

## **Geographical Range and Laboratory Studies on *Apanteles opuntiarum* (Hymenoptera: Braconidae) in Argentina, a Candidate for Biological Control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America**

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GEOGRAPHICAL RANGE AND LABORATORY STUDIES ON *APANTELES*  
*OPUNTIARUM* (HYMENOPTERA: BRACONIDAE) IN ARGENTINA, A  
CANDIDATE FOR BIOLOGICAL CONTROL OF *CACTOBLASTIS CACTORUM*  
(LEPIDOPTERA: PYRALIDAE) IN NORTH AMERICA

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ABSTRACT

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is a pest that threatens native *Opuntia* spp. in North America. Control tactics developed and implemented against this invasive pest successfully eradicated the moth in Mexico and on barrier islands in the United States. However, with the cancellation of the regional management program in the United States, no control tactics are being implemented to mitigate the expansion of the moth's geographical range. Hence, an integrated approach including biological control is proposed to regulate the population of *C. cactorum* in North America. Field surveys of the recently described parasitoid, *Apanteles opuntiarum* Martínez & Berta, were carried out within the *C. cactorum* native range in Argentina, and laboratory studies were conducted to develop a parasitoid rearing protocol. *Apanteles opuntiarum* was the most common parasitoid of *C. cactorum* and their field distributions were similar. In the laboratory, the parasitoid's reproductive success was maximized when one or two female wasps were exposed to 30 host larvae within a 500 ml container. Laboratory reared females were less successful at parasitizing hosts than field collected females. In spite of the success achieved with laboratory rearing, male bias was observed throughout the experiments. Because this bias might be related to the presence of the reproductive parasite *Wolbachia*, both laboratory colony and field collected individuals were screened and *Wolbachia* was detected. This study provides useful field and laboratory information on (1) laboratory rearing techniques for *A. opuntiarum*; (2) developing host specificity test protocols for studies under quarantine conditions; and (3) selecting parasitoid populations that best match the climatic conditions present in the *C. cactorum* invaded areas of North America.

Key Words: Cactus moth, *Apanteles*, reproductive success, field occurrence, parasitoid attack rates, *Wolbachia*

RESUMEN

La polilla de la tuna, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), es una plaga que amenaza las *Opuntia* spp. nativas de América del Norte. Se han implementado varias estrategias para su control, erradicándola de México pero no de los Estados Unidos, donde ha continuado incrementando su distribución geográfica con éxito. Por lo tanto, se propone un enfoque integrado que incluya al control biológico para regular las poblaciones de *C. cactorum* en América del Norte. Se realizaron relevamientos de campo del parasitoide recientemente descrito, *Apanteles opuntiarum* Martínez & Berta, dentro del área de distribución nativa de *C. cactorum* en Argentina, y se realizaron estudios de laboratorio para desarrollar un protocolo de cría del parasitoide. *Apanteles opuntiarum* fue el parasitoide más común de *C. cactorum* y sus distribuciones en el campo coincidieron ampliamente. En el laboratorio, el éxito reproductivo del parasitoide se maximizó cuando una o dos avispa estuvieron expuestas a 30 larvas dentro de un recipiente de 500 ml. Hembras criadas en laboratorio fueron

menos exitosas parasitando hospedadores que hembras provenientes del campo. A pesar del éxito logrado en la cría de los parasitoides, obtuvimos una proporción de sexos sesgada hacia machos en los experimentos. Como este sesgo podría estar relacionado a la presencia del parásito reproductivo *Wolbachia*, tanto la colonia de laboratorio como individuos provenientes del campo fueron analizados y poseían *Wolbachia*. El presente estudio proporcionó información útil de campo y laboratorio para (1) estandarizar una técnica de cría en laboratorio; (2) realizar estudios de especificidad de hospedadores en condiciones de cuarentena; y (3) seleccionar las poblaciones de parasitoides que mejor coinciden con el clima de las áreas invadidas por *C. cactorum* en América del Norte.

Palabras Clave: polilla de la tuna, *Apanteles*, éxito reproductivo, presencia en el campo, tasas de ataque, *Wolbachia*

The South American cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), accidentally arrived in the United States in 1989 (Dickel 1991) and in Mexico in 2006 (Hight & Carpenter 2009). In the United States, *C. cactorum* spread from Florida to the west (Bloem et al. 2007; Hight & Carpenter 2009) and was found in the state of Louisiana in May 2009. The insects' rate of migration and establishment poses a potential threat to wild and cultivated *Opuntia* spp. in southwestern United States and Mexico (Strong & Pemberton 2000; Perez-Sandi 2001; Soberón et al. 2001). In the United States, development of control tactics to address the *C. cactorum* invasion was only initiated 12 years after it was discovered in Florida (Mahr 2001; Carpenter et al. 2001), and limited resources and funding were insufficient to adequately address the infested area at the leading western edge (USDA APHIS 2009). In Mexico, the cactus moth was eradicated in 2007-2009, first on Isla Mujeres through host plant removal and sanitation (removal of eggsticks, pads and plants infested with larvae and pupae), and then on Isla Contoy by using sanitation and the Sterile Insect Technique (SIT) (Hight et al. 2008). Eradication of *C. cactorum* in Mexico benefited from an ongoing awareness campaign (Hernández et al. 2007) and the development of survey and control tactics in the United States (Bloem et al. 2007), and was accomplished by the expeditious identification of the two outbreaks and the rapid application of control tactics with a commitment of resources (funding and labor) that was sufficient to address the infested area (NAPPO 2009). But, in order to address the remaining and expanding threat of *C. cactorum* in the United States and the Caribbean, sustainable control tactics need to be developed.

Chemical control of *C. cactorum* was evaluated but has generally not been recommended due to lack of specificity, limited effectiveness against internal feeding *C. cactorum* larval stages, and difficulties of application timing (Habeck & Bennett 1990; Bloem et al. 2005). In addition, aerial applications of insecticides would not be effective given the vast area of *Opuntia* spp. host plants in

the western United States environment and the internal feeding aspect of the insect (Vigueras & Portillo 2001).

In August 2007, surveys were begun to identify natural enemies of cactophagous moths in their Argentine native range. Several gregarious *Apanteles* larval parasitoids were found, of which *A. alexanderi* Bréthes appeared to be the most common. This parasitoid was previously reported as a potentially important biological control agent against *C. cactorum* because of its widespread occurrence, prevalence, and impact (Pemberton & Cordo 2001). However, the parasitoid was considered to have a wide host range, attacking many Pyralidae-cactus feeding species in Argentina (De Santis 1979; Pemberton & Cordo 2001).

Detailed examination of the *Apanteles* specimens collected during the field surveys revealed morphological variations, suggesting the presence of more than one species. Identity was later confirmed through molecular and taxonomic studies and a new species, *Apanteles opuntiarum* Martínez & Berta, was described (Martínez et al. 2012). Collection records to date have revealed a restricted host range of *A. opuntiarum* to *C. cactorum* and *C. doddi* Heinrich (Martínez et al. 2012). This finding improved the outlook of *A. opuntiarum* as a potential biological control agent for *C. cactorum* in North America. The parasitoid was recently exported to the quarantine facility at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida, USA for host specificity testing with North American cactophagous moth species. Export permits were issued by Argentine regulatory agencies (Dirección de Fauna Silvestre and Dirección Nacional de Ordenamiento Ambiental y Conservación de la Biodiversidad; Permit # 4612/13; Servicio Nacional de Sanidad y Calidad Agroalimentaria; DNPV Permit # 87). The importation permit was issued by the US Department of Agriculture, Animal and Plant Health Inspection Service (P526P-Permit # 13-00380).

In this article we describe the distribution of *A. opuntiarum* in Argentina. We also present data on the biology of this parasitoid, providing information on laboratory rearing techniques,

parasitoid attack rates, and infection with the reproductive parasite *Wolbachia*. This information will improve laboratory rearing of *A. opuntiarum* in the United States and assist in the selection of Argentine parasitoid populations with the best climate match of the *C. cactorum* invaded areas in North America.

## MATERIALS AND METHODS

### Field Surveys for Natural Enemies of *C. cactorum*

Surveys to identify the occurrence of *C. cactorum* natural enemies were carried out between Aug. 2007 and Dec. 2011 in central and northern Argentina between parallels 23° and 40°. Samples were taken at sites every 30-50 km along highway rights-of-ways and adjacent fields upon observing *Opuntia* patches. A total of 393 sites were examined, including plantations with the Mexican prickly pear, *O. ficus-indica* (L.) Mill., and other non-native *Opuntia* spp. Searches for *C. cactorum* larvae were conducted on all native and non-native *Opuntia* species present at each site. When damaged *Opuntia* cladodes were found, they were dissected and examined for the presence of *C. cactorum* larvae. A sample of 5-10 infested cladodes from each site were taken to the laboratory. Once in the laboratory, larvae were checked every 2-3 days for the presence of parasitoid cocoons.

*Cactoblastis cactorum* larvae were identified following McFadyen (1985) and confirmed with molecular analysis performed by Travis Marsico (Arkansas State University, Jonesboro, Arkansas, USA). *Opuntia* species were identified by Fabián Font (Herbario Museo de Farmacobotánica Juan Domínguez, Buenos Aires, Argentina) and parasitoids by Juan José Martínez (Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina). Insect and plant vouch-

er specimens were deposited at the Fundación para el Estudio de Especies Invasivas (FuEDEI) collection. *Apanteles opuntiarum* holotypes are deposited at the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”.

### Laboratory Studies on *Apanteles opuntiarum*

**Insect Rearing.** To establish an *A. opuntiarum* laboratory culture at FuEDEI, *C. cactorum* larvae were collected in Quilino, Córdoba Province, Argentina (S 30° 12' 15" W 64° 28' 32"); a site with high parasitism rates based on previous surveys. Host larvae were collected from and reared on *O. ficus-indica* cladodes. Upon arrival at the laboratory, *C. cactorum* larvae were kept in 2-liter rearing containers (23 × 23 × 10 cm) with clay pellets to absorb exudates from larvae and cladodes. Containers were inspected every 2-3 days, and pellets were replaced and food added as needed. Parasitoid last instar larvae (Fig. 1) were observed just before they pupated, after leaving the dead host larva to form cocoons (Fig. 1). Cocoons from a parasitized larva were placed individually in 20-mL cups for wasp emergence. As adult parasitoids emerged, one female and two males (preferably not siblings) were placed in 3-liter jars for mating, and honey strips and moist cotton pads were added as a food and water source. After 24-48 h, individual females from mating jars were randomly selected and transferred to a 500 mL container with 10-50 first-third instar host larvae. Containers were provisioned with food for both parasitoids (honey and water) and host (*Opuntia* cladodes). Cactus cladodes were offered in thin slices to prevent moth larvae from hiding from the female parasitoid. Female wasps remained in the container until death, which usually occurred after 48-72 h. Each group of larvae that had been exposed to female parasitoids were carefully transferred to their individual 2-liter

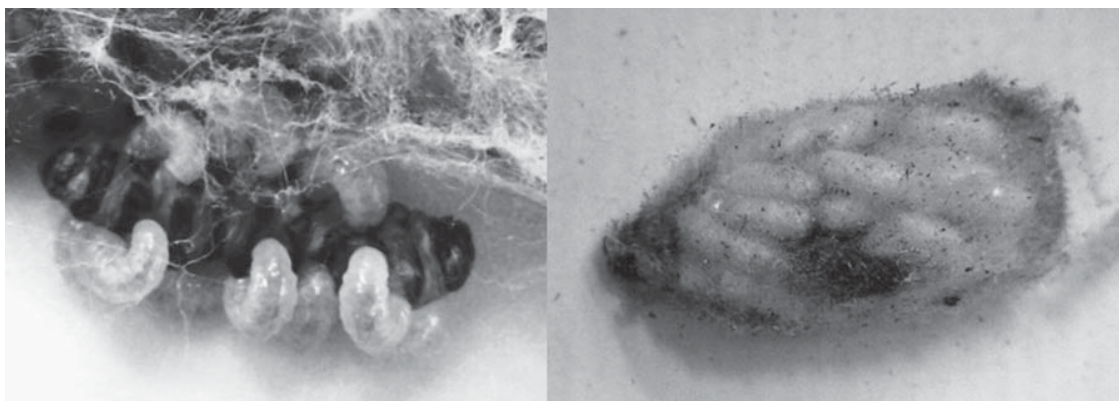


Fig. 1. Gregarious *Apanteles opuntiarum* larvae (left) outside dead *Cactoblastis cactorum* larva and cluster of parasitoid cocoons (right).



rearing containers and fed with *O. ficus-indica* cladodes until they completed development or parasitoid cocoons became present.

A laboratory culture of *C. cactorum* was maintained at FuEDEI and used to guarantee year-round provision of host larvae for the *A. opuntiarum* colony and experiments. Insect rearing and experiments were conducted under a photoperiod of 16:8 (L:D) at 25 °C and 70% RH. All rearing, mating, and oviposition containers used were plastic and vented with a fine mesh screen.

**Reproductive Success of *A. opuntiarum*.** A series of experiments were conducted to optimize the reproductive success of *A. opuntiarum* by evaluating the effect of host density, parasitoid source, and numbers of female parasitoids placed together in oviposition containers. The effect of host density was studied by exposing 10, 30, or 50 larvae of *C. cactorum* per container to one *A. opuntiarum* female, each treatment with 5-10 replications. Two sources of female parasitoids were considered; "field females", those obtained from field collected host larvae, and "laboratory females", those emerged from host larvae parasitized and reared in the laboratory. Female parasitoids were placed in containers to mate with males from the same generation and source. When assessing the effect of female source (laboratory or field), only one wasp was used to attack 30 host larvae, with 10-12 replicates. To determine the effect of the presence of another female during oviposition, either 1 or 2 laboratory wasps were placed in the container to attack 30 host larvae, with 5-10 replicates for each treatment. The variables recorded were parasitism rates, number of cocoons and emerged wasps, and wasp sex ratio.

**Longevity and Fecundity of *A. opuntiarum*.** Upon emergence, parasitoids were placed individually in Petri dishes (50 mm ID) containing moist cotton and honey threads. Insects were checked daily, and food and water were replaced as necessary until death. Dates of emergence and death were recorded to estimate longevity. Potential fecundity was estimated by dissecting 10 field females immediately after emergence and counting the mature oocytes in the ovarioles using a microscope (400x). Detergent was used to separate the mass of eggs and facilitate counting.

***Wolbachia* Detection.** Reproductive parasites like *Wolbachia* can induce a male-biased sex ratio in haplodiploid species as a consequence of cytoplasmic incompatibility (Breeuwer & Werren 1990; Breeuwer & Jacobs 1996). This is a form of conditional male sterility that occurs when infected males mate with uninfected females (Serbus et al. 2008). Total genomic DNA was extracted from adult parasitoids to screen for *Wolbachia* infection using the RED Extract-N-Amp Tissue kit (Sigma-Aldrich, Saint Louis, Missouri, USA), following manufacturer's instructions. Ten individuals from the laboratory colony and ten field

collected from Quilino, Córdoba, were assayed. Total genomic DNA from *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) naturally infected with *Wolbachia* was used as a positive control. Negative controls consisted of samples lacking DNA template from insects and *D. melanogaster* treated with tetracycline. *Drosophila melanogaster* DNA was kindly provided by Dr. Scott O'Neill (Queensland University, Australia). All experiments were repeated at least twice.

*Wolbachia* infection was diagnosed through amplification of 16S rDNA, *coxA* and *gabtB* genes, using the primers designed by O'Neill et al. (1992) and Baldo et al. (2006), respectively. Primers S1718 and A2442, specific for insect mitochondrial cytochrome oxidase subunit I (COI) gene (Rodríguez et al. 2010), were used to assess the quality of the DNA extraction. All amplifications for *Wolbachia* diagnosis were carried out in a 20 ml volume reaction with 50–100 ng of DNA used as template, 0.5 uM of each primer, 0.1 mM of each dNTP (Promega), 3.0 mM MgCl<sub>2</sub>, 1.0 unit of Taq polymerase, and 1X reaction buffer (Invitrogen, Carlsbad, California, USA). PCR reactions were performed in a Verity thermal cycler (Applied Biosystems, Foster City, California, USA) under the conditions specified by O'Neill et al. (1992) for the 16S rDNA gene, Baldo et al. (2006) for the *coxA* and *gabtB* genes, and Rodríguez et al. (2010) for the COI gene. Double-stranded PCR products were separated by electrophoresis on a 1% agarose gel with Tris-acetate-EDTA (TAE) buffer containing 0.5 mg/ml of GelRed (GenBio-technic SRL, Buenos Aires, Argentina).

*Wolbachia* strain was characterized by full MLST and also WSP typing system (Baldo et al. 2006), which is a complement to, but not a substitute for, MLST. Amplification and sequencing of *coxA*, *fbpA*, *ftsZ*, *gatB*, *hcpA*, and *wsp* fragments followed those of Baldo et al. (2006), and was carried out in a 50 ml volume reaction. PCR products were purified with a QIAquick Gel Extraction Kit (Qiagen Inc., Venlo, Netherlands). DNA was sequenced using a 3130-XL Automatic Sequencer (Applied Biosystems, Foster City, California, USA). Standard chromatographic curves of forward and reverse sequences were edited with the program BIOEDIT v. 7.0.5.3 (Hall 1999). For the MLST, allele number per locus was assigned after comparison with the *Wolbachia* MLST database (<http://pubmlst.org/wolbachia>), and the combination of MLST numbers constituted an allelic profile or sequence type (ST). For WSP typing system, the amino acid motifs of the four hypervariable regions (HVRs) were assigned after comparison with the WSP database (<http://pubmlst.org/wolbachia/wsp/>), and an allele number per HVR was given. Each WSP sequence was defined as a combination of four numbers, the WSP profile. The *Wolbachia* strain harboured by *A. opuntiarum* was characterized by the combination of the ST plus the WSP profile.

Statistical Analysis

Effects of *A. opuntiarum* female source on number of parasitized larvae, offspring cocoons, emerged adult wasps, and female proportion of offspring were analysed with Mann-Whitney U tests. The effect of *A. opuntiarum* female number used to parasitize larvae on the number of offspring cocoons produced was analysed with T-tests, and the effect on the number and female proportion of emerged offspring adult wasps was analysed with Mann-Whitney U tests. The effect of host density on the number of parasitized larvae, offspring cocoons, and emerged adult wasps, and the proportion of female offspring was analysed with Kruskal Wallis and a Duncan test for post-hoc comparisons. The effect of host density on the proportion of larvae attacked was examined with ANOVA. Differential longevity between sexes was analyzed with a Mann-Whitney U test for independent samples. Statistical analysis was performed using InfoStat (Di Rienzo et al. 2008) and the data were expressed as mean  $\pm$  SE.

RESULTS

Field Surveys for Natural Enemies of *C. cactorum*

From the 393 sites with *Opuntia* spp., 95 (24%) were infested with *C. cactorum* and 42 (11%) with *A. opuntiarum*. The parasitoid occurred at 44% of sites with *C. cactorum*, and was the most common parasitoid that emerged from *C. cactorum* larvae returned to the laboratory. Seven other parasitoid species belonging to the families Trichogrammatidae, Tachinidae, Braconidae, and Ichneumonidae were collected at 18 (19%) sites (Table 1). *Apanteles opuntiarum* was found attacking *C. cactorum* in 6 (60%) out of 10 different *Opuntia* species infested with larvae (Table 2), including native and exotic host plant species. The parasitoid was found at the highest frequency in the host species *O. ficus-indica*, *O. elata* var. *elata*

Link & Otto ex Salm-Dyck, and *O. megapotamica* Arechav., with parasitized *C. cactorum* larvae in 60, 60, and 56% of the sites, respectively.

*Apanteles opuntiarum* was collected throughout most of the distribution of *C. cactorum* in Argentina (Fig. 2). The provinces with a low number of sample sites containing *C. cactorum* (less than 3 sites in each province) were Salta, Tucumán, La Rioja, San Luis, and La Pampa, and the occurrence percentage of *A. opuntiarum* in these provinces ranged between 50 and 100. In the other provinces within the distribution of *C. cactorum*, parasitoid occurrence ranged between 16% in Chaco to 67% in Santiago del Estero (Fig. 2). The parasitoid occurred in dry and humid regions, as well as at sea level and high elevations (Table 3).

Laboratory Studies on *Apanteles opuntiarum*

*Reproductive Success of A. opuntiarum.* The reproductive success of *A. opuntiarum* was significantly affected by female source, number of females in the parasitism arena, and host density (Table 4). Field-collected females of *A. opuntiarum* produced significantly more pupae and adults than laboratory females ( $U = 85, P = 0.05$  and  $U = 77, P = 0.012$ , for pupae and adults, respectively). However, female source showed no effect on the number of parasitized larvae obtained ( $U = 96.5, P = 0.21$ ). The proportion of female progeny was significantly greater for field females than for laboratory females ( $U = 88, P = 0.03$ ). The presence of a second female in the same arena showed no significant effect on the number of larvae parasitized ( $T = -0.35, P = 0.73$ ), the number of cocoons ( $T = -1.23, P = 0.24$ ), or the number of adult wasps ( $U = 38, P = 0.29$ ). However, the use of a single female always produced male offspring only, whereas the use of two females produced mixed offspring genders ( $U = 45; P = 0.02$ ). The density of host larvae to which the parasitoid was exposed affected the parasitism rate. When 10 larvae were exposed to a single female parasitoid, significantly fewer

TABLE 1. TAXONOMIC IDENTITY OF PARASITOIDS REARED FROM FIELD COLLECTED *CACTOBLASTIS CACTORUM*, HOST STAGE OF PARASITOID ATTACK AND EMERGENCE, AND NUMBER (PERCENTAGE) OF 393 *OPUNTIA* SPP. SITES SURVEYED IN ARGENTINA CONTAINING EACH PARASITOID SPECIES.

Parasitoid species	Family	Host stage		# Sites (%)
		attacked	emerged from	
<i>Apanteles opuntiarum</i> Martínez & Berta	Braconidae	larva	pre-pupa	42 (44)
<i>Trichogramma pretiosum</i> Riley	Trichogrammatidae	egg	egg	5 (5)
<i>Pseudochaeta</i> sp.	Tachinidae	larva	larva	4 (4)
<i>Podogaster</i> sp.	Ichneumonidae	larva	pre-pupa?	4 (4)
<i>Epicoronimyia mundelli</i> (Blanchard)	Tachinidae	larva	pupa	2 (2)
unidentified	Ichneumonidae	larva	pre-pupa?	1 (1)
unidentified	Braconidae	larva	larva	1 (1)
<i>Trichogramma</i> sp.	Trichogrammatidae	egg	egg	1 (1)

TABLE 2. OCCURRENCE OF *APANTELES OPUNTiarum* EMERGING FROM FIELD COLLECTED LARVAE OF *CACTOBLASTIS CACTORUM* ON *OPUNTIA* SPP. IN ARGENTINA.

<i>Opuntia</i> species	# Sites with <i>Apanteles opuntiarum</i> / <i>Cactoblastis</i>	Prevalence (%)
<i>O. anacantha</i> Speg.	1/7	14.3
<i>O. auriantica</i> Gilles ex Lindleyand	0/1	0
<i>O. elata</i> var. <i>cardiosperma</i> (K. Schum.) R. Kiesling	6/21	28.6
<i>O. elata</i> var. <i>elata</i> Link & Otto ex Salm-Dyck	3/5	60.0
<i>O. ficus-indica</i> <sup>1</sup> (L.) Mill.	26/43	60.5
<i>O. leucotricha</i> <sup>1</sup> DC.	0/2	0
<i>O. megapotamica</i> Arechav.	5/9	55.6
<i>O. penicilligera</i> Speg.	0/1	0
<i>O. quimilo</i> K. Schum.	1/4	25.0
<i>O. robusta</i> <sup>1</sup> H. L. Wendl. ex Pfeiff.	0/2	0
<b>Total</b>	<b>42/95</b>	<b>44.2</b>

<sup>1</sup>Species native to Mexico.

host larvae were parasitized than when 30 or 50 larvae were exposed ( $H = 8.92$ ,  $P = 0.01$ ). The percentage of replicates in which any level of parasitism was obtained was 100, 34, and 29% when exposing 30, 10, or 50 larvae, respectively. Although increasing host larval density produced an increase in number of offspring (pupae and adults), the differences were not significant ( $H = 4.53$ ,  $P = 0.10$  and  $H = 3.18$ ,  $P = 0.10$ , for pupae and adults, respectively). The proportion of female offspring did not vary significantly with the density of host larvae ( $H = 1.38$ ,  $P = 0.41$ ).

**Longevity and Fecundity of *A. opuntiarum*.** Wasp longevity was significantly affected by gender. Males lived  $12.7 \pm 0.9$  days and females lived  $8.9 \pm 0.6$  days ( $U = 56.5$ ,  $P = 0.004$ ). Females not in contact with host larvae lived between 6 and 13 days, while those used in parasitism experiments lived a maximum of 3 days. Potential fecundity, measured as the number of mature oocytes in ovarioles upon emergence, was  $396 \pm 35.8$  (range 346-450). Upon dissection, the majority of the oocytes were developed, elongated in shape, with a thin stalk (Fig. 3). We also observed smaller (immature) oocytes that were not considered in the count, but their presence suggested that *A. opuntiarum* females might be synovigenic (Jervis et al. 2001).

**Wolbachia Detection.** We positively diagnosed *Wolbachia* infection for *A. opuntiarum* in both lab rearing and field collected individuals, which validated a good DNA quality ( $n = 19$ ). Sequencing of MLST genes of the parasitoid strain yielded alleles 14, 181, 9 and 13 for loci *coxA*, *fbpA* *gatB* and *hcpA*, respectively. Unfortunately, the *ftsZ* fragment could not be amplified. Sequencing of the WSP yielded HVRs 134 - 217 - 127 - 102. After comparison with the MLST database, we found a partial coincidence with the *Wolbachia* strain that infects many parthenogenetic weevils of the

South American tribe Naupactini (Coleoptera: Curculionidae). There was coincidence for every gene fragment but the sequence *ftsZ*, which remains to be tested. This strain belongs to Super-group B (see Rodriguero et al. 2010).

DISCUSSION

*Apanteles opuntiarum* was the most common parasitoid of *C. cactorum* in Argentina field surveys, and the field distribution of both host and parasitoid closely coincided. In no instance was *A. alexanderi* reared from *C. cactorum* collections, further confirming the separation of the two *Apanteles* species in their host range. The occurrence of *A. opuntiarum* under varied climatic conditions and altitudes suggested high habitat plasticity for this insect; this would facilitate the eventual establishment and spread in different habitats within the *C. cactorum* distribution range in North America. Information obtained in this study on the geographical distribution of this parasitoid in its native range will be useful to develop bioclimatic models to predict its potential geographic range in North America.

Other parasitoid species reared from *C. cactorum* collected during this survey are likely not appropriate biological control agents. Many of these species were documented by Pemberton and Cor-do (2001) as known parasitoids of *Cactoblastis* spp. in their native range, but considered unfavorable as biological control agents due to their lack of host specificity. *Trichogramma* spp. were not previously identified as natural enemies of *C. cactorum* in Argentina, but studies in Florida on *T. fuentesi* Torre found attacking eggsticks of *C. cactorum* identified a broad host range for this egg parasitoid (Paraiso et al. 2013).

In laboratory tests, the reproductive success of *A. opuntiarum* was influenced by several rearing



Fig. 2. Distribution of *Apanteles opuntiarum* which emerged from *Cactoblastis cactorum* larvae (black circles) and locations where *A. opuntiarum* did not emerge from *C. cactorum* larvae (white circles) collected in the Argentina provinces of Buenos Aires (BA), Catamarca (CA), Córdoba (CB), Chaco (CH), Corrientes (CO), Entre Ríos (ER), Formosa (FO), Jujuy (JU), La Pampa (LP), La Rioja (LR), Río Negro (RN), Salta (SA), Santiago del Estero (SE), Santa Fe (SF), San Luis (SL), and Tucumán (TU). Shaded area indicates surveyed region.

conditions, such as host and parasitoid densities, and source of the females. The highest success was obtained with 30 host larvae exposed to parasitism by two field-collected female wasps. Increasing host larval numbers ( $n = 50$ ) did not translate into higher reproductive success, and decreasing host larval numbers ( $n = 10$ ) negatively impacted the reproductive success of *A. opuntiarum*. At this lowest density, many host larvae died in earliest instar stages, probably because of superparasitism or numerous stings. Although field-collected females had a similar attack rate as laboratory-reared females, the former produced a greater number of offspring and produced both males and females, essential to the continuity of a laboratory culture.

The greater longevity of females during survival tests (no exposure to host larvae) compared with females used in the parasitism studies could be due to egg reabsorption and energy conservation. The potential fecundity (396 oocytes per female) was 2.4 times the maximum fertility recorded during the experiments (166 wasps produced/female), indicating a higher reproductive potential than obtained in the experiments. Unfortunately, most of the scarce literature available on rearing techniques and/or biology of *Apanteles* species is focused on solitary parasitoids. Longevity values reported in this study are similar to those found for *Apanteles machaeralis* Wilkison (Peter & David 1990), but lower than those found for *Apanteles taragamae* Viereck (Mohan & Sathi-



TABLE 3. GEOGRAPHIC LOCATIONS AND WEATHER INFORMATION OF TEN SELECTED LOCALITIES WITHIN THE GEOGRAPHICAL DISTRIBUTION RANGE OF *APANTELES OPUNTiarum* IN ARGENTINA.

Locality, Province <sup>1</sup>	Coordinates		Altitude (m)	Mean temperature (°C)		Annual precipi- tation (mm)
	South	West		January	July	
Laguna Yema, FO	24° 14' 47.5"	61° 14' 01.2"	156	27.9	16.6	627
El Colorado, FO	26° 24' 51.8"	59° 22'11.7"	80	27	15.7	969
San Salvador, CO	29° 16' 20.4"	57° 30' 25.6"	68	26.6	13.9	1263
Concordia, ER	31° 23'43.5"	58° 03' 55.4"	37	25.7	12.4	1175
Villa Arias, BA	38° 49' 22.2"	62° 06'12.1"	10	21.5	7.0	522
Santa Isabel, LP	36° 13' 53.1"	66° 56'16.3"	320	24.8	6.8	280
Chumbicha, CA	28° 50' 58.7"	66° 14' 44.9"	421	28.1	11.2	356
El Carmen, JU	24° 23' 37.7"	65° 16'16.6"	1150	22.1	11.0	656
El Carril, SA	25° 02' 58.5"	65° 29' 47.4"	1155	22.6	11.3	529
Merlo, SL	32° 38' 09.7"	65° 58' 49.6"	900	21.9	9.0	642

<sup>1</sup>FO: Formosa, CO: Corrientes, ER: Entre Ríos, BA: Buenos Aires, LP: La Pampa, CA: Catamarca, JU: Jujuy, SA: Salta, SL: San Luis.

amma 2007), both solitary parasitoids. However, the realized fecundity of *A. opuntiarum* was higher than *A. machaeralis* and much higher than reported for *A. taragamae*. The higher proportion of male offspring under laboratory rearing conditions is a common feature of other species of this genus (Kulkarni 1965; Kishani Farahani et al. 2012). The male-biased sex ratio might be the consequence of mating absence under laboratory conditions, as reported for many Hymenoptera insects, particularly in *Apanteles* (Allen & Smith 1958; Tagawa et al. 1987). Bacterial infections or complementary sex determination might also affect sex ratio (Heimpel & De Boer 2008). It is known that *Wolbachia* or *Cardinium*, among many other reproductive parasites, can induce male sterility (Breeuwer & Werren 1990; Breeuwer & Jacobs 1996; Serbus et al. 2008). We found a

*Wolbachia* infection in both lab and field collected insects of *A. opuntiarum* at a prevalence of 100%. This strain closely resembles the one infecting most of the parthenogenetic weevils of the South American tribe Naupactini (strain *wNau1*) (Rodrigoero et al. 2010; Rodrigoero et al. 2012). Horizontal transfer is pervasive in the *Wolbachia* genus, and there is no match between strains and phenotypes (Werren et al. 1995); two closely related strains can induce different phenotypes (Werren et al. 1995), and, even more surprising, the same strain can induce different reproductive alterations in different hosts (Werren et al. 1995; Fujii et al. 2001). Thus, geographical proximity of Argentine populations of weevils and wasps, and horizontal transfer might explain why two different insect species exhibit different phenotypes that share the same

TABLE 4. *APANTELES OPUNTiarum* REPRODUCTIVE SUCCESS ON *CACTOBLASTIS CACTORUM* LARVAE UNDER VARIOUS EXPERIMENTAL CONDITIONS IN LABORATORY CULTURE. MEAN ± SE IS REPORTED.

	<i>Apanteles opuntiarum</i>			Proportion of females
Treatment	# <i>C. cactorum</i> larvae parasitized	#Cocoons	#Adults emerged	
Experimental female origin				
Field	4.4 ± 0.8 a	77.2 ± 16.8 a	65.1 ± 13.8 a	0.4 ± 0.1 a
Laboratory	3.0 ± 0.6 a	29.7 ± 7.3 b	20.0 ± 5.7 b	0 b
Females in the arena				
1	2.9 ± 0.6 a	30.3 ± 7.1 a	20.5 ± 5.6 a	0 a
2*	3.2 ± 0.6 a	49.9 ± 18.4 a	33.3 ± 10.7 a	0.4 ± 0.1 b
Host larval density				
10	1.9 ± 0.5 a	33.0 ± 9.5 a	29.8 ± 9.3 a	0.2 ± 0.1 a
30	4.5 ± 0.9 b	77.0 ± 20.1 a	63.2 ± 16.4 a	0.4 ± 0.1 a
50	6.8 ± 1.7 b	85.7 ± 20.5 a	63.3 ± 20.8 a	0.05 ± 0.03 a

\*The values of the measured variables are expressed per female.  
Means with different letters within each column and treatment indicate significant differences (*P* < 0.05).

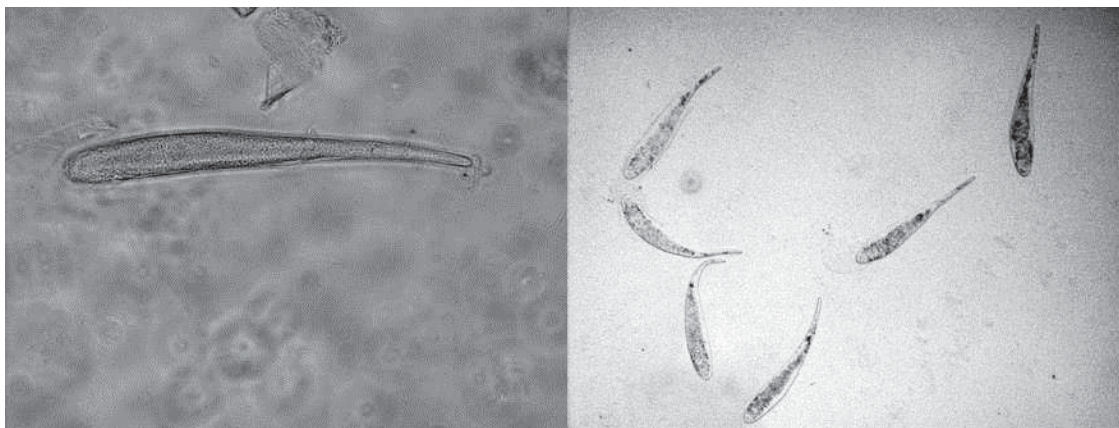


Fig. 3. Oocytes of *Apanteles opuntiarum* obtained by dissecting females soon after emergence. Left: 400 x, right: 100 x.

strain (*A. opuntiarum* is not thelytokous, while there is evidence of parthenogenesis induction by *Wolbachia* in Naupactini weevils, M. R unpublished data). Whether or not this strain is inducing cytoplasmic incompatibility in *A. opuntiarum* is still unknown and a subject of future research in order to see if *Wolbachia* is the cause of the male-biased rearing problem.

The rearing method presented in this study must be improved to ensure a continuous production of female offspring and the perpetuation of the lab culture without periodic field insect re-introductions. Additional tests are being conducted on mating behavior, reproductive biology (preliminary results showed that many lab reared males do not produce sperm), and *Wolbachia* induced phenotype to determine improved breeding conditions. Because *A. opuntiarum* is a gregarious parasitoid, its potential polyembryony is under investigation, together with the oviposition behavior of females.

#### CONCLUSIONS

The extensive field prevalence of *A. opuntiarum* in Argentina, its restricted host range, and a feasible laboratory rearing technique make this parasitoid a good potential biological control agent for *C. cactorum* in North America. The rearing protocol reported here is being used at the Gainesville, Florida quarantine laboratory and should allow the design of more reliable host specificity tests with cactophagous moth species native to North America. In addition, our initial rearing protocol will facilitate the mass rearing of *A. opuntiarum* for future field releases in *C. cactorum*-infested areas in North America.

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