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A Study of Cactoblastis cactorum (Lepidoptera: Pyralidae) in its Native Range: Further Insights into Life Cycle, Larval Identification, Developmental Parameters, Natural Enemies, and Damage to the Host Plant Opuntia ficus-indica (Caryophyllales: Cactaceae)

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Abstract

Cactoblastis cactorum Berg (Lepidoptera: Pyralidae) has been extensively studied since its initial use as a biological control agent for invasive populations of *Opuntia* Mill. The moth is native to several South American countries including Argentina where the exotic *Opuntia ficus-indica* (L.) Mill. (Cactaceae) is grown as a commercial crop. Recently *C. cactorum* has attracted considerable attention following its non-intentional establishment in Florida, because it now threatens the highly diverse and economically important *Opuntia* taxa of the southern USA and Mexico. To elucidate several aspects of this system, we recorded phenological data and parasitoid activity from Argentina across an annual cycle. We reared several generations of moths to better document the life cycle, described several formerly unpublished larval stages and morphological characters, and compared developmental parameters from samples collected from different sites. We found that *C. cactorum* has 3 overlapping generations across a 9 mo growth period with winter quiescence at the larval VI instar or pupal stage. The most common natural enemy of larvae was the parasitoid *Apanteles opuntiarum* (Martínez and Berta) (Hymenoptera: Braconidae). Information is given on its development and percentages of parasitism throughout the year. No egg parasitoids were found in field-collected eggsticks or on experimental eggsticks. There were no significant differences between developmental stages and times of *C. cactorum* from Tucumán and Córdoba in Argentina. We found intermediate *C. cactorum* damage on low-density cultivated *Opuntia*, but much lower damage in commercial plantations with high densities of plants. Surprisingly, we found that a "black spot" fungal infection (*Alternaria* Nees) (Pleosporaceae) produced a higher level of damage in commercial plantations in Córdoba, as well as in natural settings in Tucumán.

Key Words: Biological control; cactus damage; larval description; phenology; tri-trophic interactions; parasitoids

Resumen

Cactoblastis cactorum Berg (Lepidoptera: Pyralidae) ha sido extensamente estudiada desde su uso incial como un agente de control biológico de las poblaciones invasoras de *Opuntia* Mill. Su larva es nativa de Sud América, incluyendo la Argentina, donde el cactus exótico *Opuntia ficus-indica* (L.) Mill (Cactaceae) se cultiva comercialmente. *C. cactorum* ha atraído la atención recientemente, después de su establecimiento accidental en Florida, dado que amenaza a la gran diversidad de especies de *Opuntia* que son económicamente importantes y nativas de Estados Unidos y México. Con el objeto de dilucidar varios aspectos de este sistema hemos registrado datos fenológicos y la actividad de parasitoides en el campo a lo largo de un año. Hemos criado varias generaciones de polillas para documentar mejor el ciclo de vida, para describir estadios larvales y caracteres morfológicos previamente no publicados, y para comparar parámetros de desarrollo de muestras colectadas de diferentes sitios. Hemos encontrado que *C. cactorum* tiene 3 generaciones superpuestas a lo largo de 9 meses de crecimiento con una quiescencia invernal de la larva VI o pupa. El enemigo natural más común fue el parasitoide de larvas *Apanteles opuntiarum* (Martínez and Berta) (Hymenoptera: Braconidae). Se presenta información sobre su desarrollo y porcentaje de parasitismo a lo largo del año. No se hallaron parasitoides de huevo en muestras de campo ni en ristras de huevo colocadas experimentalmente. No se encontraron diferencias significativas en los estadios de desarrollo ni en sus tiempos entre muestras provenientes de los sitios de Córdoba y Tucumán. Registramos intermedio daño por *C. cactorum* en la especie de *Opuntia* cultivada en baja densidad pero mucho menor daño en plantaciones comerciales con alta densidad de plantas. Sorpresivamente, encontramos un alto nivel de daño producido por un hongo que genera la "mancha negra", tanto en plantaciones comerciales de Córdoba como en situaciones naturales de Tucumán.

Palabras Clave: Control biológico; daño en cactus; descripción larval; fenología; interacciones tri-tróficas

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Cactoblastis cactorum Berg (Lepidoptera: Pyralidae) is in the Pyralidae snout moth family, a large group that includes agriculture and forestry pests. Fifteen genera of the subfamily Phycitinae are cactus feeders (Simonsen 2008). *Cactoblastis cactorum* is 1 of 5 species in this genus native to South America, occurring in Argentina, Uruguay, Paraguay, and southern Brazil (Mann 1969). Individuals of this species were introduced originally from Argentina into Australia (Dodd 1927) and to South Africa (Pettey 1948) in successful biological control programs (Dodd 1940; Annecke & Moran 1978) in the absence of natural enemies and lack of induction of plant defenses by exotic species (Woodard et al. 2012). *Cactoblastis cactorum* was first observed in Florida in 1989 (Habeck & Bennett 1990), possibly due to accidental introductions and natural spread from Caribbean islands (Pemberton 1995), and its current range includes locations from South Carolina to Louisiana (Hight et al. 2002; Simonsen et al. 2008; Hight & Carpenter 2009; Marsico et al. 2011).

Opuntia is a keystone resource in many new world arid ecoregions. A dramatic loss of *Opuntia* is likely to have major effects on the structure and biodiversity of native and agro-ecosystems in these regions (Soberón et al. 2001; Zimmerman et al. 2004). The threat of *C. cactorum* to native and cultivated *Opuntia* is greatest in habitats where the moth is an exotic and, thus, does not face a full complement of natural parasitoids, predators, and pathogens, as well as lack of induction of host plant defenses. But the threat of introduced *C. cactorum* is most dire in regions like the southern USA and parts of México where *Opuntia* species diversity increases sharply (Jezorek et al. 2010). There are 46 native species of *Opuntia* in USA, of which 1 is federally protected, and 12 others are under review (Zimmermann et al. 2004). In its native range, *C. cactorum* is considered to negatively affect *Opuntia* fruit production, plant structure, and longevity (Lobos 2006). However, the level of damage to these cultivated plants in Argentina is not well documented apart from a brief report by Lobos and Ochoa de Cornelli (1997). In fact, several farmers experienced severe outbreaks of black spot fungal damage on the cladodes (Ayala-Escobar et al. 2006) to the point that they have burned their plantations (several farmers, personal communications). Fungal damage to *O. ficus-indica* has not been reported in Argentina to our knowledge, and there is a possibility of interactions between moth and fungal damage. Black spot is a symptom produced by several fungal infections that has been identified in Mexico (Ochoa 2013; Ochoa et al. 2015) but not in Argentina.

Much of the basic biology of *C. cactorum* is known from data gathered from its introduced ranges (Dodd 1940; Mann 1969; Zimmermann et al. 2000, 2004), whereas recent studies of *C. cactorum* in its native range describe its distribution and performance on different native and exotic *Opuntia* species (Briano et al. 2012; Varone et al. 2012; Brooks et al. 2014). However, few details of the life cycle of *C. cactorum* are known from the field in its native range other than preliminary accounts (Lobos & Ochoa de Cornelli 1997; Lobos et al. 2013). Early surveys in Argentina reported parasitoids on *Cactoblastis* (De Santis 1967; Mann 1969; Pemberton & Cordo 2001b), but most of these findings were incidental to the principal survey goals of documenting insect herbivores of *Opuntia* and refer to generalist parasitoids, precluding their use for biocontrol in the moth's introduced range (Hawkins & Marino 1997; Pemberton & Cordo 2001a; Louda et al. 2003). More recently Goñalons et al. (2014) conducted a more thorough survey for parasitoids of *C. cactorum* and reported the distribution of the braconid larval parasitoid *Apanteles opuntiarum* (Martínez & Berta) (Hymenoptera: Braconidae) in Argentina and proposed it to be specific to *C. cactorum* (Goñalons et al. 2014) or to *C. cactorum* and *Cactoblastis doddi* (Heinrich) (Lepidoptera: Pyralidae) (Gomez et al. 2015; Varone et al. 2015). Most probably, *A. opuntiarum* is part of a group of cryptic

species with specialized hosts (Martínez et al. 2012), as was found for the *Apanteles leucostigmus* (Ashmead) (Hymenoptera: Braconidae) complex, which includes 36 cryptic species (Smith et al. 2008).

The morphological distinction of juvenile forms of *C. cactorum* classically relies on information given by McFadyen (1985) based on colored spots and their pattern on the VI instar larvae. That work clearly differentiates the larvae among *Cactoblastis* species. However, several variant morphs were assigned to *C. cactorum*. Later, these morphological types were assigned to genetic haplotypes (Marsico et al. 2011) by Brooks et al. (2014), although the assignment of the 12 morphological characters used were not published in that work and were referred again to McFadyen's work. In addition, no morphological information is found for *C. cactorum* in the literature regarding larval stages other than instar VI. However, less variable characters, as well as information from other larval instars, are needed to help confirm the identity of larval stages of this species based on morphological traits.

In the context of planning biological control strategies for invasive *C. cactorum* in North America, there are several fundamental areas of knowledge that require further study. A detailed knowledge of the life cycle of *C. cactorum* from its native range is needed to facilitate a search for its natural enemies in various life stages. In addition, there are no long-term surveys of *C. cactorum* phenology from the native area. Most work done so far in the native habitat was comprised of samples gathered during limited sampling periods. The addition of systematic surveys with continuous data from the same site is needed to quantify the number of generations per yr, and the role of natural enemies across seasons and developmental stages. Furthermore, the description of larval instars other than stage VI, and additional traits beyond those given by McFadyen, is necessary to confirm the identification of *C. cactorum*. Finally, there is little quantification of damage to *O. ficus-indica* by *C. cactorum* in the native range of the moth. We have little understanding of the impacts by *C. cactorum* where its home range includes the presence of natural enemies.

Accordingly, the goals of this study were:

1) To study in the laboratory the life cycle of *C. cactorum* from its native range, and describe life stages that lack descriptions using characters besides patterns of spot markings.

2) To study *C. cactorum* in field populations across 12 mo to determine the phenology of developmental stages, the number of generations, and to assess the presence of potential biological control candidates throughout the life cycle.

3) To follow laboratory generations to evaluate developmental timing differences from samples gathered from different geographical regions.

4) To assess damage by *C. cactorum* and by black spot fungi on introduced *O. ficus-indica* within the native range of the moth.

Materials and Methods

FIELD SAMPLING

Field Sites and Survey Methods

Eight field sites (Tala Muyo, Los Arroyos, Paso del Molino, Dique Escaba, Graneros, Taco Rodeo, Lamadrid, and Taco Ralo) within a 90 km range from Alberdi, Tucumán, Argentina (between 27.5886°S, 65.6200°W and 27.6652°S, 65.7530°W) were sampled monthly from Sep 2013 to Sep 2014, except for 2 mo that were not sampled due to logistical problems. Instead, those mo (Feb and Apr 2014) were sampled

in 2015; the stages found during 2015 were intermediate between the previous and the following months of 2014, suggesting that the phenology did not change qualitatively between years.

At each site, from 2 to 12 (winter and summer, respectively) samples of *Opuntia ficus-indica* cladodes infested with *Cactoblastis* eggs or larvae were collected monthly and brought to the laboratory within 2 to 3 d of collection. *Cactoblastis cactorum* life stages in these samples were recorded and then placed with fresh *O. ficus-indica* pads for their further development. Naturally occurring eggsticks of *C. cactorum* were recovered for laboratory rearing. We searched for pupae by looking at the base of each plant within the litter. In an effort to broaden the survey for natural enemies, we also sampled more opportunistically at other provinces with *O. ficus-indica*, including Santiago del Estero (28.0980°S, 64.7375°W) where 3 sites were visited in 6 different mo, Córdoba (30.2116°S, 64.4769°W) where 7 sites were visited in 8 different mo, and Salta (25.8000°S, 64.9666°W) where 4 sites were visited in 3 different mo. Voucher specimens of larvae and adults were retained in the Ant Laboratory at the Universidad Nacional de Quilmes.

All of our studies were conducted on the globally cultivated species *O. ficus-indica*, a polyphyletic taxon originating in Central Mexico (Griffith 2004), because it is the most widely introduced species common to Argentina and USA.

Rearing of Field Collected Samples

Eggsticks and larval stages found in the field were placed on healthy *O. ficus-indica* pads brought from the same field site. In the field, eggsticks were removed manually, and when possible, with the piece of pad to which they were attached. Infested pads were easily recognized in the field by the presence of larval entry holes surrounded by larval frass. The rearing protocol was based on that of Marti et al. (2008) but modified as described here. Pads with eggs or larvae were placed in a plastic box (32 ×21 × 10 cm) on a wire mesh above a layer of commercial, absorbent cat litter to absorb the larval feces and reduce humidity. Samples were checked every other d. Pupae found in the field or those developed in the laboratory were placed in similar plastic boxes, but without absorbent cat litter and with a segment of host plant fixed high enough for emergent adults to fly and mate. Inside these boxes, we hung paper strips from the cover and mounted thin wooden sticks across the container to provide perches for emerging moths. All the pupae obtained from larvae emerging from the same pad were held together in the same container, and the males and females that emerged were left there to mate without additional handling. The only aspect we controlled was to maintain a limit of 2 eggsticks within each box. This ensured that the first instar larvae had sufficient food within the single entire pad that was placed in each rearing box. Individuals were maintained in controlled rooms at 26 °C, 70% RH, and 12:12 h (L:D) photoperiod following Marti et al. (2008). For tracking purposes, we defined a new generation for each set of new numerous eggsticks collected in the field or obtained in the laboratory.

Characterization of Life Stages

We identified the moth as *Cactoblastis cactorum* by using literature by Heinrich (1956), McFadyen (1985), and Zimmermann et al. (2004). Voucher specimens of larvae and adults were kept for reference in the Universidad Nacional de Quilmes collection. We recorded size-related and morphological features using a binocular microscope (Nikon SMZ 745, 20×, 30×) (Nikon, Tokyo, Japan) to clearly define each larval stage because they have not been described before except for larval instars I and VI (McFadyen 1985; Zimmermann et al. 2004). The number of larval instars was determined based on Dyar's rule (Gaines & Campell 1935). We also described occurrence of setae on the thorax and abdo-

men, and the number of crochets on prolegs from each larval instar following Hinton (1946) and Stehr (1987), respectively. Larvae used for taxonomic descriptions belonged to samples from Tucumán. Pupae were readily recognized in their pink-white cocoons. Sex of adults was determined by size, with females being larger and having longer labial palps than males (Heinrich 1956).

Life Stage Development Times

From 36 pupae simultaneously collected in Graneros, Tucumán, in Sep 2013, we reared adults that mated and provided a complete generation that was tracked in the laboratory. We also collected eggsticks in Sep 2014 from the Tucumán and Córdoba sites, and reared them for 2 generations.

During the rearing of these cultures, we recorded the numbers at each life stage on alternate d. For larval stages we opened the pad minimally (to avoid mortality from handling) to score the larval instar and then generalized the result for all larvae inside that pad. However, change of instar was registered as soon as it was noticed in the first individual.

Collection of Parasitoids

All field-collected samples (eggsticks, larvae within pads, and pupae) from all sites and provinces were examined for parasitoids. Such specimens were isolated and the parasitoids were reared in the laboratory at 26 °C, 70% RH, and 12:12 h (L:D) photoperiod.

To survey for egg parasitoids, eggsticks from the field were isolated in the laboratory to document parasitoid emergence. Likewise, eggsticks generated from laboratory stocks were taken to the field and mounted on *O. ficus-indica* pads and left for 15 to 20 d to attract potential egg parasitoids. At Tucumán, we set out 48 eggsticks in Mar 2013, 1 eggstick per plant, distributed at 6 different sites. On a following visit to Tucumán in May 2013, we placed 30 eggsticks across 10 different sites. At Córdoba, we set out 10 eggsticks at each of the 2 sites (a total of 20). After field exposure these were recovered for further inspection in the laboratory.

In order to assess which larval instars were infected by *A. opuntiarum*, we recorded which larval instars collected from the field later yielded *A. opuntiarum* pupae. We also offered different immature stages (eggsticks, each larval instar, and pupae) of *C. cactorum* to *Apanteles* adults in the laboratory. For each trial, we placed a number of individuals of *C. cactorum* on a cladode inside a container (35 × 28 × 20 cm) where we added from 6 to 10 *Apanteles* females, and a similar number of males, so they could mate and the females oviposit. We repeated this 3 times for each life stage, except for eggsticks where we ran 6 trials. We considered that the tested stage and instar was used as a host by the parasitoid if we obtained C. *cactorum* instar VI parasitized after rearing them.

Documenting Vegetative Damage in Wild and Cultivated *Opuntia*

In Tucumán and Córdoba provinces, we documented damage due to *C. cactorum* and black spot fungus in Jul and Aug of 2014. At Tucumán, of the 9 sites visited, 3 sites had plants cultivated for feeding pigs, and we sampled on average 36 plants per site. At the other 6 sites, *O. ficus-indica* occurred in the wild at low densities, and we sampled on average 4 plants per site. In Córdoba, all 6 sites were commercial plantations with numerous plants, ranging from 400 to 4,400 plants per site, and we searched an average of 633.3 plants per site. Plants were scored as infected by *C. cactorum* if 1 or more cladodes were damaged per plant. Similarly, cacti were scored as having fungal dam-

age if we saw 1 or more cladodes with black spot infections (Fig. 7). The fungi obtained from 20 infected cladodes were placed on water-agar, then isolates were cultured on potato dextrose agar, and later on potato carrot agar or cornmeal when necessary. The isolated fungi were identified using the Barnett and Hunter (1998) and Simmons (2007) morphological keys.

STATISTICAL ANALYSES

When 2 groups of samples were compared, we used Mann-Whitney tests. When more than 2 groups were analyzed, we used Kruskall-Wallis tests with a posteriori contrasts with alpha values adjusted by using the Bonferroni correction (Siegel 1979). We used non-parametric analyses because data did not show normality or homoscedasticity.

Results

DESCRIPTIONS OF *CACTOBLASTIS CACTORUM* LIFE STAGES

Adults

As described earlier, females are larger and have conspicuous longer and darker palpi than males. Females from Tucumán had a body length of 38 mm (± 0.03) and wing span of 40.4 mm (± 2.55), whereas males measured 28 mm (± 0.02) body length and 29.2 mm (± 1.2) wing span (n = 25). In the laboratory, adults emerged in the afternoon (Fig. 1 i, j, k, l). Mating was seldom observed and assumed to occur mainly at night.

Eggs

In the laboratory, females either deposited eggs onto a pad as an eggstick (Fig. 1a) or directly onto the boxes in which they were housed. Eggsticks resemble spines (Zimmermann et al. 2004). In the field, eggsticks had 12 to 78 eggs (n = 176), whereas in the laboratory eggsticks comprised 15 to 50 eggs (n = 1,608). Eggsticks had a length of 7 to 20 mm depending on the number of eggs per eggstick. Eggs were cylindrical and flattened, changing from a whitish color to brownish as the embryos matured. Egg size was on average 0.32 mm width (± 0.03) and 0.84 mm length (± 0.03) (n = 202). The micropylar region and sculpturing of the egg have been previously described by Baker et al. (2012).

The number of larval instars was confirmed by obtaining a geometric progression of the width of the head capsule according to Dyar's rule (Gaines & Campell 1935). All larvae were gregarious (Mann 1969).

Instar I

First instar larvae are greenish-grey in color, 3.04 mm (± 0.06) long with a fresh weight of 0.60 mg (± 0.03) (n = 45). The head (hypognathous) is dark brown with light-brown antennae and mouthparts (Fig. 1b). The width of the head capsule is 0.49 ± 0.02 mm. The following setae were found: a pair of posterior setae P1 and P2 alongside the epicranial sulcus; a pair of F1 setae on the forehead; 2 pairs of setae AF1 and AF2 on the adfrontal sclerite; a pair of setae C1 and C2 on the clipeus; anterior setae A1, A2, and A3 on the lower side of the head, along with a lateral seta L1 and 3 ocellar setae. The thorax is well differentiated into 3 segments with 1 pair of legs on each. Setae D1 and D2, SD1 and SD2, XD1 and XD2 were found in the dorsal and subdorsal part of the prothorax, L1 and L2 on the side, and SV1 and SV2 subventrally. In the mesothorax D1, D2, SD1, and SD2 setae occur dorsally, L1, L2, and L3 setae laterally, and SV1 subventrally (see Fig. 2 for position of setae). The abdomen has 10 segments, with a pair of prolegs on segments 3, 4, 5, 6, and 10. Setae on
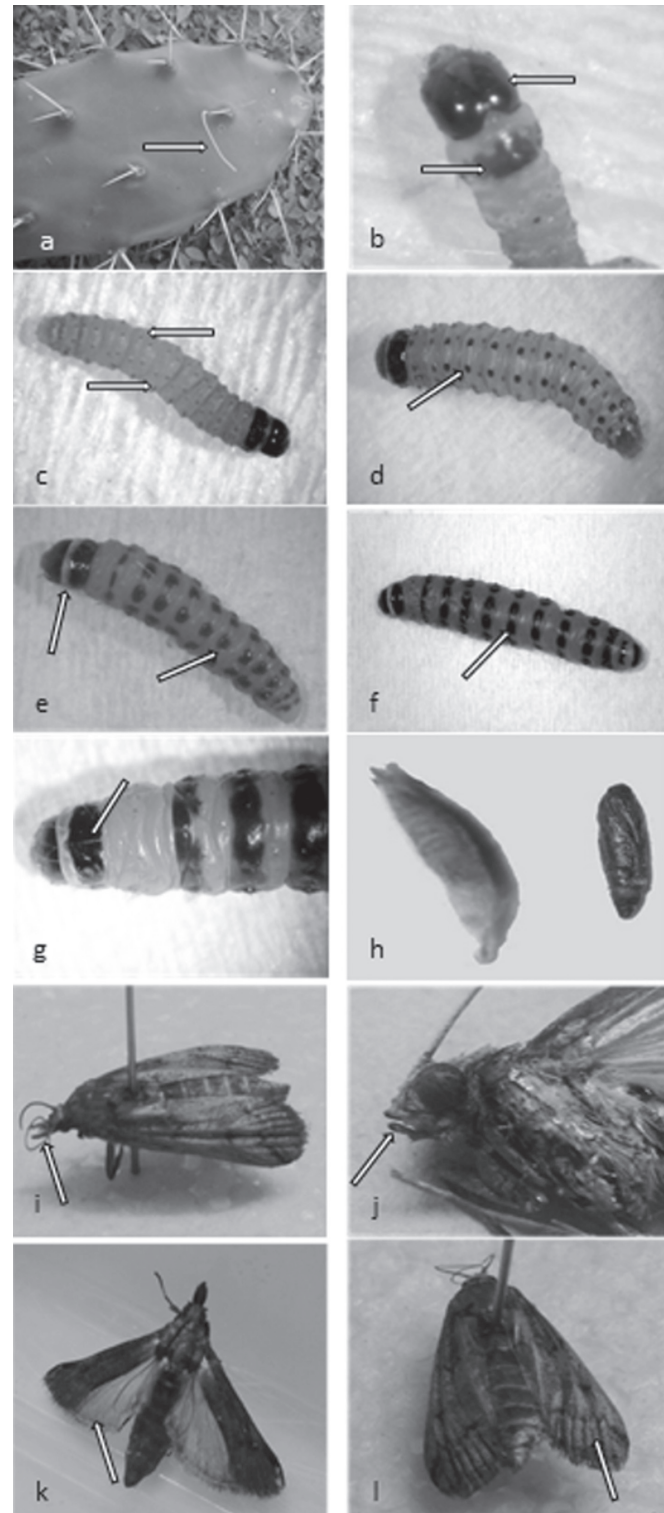

Fig. 1. (a) An eggstick oviposited on a pad of *Opuntia ficus indica*; (b) Anterior part of the larva exhibiting the cephalic capsule and prothorax starting to sclerotize; (c) Larva II has a dark shield on the prothorax and small macula at the base of each setae in the abdomen; (d) Larva III with bigger maculae with alternating color intensity on successive segments; (e) Larva IV with a white line between the head capsule and prothorax shield; (f) Larva V characterized by almost continuous black rings on the abdomen on an orange-brownish background; (g) Typical bright orange larval VI with the prothorax shield fractured in 2 and apparently continuous black rings; (h) pupa within silk cocoon and naked pupa; *Cactoblastis cactorum* females (left) and males (right); females have longer palps (i) than males (j). Both genders are characterized by a transverse line in the distal part of the wings (k, l).

the seventh abdominal segment includes D1 and D2 dorsally, L1 and L2 laterally, and SV1 subventrally. The anal segment has setae D1, D2, SD1, SD2, PPI, SV1, and SV4 (Fig. 2). Prolegs of the abdominal segments with crochets arranged in a circle, uniserial, and incipiently biordinal with 16 to 30 crochets. The proleg of the anal segment with crochets penellipse, uniserial, and ordinal with 18 to 20 crochets. The first instar larva has a light prothoracic shield (Fig. 1b, Fig. 2). This instar burrows a small hole to enter the cladode, then spins a silk web to occlude the hole, which is surrounded by ejected frass (feces).

Instar II

Second instar larvae are greenish-creamy in color and are 5.2 mm (± 0.08) long with a fresh weight of 1.12 mg (± 0.4) (n = 43). Head capsule width is 0.71 ± 0.02 mm. Larvae II begin to show spot "k" on the prothorax (surrounding setae L1 and L2) and spot "a" (between D1 and SD1) on abdominal segments (Fig. 1c). Other markings are similar to the previous instar (Fig. 2). Prolegs of the abdominal segments have crochets arranged in a circle, uniserial, and incipiently biordinal with 22 to 34 crochets. Proleg crochets of the anal segment are arranged penellipse, ordinal, and uniserial with 22 to 26 crochets.

Instar III

This instar was light orange-brown (Fig. 1d), 9.0 mm (± 0.2) long, and weighed 8.42 mg (± 0.08) (n = 43). Head capsule width 0.94 ± 0.03 mm. Compared to larvae II markings, spot "k" on the prothorax is darker, spot "a" on the abdominal segments is of greater size, with the lower part (where SD1 is placed) starting to surround the spiracles on the sides, and spot "c" (from L1 and L2) starts to appear (Fig. 2). The L1 seta occurs on the anal segment. The anal shield is clearly seen (Fig. 1d). Prolegs of the abdominal segments with crochets arranged in a circle, uniserial, and biordinal, with 32 to 44 crochets. Proleg of the anal segment has crochets penellipse, uniserial, and biordinal, with 28 to 30 crochets. Crochet arrangements remain consistent from instar III through to the final instar.

Instar IV

Instar IV larvae were of a color similar to LIII, a bit darker, but proportionately wider than the previous instar. Larval length was on average 10.7 mm (± 0.75) with a fresh weight of 32.0 mg (± 9.4) (n = 43). They had a white line between the head capsule and prothoracic shield (Fig. 1e). Head capsule width was 1.08 ± 0.04 mm. A small and light spot ("h") appears from SD1 setae. All spots were greater and darker than those in the previous instar (Fig. 2). Prolegs of the abdominal segments with 42 to 46 crochets, and of anal segment with 38 to 40 crochets.

Instar V

Each abdominal segment of this instar was orange with black interrupted spots (Fig. 1f). Larval length was 12.7 mm (± 1.7) with a fresh weight of 55.6 mg (± 14.6) on average (n = 36). Head capsule width 1.22 ± 0.03 mm. This instar was of much greater size than the previous one, and in particular broader due to the great voracity that characterized the last 3 instars. At this stage, a fracture appeared on the posterior part of the prothorax shield (Fig. 1g). All the spots mentioned in the fourth instar were very evident in the fifth instar (except "h"), all dark in coloration and larger according to the greater size of this larva (Fig. 2). Prolegs of the abdominal segments with 46 to 56 crochets, and of the anal segment with 38 to 40 crochets.

Instar VI

Sixth instar larvae were intensely orange with apparently continuous black rings on the abdominal segments, although spots did not
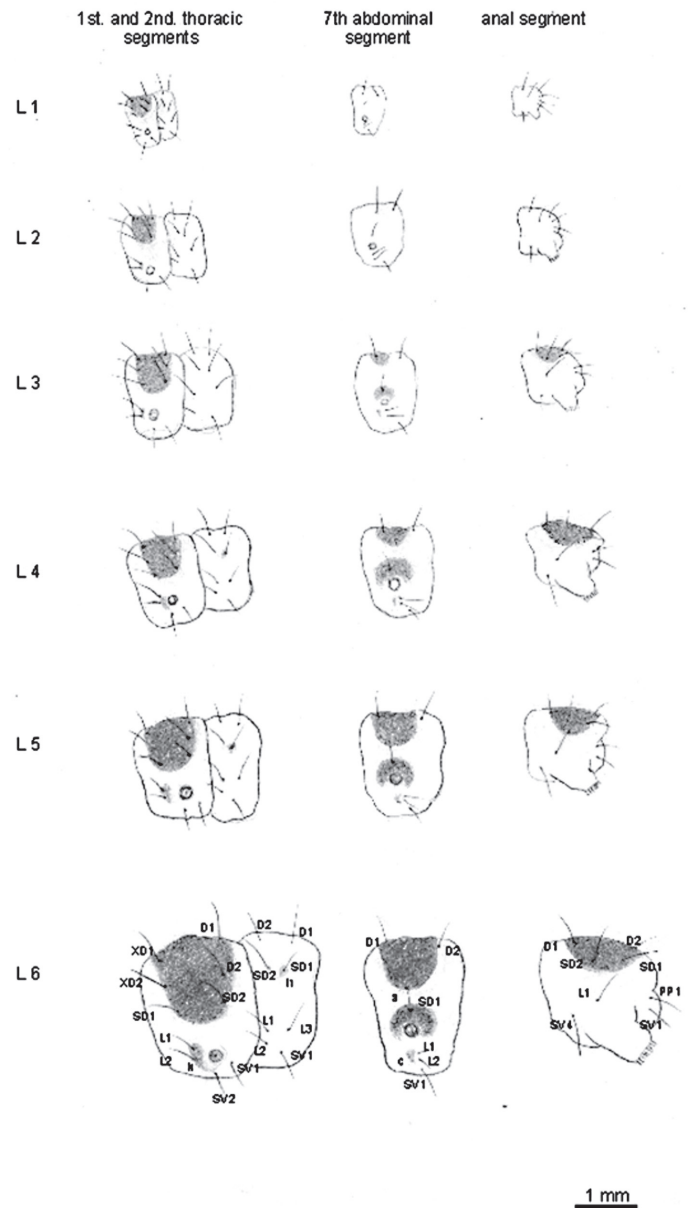

Fig. 2. Description of spots and setae from larval I to VI, shown in the pro- and meso-thoracic segment, and the seventh and anal abdominal segments: D1–2: dorsal setae; SD1–2: subdorsal setae; XD1–2: prothoracic setae; L1–3: lateral setae; SV1–2: subventral setae; PP1: posterior setae; spot "k" in prothorax, "h" in mesothorax, "a" and "c" in the seventh abdominal segment, and anal shield in the tenth and last abdominal segment.

touch laterally and barely touched in the midline (Fig. 2). This last instar had a length of 21.8 mm (± 2.5) and a fresh weight of 118.6 mg (± 28.7) (n = 36). Cephalic capsule width was 1.57 ± 0.11 mm. The fracture of the prothorax divided the shield clearly into 2 parts (Fig. 1g). All spots mentioned for instar V were very evident, dark, and of greater size in this instar, except "h" (Fig. 2). Prolegs of the abdominal segments with 54 to 62 crochets, and of the anal segment with 42 to 52 crochets.

Pupa

Cactoblastis cactorum has an obtect pupa, reddish brown in color, usually within a white silk cocoon, and covered partially or completely by small particles of debris (Fig. 1h). Plain silk cocoons were found either on the lid or corner of the container, whereas particle-covered co-

Table 1. Time in days for each developmental form of *Cactoblastis cactorum* reared in the laboratory from 36 pupae collected in Tucumán. Data shown as medians, quartiles (Q25%–Q75%), and sample sizes (N).

	Eggsticks	Larvae I	Larvae II	Larvae III	Larvae IV	Larvae V	Larvae VI	Pupae	Female	Male
Median	30	6	7.5	7	6	8.5	10	19	3	2
Q25–Q75	(27 – 31)	(4.5 – 6)	(6 – 8)	(4.7 – 8.5)	(4 – 7.7)	(8 – 9)	(5 – 12.7)	(17 – 20)	(3 – 4.5)	(2 – 2.5)
Sample Size	N: 18	N: 11	N: 10	N: 10	N: 10	N: 10	N: 6	N: 13	N: 6	N: 7

coons were found on the base of the box among the cat litter. The size of the pupa appeared to depend on the size attained by the sixth instar, with an average weight of 70.0 mg (± 16.7) (n = 36). As described by Zimmermann et al. (2004), the anal scar was found near the tip of the abdomen at the tenth segment. In female pupae, the genitalia scar was localized ventrally on the eighth and ninth segments, whereas in males the scar was placed on the ninth segment between 2 raised bullae.

PHENOLOGY OF *CACTOBLASTIS CACTORUM* LIFE STAGES

The complete life cycle of *C. cactorum* from Tucumán lasted 96.5 d for a first generation reared in the laboratory at 26 °C, 70% RH, and 12:12 h (L:D) photoperiod. The longest developmental stage was the time for eggs to hatch (30 d and 98% of all eggs from the same eggstick hatched simultaneously), whereas the shortest was the adult's lifespan (2.5 d) (Table 1).

From the monthly surveys of *C. cactorum* at Tucumán we estimated that the life cycle may include 3 generations between early spring (Sep) and late fall (May) followed by quiescence of late instar VI larvae, or pupae (Fig. 3). This is based on 2 lines of evidence; first, the generation time of about 100 d allows for 3 generations during the active months, if indeed the field populations have similar generation times. Second, the presence of eggsticks with (decreasing) peaks in Sep, Dec, and Mar occur at about 90 to 100 d intervals (Figs. 3, 4). Results of the opportunistic surveys in other provinces very distant from each other showed similar patterns. At Córdoba, we found eggsticks in Sep and Nov, and instar VI was found from May to Aug, whereas instars III and IV were found in Apr, and instar 1 in Sep. In Mar at Salta we found instars II, III, VI plus eggsticks, and in Jun instar VI. At Santiago del Estero we found eggsticks in Mar, instar VI in Jun, instar VI and pupae in Oct, and instars V, VI, and pupae in Nov.
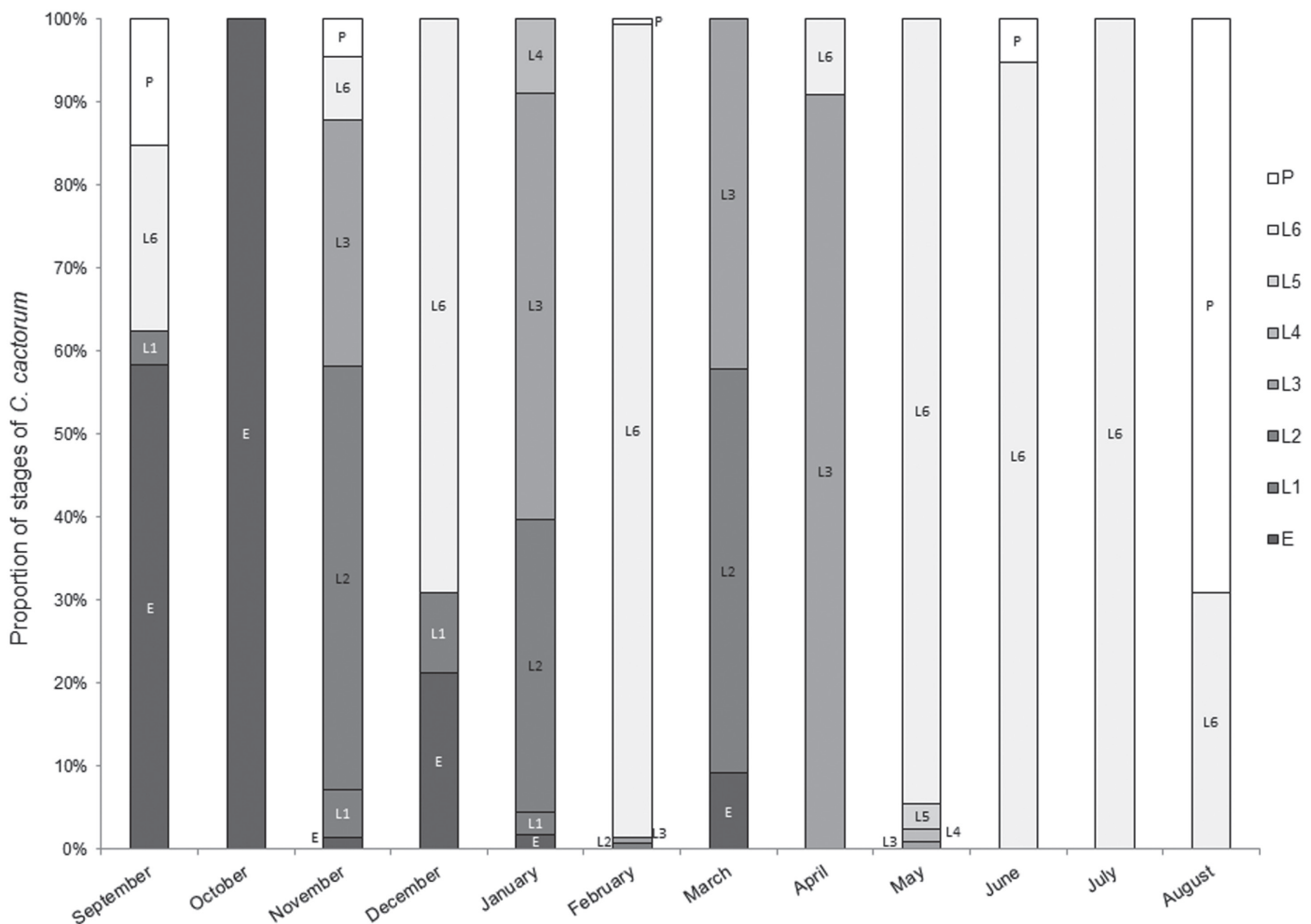

Fig. 3. Proportion of individuals of different developmental stages of *Cactoblastis cactorum* in Tucumán throughout the year. Inside the bars: E = eggsticks, L = larvae, P = pupae.
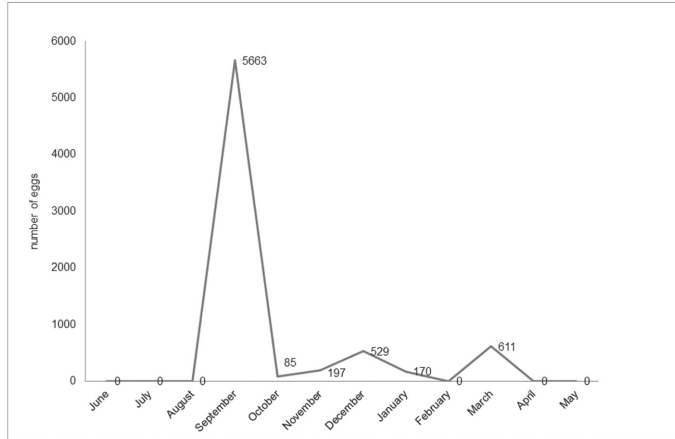
Fig. 4. Number of eggs across months for all sampling sites from Tucumán.

For the laboratory cultures of lineages from Tucumán and Córdoba, we compared the duration of developmental stages and found no significant differences between sites (all $P > 0.05$) (Table 2). Lifespan for females was, on average, 1 d longer than for males for moths from both provinces. The total development times for material from Córdoba was longer than for Tucumán and almost significant for G1 (KW = 3.19, P = 0.07; Table 2).

Parasitoids Associated with *Cactoblastis cactorum*

During the year-round field surveys in Tucumán, plus 14 locations in other provinces within the same period of time, we found very few natural enemies. We found *A. opuntiarum* parasitizing *C. cactorum* at all sites (Fig. 6), but *Apanteles alexanderi* Brèthes (Hymenoptera: Braconidae) was found parasitizing *C. cactorum* only at Tucumán in Feb and Nov. Although no systematic surveys on other lepidopteran larvae were done, we found that *A. alexanderi* also parasitized *Laetilia coccidivora* (Comstock) (Lepidoptera: Pyralidae) larvae at Santiago del Estero in Mar, Oct, and Nov. Other parasitoids found in Tucumán samples parasitizing *C. cactorum* pupae were a species of Ichneumonidae (Hymenoptera), a species of Chalcididae (Hymenoptera), and a species of Tachinidae (Diptera). We also found the fungus *Beauveria* sp. on eggsticks and larvae in the field. Due to their extremely low abundance, these specimens were not identified to the species level.

There was no clear relationship between the abundance of *A. opuntiarum* and that of the host in absolute values or percentages of parasitism. The parasitism of *A. opuntiarum* in Tucumán, calculated from the overall number of *C. cactorum* larvae collected in that mo was low, ranging from 0% to 7.9% across the yr (Fig. 5). On average, 39.2% of cladodes collected in Tucumán (34% across all sites and provinces) with apparent *C. cactorum* damage did not contain living *C. cactorum* larvae. However, from those cladodes that were positive for *C. cactorum* we calculated that 67% of them at Tucumán (64% across all sites and provinces) were also positive for *Apanteles* (Fig. 6). On average, from 5 to 18.4 parasitoid pupae were found per infected larvae VI of *C. cactorum* (Fig. 6). Despite the much higher density of plants on Córdoba sites, the percentages of infection and parasitism were similar to those found at Tucumán, and only a slightly higher number of pupae of *Apanteles* (13.2 ± 2.5) were found per cocoon at the first site in comparison to Tucumán (10.7 ± 3.3) (Fig. 6).

Apanteles opuntiarum pupated in the same cocoon, and there was 1 cocoon per instar VI host. Adults emerged in 13.6 d ± 7.2 (n = 30). All parasitoid pupae emerged simultaneously and usually generated both females and males (n = 30), except in 8 cases where all adults that emerged from the same cocoon were males (probably due to *Wolba-*

Table 2. Developmental times in days for *Cactoblastis cactorum* eggsticks collected from Córdoba and Tucumán and reared in the laboratory. Data shows median value, sample size and quartiles (25% and 75%).

		Mating – egg		Egg		Instars I – VI		Pupa		Total development time	
	Generation	Córdoba	Tucumán	Córdoba	Tucumán	Córdoba	Tucumán	Córdoba	Tucumán	Córdoba	Tucumán
Median/N	G0			10*/5	10*/9	41.5*/4	42/9	25/4	21.5/8	79/4	77/7
Q25–Q75				10 – 15	10 – 11	38.75 – 45.25	40 – 43	24.50 – 25.75	20 – 23.5	77 – 82	73.50 – 77.50
Median	G1	2.5/14	3/15	30.50/14	28.50/14	43.50/12	40/12	20/11	19/12	98/11	86.50/12
Q25–Q75		1.25 – 4	1.50 – 4.50	30 – 32	27 – 30.75	38.75 – 50.50	35 – 43.25	18.50 – 24	18.50 – 20	87.50 – 103.50	80.75 – 93.25

*time probably less because spent an unknown time in the field from where they were collected
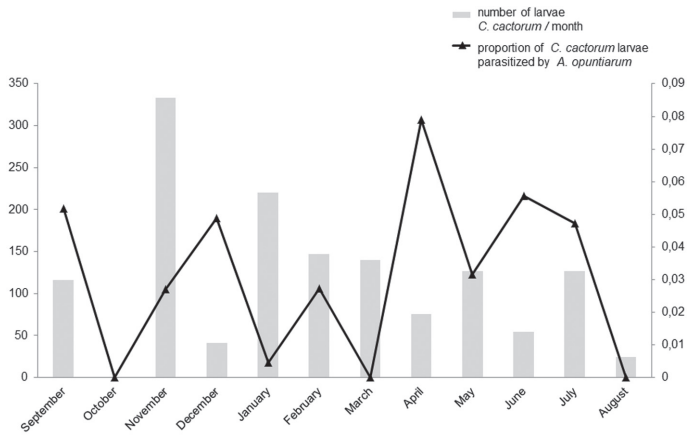
Fig. 5. Number of larvae of *Cactoblastis cactorum* per mo from all sites of Tucumán and the proportion of those that were parasitized by *Apanteles*.

chia infection, see Goñalons et al. 2014). The average number of pupae of *Apanteles* per cocoon (or instar VI) was 11.6 (± 3.1) (Fig. 6). The emergence rate was 87.1% (969 adults from 1113 pupae) with a female to male sex ratio of 1.78:1. Adult wasps did not live for more than 4 d when used in parasitism assays. We encountered parasitized larvae in all mo except from samples collected during Mar, Aug, and Sep.

In our effort to determine the life stages infected by *A. opuntiarum*, we obtained *A. opuntiarum* from instar VI in the laboratory from instars I, III, and VI collected from the field. Furthermore, the only laboratory induced oviposition occurred when instar I were exposed to *Apanteles*, from which we obtained only 1 instar VI parasitized with 6 *Apanteles* pupae.

Many of the eggsticks taken from the lab and placed in the field were recovered. In the first experiment at Tucumán, rains delayed recovery by about 1 mo and we recovered only 11 of 48 eggsticks or the emerged larvae. Among the larvae, there were 26 third instars, of which 16 survived to pupal stage, whereas 7 larvae were parasitized by *A. opuntiarum*. During the second experiment at Tucumán, 23 of 30 eggsticks were recovered after 15 d. All recovered eggsticks were dark colored indicating that they were ready to hatch, and none were parasitized. At Córdoba we recovered all 20 eggsticks close to hatching but none were parasitized.

Sources of *Opuntia* Damage

Damage to *Opuntia* pads by *C. cactorum* and various fungi was easy to distinguish (Fig. 7) and did not differ much in time of occurrence. We documented 2 forms of black spot fungal damage, namely circular and map-shaped, with distinctive shapes of fungal growth. Between 60 to 70% of the plants showed some damage by either *Cactoblastis* or black spot fungus, with a maximum level of 46% damage by *C. cactorum*
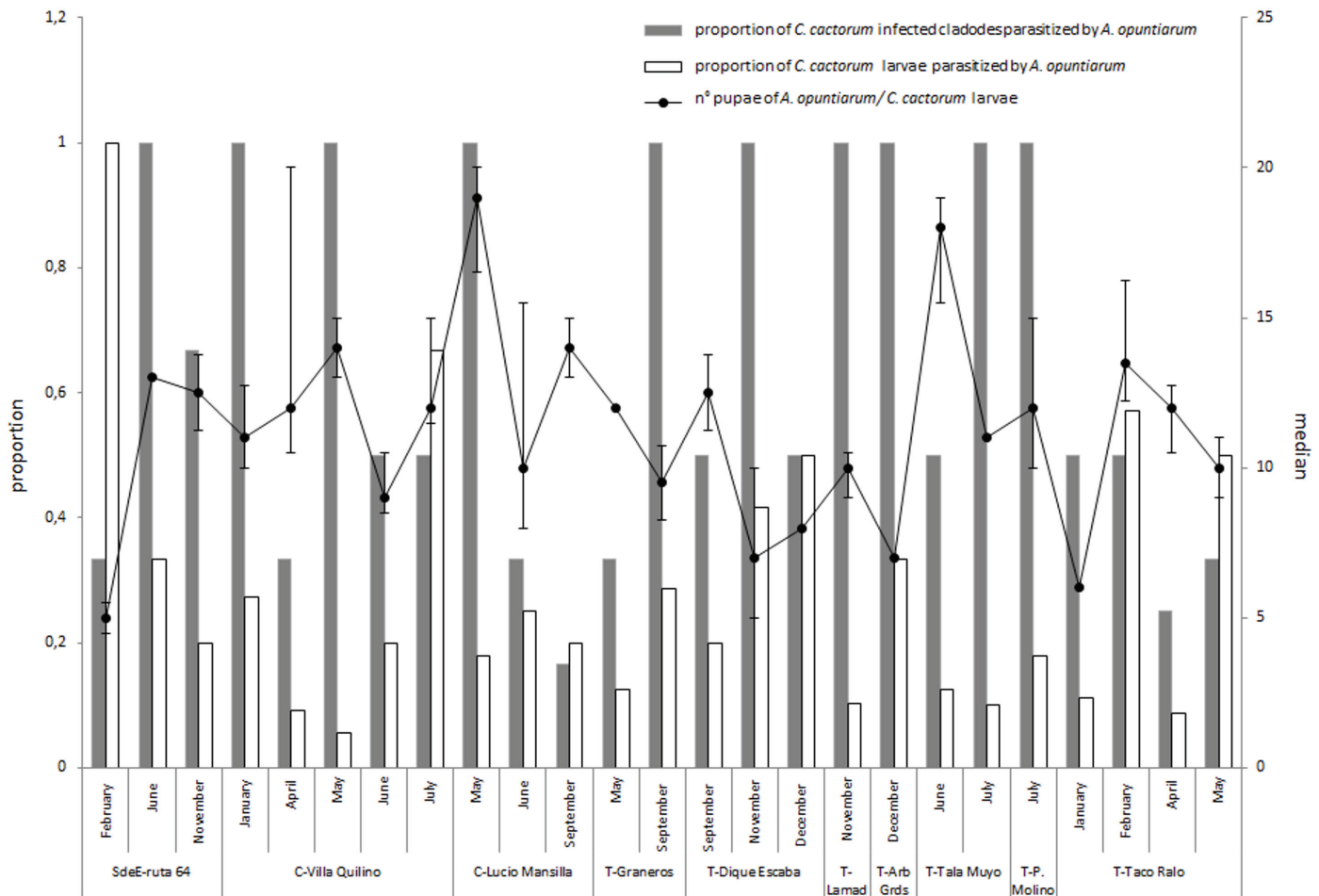

Fig. 6. Proportion of *Cactoblastis cactorum* infected cladodes parasitized by *Apanteles opuntiarum* and proportion per cladode of *C. cactorum* larvae parasitized by *A. opuntiarum* throughout the yr for sites from Santiago del Estero, Córdoba, and Tucumán provinces. The average and standard deviation of the number of pupae of *A. opuntiarum* per *C. cactorum* larvae also is shown.
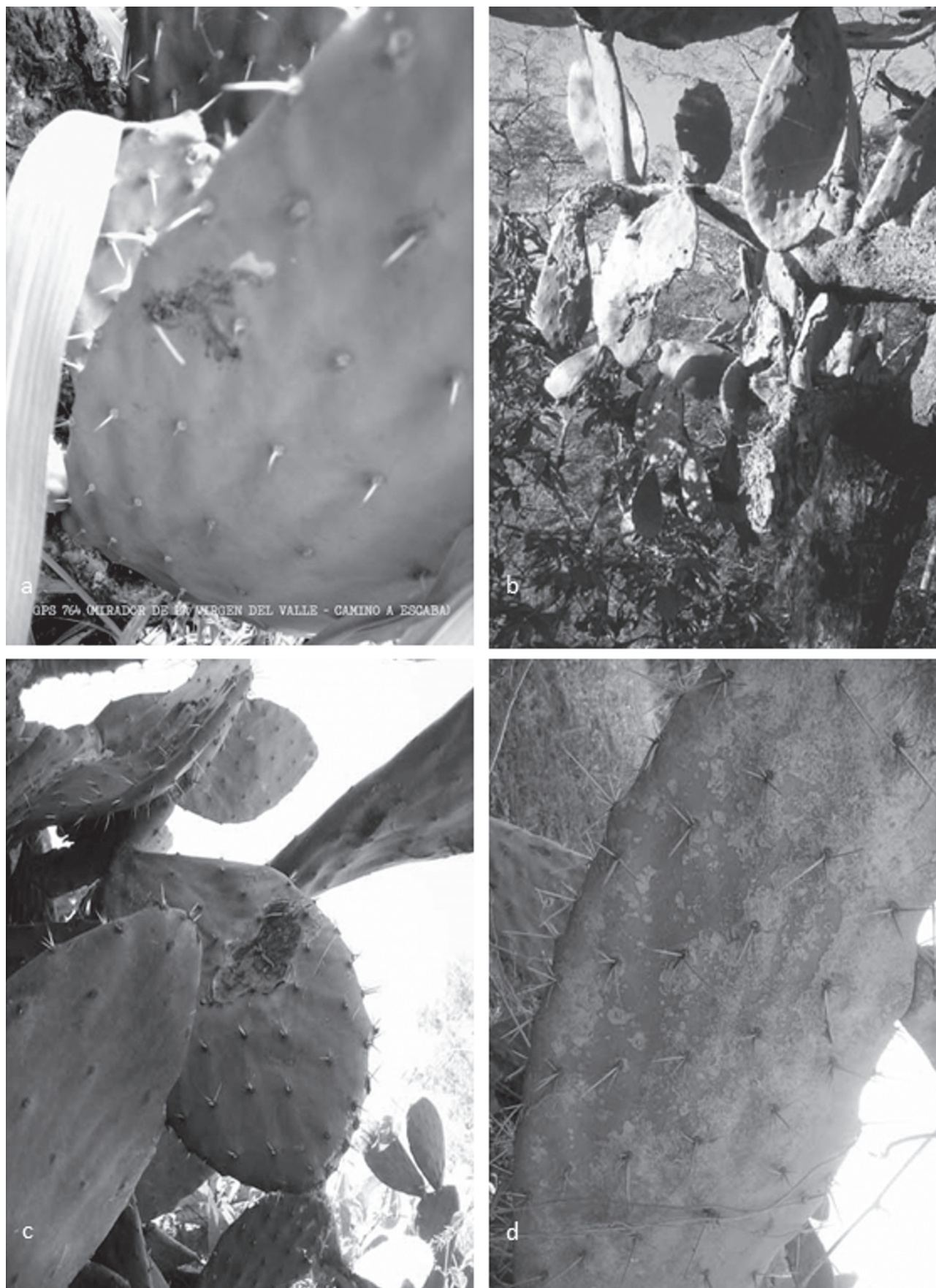
Fig. 7. Comparison of 3 characteristic forms of damage: (a) hole and feces coming from inside the pad, useful to distinguish pads with *Cactoblastis cactorum*; (b) typical damage observed in plants that were attacked by *C. cactorum*; (c) circular black spot fungal damage; (d) map black spot fungal damage.

in low density plots and 68% damage by the 'circular spot' fungus in high density plots (Table 3). The percentage of plants with *C. cactorum* damage was inversely proportional to plant density, whereas the 'map-spot' fungus was represented more frequently in low density plots (60%) and the 'circular spot' fungus in high density plots (68%). There was greater damage by *C. cactorum* in plants from Tucumán (approximately 40%) than in those from Córdoba (10%), although the effect of site and density cannot be isolated from each other. The proportion of plants with both *C. cactorum* and fungal infection simultaneously was similar to that of plants with *C. cactorum* alone, and the proportion of damaged plants decreased as plant density increased.

We recovered several fungi from the black spot injuries including *Fusarium*, *Cercosporella*, *Alternaria*, and *Micelia sterilia*. Several species of *Alternaria* (see section on *Alternaria*) seemed to produce both circular and map-spot symptoms in the laboratory (Folgarait et al., unpublished results).

Discussion

In our broad study of *C. cactorum* life history, we have elucidated previously undescribed life stages and morphological larval characters, documented the phenology of field populations, quantified the developmental stages of *C. cactorum* from 2 sites in Argentina, and conducted surveys of natural enemies of *C. cactorum* and sources of damage and infections in the *O. ficus-indica* host plant.

DESCRIPTIONS OF *CACTOBLASTIS CACTORUM* LIFE STAGES

Our specimens agree mostly with the larvae description of *C. cactorum* by McFadyen (1985). We disagree only in a potentially variable character, i.e. if spot "a" from the abdominal segments is fused in the midline or not. In some cases we found a slight fusion of spot "a" at the midline instead of being clearly divided, as in McFadyen's species A, whereas in other cases the fusion was absent, matching McFadyen's species B and C, but in all cases the adults consistently matched *C. cactorum*. However, Zimmermann et al. (2004) mentioned that the spots do not fuse at the midline. We also found a light and small spot "h" in the mesothorax that was not reported by McFadyen. The rest of the characters including spot "k" in the prothorax, spot "c" in the mesothorax, as well as shields in the prothorax and anal segment were quite stable in all descriptions. We also documented other traits that are very characteristic of lepidopteran larvae, in particular the body chaetotaxy and the number and arrangement of the crochets found in the prolegs. It will be interesting to further study the variation of these characters across the haplotypes described by Marsico et al. (2011).

A table of diagnostic characters of *C. cactorum*, *C. doddi*, and *Cactoblastis bucyrus* Dyar (Lepidoptera: Pyralidae) is given to show traits that can be used to confirm species identification (Table 4). *Cactoblastis bucyrus* is mainly found on *Opuntia* from the subgenus *Cereaneae* whereas the other 2 species are found on the *Platyopuntia* subgenus (McFadyen 1985). Generally, *C. cactorum* does not develop on *O. sulfurea* in the field, whereas *C. doddi* uses it as its preferred host (Mann 1969; Varone et al. 2014; Gomez et al. 2015). The final instar of *C. bucyrus* may reach twice the size of the other 2 species (Table 4). Accordingly, it has many more larval instars and only 1 generation per year. Although the size of the spots given by McFadyen (1985) may vary among individuals and populations, the presence of certain spots ("I" and "f") in the final instar can be compared to distinguish the 3 species mentioned (Table 4). Similarly, the arrangements and number of crochets, as well as the number of mesothorax setae, can be used to tease apart the 3 species (Table 4).

PHENOLOGY OF *CACTOBLASTIS CACTORUM* LIFE STAGES

We found that similar developmental stages are present in the same mo in most of our study provinces. Mann (1969) proposed that *C. cactorum* in Australia had 2 annual generations, a summer cycle (100–120 d), and a winter cycle (235–265 d), with total development times of 71 to 78 d and 85 to 92 d for each generation, respectively. Up to 3 generations were found in Florida, Georgia, and South Carolina in the US (Hight & Carpenter 2009). Lobos and Cornelli (1997) described 2 generations in populations from Santiago del Estero, 1 during winter–spring (Aug–Sep) and the other during summer–autumn (Mar–Apr). Lobos et al. (2013) mentioned 3 generations for northern Argentina. Our systematic survey at Tucumán demonstrated that the life cycle may include up to 3 overlapping generations (Fig. 4) between early spring (Sep) and late fall (May) followed by a quiescence at the late instar VI or pupal stages. This observation is supported by the 3 pulses of eggstick production (as demonstrated by Hight & Carpenter 2009), at intervals corresponding to approximate laboratory generation times, and the presence of multiple life stages through most monthly surveys.

For laboratory-reared populations, Varone et al. (2012) reported a 30-d larval development time at 25 °C of *C. cactorum* on *O. ficus-indica*, whereas our observations of larvae development on *O. ficus-indica* at 26 °C ranged from 40 to 45 d. Total development times from our data varied from 87 to 98 d at 26 °C compared to 84 d reported from *O. ficus-indica* in a laboratory under unknown temperature conditions (Varone et al. 2014). In contrast, development times of *C. cactorum* from Australia and South Africa were longer even for their summer generations (100–120 d and 113–132 d, respectively) (Robertson 1988; Zimmermann et al. 2004). Prolonged development may be a response to sub-optimal abiotic conditions, or biotic factors such as host plant mismatches or microbial interactions.

PARASITOIDS ASSOCIATED WITH *CACTOBLASTIS CACTORUM*

The principal parasitoid encountered across all sites was *A. opuntiarum*, in agreement with previous surveys (Goñalons et al. 2014). *Apanteles opuntiarum* seemed to be well adapted to a variety of climates as we found it at all sites surveyed across Argentina (Gomez et al. 2015; Varone et al. 2015).

Despite finding low levels of natural parasitism by this parasitoid, the low level of damage quantified in *Opuntia* by *C. cactorum* suggested an important regulatory effect of the moth population by natural enemies such as parasitoids, ants (Robertson 1988), or pathogens such as microsporidia (Pemberton & Cordo 2001b).

An intriguing feature of the life cycle of *C. cactorum* was its long time for egg development before hatching, during which time the eggs are exposed to desiccation, predation, and parasitism. Given this, one would expect that parasitoids may take advantage of resources exposed for long periods, and we anticipated finding egg parasitoids. Egg parasitoids (*Trichogramma* sp.) (Hymenoptera) are found occasionally (Lobos 2006; Goñalons et al. 2014), but they are likely to be generalist parasitoids (Paraiso et al. 2012). However, throughout continuous sampling in 1 province plus non-systematic sampling done at many other times and sites, we found no evidence of egg parasitism. We assume that discovery of eggsticks by egg parasitoids is likely due to odor cues, not visual (Peñaflor et al. 2011), and may occur soon after oviposition when odors are fresh and before the egg shells harden. In South Africa, over 50% of eggs were lost to ant predation (Robertson 1988), and discovery by ants is also likely to be odor based. To further elucidate this issue, studies such as those of Robertson and Hoffman (1989) should be continued.

Table 3. Damage by *Cactoblastis cactorum* and black spot on *Opuntia ficus-indica* discriminated by location and density. Data show counts and proportions. Low density is defined as 4 plants per ha, medium density is 36 plants per ha, high density is 633 plants per ha.

Province	Plants searched	Plants with C. cactorum	Plants with map spot fungi	Plants with circular spot fungi	Plants with both fungi	Plants with map spot fungi and C.cactorum	Plants with circular spot fungi and C. cactorum	Plants with both fungus and C. cactorum
Tucumán								
Low density	1 (0)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
	1 (0)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	13 (0)	4 (0.31)	13 (1)	13 (1)	13 (1)	4 (0.31)	4 (0.31)	4 (0.31)
Mean	4.80 (0.40)	1.20 (0.46)	3.00 (0.60)	3.00 (0.60)	3.00 (0.60)	1.20 (0.46)	1.20 (0.46)	1.20 (0.46)
SD	5.02 (0.55)	1.64 (0.51)	5.61 (0.55)	5.61 (0.55)	5.61 (0.55)	1.64 (0.51)	1.64 (0.51)	1.64 (0.51)
Intermediate density	30 (0)	4 (0.13)	22 (0.73)	18 (0.6)	18 (0.6)	4 (0.13)	4 (0.13)	4 (0.13)
	30 (0)	0 (0)	30 (1)	30 (1)	30 (1)	0 (0)	0 (0)	0 (0)
	50 (1)	50 (1)	11 (0.22)	7 (0.14)	7 (0.14)	18 (0.36)	7 (0.14)	7 (0.14)
	200 (0.75)	4 (0.02)	22 (0.11)	12 (0.06)	6 (0.03)	3 (0.01)	1 (0.01)	1 (0.01)
Mean	77.50 (0.44)	14.50 (0.29)	21.25 (0.50)	16.75 (0.45)	15.25 (0.44)	6.25 (0.13)	3.00 (0.07)	3.00 (0.07)
SD	82.21 (0.52)	23.74 (0.48)	7.80 (0.42)	9.91 (0.44)	11.24 (0.45)	8.02 (0.17)	3.16 (0.08)	3.16 (0.08)
Córdoba								
High density	800 (0.35)	90 (0.11)	200 (0.25)	600 (0.75)	200 (0.25)	30 (0.04)	60 (0.07)	10 (0.01)
	100 (0.03)	38 (0.38)	100 (1)	100 (1)	100 (1)	38 (0.38)	38 (0.38)	2 (0.02)
	500 (0.54)	37 (0.07)	100 (0.20)	200 (0.40)	100 (0.20)	11 (0.02)	26 (0.05)	0 (0)
	400 (0.64)	2 (0.01)	40 (0.10)	60 (0.15)	40 (0.10)	0 (0)	1 (0.01)	0 (0)
	1000 (0.19)	3 (0.01)	400 (0.40)	900 (0.90)	400 (0.40)	3 (0.01)	3 (0.01)	1 (0.01)
	1000 (0.19)	0 (0)	800 (0.80)	900 (0.90)	800 (0.80)	0 (0)	0 (0)	0 (0)
Mean	633.33 (0.32)	28.33 (0.10)	273.33 (0.46)	460.00 (0.68)	273.33 (0.46)	13.6 (0.07)	21.33 (0.09)	2.17 (0.01)
SD	361.48 (0.23)	34.96 (0.15)	287.52 (0.36)	390.90 (0.34)	287.52 (0.36)	16.45 (0.15)	24.49 (0.15)	3.92 (0.01)

Table 4. Comparison of biological and morphological characteristics among larvae of *Cactoblastis cactorum*, *Cactoblastis doddi*, and *Cactoblastis*.

Species	Preferred host	No instars	No generations per year	Size* (mm)	Spots h & i* mesothorax	Spot f* anal segment	Setae on mesothorax*	No crochets 7th segment*	No crochets 10th segment*
C. cactorum	On *Opuntia*; depends on species availability	6	3	21.8 ± 2.5	"h" present; "i" absent	absent	D1, D2, SD1, SD2, L1, L2, L3, SV1	54 – 62	42 – 52
C. doddi	*Opuntia sulfurea*	7	2	24.2 ± 2.0	both present	present	D1, D2, L1, L2	50 – 9	39 – 40
C. bucyrus	*Trichocereus EchinopsisDenmoya*	9	1	52.0 ± 3.0	both absent	present	D1, D2, SD1, SD2, L1, L2, L3, SV1	72 – 76	44 – 54

*In last instar Information for *C. cactorum*: this study; for *C. doddi* and *C. Bucyrus*, taken from Arce de Hamity and Nader 1999, Gomez et al. 2015. Information on preferred hosts taken from McFayden 1985 and Varone et al. 2014.

SOURCES OF *OPUNTIA* DAMAGE

A previous study of damage and pests on *Opuntia* in Argentina (Lobos 2006) indicated that *C. cactorum* was the principal pest; however, we found that the major source of cladode damage seemed to be from fungal infections, although we did not measure impacts on fruit production. Our study was focused on the introduced and widely cultivated *O. ficus-indica* and we consider our findings to reflect an overall intermediate to low level of plant damage by *C. cactorum*, even where host plants occurred at high densities, implying some population regulation by native parasitoids, predators, or pathogens.

There was small variation (approximately 50 to 60%) in the extent of damage to plants by fungal infections between sites in different provinces, despite being at substantially different plant densities. In addition, only at low densities of host plants in Tucumán, we found a suggestive pattern of intermediate co-occurrence (46%) between the moth and the black spot fungus. Possibly females of *C. cactorum* have limited choices under low host plant densities and may be able to respond to differences in host plant quality at higher host densities where females may discriminate plants with substantial black spot damage from those that are not affected. If true, then our results contrast to a previous suggestion that *C. cactorum* may be a vector of fungal pathogens (Martin & Dale 2001). Furthermore, we did not find reports of black spot fungal damage to cactus in introduced ranges where *C. cactorum* is now present, implying either an absence of infective fungi, or a lack of vectoring by *C. cactorum*. The dominant fungi we encountered were identified as *Alternaria*, and not one of the fungal species previously reported as black spot in Mexico (i.e., *Pseudocercospora*) (Ochoa 2013). Further studies of fungal infections are needed to confirm if this result holds elsewhere in Argentina, and attention should be given to commercial plantations where this fungal disease is prevalent and causes economic costs.

So far, the single candidate for introducing a biological control of *C. cactorum* to the US seems to be *Apanteles opuntiarum*. However, 2 important pieces of information still are lacking. On the one hand, it is necessary to confirm that instar I is used by this parasitoid to oviposit and, if so, then introductions of mated females of *A. opuntiarum* should be made 30 d after eggstick pulses, when instar I would have emerged in the field. On the other hand, it is necessary also to document the effect on non-target individuals, such as experiments of possible parasitism of this wasp on other US native larvae species, as well as the presence and action of pathogens that may be transmitted by the wasp.

Acknowledgments

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