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EGG PARASITOIDS ATTACKING *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE) IN NORTH FLORIDA

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ABSTRACT

Interest in the natural enemies of *Cactoblastis cactorum* (Berg) has increased since the moth was found in Florida in 1989. Previous surveys for natural enemies in Argentina identified egg parasitoids in the family Trichogrammatidae as potentially important control agents of *C. cactorum*. A study was conducted in north Florida to identify and to assess occurrence of egg parasitoids attacking this invasive moth in its new homeland. Surveys undertaken at 6 locations in north Florida from Jul 2008 to Dec 2009 revealed that eggsticks of *C. cactorum* were attacked by egg parasitoids from the *Trichogramma* genus: *T. pretiosum* Riley, *T. fuentesi* Torre, and an additional unidentified *Trichogramma* species belonging to the *T. pretiosum* group. In order to assess the importance of these egg parasitoids, the fate of individual *C. cactorum* eggsticks was determined during weekly visits to each site. This assessment showed that the combined level of parasitism of *C. cactorum* eggsticks was very low with less than 0.2% of host eggs attacked at any one of the 6 sites. While parasitoids attacked smaller eggsticks, there was no correlation between the numbers of eggs in an eggstick attacked with increasing number of eggs/eggstick. Comparing the mean number of eggs/eggstick, there was no difference between the 3 flight periods of *C. cactorum*, but there was a difference between the 6 sites. Based on these results, the use of *Trichogramma* wasps as an inundative biological control agent, complementary to the Sterile Insect Technique application, is discussed.

Key Words: *Cactoblastis cactorum*, cactus moth, *Trichogramma*, egg parasitoids, North Florida

RESUMEN

El interés en los enemigos naturales de *Cactoblastis cactorum* (Berg) ha aumentado desde que esta especie fue encontrada en el estado de Florida en 1989. Busquedas de enemigos naturales de *C. cactorum* hechas en años pasados en Argentina identificaron a parasitoides de huevos de la familia Trichogrammatidae como enemigos naturales de posible importancia para esta especie. Llevamos a cabo un estudio en seis localidades en el norte del estado de Florida con el objetivo de identificar y evaluar la ocurrencia de parasitoides de huevos atacando a esta especie en su nueva área de distribución. La busqueda de parasitoides llevada a cabo entre julio del 2008 y diciembre del 2009 indico que los bastoncitos de huevos de *C. cactorum* son atacados por parasitoides del genero *Trichogramma*: *T. pretiosum* Riley, *T. fuentesi* Torre, y una especie adicional no identificada perteneciente al grupo taxonómico de *Trichogramma pretiosum*. Para evaluar la importancia de estos parasitoides en el control de *C. cactorum*, seguimos el destino de bastoncitos individuales a través de visitas semanales a cada una de las localidades. Estas observaciones demostraron que el grado de parasitismo en esos basoncitos es muy bajo, con menos de 0.2% de los huevos parasitados en cualquiera de las seis áreas. Mientras que observamos que los parasitoides atacaron bastoncitos de huevos de tamaño pequeño, no hubo correlación entre el número de huevos parasitados por bastoncito y el tamaño del mismo. Comparando el numero promedio de huevos por bastoncito, no detectamos diferencia en los bastoncitos ovipositados en las tres generaciones anuales de *C. cactorum* pero detectamos diferencias dependiendo del área. Basado en estos resultados, discutiremos el uso de parasitoides del genero *Trichogramma* como agentes inundativos de control biológico, complementando la aplicación de la Técnica del Insecto Estéril contra *C. cactorum*.

Translation provided by the authors.

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is often cited as the perfect example of a successful weed biological control agent (Moran & Zimmermann 1984). In 1925, the cactus moth was introduced from its native Argentina into Australia to control prickly pear cactus, *Opuntia* spp., which had originally been brought into Australia for commercial purposes (Dodd 1940; Mann 1970). The cactus had become invasive and made large tracts of rangeland unfit for grazing cattle. Within a few years after the introduction of *C. cactorum* into Australia, US $6 million worth of rangeland was restored, equivalent to more than US $60 million in today's dollars (Dodd 1940; Williamson 2009). Based on these promising results, *C. cactorum* was imported from Australia to South Africa, Mauritius, and Hawaii to manage other non-native and invasive *Opuntia* spp. (Moran & Zimmermann 1984). In 1957, *C. cactorum* was introduced into several Caribbean islands (Nevis, Montserrat, and Antigua) to control non-native as well as native *Opuntia* spp. (Simmonds & Bennett 1966). Unfortunately, the implementing agencies did not fully consider the potentially injurious environmental impacts of *C. cactorum* if this insect were to move to neighboring countries where some species of *Opuntia* are important native species and some are commercially important (Stiling et al. 2004).

The first record of *C. cactorum* in the U.S. was from Bahia Honda Key, Florida, in Oct 1989 (Dickel 1991). It is uncertain how the moth arrived in Florida, but several interceptions of Caribbean ornamental *Opuntia* spp. infested with *C. cactorum* were found at ports of entry in south Florida during the 1980s and 1990s (Pemberton 1995; Zimmermann et al. 2001; Stiling 2002; Simonsen et al. 2008). Since its appearance in Florida, *C. cactorum* has become a threat to native *Opuntia* spp. in North America. Current management options include the use of Pherocon 1-C Wing traps (Trécé Incorporated, Salinas, CA) baited with a 3-component synthetic sex lure (Suterra, LLC, Bend, OR) to identify the presence of the moth, coupled with removal of infested plants to reduce *C. cactorum* populations (Bloem et al. 2005; Hight & Carpenter 2009). Complementary to the detection, monitoring, and removal efforts, implementation of the Sterile Insect Technique (SIT) is being used to slow the geographic expansion of *C. cactorum* in the U.S. (Hight et al. 2002; Bloem et al. 2005; Bloem et al. 2007). In Mexico, localized invasions of *C. cactorum* on 2 islands were eradicated in 2008 with a program of pheromone traps, host removal, and SIT (NAPPO 2006; NAPPO 2008; NAPPO 2009).

Bennett & Habeck (1995) suggested biological control as an additional control option that should be considered for *C. cactorum*. Pemberton & Cordo (2001) reported that several larval and pupal parasitoids attacked the cactus moth in South America, including species of Hymenoptera (Braconidae, Chalcidae, and Ichneumonidae), and 1 Diptera (Tachinidae). They also reported on 2 chalcid species (*Brachymeria ovata* (Say) and *B. pedalis* Cresson) and 1 unidentified egg parasitoid from the family Trichogrammatidae attacking *C. cactorum* in Florida. Logarzo et al. (2008) found the larval parasitoid *Apanteles alexanderi* Brethes (Hymenoptera: Braconidae) and the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) attacking *C. cactorum* in Argentina.

Trichogrammatid egg parasitoids have been used successfully for inundative biological control against major lepidopteran pests such as corn borers, sugarcane borers, and cotton bollworm (Li-Ying 1994; van Lenteren 2000). Egg parasitoids are easy to rear in mass quantity in laboratory conditions and to release over wide areas. Biological control can be used to complement and synergize the application of SIT (Gurr & Kvedaras 2010). Recent studies showed that the combination of both techniques was more efficient in controlling pest population of the codling moth, *Cydia pomonella* (L.) (Bloem et al. 1998). Synergistic interactions between SIT and fruit fly biological control with parasitoids increased the suppression of pest fruit flies, even leading to eradication (Sivinski 1996; Rendon et al. 2006). SIT and biological control have been successfully combined to combat several lepidopteran pests, including *C. pomonella* (Bloem et al. 1998) and painted apple moth, *Orgyia anartoides* (Walker) (Suckling et al. 2007). Radiation doses for sterilizing *C. cactoblastis* adults have been determined to produce partially sterile but more fit males which, when mated with wild females, generate sterile offspring (Carpenter et al. 2005). The combination of egg parasitoid releases and SIT has the advantage that parasitoids manage high pest densities, while SIT works best at low pest densities. In addition, release of sterile insects provides an egg resource for egg parasitoids increasing the ratio of natural enemies to adult hosts. Egg parasitoids and sterile insects have the characteristic of being self dispersing and consequently are able to cover wide areas (Sivinski 1996).

We conducted field surveys in order to identify egg parasitoids already established in North Florida that attack *C. cactorum*. *Cactoblastis cactorum* adults have 3 annual flight periods in north Florida (Apr-May, Jul-Aug, and Oct-Nov) (Hight et al. 2005; Hight & Carpenter 2009). We report on the distribution, seasonality, and parasitism parameters of the *Trichogramma* species attacking *C. cactorum* in northern Florida. The number of eggs/eggstick was compared between different flight periods and sites to assess host egg resource for egg parasitoids. The effect of *C. cactorum* eggstick size on level of parasitism was evaluated by comparing number of eggs from parasitized versus un-parasitized eggsticks. These data will be beneficial in promoting discussions on possible implementation of biological control for the cactus moth and, in particular, assessing the potential of an inundative biological control program against *C. cactorum* in North America.

MATERIALS AND METHODS

Field surveys were carried out at 6 locations (Fig. 1) in north Florida from Jul 2008 to Dec 2009. The selection of study sites was based on existing records of infestations from the literature, personal observations from preliminary surveys, and information provided by experts. Female *C. cactorum* place their eggs end to end to form a chain that looks like a short "stick", and the egg mass is referred to as an eggstick. Although no extensive field surveys were conducted from May to Jul 2008 at St. Marks and St. George Island, eggsticks with eggs that appeared parasitized were collected and held in laboratory conditions until parasitoids emerged. At survey locations, 20 to 30 healthy *Opuntia* spp. plants were chosen with no to minor feeding damage by cactus moth larvae and an average of at least 50 pads per plant. During weekly visits throughout all 3 flight periods, any new eggstick was identified by plant, pad, and its general location on the plant so the eggstick could be found during subsequent checks. A mark was made on the plant at the base of the eggstick with a felt tip pen and a red tape "flag" affixed to an insect pin placed near the eggstick to aid in finding the eggstick. The flag was labeled with a unique number to identify each eggstick. The oviposition preferences of *C. cactorum* females on host plants were recorded by classifying the attachment of the eggstick to either a glochid at an areole, to a spine, or on the fruit. Observations on plant habitat and host eggstick distribution within the surveyed site and within the selected plant were collected to provide additional information on the host finding behavior of egg parasitoids. The number of eggs per eggstick was determined either by a direct count or by a correlated estimate of eggstick length to egg number (2.62 ± 0.013 eggs/mm). The ratio of eggstick length to egg number was calculated in this study by counting the number of eggs in a segment of eggstick, replicated on 20 eggsticks. Eggstick length was estimated *in situ* by placing a plastic string next to the eggstick and cutting a piece of equivalent length. The length of the piece of string was then measured to the nearest 0.01 mm with a metric micrometer. Measurements of eggsticks were obtained so that the number of eggs per eggstick could be estimated if the eggstick was lost before it could be collected and directly counted. The fate of each eggstick was determined by making weekly visits to each site to evaluate the status of previously tagged eggsticks. The fate of each eggstick was categorized as follows: eggstick lost; predated (visible chewing damage) eggs in the eggstick versus non predated eggs; or parasitized eggs in the eggstick (black eggs formed before *C. cactorum* larvae successfully developed). Eggsticks were collected if they were damaged during evaluation or measurement, eggs of the eggstick had hatched, or eggs appeared predated or parasitized. Eggsticks with viable eggs were collected and held in small plastic cups (30 mL) under laboratory conditions (25 ± 1 C°, 16:8 L:D and 40-60% RH) to record hatch rate. Eggsticks with parasitized eggs were collected and monitored in the laboratory to determine the emergence rate, number of eggs/eggstick attacked by parasitoids, number of parasitoids emerging per parasitized egg, and to ascertain the identity of the parasitoids. Parasitoid specimens were submitted to R. Stouthamer, Department of Entomology, University of California, Riverside, for molecular identification. The sequencing of ribosomal DNA Internal Transcribed Spacer 2 (ITS 2) was used to identify the different species of egg parasitoids.

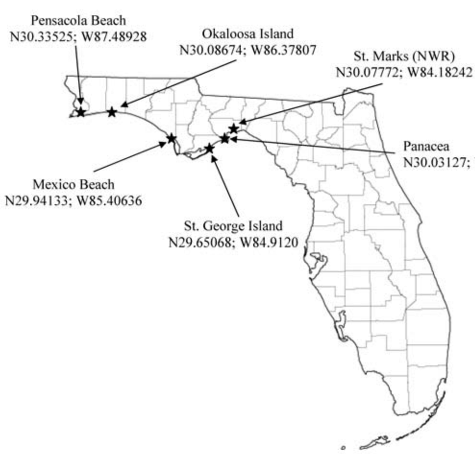

Fig. 1. Locations and their coordinates surveyed for egg parasitoids of *Cactoblastis cactorum* in North Florida.

Data Analysis

The numbers of eggs/eggstick at different flight periods for each surveyed location and the average number of eggs/eggstick at each site were log transformed before analyses to satisfy the assumptions of the analysis of variance. One way analysis of variance (PROC GLM) was applied to the log transformed data and the separation of means was made with the least significant difference (LSD) test. Comparison of number of eggs/eggstick that was parasitized versus number of eggs/eggstick not parasitized was also evaluated with a one-way analysis of variance (PROC GLM). Since only a few eggsticks with parasitized eggs were collected in this study (see text below), comparisons between eggsticks with parasitized eggs were made against the same number of randomly selected eggsticks with un-parasitized eggs. Variation between the number of eggs for parasitized eggsticks and the number of eggs for the randomly selected un-parasitized eggsticks was analyzed by a folded *F* test (Davis 2007). Because the variances in numbers of eggs for eggsticks with parasitism and number of eggs in eggsticks without parasitism were not significantly different, means of these 2 groups were compared with a two-sample *t*-test. A Pearson's Correlation Coefficient (r) was calculated to determine whether the numbers of eggs parasitized were dependent on the number of eggs/eggstick. The SAS Statistical Software Version 9.2 (SAS Institute, Cary, North Carolina) was used to perform the statistical analyses. Estimates of central tendencies were reported as mean ± standard error of mean.

Results and Discussion

Although host plant species of *Opuntia stricta* (Haworth) Haworth, *O. humifusa* (Rafinesque) Rafinesque, and *O. ficus-indica* (L.) P. Miller varied among the different geographic regions surveyed, the oviposition preferences of *C. cactorum* females was similar on the various species (Table 1). In this study, parasitized eggsticks of *C. cactorum* appeared mostly on the areole/glochid structure of the pads (Table 1).

Altogether, 1,527 eggsticks with 91,013 *C. cactorum* eggs, not including 344 eggsticks missing from the field or lost during collection, were tagged on plants of *Opuntia* spp. (Table 2). Of all the eggsticks checked, 62% were collected on Okaloosa Island and had a mean of 59 (+/- 1.83) eggs/eggstick. The proportion of eggsticks examined in the laboratory as percentage of all eggsticks surveyed at the 6 field sites ranged from 53 to 100%, except for summer 2008 at St. George Island and St. Marks National Wildlife Refuge (NWR) in which only 30% and 24%, respectively, of the monitored eggsticks were examined (Table 2). The majority of the eggsticks from these 2 locations for this flight period were recorded as lost (Table 2). The cause for this high number of lost eggsticks is not clear. Several biotic and abiotic factors could have contributed to the high number of lost eggsticks. During summer 2008, 23% of eggsticks examined from St. Marks had eggs that were preyed upon compared with less than 3% in other locations. Although not directly observed at St. George Island or St. Marks, substantial predation of *C. cactorum* eggs by ants has been recorded in South Africa (Robertson 1984). Because the plants surveyed at St. Marks were located within 100 m of the waters of the Gulf of Mexico, strong winds characteristic of coastal regions could have knocked eggsticks off the plants. All other study sites were along the Gulf Coast; in none of them were the plants as close to the water as at St. Marks. In addition, heavy rainfall may have separated the eggsticks from plants, but we do not have any data on the severity of the rain storms at different study sites. *Cactoblastis cactorum* life table studies in Argentina (Logarzo et al. 2009) and South Africa (Robertson & Hoffmann 1989) identified rain and wind as major factors contributing to mortality of eggs.

Surveyed sites and oviposition periods were analyzed to evaluate their influence on number of eggs/eggstick. Eggsticks were collected for multiple oviposition periods at 3 sites (St. George Island, St. Marks, and Okaloosa Island) (Table 2). The numbers of eggs/eggsticks for the different oviposition periods were not significantly different for St. George Island ($F = 1.84$, $df = 1$, $P = 0.18$), St. Marks ($F = 93.86$, $df = 3$, $P = 0.07$), or Okaloosa Island ($F = 0.22$, $df = 3$, $P = 0.88$). Because the numbers of eggs/eggstick for multiple oviposition periods were not different, eggsticks from all flight periods were pooled to calculate the means for those sites (St. George Island (62 ± 2.8), St. Marks (53 ± 2.8), and Okaloosa Island (59 ± 1.8)). The pooled eggsticks were used to compare the number of eggs/eggstick between all 6 sites and significant differences were found ($F = 11.44$, $df = 5$, $P < 0.0001$) (Table 2).

Female *C. cactorum* laid similar numbers of eggs/eggstick for each of the 3 oviposition periods but not at all 6 survey sites along the Florida panhandle. The longest eggsticks were observed at St. George Island, Pensacola Beach, and Okaloosa Island (Table 2). Significantly smaller eggsticks were recorded at St. Marks and Mexico Beach (Table 2). Panacea had significantly smaller number of eggs/eggstick than all other sites (Table 2). The cause of differences between eggsticks at the various sites was unclear. Studies in South Africa identified differences in total fecundity of *C. cactorum* due to host plant species, the flight period when eggs were laid, and the temperature during oviposition (Robertson 1989). We did not distinguish eggsticks collected from different host plants (Table 1). While South African female *C. cactorum* had significantly higher fecundity during the summer flight (Robertson 1989), our study did not show any difference in number eggs/eggstick between flight periods in north Florida. *Cactoblastis cactorum* has a tendency to oviposit on plants with high nitrogen (Myers et al. 1981; Robertson 1987), but we have no direct measurements of plant quality at our sites.

Comparing the number of eggs/eggstick for eggsticks that were parasitized (38 ± 13.7) (Table 3) against un-parasitized eggsticks (61 ± 13.1) revealed a significant difference (pooled t test = 3.14, $df = 12$, $P = 0.0085$). Although the number of eggs/eggstick was highly variable, the variation of the number of eggs/eggstick for parasitized versus the randomly selected un-parasitized group

TABLE 1. SITES SURVEYED IN NORTH FLORIDA FOR *TRICHOGRAMMA* EGG PARASITOIDS OF *CACTOBLASTIS CACTORUM* EGGSTICKS ON *OPUNTIA* SP. AND ADDITIONAL INFORMATION ON MOTH OVIPOSITION PREFERENCE.

Site	GPS Coordinate	Dates Eggsticks Surveyed	Total Number Surveys	Species of *Opuntia* Host Plant	Number Host Plant Examined	Total Number Eggsticks Evaluated	Percent Eggsticks at Attachment Location[1]			
							Areole/ Glochid	Spine	Fruit	Missing[2]
Pensacola Beach	N30.33525 W87.48928	Summer 2008 (Jul 10-Sep 10, 08)	10	*O. stricta* *O. humifusa* *O. ficus-indica*	20	120	50	34	16	0
St. George Island	N29.65068 W84.9120	Summer 2008 (Jul17-Sep19, 08)	10	*O. stricta*	23	105	63	30	7	0
		Fall 2008 (Sep 25, 08-Feb 25, 09)	20		27	28	89	11	0	0
St. Marks (NWR)	N30.07772 W84.18242	Summer 2008 (Jul 15,-Sep 12, 08)	18	*O. stricta* *O. humifusa*	30	45	80	13	7	0
		Fall 2008 (Oct 01, 08-Feb 25, 09)	20		30	9	78	0	22	0
		Spring 2009 (Apr 17-Jul 15, 09)	13		35	47	88	2	0	10
		Fall 2009 (Oct 07, 09-Jan 12, 10)			35	151	n/a	n/a	n/a	n/a
Mexico Beach	N29.94133 W85.40636	Fall 2009 (Oct 21-Nov12, 09)	3	*O. ficus-indica*	n/a	29	n/a	n/a	n/a	n/a
Panacea	N30.03127 W84.39353	Fall 2009 W84.39353	3	*O. stricta* *O. ficus-indica*	n/a	65	n/a	n/a	n/a	n/a
Okaloosa Island	N30.08674 W86.37807	Fall 2008 (Oct 08, 08-Feb 27, 09)	21	*O. ficus-indica*	10	186	81	18.5	0.5	0
		Spring 2009 (Apr 08-Jul 08, 09)	14		18	308	79	15	1	4
		Summer 2009 (Jul 01-Sep 25, 09)	18		18	280	77	19	2	2
		Fall 2009 (Sep 18, 09-Jan 12, 10)			25	151	n/a	n/a	n/a	n/a

[1]Attachment locations of eggsticks that were not determined is indicated by “n/a”.
[2]Information about eggstick attachment failed to be recorded.

TABLE 2. NUMBER OF *CACTOBLASTIS CACTORUM* EGGSTICKS COLLECTED, LOST IN THE FIELD, EXAMINED IN THE LABORATORY, AND MEAN NUMBER OF EGGS PER EGGSTICK ± SE AT DIFFERENT SITES IN NORTH FLORIDA FOR DIFFERENT OVIPOSITION PERIODS.

Site	Flight Period	Total Number Eggsticks Tagged	Total Number (Percent) Eggsticks Lost	Percent Eggsticks Examined	Total Number Moth Eggs Examined	Mean Number Eggs/Eggstick ± SE	Overall Mean Eggs/ Eggstick ± SE at Each Site[1]
Pensacola Beach	Summer 2008	120	69 (58)	42	7,402	62+/-1.5	62 ± 1.5 a
St. George Island	Summer 2008	105	84 (70)	30	6,685	64+/-2.0	62 ± 2.8 a
	Fall 2008	28	13 (46)	53	1,614	58+/-3.6	
St. Marks (NWR)	Summer 2008	45	35 (77)	24	3,088	68+/-2.9	53 ± 2.8 b
	Fall 2008	9	4 (44)	55	513	57+/-3.3	
	Spring 2009	47	23 (46)	54	2,561	54+/-3.1	
	Fall 2009	151	0 (0)	100	6,917	46+/-1.4	
Mexico Beach	Fall 2009	29	0 (0)	100	1,522	52+/-3.0	52 ± 3.0 b
Panacea	Fall 2009	65	0 (0)	100	2,892	45+/-1.9	45 ± 1.9 c
Okaloosa Island	Fall 2008	186	61 (29)	71	11,118	60+/-1.4	59 ± 1.3 a
	Spring 2009	308	3 (1)	99	20,527	61+/-1.2	
	Summer 2009	280	21 (8)	92	17,126	60+/-1.1	
	Fall 2009	151	1 (0.6)	99	8,638	57+/-1.2	

[1]Means with different letter are significantly different ($P < 0.05$).

TABLE 3. LOCATION AND DATE PARASITIZED *CACTOBLASTIS CACTORUM* EGGSTICK WAS COLLECTED, IDENTITY OF PARASITOID SPECIES, NUMBER OF EGGS PER EGGSTICK, NUMBER OF PARASITIZED EGGS, NUMBER OF PARASITOIDS EMERGED, FEMALE RATIO, AND PARASITISM LEVEL OF EGG PARASITOIDS ATTACKING *C. CACTORUM* IN NORTH FLORIDA.

Site	Collection Date	Flight Period	*Trichogramma* sp.	Number Eggs/ Eggstick	Number Parasitized Eggs/Eggstick # (%)	Number Parasitoids Emerged	Percent Females	Level of Parasitism (%)
St. Marks	05/15/08	Spring 08	*T. pretiosum*	73	5 (7)	8	75	n/a[1]
	05/15/08		*T. pretiosum*	78	17 (22)	34	85	
Pensacola Beach	04/22/08	Spring 08	unknown	88	19 (22)	18	77	n/a
	08/06/08	Summer 08	*T. pretiosum*	44	8 (18)	5	40	0.2
	08/13/08		*T. pretiosum*	18	6 (33)	10	70	
Okaloosa Island	10/16/08	Fall 08	*T. fuentesi*	20	2 (10)	11	73	0.1
	10/16/08		*T. pretiosum*	42	10 (24)	7	71	
	10/16/08		*T. fuentesi*	56	6 (11)	16	62	
	11/03/08		unknown	52	3 (6)	9	56	
	10/23/09	Fall 09	*T. fuentesi*	25	13 (52)	63	89	0.1

[1]Indicates that the level of parasitism was not determined.

was similar (folded F test = 1.10, df = 6, P = 0.91), suggesting that the difference found between the two groups was not driven by unequal or extreme variation. However, there was not a significant correlation between the number of eggs/eggstick and number of eggs parasitized by *Trichogramma* spp. (n = 7, r = -0.16, P = 0.74). Therefore, while female *Trichogramma* spp. parasitized eggsticks with fewer eggs, they did not parasitize more eggs as the number of eggs in an eggstick increased. The average number of eggs parasitized in an eggstick was 9 (±5.8).

Ten eggsticks were found parasitized at 3 of the 6 sites surveyed (Pensacola Beach, St. Marks, and Okaloosa Island). Five of the parasitized eggsticks were found at Okaloosa Island. Parasitized eggsticks were found during all 3 oviposition periods of *C. cactorum* females: the spring flight (St. Marks and Pensacola Beach), summer flight (Pensacola Beach), and fall flight (Okaloosa Island). Of the 496 eggs in the 10 parasitized eggsticks, a total of 89 eggs (or 18%) were parasitized, resulting in the emergence of 181 adult parasitoids with a sex ratio of 70% (±14) females (Table 3). The level of parasitism by *Trichogramma* spp., relative to the total number of eggs examined during the different flight periods for each site, was less than 0.2% of total *C. cactorum* eggs collected (Table 3). We did not observe any parasitized eggsticks at St. George Island, Mexico Beach, or Panacea.

Two species of *Trichogramma* were reared from *C. cactorum* eggsticks in north Florida (Table 3) and identified by differences in IST2 sequences. *Trichogramma pretiosum* was collected at St. Marks, Pensacola Beach, and Okaloosa Island, while *T. fuentesi* Torre was recovered only from Okaloosa Island. It was not possible to identify 2 collections of *Trichogramma* spp. from Okaloosa Island; one because a good molecular sequence could not be obtained and for the other the sequence was not in the database and possibly represents a new species in the *T. pretiosum* group (R. Stouthamer, UC—Riverside, personnel communication).

More than 15 million ha of agriculture and forestry worldwide are treated annually with *Trichogramma* egg parasitoids (van Lenteren 2000). Trichogrammatid wasps have been used successfully in inundative release programs against lepidopteran pests in greenhouses and crop production worldwide (Smith 1996). Inundative releases of *Trichogramma* spp. have been implemented in Florida to control major lepidopteran pests of collards, cabbages, soybeans, bell peppers, tomatoes, corn, and tobacco production (Martin et al. 1976). *Trichogramma pretiosum* is commonly found in the Western hemisphere. This *Trichogramma* species has been released commercially against major lepidopteran pests such as cotton leafworm (*Alabama argillacea*) (Hübner), corn earworm (*Helicoverpa zea*) (Boddie), tomato pinworm (*Keiferia lycopersicella*) (Walshingham), sugarcane borers (*Diatraea* spp.), and cabbage looper (*Trichoplusia ni*) (Hübner) (Pinto et al. 1986; Hassan 1993; Li-Ying 1994; Monje et al. 1999). *Trichogramma fuentesi* have been recorded in countries in South America (Argentina, Columbia, Mexico, Peru, and Venezuela) and in the U.S. (Alabama, California, Florida, Louisiana, New Jersey, South Carolina and Texas) (Fry 1989, Pinto 1999). Its primary hosts are species from the Noctuidae family such as *H. zea* and *Heliothis virescens* (F.) and from the Pyralidae family such as *Diatrea saccharalis* (F.), *Ephestia kuehniella* Zeller, and *Ostrinia nubilalis* (Hübner) (Fry 1989; Wilson & Durant 1991; Pintureau et al. 1999; Querino & Zucchi 2003). *Trichogramma* parasitoids also are widely used for pest control in orchards (Olkowski & Zang 1990). The observed low incidence of the wasps in natural areas might be explained by unfavorable environmental factors or natural plant chemicals (Smith 1996; Romeis et al. 1997, 1999). However, contrary to other natural enemies, *Trichogramma* can be easily and cheaply mass-reared for the implementation of an inundative biological control program.

The potential for inundative releases of *Trichogramma* spp. as a strategy against *C. cactorum* is currently being investigated with sustainable laboratory colonies of *T. fuentesi* originating from field collected insects reared from parasitized *C. cactorum* eggsticks. Biological characteristics (sex ratio, egg load, and longevity) and different behavioral mechanisms (influence of parasitoid age, density, and host age on parasitism) involved in host finding of *T. fuentesi* reared on *C. cactorum* eggs are being evaluated. The inundative releases of *Trichogramma* wasps could be integrated in the current pest management system based on SIT applications during the 3 flight periods by building *Trichogramma* populations. This field survey was useful in identifying a potential inundative biological control agent that could be integrated within a pest management strategy against *C. cactorum*.

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