BIOLOGICAL CONTROL

Comparing Potential as Biocontrol Agents of Two Neotropical Parasitoids of *Liriomyza sativae*

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Abstract

We evaluated the potential of two parasitoids as biocontrol agents of *Liriomyza sativae* Blanchard in northeastern Brazil. The two species were the koinobiont larval-pupal endoparasitoid *Phaedrotoma scabriventris* Nixon (Braconidae) and the idiobiont larval endoparasitoid *Chrysocharis vonones* (Walker) (Eulophidae). The biological parameters evaluated were survivorship, parasitism, and host-feeding, at 25 and 30°C. Differences between the species were observed at 25°C, but not at 30°C. At 25°C, the total parasitism for *P. scabriventris* (196.1 ± 17.7) and *C. vonones* (176.6 ± 7.24) was similar and with higher values compared to 30°C, 102.5 ± 8.81 and 89.1 ± 5 66 parasitized larvae, respectively. However, *C. vonones* showed a 3.97 lower survivorship as well as higher daily parasitism (1.4-fold) and host-feeding means (1.9-fold) than the braconid at 25°C. The results indicate a potential for both natural enemies to be used as biocontrol agents of *L. sativae*. The differences between species detected at 25°C suggest the best conditions for the application of each parasitoid.

Introduction

Leafminers of the genus *Liriomyza* are important pests of vegetables and ornamental plants. Three species of this genus, *Liriomyza trifolii* (Burgess), *Liriomyza sativae* Blanchard, and *Liriomyza huidobrensis* (Blanchard), cause crop damage worldwide because of their high degree of polyphagy and capacity to invade new areas (Murphy & Lasalle 1999).

The most common control method for *Liriomyza* spp. is the use of insecticides with different modes of action aiming the larval and adult stages (Hernández *et al* 2011, Devkota *et al* 2016). However, the selection for resistance to many insecticides in a large number of *Liriomyza* populations has been reported (Ferguson 2004, Wei *et al* 2015), as also the impact of these chemical products on the natural enemies of leafminers (Matsuda & Saito 2014, Guantai *et al* 2015). Thus, there is growing interest in applying agricultural practices that minimize such imbalances, and biological control has an important role (Liu *et al* 2009).

Comparing different feeding guilds, the leaf-mining insects show the most parasitoid communities associated (Connor and Taverner 1997). Only using *Liriomyza* genus as hosts, more than 150 parasitoids species were recorded (Liu *et al* 2010). Meanwhile, several predators preying on leafminers have been registered; however, they are not considered as important as parasitoids as biocontrol agents (Chow & Heinz 2004, van der Linden 2004). Most published data about parasitoids of leafminers were obtained in Europe, North America, and Southeast Asia (Murphy & Lasalle 1999, Liu *et al* 2009). Knowledge of natural enemies of *Liriomyza* spp. in the Neotropical region, mostly in South America, is limited. Among the few exceptions are from Argentina, Peru, and Colombia where Salvo and Valladares (1997, 1998), Cisnero & Mujica (1999) and Cure & Cantor (2003) carried out studies about leafminer parasitoid diversity and biology. In Brazil, research with parasitoids of *Liriomyza* spp. begun to increase, mostly because of economic damage caused by the pest in several crops including melon, tomato, onion, lettuce,

potato, and ornamental plants (Carvalho *et al* 2011, Costa-Lima *et al* 2014, Araujo *et al* 2015).

Many countries invaded by polyphagous leafminers, such as Japan, have chosen to import two species of parasitoids, *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga (Abe 2017). Both biocontrol agents have been widely marketed since the 1980s, achieving distribution in more than 20 countries, mostly in Europe and North America, for use in greenhouse crops (van Lenteren 2012). However, nowadays the environmental risks of introducing natural enemies are much discussed, as the direct and indirect effects over non-targets, dispersal of biocontrol agents to new areas, and changed relationship between biocontrol agent and native host (Simberloff 2012), therefore suggesting the necessity to explore biological agents that may be native to a particular area.

Considering that *L. sativae* was first described in South America by Blanchard in 1938, the most suitable procedure would be to improve knowledge of the potential natural enemies to be applied in biological control programs in the region, rather than to introduce new species. This is currently important in Brazil, where the number of biological control companies has increased (Parra 2014) and there is a growing interest in *Liriomyza* spp. parasitoids, particularly to melon crops (Costa-Lima TC, personal information).

In previous studies, we identified four species of hymenopteran parasitoids associated with *L. sativae* in melon and cowpea plants in the state of Rio Grande do Norte, Brazil (unpublished data). For the present study, we selected two of these parasitoid species with different effects on host development, a koinobiont and an idiobiont, that were abundant and could also be maintained in the laboratory. The first species is the braconid *Phaedrotoma scabriventris* Nixon, a koinobiont larval-pupal endoparasitoid; and the second is the eulophid *Chrysocharis vonones* (Walker), an idiobiont larval endoparasitoid.

The braconid, *P. scabriventris*, is an agromyzid parasitoid with natural occurrence only in South America, with records in Argentina (Achterberg and Salvo 1997), Brazil (Campos *et al* 1984; Costa-Lima *et al* 2014), Chile (Nixon 1955), and Peru (Cisnero and Mujica 1999). This species was introduced to Kenya in 2008, from Peru, for biological control of leafminer flies and recent studies were conducted about the biology of *P. scabriventris* over different leafminer species (Akutse *et al* 2014), the effect of endophytes over the parasitoid life-history (Chabi-Olaye *et al* 2013) and field parasitism in different agroecological regions (Foba *et al* 2015). The eulophid, *C. vonones*, has 21 agromyzid hosts registered from nine genera and has a Neotropical distribution, from Argentina to the USA (Noyes 2017). Comparing the biology of *P. scabriventris* and *C. vonones* in different temperatures, Costa-Lima *et al* (2014) showed that both species could develop from 15 to 32°C, but 35°C proved to be lethal. Also, the

eulophid had a juvenile development shorter than the braconid in most of the studied temperatures.

To select a *Liriomyza* biocontrol agent, besides the parasitism, the host-feeding evaluation is essential. This behavior is very common within leafminer parasitoids (Minkenberg 1989, Chien and Ku 2001) and can cause higher host death than parasitism (Allen & Charlton 1981, Tran *et al* 2007). The temperature influence over the parasitoid life span and reproduction is another important factor for the natural enemy evaluation (Klapwijk *et al* 2005). For the leafminer parasitoids *P. scabriventris* and *C. vonones*, all these parameters are still not known.

In the present study, we evaluated the potential of *P. scabriventris* and *C. vonones* as biocontrol agents of the leafminer *L. sativae*, through evaluations of their survivorship, parasitism, and host-feeding capacity. These parameters were observed in two thermal conditions, 25 and 30°C, which are the mean annual minimum and maximum temperatures in the melon crop producing area in northeastern Brazil. The results provided important information for future use of these natural enemies in field conditions.

Material and Methods

Initial population and rearing system of the parasitoids

The initial population of the leafminer *L. sativae* was obtained in the larval stage, in melon (*Cucumis melo* L.) leaves in the state of Rio Grande do Norte, Brazil. The parasitoid species *P. scabriventris* and *C. vonones* were collected in the same state, associated with *L. sativae* in melon and cowpea plants (*Vigna unguiculata* (L.) Walp.), respectively. Leaves with *L. sativae* larvae were collected and brought to the laboratory to obtain the adult parasitoids. All crops accessed had high insecticide pressure, with an average of two applications per week, directed mainly for leafminers and whiteflies.

Voucher specimens of *C. vonones* and *P. scabriventris* were deposited in the "Oscar Monte" Entomophagous Insect Collection, in the Instituto Biológico (Campinas, Brazil), and in the Vienna Museum of Natural History (Vienna, Austria), respectively. The voucher specimen of *L. sativae* was deposited at the Museum of Entomology and Acarology of ESALQ/USP (Piracicaba, Brazil).

Cowpea plants were used for the maintenance of the *L. sativae* rearing system, which served as the host for multiplying the parasitoids. Cowpea was chosen because it is also a natural host for *L. sativae* in Rio Grande do Norte (Costa-Lima *et al* 2009) and it is easier to grow than melon plants. The adult leafminer and parasitoid cages were kept in climatized rooms (25 ± 2°C, 50 ± 25% RH, and a 12 L:12 D photoperiod) and the plants with the insect immature stages

allocated in greenhouses. The *P. scabriventris* rearing followed procedures according to Costa-Lima *et al* (2017). For *C. vonones*, plants with mid- and large-size *L. sativae* larvae were offered for parasitism inside cages for 24 h. After this period, the plants were maintained in greenhouses until the parasitoid pupae formation. In this stage, the leaves were detached and transferred to plastic recipients with a fine-mesh cover. With adult emergence, the parasitoids were directed to rearing cages. The *L. sativae*, *P. scabriventris*, and *C. vonones* populations used in the experiments had approximately 55, 13, and 14 generations, respectively.

Survivorship, parasitism, and host-feeding capacity in two temperatures

Cowpea plants with two true leaves were exposed to *L. sativae* adults for 24 h. Pilot experiments were conducted to identify the larval instar of *L. sativae* and the parasitoid instar preference for parasitism. The first instar larvae occurred only until day 3 after eclosion, detected by measuring the growth ratio of the larva cephalopharyngeal skeletons, according to Petitt (1990). While the second instar could be found at days 3, 4, and 5, and the third instar at days 4, 5, and 6, after eclosion (25°C). Both parasitoid species had their best parasitism results in the last two instar days.

To evaluate life span, parasitism, and host-feeding capacity of *P. scabriventris* and *C. vonones* at two temperatures, a single experiment was conducted. Cowpea plants with two true leaves were exposed in cages with *L. sativae* adults for 24 h. Larvae with 4 to 5 days after larvae eclosion (second- and third-instar) were used in the study. Newly emerged female and male parasitoids were separated under a stereoscopic microscope. Pairs of each parasitoid species were placed in individual cages with *L. sativae* larvae in cowpea leaves. To reduce the influence of larval density, we only used leaves with widths from 7 to 10 cm, with 25 to 35 larvae. The cage was made of plastic, 12 cm in diameter in the lower part, with a fine mesh on the upper surface (9.5 cm in diameter). The base was composed of moistened florist's sponge in which the leaves containing the larvae were inserted. Pure honey was offered as the diet for the adults, on the sides of the cage. The cages were maintained in climate-controlled chambers at 25 and 30 ± 1°C, 70 ± 10% RH and a 12 L:12 D photoperiod. These two thermal conditions were chosen according to the annual mean temperatures in the region where the leafminers were collected (Mossoró, Brazil), which range from 25 to 30°C (Amorim *et al.* 1983).

Daily, the cowpea leaves with leafminers were changed, and the presence of any dead parasitoid was noted. If a male died or escaped before the female, the male was replaced by a male that emerged on the day of the evaluation. Only the males present from the beginning of the experiment were used for calculating the mean longevity. The evaluation continued until the last parasitoid died.

To evaluate the parasitism and host-feeding, the leaves with *L. sativae* larvae that were exposed to *P. scabriventris* were placed in individual Petri dishes (9 cm in diameter and 1.5 cm high) and kept in climate-controlled chambers at 25 ± 1°C, 70 ± 10% RH, and a 12 L:12 D photoperiod. After 2 days, the leaves in the Petri dishes were evaluated under a stereoscopic microscope (× 40 magnification) with transmitted light, to observe the presence of dead larvae that had succumbed to host-feeding. These larvae could be identified by having necrotic tissue on a portion of their body, the probable spot where the parasitoid ovipositor was introduced, not observed in larvae that died by natural mortality. The feeding behavior of both parasitoids studied was nonconcurrent, i.e., different larvae were used for oviposition and feeding (Costa-Lima, personal communication). Most larvae, parasitized or not, left the leaf for pupation and a minor part pupated inside the leafmine. We collected all pupae and maintained in the Petri dish with a fine-mesh cover until the emergence of the parasitoids and the flies. The *P. scabriventris* adults were killed in alcohol 70% and separated by sex. The unviable pupae were dissected to evaluate the presence of dead parasitoid pupae.

For *C. vonones*, the leaves with *L. sativae* larvae that were exposed to parasitism and host-feeding were maintained in similar cages with a moistened florist's sponge base, to maintain the turgidity of the leaves. These leaves were kept in climate-controlled chambers at 25 ± 1°C, 70 ± 10% RH and a 12 L:12 D photoperiod. The host-feeding evaluation was similar to that of *P. scabriventris*. Daily, we observed the presence of *C. vonones* larvae, by means of a stereoscopic microscope (× 40 magnification) with transmitted light. When pupae were present, the part of the leaf containing insect was cut out and isolated in test tubes 6.5 cm high and 1 cm diameter. The leaves were observed daily, to verify the *C. vonones* emergence. The parasitoids were killed in alcohol 70% and separated by sex.

Statistical Analyses

The experiments in this study used a completely randomized design, with each cage having a pair of the parasitoid, corresponding to one replicate. The experiment had 18 replicates for each parasitoid species in each temperature.

Statistical analyses were performed using the R statistical software (R Development Core Team 2017). The graphs were also built with the same software using the *ggplot2* package (Wickham 2009). The total parasitism and host-feeding data was registered as the absolute value obtained for each repetition. The daily mean parasitism and host-feeding data was obtained by the ratio between the total value from each behavior and the female

life span for the respective repetition. The parasitism and host-feeding total and daily mean data were analyzed by a quasi-Poisson generalized linear model (GLM) with a log link function. The data were compared using a factorial analysis with parasitoid species and temperature as sources of variation (2 by 2). Pairwise tests were performed at $p < 0.05$ (*multcomp* package). The sex ratio was analyzed by a binomial generalized linear model (GLM) with a logit link function, followed by chi-square test. The data also was compared using a factorial analysis with parasitoid species and temperature as sources of variation (2 by 2). The survivorship data had a factorial analysis with temperature, parasitoid species, and sex as sources of variation (3 by 3). To evaluate the influence of these parameters and the possible interactions, a Cox proportional-hazards model was designed with the *survival* package. Pairwise comparisons between group levels were done using the log-rank test, with the *pairwise_survdiff()* function of the *survminer* package ($p < 0.05$).

Results

According to the Cox proportional-hazards model analysis, the factors evaluated (sex, parasitoid species, and temperature) influenced the parasitoid survivorship and no interaction was observed among them ($p < 0.05$)(Table 1). The cox model compares the second group relative to the first, e.g., for sex, female was the latter group. The exponentiated coefficient [exp(β) – hazard ratios (HR)] gives the effect size of the covariate. Being female reduced the hazard, the death risk, by 0.53, or 47%. The higher HR occurred for parasitoid species, considering *C. vonones* the second group, i.e., with 3.97 higher death risk than *P. scabriventris*. For the two studied temperatures, the parasitoids' chance of dying at 30°C was 1.41 times higher than at 25°C (Table 1).

The sex ratio was not influenced by species ($X^2 = 1.96$; *d.f.* = 3; $p = 0.161$), temperature ($X^2 = 0.0001$; *d.f.* = 3; $p = 0.991$) and by the interaction between these two levels ($X^2 = 0.09$; *d.f.* = 3; $p = 0.757$). For the braconid, it ranged from 0.52 ± 0.09 and 0.50 ± 0.10, and for the eulophid, from 0.40 ± 0.11 to 0.42 ± 0.09, at 25 and 30°C, respectively.

No difference in survivorship between sex was observed for *P. scabriventris* at 25°C ($p = 0.419$) and 30°C ($p = 0.419$). For *C. vonones*, the females had a higher survivorship than the males at 25°C ($p < 0.001$) and no difference was observed at 30°C ($p = 0.148$). The temperature increase from 25 to 30°C reduced the survivorship of males and females for *P. scabriventris* (male: $p < 0.001$; female: $p < 0.001$) and *C. vonones* (male: $p < 0.05$; female: $p < 0.001$) (Fig 1).

Females of *P. scabriventris* and *C. vonones* initiated the parasitism of *L. sativae* larvae from the first day, at both 25 and 30°C. The peak of parasitized larvae at 25°C occurred on day 6

Table 1 Results of Cox proportional hazard analysis in relation to parasitoid species, temperature, and sex.

Fixed effect	β	SE	exp(β)	z	p
Sex	– 0.63	0.19	0.53	– 3.32	< 0.001
Species	1.38	0.25	3.97	5.54	< 0.001
Temperature	0.34	0.04	1.41	7.68	< 0.001

Estimated regression coefficients (β), standard errors (SE), and hazard ratios exp(β) of covariates that have a significant effect ($p < 0.05$).

(8.9 ± 1.22) for the braconid and on day 8 (19.1 ± 3.47) for the eulophid. At 30°C, the parasitism peak for *P. scabriventris* and *C. vonones* was reached on day 4 (10.4 ± 1.11) and day 8 (12.7 ± 1.65), respectively. At 25°C, *C. vonones* reached 50% of the total larvae parasitized between days 6 and 7; and *P. scabriventris* reached this level between days 14 and 15. At 30°C, this mark was reached by the eulophid between days 5 and 6, and the braconid by days 8 and 9 (Fig 2).

The daily mean parasitism (parasitized larvae per day) was influenced by temperature ($F_{(1,60)} = 8.21$; $p = 0.004$), species ($F_{(1,59)} = 17.20$; $p < 0.001$) and by the interaction between these two factors ($F_{(1,58)} = 16.37$; $p < 0.001$). The *C. vonones* daily mean parasitism at 25°C (7.6 ± 0.26 larvae/day) was higher than for *P. scabriventris* (5.4 ± 0.51 larvae/day) ($p < 0.001$). However, at 30°C, no difference was observed between the species ($p = 0.411$). The braconid had a higher daily mean parasitism at 30°C ($p = 0.016$) and the eulophid at 25°C ($p = 0.024$).

The total mean number of larvae parasitized was influenced by temperature ($F_{(1,60)} = 99.96$; $p < 0.001$), but not by parasitoid species ($F_{(1,59)} = 3.11$; $p = 0.083$) or by the interaction between these two levels ($F_{(1,59)} = 0.06$; $p = 0.799$). The braconid *P. scabriventris* showed a mean of 196.1 ± 17.70 total parasitized larvae at 25°C, higher than that observed at 30°C, 102.5 ± 8.81 ($p < 0.001$). The eulophid *C. vonones* behaved similarly to the braconid, with a higher number of parasitized larvae at 25°C (176.6 ± 7.24) compared to 30°C (89.1 ± 5.66) ($p < 0.001$) (Fig 3).

The *P. scabriventris* females showed an increased survivorship compared to *C. vonones* at 25°C. Thus, even with a similar total parasitism in the two species, the daily mean parasitism of the eulophid at 25°C was 29% higher than the braconid. This difference is evident in the daily parasitism mean along the life span of each species, with higher peaks in a shorter time for *C. vonones* (Fig 1). At 30°C, no difference was observed in survivorship, resulting in a similar daily mean parasitism between the species.

Host-feeding was observed since the first day for both species of parasitoids in both thermal conditions. At 25 and 30°C, the host-feeding larvae peak occurred on day 4 (7.2 ± 1.11) and day 1 (4.4 ± 0.77) for *C. vonones*, and on day 6 (3.3 ± 0.48) and day 9 (4.4 ± 0.88) for *P. scabriventris*, respectively (Fig 2).
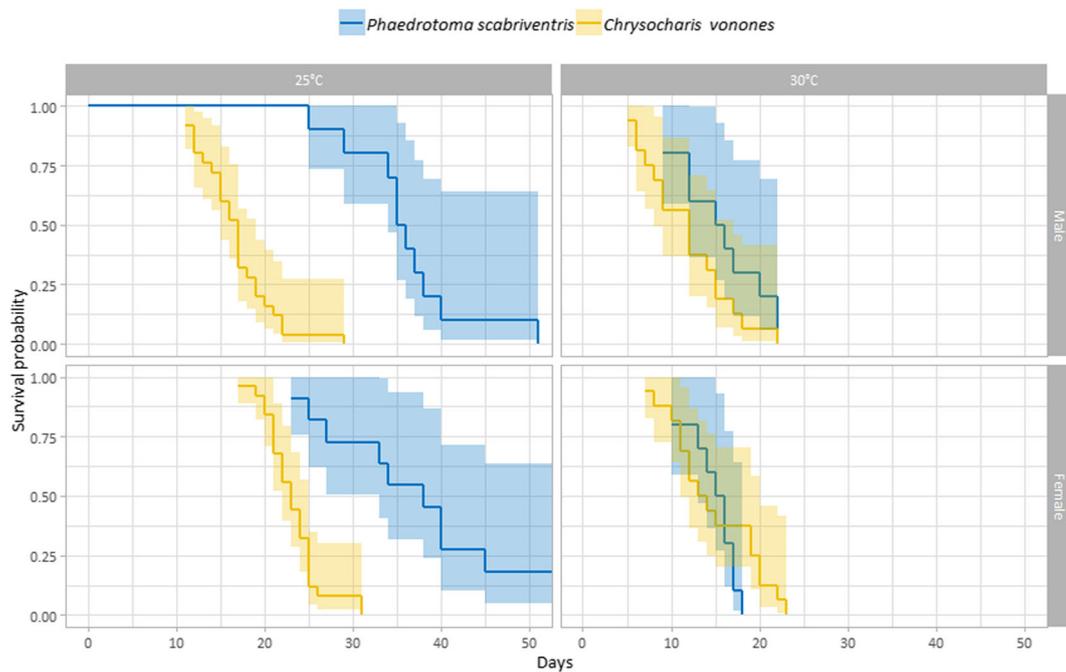
Fig 1 Survival curves of males and females of *Phaedrotoma scabriventris* and *Chrysocharis vonones* in two temperatures (70 ± 20% RH and 12 L:12 D photoperiod) (log-rank test, *p* < 0.05). The shaded area represents the confidence interval.

The host-feeding daily mean (larvae fed per day) was also affected by temperature ($F_{(1,60)}$ = 21.15; *p* < 0.001), species ($F_{(1,59)}$ = 26.36; *p* < 0.001) and by the interaction between these two factors ($F_{(1,60)}$ = 30.41; *p* < 0.001). The *C. vonones* daily mean host-feeding was higher than that of the braconid at 25°C (*p* < 0.001), and at 30°C, no difference was observed between the species (*p* = 0.900). With a rise in the temperature, the *C. vonones* daily host-feeding decreased (*p* < 0.001), and the inverse occurred for *P. scabriventris* (*p* = 0.049).

The total host-feeding mean was influenced by temperature ($F_{(1,59)}$ = 105.25; *p* < 0.001), species ($F_{(1,58)}$ = 4.12 ; *p* = 0.041), and the interaction between these two factors ($F_{(1,58)}$ = 4.90; *p* = 0.030). The *P. scabriventris* total host-
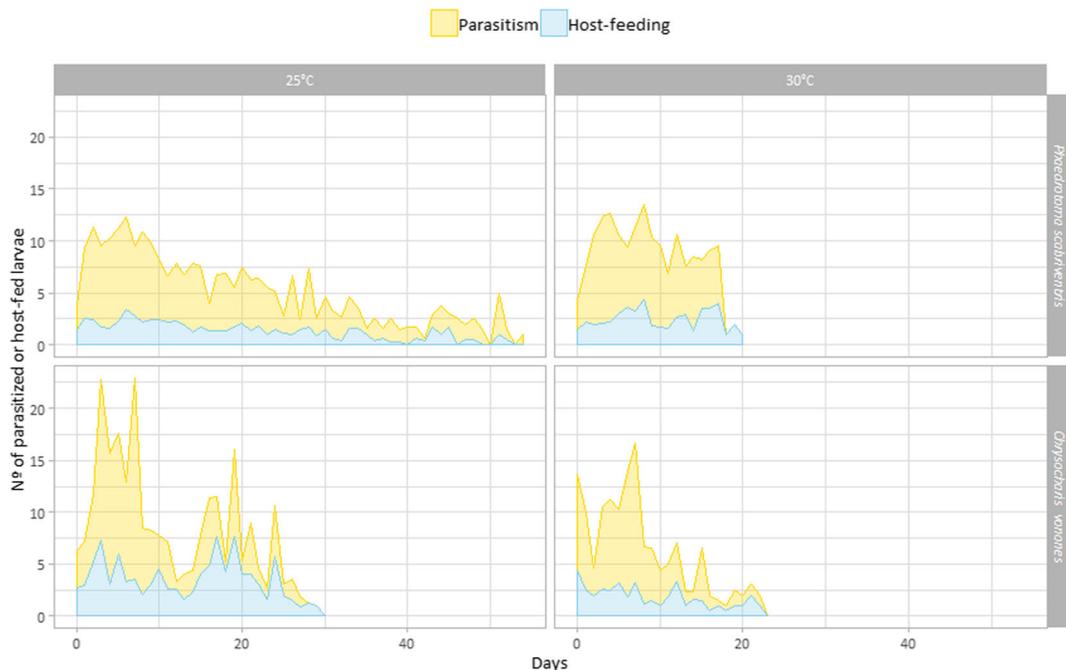

Fig 2 Parasitism and host-feeding rhythm of *Phaedrotoma scabriventris* and *Chrysocharis vonones* on *Liriomyza sativae* in cowpea plants at 25 and 30°C (70 ± 20% RH and 12 L:12 D photoperiod).
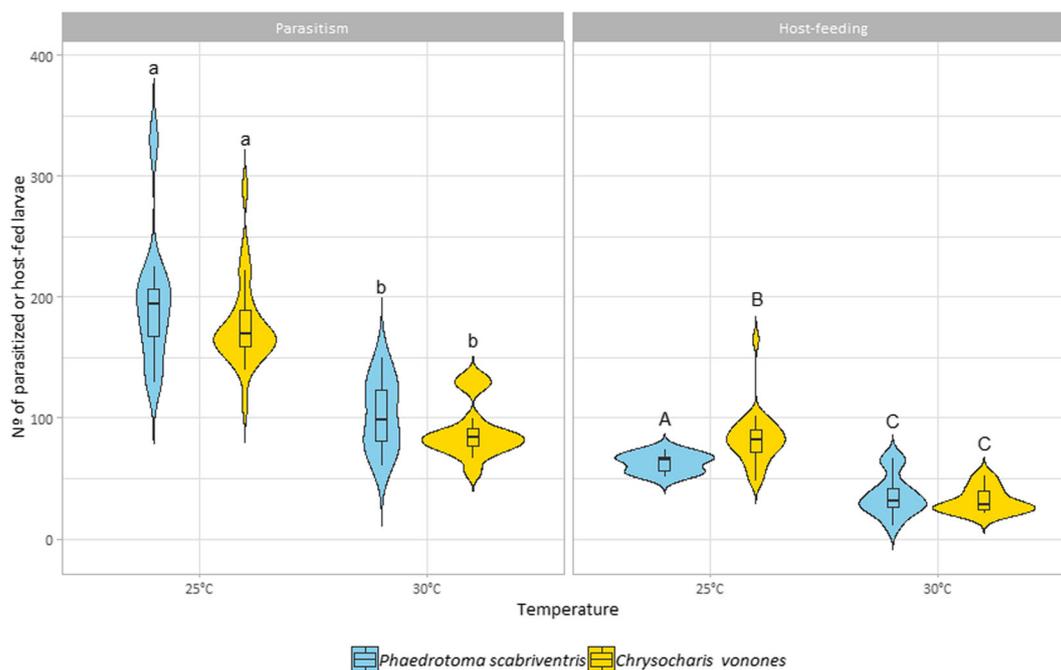
Fig 3 Parasitism and total host-feeding of *Liriomyza sativae* larvae by *Phaedrotoma scabriventris* and *Chrysocharis vonones* at 25 and 30°C. Means followed by the same letter and size did not differ at *p* < 0.05 (logistic regression). The shaded area outside the boxplot shows the probability density of the data at different values.

feeding mean was higher at 25°C (62.9 ± 2.52 larvae) compared to 30°C (35.9 ± 5.11 larvae) (*p* < 0.001). The same was observed for the eulophid host-feeding behavior, which decreased from 82.5 ± 4.50 (25°C) to 31.6 ± 2.55 (30°C) (*p* < 0.001). The host-feeding of *L. sativae* larvae was more pronounced at 25°C for *C. vonones* (*p* = 0.033), while it did not differ between the species at 30°C (*p* = 0.826) (Fig 3).

When combining the mean values of total parasitism and host-feeding, *P. scabriventris* and *C. vonones* were capable of killing a similar quantity of leafminer larvae per female (~ 259) at 25°C. While at 30°C, the total killed *L. sativae* larvae by the braconid was 138 and 120 for the eulophid.

Discussion

The total parasitism mean observed for *P. scabriventris* and *C. vonones* are close to what was registered for *D. isaea* and *Neochrysocharis formosa* (Westwood), in an experiment with similar methods (Hondo *et al* 2006). Aiming to study the potential as biocontrol agents of these two Neotropical parasitoids, achieving similar results to natural enemies that are already commercialized (van Lenteren 2012), is an initial positive finding.

The parasitoids caused larval mortality of the leafminers both by parasitism and by feeding on them. This latter behavior was highly important in both studied temperatures. At 25°C, host-feeding was responsible for 24% and 32% of the total mortality of *L. sativae* larvae by *P. scabriventris* and *C. vonones*,

respectively. At 30°C, 26% of the total larval mortality was due to host-feeding in both species. Differently than observed for *P. scabriventris*, the *Liriomyza* braconid parasitoids, *D. sibirica* and *Opius pallipes* (Wesmael), did not host-feed (Minkenberg and van Lenteren 1986). However, this behavior is common for many eulophid parasitoids of *Liriomyza*, including members of the genera *Diglyphus*, *Chrysocharis*, *Neochrysocharis*, *Hemiptarsenus*, and *Chrysonotomyia* (Minkenberg 1989, Chien & Ku 2001). From the practical point of view, this behavior is a positive aspect for a biocontrol agent in the field, where the parasitoid will be able to kill the host by two different ways (Jervis *et al* 1996). However, it becomes a problem for large-scale rearing of the natural enemy (Waage *et al* 1985), where high proportions of host-feeding will lead to elevated costs for multiplying the biocontrol agent. Based on our results and the previously cited studies, the host-feeding is certainly an important biological trait to consider when choosing a *Liriomyza* parasitoid species for a biological control program.

The host-feeding observed in *P. scabriventris* and *C. vonones* is a common behavior in female parasitoids. Kidd & Jervis (1989) estimated that one third of parasitoids host-feed. Some studies indicate that host-feeding by the parasitoids provide nutrients for egg development (Jervis *et al* 2008). In the present study, the adults were offered only honey, which is composed mainly of sugars, water, and small amounts of proteins and other substances (Da Silva *et al* 2016). Probably, the parasitoids host-fed to obtain the protein necessary for their egg maturation and to allow a longer life span.

In a modeling study considering the augmentative biological control improvement, Plouvier & Wajnberg (2018) concludes that fecundity, longevity, host handling time, and dispersal capacity are the most important life-history traits to focus on. Our work brings important results about two of these biological traits; the total parasitism is the insect fecundity and the survivorship is related to the longevity. At 25°C, *P. scabriventris* showed a higher survivorship; however, *C. vonones* achieved higher parasitism. When combining these two parameters, *C. vonones* shows a more concentrated parasitism rhythm in relation to time at 25°C compared to *P. scabriventris* (Fig 1). The latter species achieved 50% of the parasitism capacity in 2 weeks, while *C. vonones* in only 1. The two different parasitism patterns along life span aid information on how to use these parasitoids in the field. For example, the melon in northeastern Brazil has a very short cycle (~ 60 days), and in nearly 50% of these periods, the crop is covered with a physical barrier, which is removed for polinization (Guimarães *et al* 2008). Thus, the plants are exposed to leafminers in the last 30 days remaining, a short period to control the pest. Therefore, a biocontrol agent with a more concentrated parasitism rhythm, as *C. vonones*, could give a faster control response. Besides that, the eulophid shows the advantage that is capable of stopping the leafminer development at the moment it parasitizes the larvae, different from *P. scabriventris* that is a koinobion larval-pupal parasitoid (Costa-Lima *et al* 2014), which the leafminer control would depend on the capability to increment its population in the field.

Based on the present study, we can state that both natural enemies have potential as biocontrol agents of *L. sativae*. The biological results obtained at 25°C indicated that *C. vonones* showed better daily mean parasitism performance. However, this species also showed a higher daily mean host-feeding and lower survivorship than *P. scabriventris* at the same temperature, which would result in higher costs for the rearing process. For this reason, attention must be directed to the production cost-benefit ratio and performance of the parasitoid, in order to select the best biocontrol agent. The performance of *P. scabriventris* and *C. vonones,* in the thermal conditions studied, added to the biology of these species (Costa-Lima *et al* 2014) and their host (Costa-Lima *et al* 2009, 2010), are also important information for rearing procedures. The next step is to conduct field experiments with both parasitoid species and evaluate whether the results will corroborate with the findings in the present study.

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