the area of natural enemy release. Results from the no-choice tests in this study provided data on the physiological host range of *T. fuentesi*. Multiple choice tests in field conditions could be conducted to provide additional information on the ecological host range of this egg parasitoid. However, *Trichogramma* spp. are believed to be much more habitat specific than host specific (Smith 1996; Pinto 1999). The native cactus moth often shares the same niche on the host plant with *C. cactorum* and both species have overlapping oviposition periods. Since our no-choice tests identified a higher rate of parasitism and more parasitoids per host egg on *M. prodenialis* than on *C. cactorum*, there is a high likelihood that the native host would be negatively affected during inundative field releases of *T. fuentesi*. In addition, *T. fuentesi* parasitized a significantly higher proportion of butterfly eggs compared to *C. cactorum* eggs (Table 1). The inefficient reproductive strategy, high proportion of parasitized eggs for all non-target species tested, and the lowest rate of parasitism for the target species suggested that inundative releases of *T. fuentesi* should not be conducted as a biological control approach against *C. cactorum*.

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