




Lethal effect of *Goniozus legneri* on *Cactoblastis cactorum*: A potential biocontrol agent for inundative releases

Laura Varone^{1,2} , Ana Faltlhauser^{1,2}, Malena Fuentes Corona^{1,2},
Silvina Garrido³, Liliana Cichón³, María Carla Cecere^{2,4}, Stephen D. Hight⁵
and Octavio Bruzzone^{2,6}

Research Paper

Cite this article: Varone L, Faltlhauser A, Fuentes Corona M, Garrido S, Cichón L, Cecere MC, Hight SD, Bruzzone O (2024). Lethal effect of *Goniozus legneri* on *Cactoblastis cactorum*: A potential biocontrol agent for inundative releases. *Bulletin of Entomological Research* **114**, 149–158. <https://doi.org/10.1017/S000748532300069X>

Received: 15 August 2023
Revised: 22 November 2023
Accepted: 9 December 2023
First published online: 25 January 2024

Keywords:

Apanteles opuntiarum; biological control; cactus moth; *Opuntia ficus-indica*; parasitoid

Corresponding author:

Laura Varone;
Email: lauvarone@fuedei.org

¹Fundación para el Estudio de Especies Invasivas, Hurlingham, Buenos Aires, Argentina; ²Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina; ³Estación Experimental Agropecuaria, INTA – Alto Valle, General Roca, Río Negro, Argentina; ⁴FCEyN-EGE-IEGEB. Universidad de Buenos Aires, Buenos Aires, Argentina; ⁵USDA-ARS (retired), Tallahassee, Florida, USA and ⁶IFAB, Instituto de Investigaciones Forestales y Agropecuarias Bariloche, Bariloche, Argentina

Abstract

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae), the cactus moth, is native to South America with a widespread distribution in Argentina. The larvae consume the interior of *Opuntia* spp. (Cactaceae) plants. The moth was used as a biocontrol agent against invasive non-native *Opuntia* spp. in many countries around the world. The cactus moth arrived unintentionally in Florida, USA, expanded its range and threatened *Opuntia*-based agriculture and natural ecosystems in southern North America. The insect is also a pest of cultivated *O. ficus-indica* L. in Argentina. An endemic South American parasitoid, *Goniozus legneri* Gordth (Hymenoptera: Bethyilidae), is used in inundative biological control programmes against lepidopteran pests. The goal of this work was to evaluate *G. legneri* as a biocontrol agent to be used in inundative releases against *C. cactorum*. Mortality of *C. cactorum* by *G. legneri* was assessed at different spatial scales, as well as the interactions with *Apanteles opuntiarum* Martínez & Berta (Hymenoptera: Braconidae), a common Argentine natural enemy of *C. cactorum*. The ability of *G. legneri* to paralyse, parasitise and kill *C. cactorum* was confirmed. The paralysis inflicted on *C. cactorum* larvae reduced larval damage to the plants by 85%. Using two parasitoid species increased the mortality of *C. cactorum* larvae, but it was highly dependent on the order of their arrival. The combined mortality caused by both parasitoids was higher than a single one, in particular when *G. legneri* arrived first (56 ± 1%), suggesting asymmetric competition due to the preference of *G. legneri* attacking previously parasitised larvae. *Goniozus legneri* has potential as an inundative biocontrol agent of *C. cactorum*, but its interaction with the classical biocontrol agent *A. opuntiarum* needs to be considered.

Introduction

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is native to South America and has a wide distribution in Argentina (Briano *et al.*, 2012; Varone *et al.*, 2014). The larvae feed gregariously on *Opuntia* spp. (Cactaceae) plants, consuming the interior tissues. Burrowing activity usually causes secondary bacterial activity which hastens the destruction of cladodes (a modified flattened stem) and can kill the plant (Starmar *et al.*, 1988). *Opuntia ficus-indica* L. is native to Mexico (Kiesling, 1998), but has been introduced in many parts of the world as a crop and ornamental (Ervin, 2012). The fruit and cladodes are used for human and cattle consumption; the cladodes also have multiple uses (Pimienta-Barrios and Muñoz-Urias, 1995; Soberón *et al.*, 2001; Rodrigues *et al.*, 2023). In the 1980s, *C. cactorum* arrived unintentionally in Florida, USA (Dickel, 1991), and expanded its range representing a threat to *Opuntia*-based agriculture and natural ecosystems in the southern USA and Mexico (Solis *et al.*, 2004; Hight and Carpenter, 2009). In Argentina, this pest is present in around 40% of the orchards with cultivated *O. ficus-indica* (Varone *et al.*, 2014), and produces a detrimental impact on its fruit production (Fuentes Corona *et al.*, 2021).

Sustainable and low-environmental impact management tools are being developed to control *C. cactorum*, including classical biological control in invaded areas (Varone *et al.*, 2015; Srivastava *et al.*, 2019). Field surveys conducted in Argentina to search for natural enemies identified the koinobiont larval endoparasitoid *Apanteles opuntiarum* Martínez & Berta (Hymenoptera: Braconidae) (Mengoni Goñalons *et al.*, 2014). Field experiments determined that it has the potential as a biological control agent, accounting for 80–90% of the total parasitism of *C. cactorum* (Varone *et al.*, 2019) in almost half of the natural populations of

C. cactorum (Varone *et al.*, 2015). Highly specific to the genus *Cactoblastis* in Argentina (Varone *et al.*, 2015), *A. opuntiarum* was exported to the USA where underwent host specificity testing on North American potential hosts (Srivastava *et al.*, 2019). The parasitoid is considered host specific, not a risk to non-target species, and a request was submitted seeking approval to release it as a biological control agent in the North America against *C. cactorum* (Personal Communication, N. Benda, Florida Department of Agriculture and Consumer Services, Bureau of Methods Development and Biological Control, Gainesville, FL, USA).

Cactoblastis cactorum is a naturally occurring *Opuntia* pest in South America. In spite of the fact that *A. opuntiarum* is found attacking half of the *C. cactorum* populations in Argentina, larval damage to some *Opuntia* plantations can be serious (Lobos, 2006; Folgarait *et al.*, 2018). A second parasitoid is under evaluation as an additional control measure against *C. cactorum* in Argentina; *Goniozus legneri* Gordh (Hymenoptera: Bethyridae), an endemic idiobiont ectoparasitoid (Legner and Silveira-Guido, 1983). *Goniozus legneri* is largely used in inundative biological control programmes in Argentina against lepidopteran pests on apple, pear, stone fruits and in nut orchards (Garrido *et al.*, 2018a; Garrido *et al.*, 2019). Some habits such as paralysing larvae and host-feeding, are considered positive attributes for a biocontrol agent (Skinner *et al.*, 1990; Legner and Gordh, 1992; Balasubramanian, 2017). *Goniozus legneri* was introduced into California in 1979 to control the almond pest (Legner and Silveira-Guido, 1983; Legner and Gordh, 1992). The parasitoid is currently available in the USA for purchase and release from commercial insectaries (Wilson *et al.*, 2020).

In general, koinobiont parasitoids (such as *A. opuntiarum*) do not paralyse or kill their hosts immediately after oviposition, and because parasitised hosts continue to develop for some time, it is possible for another parasitoid to attack the previously parasitised host (Wang *et al.*, 2008; Magdaraog *et al.*, 2012). Interspecific competition among parasitoids can occur when their larvae develop in or on the same host (Wang *et al.*, 2008; Magdaraog *et al.*, 2012; Yang *et al.*, 2013; Costi *et al.*, 2022). This type of competition can affect the establishment of a biocontrol agent and decrease the efficacy of parasitoids released for biological control (Denoth *et al.*, 2002; Wang *et al.*, 2008). Therefore, the effects of multiparasitism have received attention in many biological control programmes that involved the release of multiple parasitoid agents (Pedata *et al.*, 2002; Roszbach *et al.*, 2008; Ulyshen *et al.*, 2010). Adding multiple species of biocontrol agents can significantly increase pest mortality and lead to a greater extent of control (DeBach, 1966; Stiling and Cornelissen, 2005; Aguirre *et al.*, 2021). The justification to add *G. legneri* with *A. opuntiarum* into a biological control programme against *C. cactorum* will be influenced by the type of interaction between the parasitoid species and their overall impact on the target host population.

The goals of this study were to assess the lethal effect of *G. legneri* on larvae of *C. cactorum*, as well as to evaluate its interaction with *A. opuntiarum*, to determine the potential of *G. legneri* as a biological control agent for inundative releases against *C. cactorum*. To explore the interactions between parasitoids, we performed a series of laboratory and cage studies, coupled with black-box survival and functional response models, to investigate the effect of sequential exposure of host *C. cactorum* to different parasitoid species. Use by the two parasitoids of the same host larva will be determined, and the potential for competition between the two parasitoid species will be evaluated.

Materials and methods

Insect colonies and life cycles

Laboratory colonies of *C. cactorum* were maintained to provide host larvae for testing parasitism by *G. legneri* and *A. opuntiarum*. Insect rearing was conducted under 25°C, 70% RH, and a photoperiod of 16:8 (L:D) at the Fundación para el Estudio de Especies Invasivas (FuEDEI), Hurlingham, Buenos Aires, Argentina. *Cactoblastis cactorum* larvae were originally collected in a large *O. ficus-indica* plantation located near Santiago del Estero city, Santiago del Estero province, Argentina (S 27° 62' 24.2", W 62° 34' 39.1"). Larvae were transported to the laboratory to initiate a laboratory colony of the cactus moth. Females lay eggs as a linear structure called an 'eggstick' (Dodd, 1940) that hatch simultaneously; larvae gregariously chew a hole to enter the plant and tunnel through the cladode while feeding. Larvae complete their development by building galleries as they feed on soft interior tissues and discard their faeces outside the plant. The final (sixth) instars leave the cladode, spin a cocoon, and pupate inside the cocoon mostly within plant litter near the base of the host plant. The complete life cycle takes from 4 to 6 months, depending on the host plant species, giving rise to two or three generations per year (Zimmermann *et al.*, 2004; Varone *et al.*, 2019).

Females of *A. opuntiarum* came from wild larvae of *C. cactorum* collected in the same *O. ficus-indica* plantation during the last host larval generation before winter, previously determined to have high parasitism rates (Varone *et al.*, 2019). Larvae were lab-reared in groups of 50 until pupation when parasitised larvae were identifiable. Parasitised *C. cactorum* were individually stored in 20 ml plastic cups and checked daily until emergence of the adult parasitoids. Upon wasp emergence, females were confined with double the amount of males (4–8 females with 8–16 males) for 24 h to allow mating in a 3 L plastic canning jar with a modified 80-mesh lid. Added to the mating jar were a slice of *O. ficus-indica* (6 × 10 cm), a tablespoon of *C. cactorum* larval frass to stimulate female oviposition (Varone *et al.*, 2020), and a damp strip of paper towel (1 × 3 cm) saturated with water and honey to provide moisture and food.

Individuals of *G. legneri* were obtained from the Instituto Nacional de Tecnología Agropecuaria mass-rearing institute located in Alto Valle, Río Negro, Argentina. Mass-rearing of this parasitoid is carried out with fifth instar larvae of *Galleria melonella* (L.) (Lepidoptera: Pyralidae) as hosts because oviposition is higher in this species and produces a more efficient generational increase (Garrido *et al.*, 2018b). Parasitoids were received at FuEDEI as pupae in 20 ml plastic cups. When female parasitoids emerged, they were immediately used in the experiments. Copulation was ensured because males of *G. legneri* emerge before females and enter the female cocoons for mating. After mating, the female seeks out a host larva and injects a poison through its stinger, causing paralysis that facilitates oviposition (Gordh *et al.*, 1983).

Study 1: Lethal effect of *G. legneri* on *C. cactorum*

Three experiments were conducted, with increasing experimental spatial scale, to assess the lethal effect of *G. legneri* on *C. cactorum* larvae (fig. 1a–c). The first experiment was performed to test the ability of *G. legneri* to attack (kill and/or parasitise) *C. cactorum* in the presence or absence of a small refuge for the host larvae, and with different larval instars exposed to the parasitoid. Although *C. cactorum* larvae have internal feeding habits in the cladodes,

300 ml test arena because they could survive starvation during the parasitoid exposure time. Female parasitoids were confined with larvae for 24 h and removed. Between 10 and 12 replicates of each treatment were conducted. An additional set of five replicates with three to five L4 host larvae plus cactus cubes, without parasitoids, served as controls. *Cactoblastis cactorum* larvae were fed with fresh *O. ficus-indica* cladodes as needed until they either completed development and pupated, or died. Containers were checked three times a week to record parasitised, dead, or pupated larvae. Parasitised larvae were isolated in 20 ml plastic cups until the emergence of *G. legneri* adults. The number and sex ratio of adult wasps per container were recorded.

A second experiment tested the mortality of *C. cactorum* with increasing numbers of *G. legneri* females and larger pieces of *O. ficus-indica* (fig. 1b). Because *C. cactorum* larvae reduced cactus mass by feeding inside the cladode, the piece of cactus was weighed before and after the trial to estimate weight loss due to feeding. Groups of 20 L4 *C. cactorum* larvae were placed in an 8 l plastic vented container with half a cladode of *O. ficus-indica* of similar size, and larvae were allowed to penetrate the plant for 24 h. After 24 h, 5, 10, 15, or 20 *G. legneri* mated females were added to the containers with a solution of honey and water as a food source. After the death of all the females, containers were checked three times a week to record parasitised, dead, and pupated *C. cactorum* larvae until all larval hosts completed development or died. Groups of 20 larvae feeding on similar sized cladodes, unexposed to parasitoids, were placed in 8 l containers and served as controls for larval survival. Ten replicates of each treatment (and control) were conducted.

The third experiment was conducted in cages to evaluate the lethal effect of *G. legneri* on *C. cactorum* when the larvae were feeding inside live prickly pear plants (fig. 1c). Two-year-old *O. ficus-indica* potted plants (with two cladodes each) were infested with 40 L4 larvae of *C. cactorum*. Prior to the release of the parasitoids, and so that they could penetrate into the plant, larvae were placed in an open 20 ml plastic cup, and attached to the plant in a manner that the larvae were in contact with the surface of a cladode. Potted plants were placed inside a 120 × 50 × 50 cm rectangular mesh cage. After larvae feed for 48 h, 40 *G. legneri* mated females were released in the centre of the cage. A solution of honey and water was also provided as food for the females. Five replicates were conducted and five plants with *C. cactorum* larvae, without parasitoids, were used as controls. Since larvae leave the plant to pupate, a piece of cloth was placed at the base of each plant, which served as a pupation substrate. After the parasitoids' death, cages were checked three times a week to record the number of pupating *C. cactorum*, meaning that those larvae survived exposure to the parasitoids. Parasitised or dead larvae remaining inside the plants were not recorded since this would have required the destruction of the entire living plant system. After all larvae pupated (or died), damage to the plants produced by larval feeding was estimated as the percentage of visible surface damage to plant tissue (categories of 0, 25, 50, and 100% surface damage). The damage estimate, along with the number of successfully pupated larvae, were compared between treatments with and without *G. legneri* to identify the impact of the parasitoid on the test host larvae.

Study 2: Interaction between *G. legneri* and *A. opuntiarum*

The mortality produced by *G. legneri* and *A. opuntiarum*, acting together or separately, was tested with increasing amounts of *C. cactorum* host larval densities. In addition, the influence of

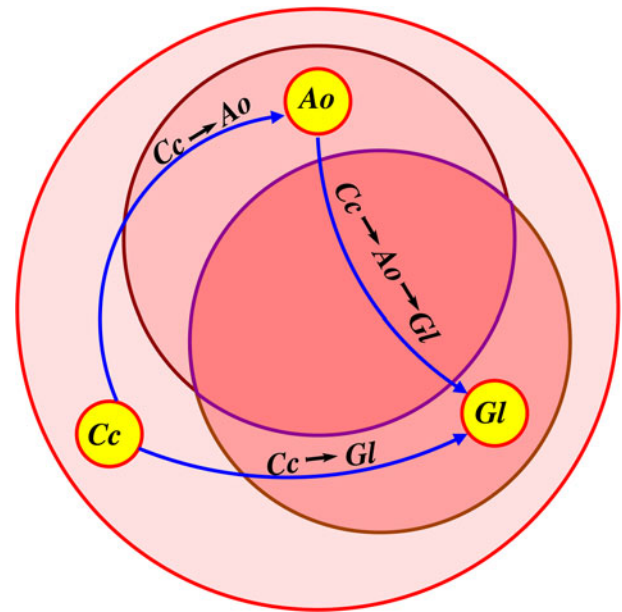


Figure 2. Venn diagram of trophic relationships in this study. The yellow circles are the species, and the blue lines indicate the trophic relationships between species. The light pink filled circle indicates the entire group of hosts/prey of *Cactoblastis cactorum* (Cc) exposed to natural enemies. The medium-light pink circle indicates the subgroup attacked only by *Apanteles opuntiarum* (Ao, shown as Cc → Ao), while the medium-dark pink circle indicates the subgroup attacked only by *Goniozus legneri* (Gl, shown as Cc → Gl). The dark pink area indicates the Cc larvae parasitised by Ao and attacked by Gl (Cc → Ao → Gl).

refugia on parasitoid interaction was included in the host density tests; with a piece of cactus that served as a refuge for the larvae or without a piece of cactus (no refugia) (fig. 1d). Figure 2 highlights the trophic relationships between species that were investigated in this study.

The interaction study consisted of four treatments: host larvae exposed to only one species of the two parasitoids, host larvae exposed to one parasitoid species for 24 h and then the sequential exposure to the other parasitoid species (*G. legneri* followed by *A. opuntiarum*, and vice versa). In all four treatments, five larval densities (one repetition each) of 20, 25, 30, 35, and 40 L3 *C. cactorum* were confined with a single female parasitoid for 24 h in a 500 ml vented arena. In the treatments with sequential exposure of the two species of parasitoids, the female was also with the larvae for 24 h. After the first 24 h with one parasitoid species, the parasitoid was removed and the other species was immediately introduced for another 24 h. A piece of cactus was added in the refugia-present design when host larvae were placed in the arena, allowing them to enter and use it as food and refuge (fig. 1d). The same design was repeated (four treatments of parasitoids exposed to five larval densities) but only a slim piece of cactus was available as a food source, insufficient in thickness to be used as a refuge. At the end of all parasitoid 24 h exposures, host larvae were transferred to 3 l vented containers with a more significant piece of *O. ficus-indica* cladode for feeding and development. Larvae were checked thrice weekly to record parasitised, dead, or pupated larvae.

Data analysis

The lethality of *G. legneri* on *C. cactorum* was analyzed in the first and third experiments of Study 1 which compared larval mortality among treatments. For the first experiment, the number of

G. legneri adults and the sex ratio of the F1 that emerged from the parasitised *C. cactorum* larvae were compared. All comparisons were made using a Kruskal–Wallis test because of the non-normal distribution of the data, and groups were identified post hoc using Duncan’s test. For the second experiment of Study 1, a linear regression was conducted between larval survival, or relative loss of cladode weight, and the increasing number of female *G. legneri* exposed. All the analyses were conducted in Infostat version 2020e (Di Rienzo *et al.*, 2008).

The interaction between *G. legneri* and *A. opuntiarum* of Study 2 was evaluated by measuring the effectiveness in the order of the release of the species causing host mortality through the development of a series of multiplicative models of cumulative mortality coupled with functional responses. The best-explaining model in terms of more explanatory power and less complexity was selected by means of the Schwartz criterion, or Bayesian Information Index, called BIC hereon (Schwarz, 1978).

Model formulation

The starting point of the model development was a *null model* in which the host mortality is constant:

$$N_d \sim B(N_o, p_d) \tag{1}$$

where N_o is the number of offered hosts and the number of dead hosts (N_d) is a random variable which follows a binomial distribution (B), with a probability of death equal to p_d . To add the effect of parasitoids, we modified the parameter p_d . The mortality caused by a given parasitoid i is:

$$p_r(q) = (q_i > 0)p_{di} \tag{2}$$

where p_r is the resulting mortality caused by parasitoid i , q_i is the number of parasitoids of species i in the experimental arena (zero or one), and p_{di} is the mortality caused by parasitoid i . If parasitoid i is absent, q_i is zero, and then, the resulting mortality is zero, otherwise, p_r is p_{di} .

As presented here, equation 2 assumes that the parasitoids follow a type-I functional response, with a constant attack rate resulting in a constant proportion of hosts attacked regardless of the number of hosts offered (Holling, 1959). More realistically, if the proportion of attacked hosts decreases with the number of hosts (N) because the handling time of the host is required, it reaches a saturation curve. Replacing equation 2 with a type-2 functional response model (Holling, 1959):

$$p_r(q_i, N) = q_i p_p / (1 + p_p h N q_i) \tag{3}$$

where q_i is the number of parasitoids in the experiment, p_p is the attack rate, and h is the handling time. Because at very low N , p_r is almost the same as p_r from equation 2, in the following equations all the $p_r(q_i)$ or $p_r(q_i, N)$ will be called p_r for simplicity.

If the ‘natural’ mortality of equation 1 is combined with mortality caused by parasitoids from equations 2 and 3, the following model is:

$$p_{di}(q_i) = 1 - (1 - p_o)(1 - p_r) \tag{4}$$

with

$$N_d \sim B(N_o, p_{di}(q_i))$$

where p_o is the natural mortality of the hosts. Consequently, the proportion of the dead hosts is given by those that remain after

removing hosts that survived the other causes of mortality and the action of parasitoids.

The next model considers that both parasitoid species cause different mortality levels, thus the second term from equation 2 is incorporated into a new equation:

$$p_{d12}(q_1, q_2) = 1 - (1 - p_o)(1 - p_{r1}(1 - p_{r2})) \tag{5}$$

$$N_d \sim B(N_o, p_{d12}(q_1, q_2))$$

where p_{r1} and p_{r2} are the proportion of hosts attacked by parasitoids 1 and 2, respectively. Given there are two parasitoids q_1 and q_2 acting sequentially the expected proportion of dead hosts is given by the remaining hosts that survived the natural cause of mortality and both parasitoids.

The following model considered that the mortality might also be affected by the availability of refuges (R) (pieces of cacti available in the experimental arena) and that the presence of refuges decreases the hosts’ finding by the parasitoid at a certain ratio r . According to the function $r(R) = r$ ($R = 0$), if there are no refuges R is zero, otherwise, R is one. In the case of $R = 0$, the mortality increases by r . Equation 4, is then modified as follows:

$$p_{d12}(q_1, q_2) = (1 - r(R))(1 - (1 - p_o)(1 - p_r(q_1)(1 - p_r(q_2)))) \tag{6}$$

Finally, it is considered that the mortality might increase more than proportionally by the effect of the second parasitoid in the sequence:

$$p_{d12}(q_1, q_2) = (1 - r)(1 - (1 - p_o)(1 - p_r(q_1)(1 - p_r(q_2)))p_{e12}) \tag{7}$$

where p_{e12} , is the extra mortality caused by the parasitoid 1, given that the host was first exposed to the parasitoid 2. Alternatively, the mortality might increase less than proportionally after the second parasitoid is added, for example, if there is a preference to attack hosts previously attacked as a form of interference competition. Thus the equation modifies as follows:

$$p_{d12}(q_1, q_2) = (1 - r)(1 - (1 - p_o)(1 - p_r(q_1)(1 - p_r(q_2)p_{e12}))) \tag{8}$$

where p_{e12} is the mortality caused by the second parasitoid, given that the attacked hosts were previously attacked by the first parasitoid.

Numerical methods (model selection and parameter calculation)

The data analysis was performed in two steps; first, a model selection procedure was performed to select the combination that produced the best explanatory power in terms of the log-likelihood of the model, and less complexity, in terms of the number of parameters. Second, the parameters of the selected model and their statistical distribution were calculated using Monte Carlo methods. A list of candidate models was created using different combinations of the equations described above (table 1). A Markov Chain Monte Carlo procedure using the Metropolis-Hastings algorithm was performed (MCMC) for all the candidate models, according to Gelman *et al.* (2003). A total of 20,000 iterations were discarded, as a *burn-in* for optimisation of the parameters, and 50,000 iterations for the parameters and information indexes calculation, named *traces*.

The BIC information criterion was calculated using these traces that contained the values of the parameters and the log-likelihood

Table 1. List of models proposed to evaluate the interaction between the parasitoids *Goniozus legneri* and *Apanteles opuntiarum* on the mortality of *Cactoblastis cactorum* (see model selection procedure)

Model name	Mortality equation	p_r	# Parameters estimated	Likelihood	BIC	gcd
1. Null	1	-	1	-305.18	622.10	0.0000
2. Parasitoid mortality (PM)	4	$p_r(P)$	2	-251.22	518.09	0.8845
3. Sequential PM (SPM)	5	$p_r(P)$	3	-232.77	485.11	0.9448
4. SPM and refuge (SPMR)	6	$p_r(P)$	4	-231.73	486.93	0.9470
5. SPMR + extra mortality <i>G. legneri</i> (EG)	7	$p_r(P)$	5	-231.73	490.84	0.9470
6. SPMR + extra mortality <i>A. opuntiarum</i> (EA)	7	$p_r(P)$	5	-228.13	483.64	0.9541
7. SPMR + reduced mortality <i>G. legneri</i> (RG)	8	$p_r(P)$	5	-226.53	480.43	0.9570
8. SPMR + reduced mortality <i>A. opuntiarum</i> (RA)	8	$p_r(P)$	5	-231.72	490.84	0.9470
9. SPMR + RG + RA	8	$p_r(P)$	6	-226.15	483.60	0.9576
10. SPMR and functional response (FR) (SPMRFR)	6	$p_r(P, N)$	6	-231.73	494.75	0.9470
11. SPMFR + EG	7	$p_r(P, N)$	7	-231.73	498.67	0.9470
12. SPMFR + EA	7	$p_r(P, N)$	7	-228.13	491.47	0.9541
13. SPMRFR + OA + RG	8	$p_r(P, N)$	7	-225.12	485.45	0.9593

P_r is the mortality caused by a parasitoid, BIC is the Schwarz criterion, and gcd is the generalized coefficient of determination. In bold is the selected model after the procedure.

function at each iteration (Schwarz, 1978). The model with the lowest BIC value was chosen, and the trace of the iterations was used to calculate the parameters. Additionally, to describe how well the model described the data, we used a generalised r^2 calculated according to Cox and Snell (2018), because the variable was binomially distributed and therefore the classical r^2 was not applicable.

The model was written in the Python programming language version 3.10 (Van Rossum and Drake, 2009), with the libraries NumPy version 1.21.5 (Harris et al., 2020), and scipy version 1.7.3 (Virtanen et al., 2020).

Results

Study 1: lethal effect of *G. legneri* on *C. cactorum*

Goniozus legneri showed an overall ability to mainly kill, but also parasitise larvae of *C. cactorum*. This was the first record of the interaction between these two species. As a general trend, females of *G. legneri* stung the host larvae to first paralyse the host and then oviposited. However, paralysis was much more common than parasitisation. Host feeding was also observed, but not quantified, and likely contributed to host mortality. In the first experiment, all the larvae of *C. cactorum* were killed by *G. legneri*, independent of the larval instar exposed and the presence of a refuge (fig. 3, $H = 13.04$, $p < 0.01$). The proportion of parasitised larvae did not differ between treatments with different larval stages or the presence of refuge for the larvae (fig. 3, $H = 1.11$, $p = 0.56$), and ranged between 0.27 and 0.37. Neither the amount of *G. legneri* F1 adults nor the sex ratio obtained was different between treatments ($H = 0.22$, $p = 0.23$ and $H = 0.95$, $p = 0.64$, respectively). When L2 *C. cactorum* hosts were exposed, 0.56 ± 1.33 adults *G. legneri* developed, with a 0.87 ± 0.17 proportion of females. In the case of L4 hosts with refuge, 2.0 ± 1.93 adults developed, with 0.93 ± 0.12 proportion of females, while without a refuge, 1.42 ± 3.06 adults emerged and were all females.

In the second experiment, the increasing amount of *G. legneri* females generated increasing mortality of *C. cactorum* larvae.

When 15 or more female parasitoids were exposed per 20 *C. cactorum* larvae, all larvae died in all replicates (fig. 4, $F_{1, 23} = 25.26$, $p < 0.01$). Additionally, the weight loss of the cladodes decreased with increasing amounts of female parasitoids in the experimental arena (fig. 5, $F_{1, 23} = 23.01$, $p < 0.01$) because the larvae of *C. cactorum* died as a consequence of parasitism or paralysis and stopped eating the plant.

In the third experiment, the capacity to kill and paralyse of *G. legneri* was also confirmed when *C. cactorum* larvae were completely inside the cactus cladode in a live-standing plant. In the presence of *G. legneri*, the proportion of dead *C. cactorum* larvae (0.96 ± 0.05) was significantly higher than in the absence of parasitoids (0.54 ± 0.12) ($H = 6.82$, $p < 0.01$). The percentage of visible damage generated to the cladodes by the larval feeding without the presence of the parasitoids was $90.0 \pm 22.36\%$, while with the release of *G. legneri*, only $6.0 \pm 13.41\%$ of the cladode surface showed visible feeding damage.

Study 2: Interaction between *G. legneri* and *A. opuntiarum*

The best model in terms of explained variability and complexity using the BIC criterion was the 7th of the 13 models proposed (table 1). According to model 7, the interaction between parasitoids was explained by equation 8, and the equation was expressed as follows based on the estimated parameters:

$$\begin{aligned}
 p_{dag}(q_g, q_a) &= (1 - r)(1 - (1 - p_o)(1 - p_r(q_g)(1 - p_r(q_a)))) \\
 &\text{with} \\
 sp1 &= g(G.legneri) \\
 sp2 &= a(A.opuntiarum) \\
 &\text{and } p_{ga} = 1 \\
 p_{dga}(q_a, q_g) &= (1 - r)(1 - (1 - p_o)(1 - p_r(q_g)(1 - p_r(q_a)p_{ag}))) \\
 &\text{with} \\
 sp1 &= a(A.opuntiarum) \\
 sp2 &= g(G.legneri)
 \end{aligned} \tag{9}$$

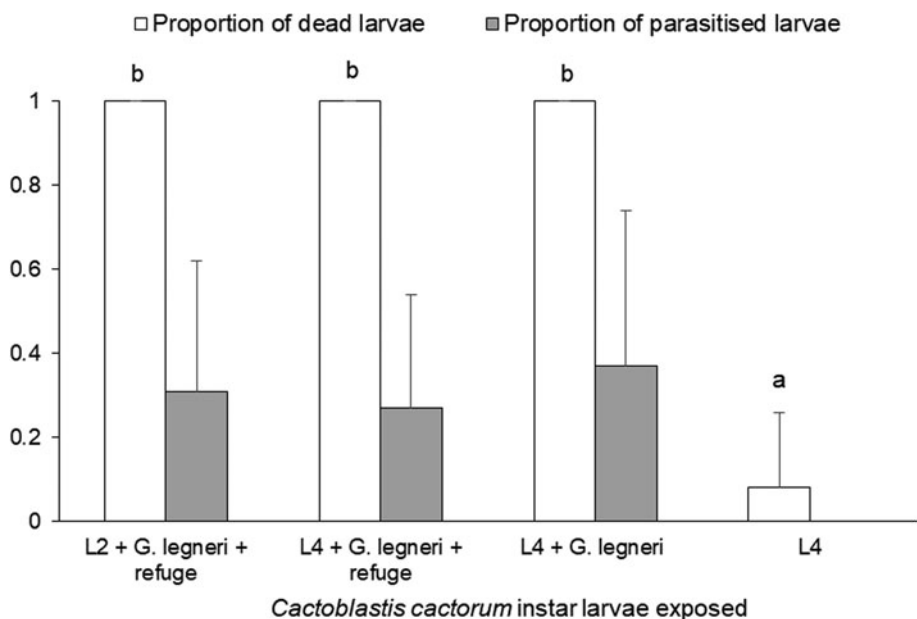


Figure 3. Proportion of dead and parasitised larvae of different instars of *Cactoblastis cactorum* exposed to a single female of *Goniozus legneri*, with or without refuge for the host larvae. Data were analyzed with a Kruskal–Wallis test. Different letters indicate statistically significant differences.

The first line is for the experiment where *G. legneri* (*sp1*) attacked first, and the second is when *A. opuntiarum* (*sp2*) attacked first. As there was no effect of *A. opuntiarum* on *G. legneri* (the mortality inflicted by *G. legneri* does not change if *A. opuntiarum* attacked first), the parameter p_{ga} was equal to one and was eliminated from the first line of equation 9. As a result of this equation, the mortalities caused by both parasitoids simultaneously, after correcting for the presence of the refuges and the natural mortality, was estimated as:

$$\begin{aligned}
 P_g P_a &= (1 - p_r(q_g))(1 - p_r(q_a)) \\
 P_a P_g &= (1 - p_r(q_g)(1 - p_r(q_a)p_{ag}))
 \end{aligned}
 \tag{10}$$

where $P_g P_a$ is the mortality caused by both parasitoids when *G. legneri* attacked first, and $P_a P_g$ is the mortality caused by both when *A. opuntiarum* attacked first.

Most of the proposed models had a high explanatory power with a *gcd* higher than 90%, and even some higher than 95%. While the selected model also had a *gcd* of more than 95%, it contained fewer parameters. The selected model revealed three

findings: the host larvae had a constant baseline natural mortality of near 20% (parameter p_o = without the action of parasitoids); the addition of an *Opuntia* cladode piece produced a refuge effect that decreased the mortality by nearly 5% (parameter r) (table 1); and within the range of offered hosts, the parasitoids attacked the larvae proportionally to the offered number, as occurs in a type I functional response.

Mortality caused by *G. legneri* was nearly double that caused by *A. opuntiarum* (parameters P_{pg} and P_{pa} , respectively) and the highest mortality was achieved when both parasitoids were acting together, with *G. legneri* arriving first (parameter $P_a P_g$) (table 2). This competitive interaction between both parasitoids was asymmetric, evidenced by the differences in mortalities related to the order in which the parasitoids were released in the experimental arena. The additional mortality caused by using *G. legneri* in the second place (according to the parameter P_{ag}) was almost half compared to the expected additional mortality caused if this species was used in the first place (P_{ga}) (table 2). A mortality of five percentile points higher was determined when *A. opuntiarum* attacked second, because that parasitoid attacked

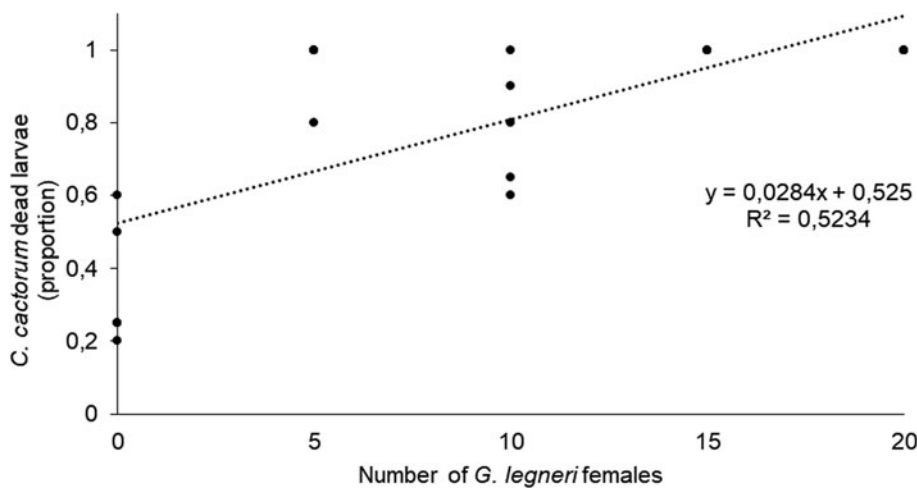


Figure 4. *Cactoblastis cactorum* larvae killed by different numbers of *Goniozus legneri* female parasitoids.

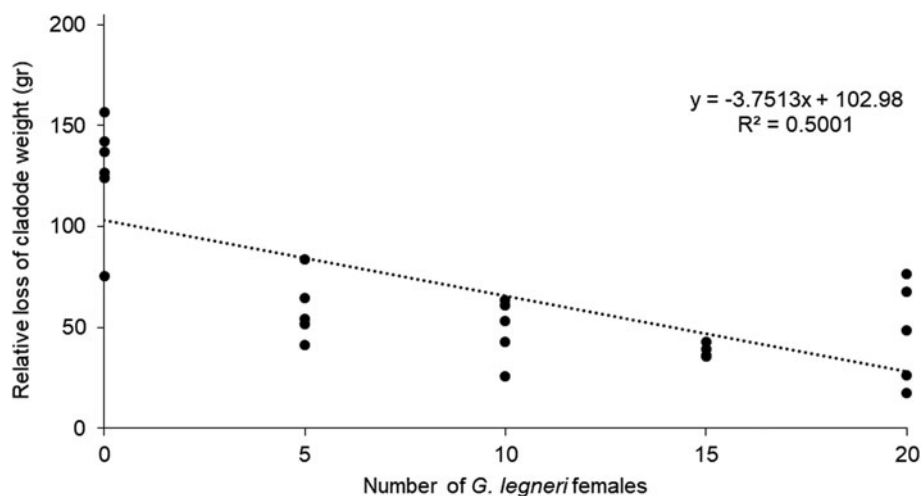


Figure 5. Weight loss (in grams) of *Opuntia ficus-indica* cladodes due to feeding by 20 *Cactoblastis cactorum* larvae exposed to increasing numbers of *Goniozus legneri* female parasitoids.

only the hosts that previously survived *G. legneri*. *Goniozus legneri* preferred to attack hosts previously attacked by their competitor *A. opuntiarum*, killing them in the process.

Discussion

This work presented the first record of the parasitoid *G. legneri* successfully parasitising the cactus moth, *C. cactorum*, and confirmed the lethality of the attack on host larvae. Also supported under laboratory conditions, the potential of using two parasitoid species, *G. legneri* and *A. opuntiarum*, together as biological control agents against *C. cactorum* without diminishing the efficacies of either parasitoid species.

The ability of *G. legneri* to paralyse, host feed, parasitise and kill *C. cactorum* was demonstrated even when larvae were feeding inside the cladodes. One of the more promising abilities that produced the lethal effect of *G. legneri* on *C. cactorum* larvae was the paralysis inflicted on hosts, followed by death of larvae. Damage to *O. ficus-indica* plants from larval feeding was decreased by

G. legneri attack, likely enhancing plant health and survival. Because *C. cactorum* has two or three discrete generations per year (Varone et al., 2019), inundative releases of *G. legneri* during early larval stages could prevent *Opuntia* plants from severe damage.

Previous parasitisation studies of *G. legneri* attacking lepidopteran larvae under laboratory conditions reported higher mean numbers of parasitised larvae per female, as in the case of *C. pomonella* (13 larvae/female) (Laumann et al., 2000), and *A. transitella* (23 larvae/female) (Gordh et al., 1983). Given the low parasitisation rates found in the present work, the main mechanisms involved in the death of *C. cactorum* larvae appeared to be paralysis and host feeding. Female bethylid wasps paralyse their hosts by repeated stinging (Steiner, 1986) and produce toxins that permanently paralyse larvae (Skinner et al., 1990). The number of *G. legneri* stings was quite variable and when paralysis occurred (usually within 30 min), the *C. cactorum* larvae did not recover. In the present research, host feeding, another mechanism reported for bethylid wasps (Gordh et al., 1983) was observed, but not quantified.

Since *G. legneri* was first reported in southern Argentina in 2005 (Garrido et al., 2005), a *G. legneri* mass-rearing protocol was developed using the pyralid host *G. mellonella*. Numerous successful inundative biocontrol programmes were carried out as part of integrated management approaches to control pests of fruit trees with low negative environmental impact (non-target attacks). *Goniozus legneri* was also successfully used in inundative biological control programmes against lepidopteran pest species in the US (Gordh et al., 1983; Butler and Schmidt, 1985). In central California, the parasitoid was capable of significant population regulation of the navel orangeworm, *A. transitella*, due to its ability to increase the percentage of parasitisation with rising host densities (Legner and Silveira-Guido, 1983; Legner and Gordh, 1992). Given the potential of *G. legneri*, studies were also conducted with the parasitoid to evaluate behavioural responses to the carob moth, *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), a major pest of pomegranate in Iran (Aleosfoor et al., 2014), and to the jasmine moth, *Palpita unionalis* Hb. (Lepidoptera: Crambidae), a destructive pest of young olive farms in Egypt and in most of the Mediterranean basin countries (El-Basha and Mandour, 2006). Currently, the commercial biological control industry has developed mass production, shipment and release methods for *G. legneri* against a variety of

Table 2. A posteriori mean (μ) and standard deviation (σ) of the estimated parameters of the selected model PMSR+EA, the log-likelihood, and the probability of being killed by both biological control agents

Parameter	Meaning	μ	σ
p_o	Natural mortality	0.2125	0.0083
r	Refuge effect	0.0520	0.0209
p_{pa}	Mortality by <i>A. opuntiarum</i>	0.2301	0.0140
p_{pg}	Mortality by <i>G. legneri</i>	0.4279	0.0149
p_{ag}	Additional mortality by <i>A. opuntiarum</i> first	0.5496	0.0691
p_{ga}	Additional mortality by <i>G. legneri</i> first	1.0000	–
LL	Log-Likelihood	–227.12	0.7183
$P_{\alpha}P_g$	Mortality by both, <i>G. legneri</i> first	0.5595	0.0140
P_gP_{α}	Mortality by both, <i>A. opuntiarum</i> first	0.5002	0.0392

Parameters $P_{\alpha}P_g$ and P_gP_{α} are according to equation 10.

Parameter p_{ga} was not estimated and assumed to be equal to 1.0, thus it has no estimation of error.

pests. In several areas of agriculture, inundative biological control has obtained considerable success and is now a reliable and appreciated element of IPM programmes (van Lenteren, 2012).

We found promising results for considering *G. legneri* as a biocontrol agent for inundative releases in a biological control programme. The next step will be to conduct field experiments to estimate the extent of control this parasitoid exerts over *C. cactorum* larvae and the outcome of the interaction with *A. opuntiarum*. Maximum mortality and improved control of the pest would be achieved with release ratios of 0.75–1 *G. legneri* females per larva of *C. cactorum*. Current inundative biological control programmes using *G. legneri* against fruit pests in northern Patagonia, Argentina, revealed that release ratios of 1:1 were obtainable and optimally controlled the pests (Garrido unpublished data).

In the absence of parasitoid competition, *G. legneri* produced higher mortality rates than *A. opuntiarum*. When the two parasitoid species interacted together with the same group of host larvae, *C. cactorum* larval mortality was increased compared to the use of a single parasitoid species. Although this increment was not additive, since there was asymmetric competition due to the preference of *G. legneri* to attack previously parasitised *A. opuntiarum* larvae, introducing both parasitoid species together as biological control agents was recommended because of greater mortality of the hosts. In plantations where *A. opuntiarum* does not occur naturally, larval mortality would be maximised by releasing *G. legneri* first, followed by *A. opuntiarum*. This superior performance of *G. legneri* was found in other idiobiont ectoparasitoids that paralyse the host by making it less likely for koinobiont endoparasitoids to find the host (Ulyshen *et al.*, 2010; Wang *et al.*, 2010). Moreover, the observed preference of parasitoids to oviposit in already parasitised hosts has previously been documented and can occur given the different competition strategies, competitive power, functional responses, advantages of arriving first, etc. (Bruzzone *et al.*, 2018).

In summary, the results presented here support the idea that despite having found competition between parasitoids, the mortality caused by the two species continues to be higher than the use of only one. However, competition experiments under laboratory conditions need to be coupled with field investigations. The evaluation of host finding ability and adult mobility of the two parasitoids will be critical because it will directly influence the probability of sequential access to the host and confirm the outcome of combined attacks and the competitive interactions. The present work constitutes baseline information for the consideration of *G. legneri* as an inundative biocontrol agent of *C. cactorum*, and to further evaluate multiple biocontrol strategies.

Acknowledgements. We are grateful to Fernando Mc Kay for reviewing earlier versions of the manuscript, and Arabella Peard for improving the final version. Research funding was granted by USDA, APHIS-PPQ, Grant/Award Number: Farm Bill Section 10201; Fondo para la Investigación Científica y Tecnológica, Argentina, Grant/Award Number: 2016-1447.

Competing interests. The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there are no financial interests to report.

References

- Aguirre MB, Bruzzone OA, Tripietsyn SV, Hight SD and Logarzo GA (2021) Influence of competition and intraguild predation between two candidate biocontrol parasitoids on their potential impact against *Harrisina cactus* mealybug, *Hypogeococcus* sp. (Hemiptera: Pseudococcidae). *Scientific Reports* **11**, 1–11.
- Aleosfoor M, Ehteshami F and Fekrat L (2014) A six-arm olfactometer for analysing olfactory responses of *Goniozus legneri* Gordh (Hymenoptera: Bethyliidae), the larval ectoparasitoid of carob moth. *Journal of Entomological and Acarological Research* **46**, 119–122.
- Balasubramanian J (2017) Host–parasitoid interaction and behavior of *Goniozus legneri* (Gordh), an external parasite of the mango leaf webber, *Orthaga exvinacea* (Hampson). *Indian Journal of Scientific Research* **12**, 036–040.
- Briano J, Varone L, Logarzo G and Villamil C (2012) Extended geographical distribution and host range of the cactus moth, *Cactoblastis cactorum* (Lepidoptera: Pyralidae), in Argentina. *The Florida Entomologist* **95**, 233–237.
- Bruzzone OA, Logarzo GA, Aguirre MB and Virla EG (2018) Intra-host interspecific larval parasitoid competition solved using modelling and Bayesian statistics. *Ecological Modelling* **385**, 114–123.
- Butler Jr GD and Schmidt KM (1985) *Goniozus legneri* (Hymenoptera: Bethyliidae): development, oviposition, and longevity in relation to temperature. *Annals of the Entomological Society of America* **78**, 373–375.
- Costi E, Bella ED, Iotti D and Maistrello L (2022) Biocontrol implications of multiparasitism by *Trissolcus mitsukurii* and *Trissolcus japonicus* on the invasive brown marmorated stink bug. *Entomologia Experimentalis et Applicata* **170**, 772–781.
- Cox DR and Snell EJ (2018) *Analysis of Binary Data*. New York: Routledge. <https://doi.org/10.1201/9781315137391>
- DeBach P (1966) The competitive displacement and coexistence principles. *Annual Review of Entomology* **11**, 183–212.
- Denoth M, Frid L and Myers JH (2002) Multiple agents in biological control: improving the odds? *Biological Control* **24**, 20–30.
- Di Rienzo JA, Casanoves E, Balzarini MG, Gonzalez L, Tablada M and Robledo CW (2008) InfoStat, versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Dickel TS (1991) *Cactoblastis cactorum* in Florida (Lepidoptera: Pyralidae: Phycitinae). *Tropical Lepidoptera* **2**, 117–118.
- Dodd AP (1940) The biological campaign against prickly pear. Commonwealth Prickly Pear Board, Brisbane, Australia. 177 pp.
- El-Basha NA and Mandour NS (2006) Effect of *Goniozus legneri* Gordh (Hymenoptera: Bethyliidae) on the life table of *Palpita unionalis* Hb. (Lepidoptera: Pyralidae). *Egyptian Journal of Biological Pest Control* **16**, 5–11.
- Ervin GN (2012) Indian fig cactus (*Opuntia ficus-indica* (L.) Miller) in the Americas: an uncertain history. *Haseltonia* **17**, 70–81.
- Folgarait PJ, Montenegro GA, Plowes RM and Gilbert L (2018) 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). *Florida Entomologist* **101**, 559–572.
- Fuentes Corona M, Varone L and Cecere MC (2021) ¿Cuánto pierde un cultivo de *Opuntia ficus-indica* si lo visita el gusano de la tuna?. XXIX Reunión Argentina de Ecología. <https://www.asaeArgentina.com.ar/RAE>
- Garrido S, Cichón L, Fernández D and Azevedo C (2005) Primera cita de la especie *Goniozus legneri* (Hymenoptera: Bethyliidae) en el Alto Valle de Río Negro, Patagonia Argentina. *Revista de la Sociedad Entomológica Argentina* **64**, 14–16.
- Garrido S, Cichón L, Lago J and Navarro D (2018a) El control biológico inundativo y por conservación mediante la utilización de especies nativa. X Congreso Argentino de Entomología. Universidad Nacional de Cuyo. Secretaría de Ciencia, Técnica y Posgrado, 21–24 May 2018 Mendoza, Argentina.
- Garrido S, Cichón L, Lago J, Navarro MD, Herrera ME and Becerra V (2018b) Evaluation of the oviposition of *Goniozus legneri* (Hymenoptera: Bethyliidae) on different Lepidoptera of fruit and vegetable interest. *Acta Zoologica Lilloana* **62**(Suplemento), 118–121.
- Garrido S, Cichón L, Claps L, Lago J, Navarro D, Gomez C and Leonelli E (2019) Uso de *Goniozus legneri* (Hymenoptera: Bethyliidae) en control biológico inundativo y su incidencia en la reducción de insecticidas en perales. Resúmenes de la VII Reunión Argentina de Parasitoidólogos. Santa Rosa, La Pampa, Argentina. 11–13 Septiembre 2019. Revista de la Facultad de Agronomía UNLPam, pp. 27–30.

- Gelman A, Carlin JB, Stern HS and Rubin DB** (2003) Hierarchical models. In Chatfield C, Tanner M and Zidek J (eds), *Bayesian Data Analysis*, 2nd Edn. New York: Chapman and Hall/CRC Press, pp. 120–160.
- Gordh G, Woolley JB and Medved RA** (1983) Biological studies on *Goniozus legneri* Gordh (Hymenoptera: Bethyilidae) a primary external parasite of the navel orangeworm *Amyelois transitella* and pink bollworm *Pectinophora gossypiella* (Lepidoptera: Pyralidae, Gelechiidae). *Contributions of the American Entomological Institute* **20**, 433–468.
- Harris CR, Millman KJ and van der Walt SJ** (2020) Array programming with NumPy. *Nature* **585**, 357–362.
- Hight SD and Carpenter JE** (2009) Flight phenology of male *Cactoblastis cactorum* (Lepidoptera: Pyralidae) at different latitudes in the southeastern United States. *Florida Entomologist* **92**, 208–216.
- Holling CS** (1959) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist* **91**, 385–398.
- Kiesling R** (1998) Origen, domesticación y distribución de *Opuntia ficus-indica*. *Journal of the Professional Association for Cactus Development* **3**, 1–9.
- Laumann RA, Ferrero AA and Stadler T** (2000) Evaluación en laboratorio de *Goniozus legneri* Gordh (Hymenoptera: Bethyilidae) enemigo natural de *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) en cultivos de nogal de la provincia de Catamarca, República Argentina. *Boletín de Sanidad Vegetal* **26**, 537–550.
- Legner EF and Gordh G** (1992) Lower navel orange worm (Lepidoptera: Phycitidae) population densities following establishment of *Goniozus legneri* (Hymenoptera: Bethyilidae) in California. *Journal of Economic Entomology* **85**, 2153–2160.
- Legner EF and Silveira-Guido A** (1983) Establishment of *Goniozus emigratus* and *Goniozus legneri* (Hym: Bethyilidae) on navel orangeworm, *Amyelois transitella* (Lep: Phycitidae) in California and biological control potential. *Entomophaga* **28**, 97–106.
- Lobos E** (2006) Control de *Cactoblastis cactorum* Berg, principal plaga de la tuna en Argentina. In Aprovechamiento integral de la tuna. *CactusNet* **10**, 26–32.
- Magdaraog PM, Harvey JA, Tankaka T and Goles R** (2012) Intrinsic competition among solitary and gregarious endoparasitoid wasps and the phenomenon of ‘resource sharing’. *Ecological Entomology* **37**, 65–74.
- Mengoni Goñalons C, Varone L, Logarzo GA, Guala ME, Rodríguez M, Hight SD and Carpenter JE** (2014) 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. *Florida Entomologist* **97**, 1458–1468.
- Pedata PA, Giorgini M and Guerrieri E** (2002) Interspecific host discrimination and within-host competition between *Encarsia formosa* and *E. pergandiella* (Hymenoptera: Aphelinidae), two endoparasitoids of whiteflies (Hemiptera: Aleyrodidae). *Bulletin of Entomological Research* **92**, 521–528.
- Pimienta-Barrios E and Muñoz-Urias A** (1995) Domestication of opuntias and cultivated varieties. Barbera G, Inglese P and Pimienta-Barrios E (eds), *Agro-ecology, Cultivation, and Uses of cactus-Pear*. Italy: Food and Agriculture Organization of the United Nations, FAO Plant Production and Protection Rome, pp. 58–63.
- Rodrigues C, Paula CD, Lahbouki S, Meddich A, Outzourhit A, Rashad M, Pari L, Coelho I, Fernando AL and Souza VG** (2023) *Opuntia* spp.: an overview of the bioactive profile and food applications of this versatile crop adapted to arid lands. *Foods (basel, Switzerland)* **12**, 1465.
- Rossbach A, Löhner B and Vidal S** (2008) Interspecific competition between *Diadegma semiclausum* Hellen and *Diadegma mollipla* (Holmgren), parasitoids of the diamondback moth, *Plutella xylostella* (L.), feeding on a new host plant. *Bulletin of Entomological Research* **98**, 135–143.
- Schwarz G** (1978) Estimating the dimension of a model. *The Annals of Statistics* **6**, 461–464. Available at <http://www.jstor.org/stable/2958889>
- Skinner WS, Dennis PA and Quistad GB** (1990) Partial characterization of toxins from *Goniozus legneri* (Hymenoptera: Bethyilidae). *Journal of Economic Entomology* **83**, 733–736.
- Soberón J, Golubov J and Sarukhán J** (2001) The importance of *Opuntia* in México and routes of invasion and impact of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Florida Entomologist* **84**, 486–492.
- Solis MA, Hight SD and Gordon DR** (2004) Tracking the cactus moth, *Cactoblastis cactorum* Berg., as it flies and eats its way westward in the U.S. *News of the Lepidopterist's Society* **46**, 3–4.
- Srivastava M, Srivastava P, Karan R, Jeyaprakash A, Whilby L, Rohrig E and Varone L** (2019) Molecular detection method developed to track the koinobiont larval parasitoid *Apanteles opuntiarum* (Hymenoptera: Braconidae) imported from Argentina to control *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Florida Entomologist* **102**, 329–335.
- Starmer WT, Aberdeen V and Lachance MA** (1988) The yeast community associated with decaying *Opuntia stricta* (Haworth) in Florida with regard to the moth, *Cactoblastis cactorum* (Berg). *Florida Scientist* **51**, 7–11.
- Steiner AL** (1986) Stinging behaviour of solitary wasps. In Piek T (ed.), *Venoms of the Hymenoptera. Biochemical, Pharmacological and Behavioural Aspects*. Amsterdam, The Netherlands: Universitet van Neatherlands, pp. 63–148.
- Stiling P and Cornelissen T** (2005) What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control* **34**, 236–246.
- Ulyshen MD, Duan JJ and Bauer LS** (2010) Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennis* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae). *Biological Control* **52**, 188–193.
- van Lenteren JC** (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **57**, 1–20.
- Van Rossum G and Drake FL** (2009) *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace.
- Varone L, Logarzo GA, Briano JA, Hight SD and Carpenter JE** (2014) *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) use of *Opuntia* host species in Argentina. *Biological Invasions* **16**, 2367–2380.
- Varone L, Logarzo GA, Martínez JJ, Navarro FJ, Carpenter E and Hight SD** (2015) Field host range of *Apanteles opuntiarum* (Hymenoptera: Braconidae) in Argentina, a potential biocontrol agent of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. *Florida Entomologist* **98**, 803–806.
- Varone L, Aguirre MB, Lobos E, Ruiz Pérez D, Hight SD, Palottini F, Guala M and Logarzo GA** (2019) Causes of mortality at different stages of *Cactoblastis cactorum* in the native range. *BioControl* **64**, 249–261.
- Varone L, Mengoni Goñalons C, Faltthausen AC, Guala ME, Wolaver D, Srivastava M and Hight SD** (2020) Effect of rearing *Cactoblastis cactorum* on an artificial diet on the behaviour of *Apanteles opuntiarum*. *Journal of Applied Entomology* **144**, 278–286.
- Virtanen P, Gommers R and Oliphant TE** (2020) SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods* **17**, 261–272.
- Wang XG, Bokonon-Ganta AH and Messing RH** (2008) Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: effect of coevolutionary history on competitive superiority. *Biological Control* **44**, 312–320.
- Wang X-Y, Yang Z-Q, Gould JR, Wu H and Ma J-H** (2010) Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. *Biological Control* **52**, 24–29. <https://doi.org/10.1016/j.biocontrol.2009.09.008>
- Wilson H, Burks CS, Reger JE and Wenger JA** (2020) Biology and management of navel orangeworm (Lepidoptera: Pyralidae) in California. *Journal of Integrated Pest Management* **11**, 25.
- Yang S, Duan JJ, Lelito J and Van Driesche R** (2013) Multiparasitism by *Tetrastichus planipennis* (Hymenoptera: Eulophidae) and *Spathius agrili* (Hymenoptera: Braconidae): implication for biological control of the emerald ash borer (Coleoptera: Buprestidae). *Biological Control* **65**, 118–123.
- Zimmermann H, Bloem S and Klein H** (2004) Biology, history, threat, surveillance and control of the cactus Moth, *Cactoblastis cactorum*. Austria: Food and Agriculture Organization of the United Nations (FAO).