UNIVERSIDAD NACIONAL DE COLOMBIA

Eficiencia de tres especies del género *Trichogramma* (Hymenoptera: Trichogrammatidae) para el control de *Spodoptera frugiperda* Smith y *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) en el cultivo de uchuva.

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A mi padre, mi madre, mi hermana y mi abuela (Q.E.P.D) por su amor y su apoyo incondicional.

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Eficiencia de tres especies del género *Trichogramma* (Hymenoptera: Trichogrammatidae) para el control de *Spodoptera frugiperda* Smith y *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) en el cultivo de uchuva

María Fernanda Díaz Niño[1], Augusto Ramírez Godoy[1], Katja Poveda Morciniec[1,2]

RESUMEN:

El control biológico aumentativo de insectos es una herramienta de los programas de Manejo Integrado de Plagas. En los agroecosistemas, el control biológico es ejercido ampliamente por especies parasitoides, por lo cual es posible predecir que la presencia de recursos alimenticios, como los aportados por las plantas con flores, pueden tener un efecto positivo en la supervivencia, capacidad de búsqueda y tasa de parasitismo de estas especies. En Colombia el cultivo de la uchuva, recientemente establecido para exportación, es continuamente atacado por un complejo de especies de lepidópteros de la familia Noctuidae. El objetivo de la presente investigación fue evaluar la eficiencia de tres especies de *Trichogramma* (*T. atopovirilia* Oatman & Platner*, T. exiguum* Pinto & Platner, y *T. pretiosum* Riley) para el control de *Spodoptera frugiperda* Smith y *Copitarsia decolora* Guenée. Se evaluó la longevidad y capacidad de parasitismo de las avispas sobre las dos especies plaga hospederas. Los resultados sugieren que *T. atopovirilia* y *T. pretiosum* son especies parasitoides promisorias para el control de *S. frugiperda* y *C. decolora* respectivamente. A partir de esta conclusión se seleccionó a *T. atopovirilia* como modelo para evaluar el efecto de la presencia de plantas con flores en experimentos de "no selección" y "selección múltiple" bajo condiciones de laboratorio y campo. Los resultados, sugieren que la presencia de trébol rojo *(Trifolium pratense* L) puede incrementar la longevidad y la tasa de parasitismo de *T. atopovirilia,* lo que indica que el suministro de recursos alimenticios florales para parasitoides, en el cultivo de la uchuva, debe ser tenido en cuenta como parte de una estrategia de diversificación de hábitat para el control de noctuidos plaga.

Palabras claves: Parasitoides, Noctuidos plaga*,* plantas con flores, trébol rojo, *Trichogramma atopovirilia,* uchuva

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Efficiency of three species of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) for the control of *Spodoptera frugiperda* Smith and *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) in *Physalis peruviana* crops.

ABSTRACT:

Augmentative biological control of insects is a tool of Integrated Pest Management programs. In agroecosystems, biological control is exercised largely by parasitoids, and it is found that the presence of food resources, as provided by flowering plants, can have a positive effect on survival, search ability and parasitism rate of parasitoid species. In Colombia, a recently established export crop, the cape gooseberry, is under continuous attack by a lepidopteran species complex in the family Noctuidae. The objective of the present study was to test the efficiency of three species of *Trichogramma* (*T. atopovirilia* Oatman & Platner*, T. exiguum* Pinto & Platner, and *T. pretiosum* Riley) to control *Spodoptera frugiperda* Smith and *Copitarsia decolora* Gueené. The longevity and the parasitism rate of these wasps on the two pest hosts were tested. The results suggest that *T. atopovirilia* and *T. pretiosum* could be promising parasitoids for the control of *S. frugiperda* and *C. decolora* respectively. *T. atopovirilia* was selected as model species to evaluate the effect of the presence of flowering plants on these variables in both no choice and multiple choice experiments under laboratory and field conditions. The results showed that the presence of red clover (*Trifolium pratense* L) can increase the longevity and parasitism rate of *T. atopovirilia*, suggesting that providing food resources to parasitoids in cape gooseberry fields should be part of a habitat diversification strategy to control noctuid pests.

Key words: Cape gooseberry, flowering plants, noctuids pests, parasitoid wasp, red clover, *Trichogramma atopovirilia.*

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1. INTRODUCTION

Current agricultural practices such as those that include the chemical control of weedy competitors of crops and high application of fertilizer and pesticides can increase toxin levels in ground waters and surface waters, contributing to the degradation of natural habitats (Tilman et al., 2002). Deterioration of natural habitats has reduced the ability of ecosystems to provide goods and services (Gurr et al., 2003; Landis et al., 2000). For example in annual cropping systems, with their inherent ecological imbalance, the regulation of herbivorous pests by natural enemies is poor, often leading to pest outbreaks (Landis et al., 2000). This disruption of natural functions, such as natural pest control, generates the need for alternative management strategies. These alternative strategies would also help to reduce the serious effects that chemical pesticides have on the environment and on human health (Gurr et al., 2003). Pest control can be improved by manipulating the agricultural plant community, mainly with the introduction of flowering plants that may favour natural enemies through the provision of suitable microclimate, alternative prey, and floral resources for adult natural enemies (Ellis et al., 2005; Fadamiro and Chen, 2005; Kennedy and Storer, 2003; Landis et al., 2000; Smith, 1996).

The underlying motivation for the addition of floral resources within the agroecosystem was developed by Root (1973), who proposed the “enemies hypothesis”. He argued that predators and parasitoids would control herbivores more effectively in diverse rather than simple habitats because they would have greater access to resources like pollen and nectar (Root, 1973). Most adult hymenopteran parasitoids benefit from carbohydrate food resources and it has been reported that nectar or other sugar sources satisfy their energetic needs (Bianchi and Wackers, 2008; Virk and Brar, 2004) and increase both longevity and fecundity (Witting-Bissinger et al., 2008). Usually parasitoids emerge with a limited supply of energy and without access to food and they may die within a few days. Sugar feeding can increase parasitoid longevity up to 20-fold (Wackers, 2001), and nectar

feeding can also enhance parasitoid fecundity, increasing the reproductive lifespan and/ or accelerating the rate of egg maturation (Winkler et al., 2006).

Studies in agricultural fields have shown that increasing flowering plants diversity can decrease pest problems (Bianchi and Wackers, 2008; Ellis et al., 2005). Begum, et al (2006) identified the importance of plant species such as *Lobularia maritima* and *Coriandrum sativum* to provide food resources to the parasitoid species *Trichogramma carverae.* In treatments with flowering plants they observed a 6.5-fold increase in the parasitism rate of the pest *Epiphyas postvittana* (Lepidoptera: Tortricidae) in commercial vineyards in Australia. Tylianakis, et al(2004) reported that flowering plant patches significantly increases the rates of parasitism by the species *Aphidius rhopalosiphi* and *Diaeretiella rapae* (Hymenoptera: Aphidiidae) in wheat fields. However, parasitism rates declined exponentially with increasing distance from floral patches, which suggests that the proximity of resource patches is important in the ecological enhancement of biological control agents (Tylianakis et al., 2004).

The cape gooseberry (*Physalis peruviana* L) is a native plant of tropical America, principally of Colombia, Peru and Ecuador (Florez and Fisher, 2000). Due to its tasty fruits, and rich content Vitamin C it has recently started to be planted for national consumption and for exportation. In Colombia this fruit is economically important. It ranks third in importance for fresh fruit export after banana and plantain, and renders revenues of US $17.000.000/year (Zapata et al., 2002). A major problem for its cultivation is that it is under continuous attack by a Lepidoptera species complex in the family Noctuidae. The larvae of *Spodoptera frugiperda* are cutworms that chew off the seedlings slightly above the soil line, generating substantial economic losses, while a second species, *Copitarsia decolora,* feeds on fruits and leaves of older plants (Jerez and Ramírez, 2006; Rozo and Godoy, 2006; Zapata et al., 2002). Pest management practices in *Physalis* fields are mainly based on the use of chemical control. These practices not only affect the population of pest arthropods, but can also drastically distort natural pest control (Crowder et al., 2010) and have harmful implications for ecosystems and human health (Pingali and Roger, 1995; van der Werf, 1996). Alternative control practices rely on the use of biological control agents. In Colombia natural enemies of noctuid pest such as *Apanteles* sp (Hymenoptera: Braconidae) and *Telenomus* sp (Hymenoptera: Scelionidae) (Ochoa et al., 2003) have been used for pest management in crops such as maize and sorghum among others. However, those crops are grown mainly in lowlands. For *P. peruviana,* a

highland crop, the use of biological controllers of noctuids pests has not yet been studied and effective natural enemies that can adapt to the crop conditions have to be identified. Promising control agents are egg parasitoids of the genus Trichogramma (Hymenoptera: Trichogrammatidae). *Trichogramma* wasps have been widely used for biological control of Lepidopteran pests (Bueno et al., 2010; Smith, 1996). In Colombia there are three species of *Trichogramma* commercially available *(Trichogramma atopovirilia, Trichogramma exiguum* and *Trichogramma pretiosum)* that could adapt to the climatic growing conditions of *Physalis* crops. Inundative release to augment natural populations of biological control agents in the field is expensive (Mills et al., 2000) so it is important to know which species of flowering plants may promote the establishment of natural enemies in crops. The aim of the study was to identify an egg parasitoid that could be used for inundative release in *P. Peruviana* fields and to determine if flowering plants would increase the longevity and parasitism rate of the suitable parasitoid. To accomplish this the goals of this study were to (1) evaluate the effectiveness of the different commercially available egg parasitoids of the genus *Trichogramma* on two different Lepidopteran pests of *P. peruviana*, (2) test how different flowering species affect lifespan and fecundity of *Trichogramma* suitable to control noctuids in *Physalis* fields, and (3) determine if the presence of flowering plants in the field can increase egg parasitism rates.

2. MATERIALS AND METHODS

2.1 Study system:

2.1.1 Pest species:

Spodoptera frugiperda and *Copitarsia decolora* (Lepidoptera: Noctuidae) are two prominent insect pests in *Physalis* fields. *S. frugiperda* larvae, also known as cutworms, consume the newly emerged seedlings, while the larvae of *C. decolora* consume buds and fruits (Florez and Fisher, 2000; Jerez and Ramírez, 2006). Eggs of *S. frugiperda* and *C. decolora* were obtained from colonies established in the pest management laboratory of the Agronomy department at the Universidad Nacional de Colombia. The two host species were reared on an artificial diet based on corn cob manufactured and distributed by Bio-Serv™ (references product #F0635). The colonies were maintained in the lab at a temperature of 18-20°, relative humidity between 60-70% and photoperiod of 12 hours.

Adult moths oviposited on strips of towel paper, which were cut in pieces bearing the number of eggs needed for experiments. Eggs of *Sitotroga cerealella* (Lepidoptera: Gelechiidae), that were used in green house and field experiments as hosts species of parasitoids, were purchased at Perkins, Ltda., a Colombian laboratory specialized in biological control agents.

2.1.2 The parasitoid species: *Trichogramma* spp:

The species of the genus *Trichogramma* have several characteristics that make them a good egg parasitoids for biological control programs. Among the principal characteristics is their long adult longevity in the field and their ability to parasitizing eggs of multiple Lepidopteran pest species, which makes them ideal for inundative control of lepidopteran crop pests (Bueno et al., 2010; Kalyebi et al., 2005; Ma and Chen, 2006). In addition they

are easily reared on alternative hosts (Milonas et al., 2009). Studies of *Trichogramma* have been carried out in more than 50 countries and commercial releases are successfully performed in approximately 32 million hectares of agricultural fields every year (Pizzol et al., 2010; Smith, 1996).

The species of *Trichogramma* evaluated were *T. atopovirilia, T. exiguum*, and *T. pretiosum* which are commercially available in Colombia. *T. atopovirilia* is distributed through Northeast Mexico, Southern Salvador, Venezuela, Colombia and Brazil. *T. exiguum* is present in the Neotropical region from Western North America, south of Québec to Florida, Mexico, Central America and South America (Chile, Uruguay, Colombia and Peru). *T*. pretiosum is the most widely distributed and is present from southern Canada to Argentina (Morales et al., 2007; Ríos and Terán, 2003). These parasitoid species are used commercially in Colombia to control pests such as *Alabama argillacea*, *Heliothis virescens*, *Diatraea* spp, *Pseudoplusia includens*, *Agrotis* spp, *Spodoptera frugiperda* and *Copitarsia spp* in crops such as cotton, corn, sugar cane, potatoes, and asparagus (Amaya, 1998)

Cardboard with *Sitotroga cerealella* parasitized eggs were purchased at the Perkins, Ltda. Laboratory, each time that they were needed for the experiments. For each experiment the cardboard was cut in small pieces that just contained the number of eggs visibly parasitized necessary for the experiments.

2.2 Flowering plants:

In order to test which flowering plants would increase the parasitoid efficiency, plants which had been reported in the literature to provide food for *Trichogramma* wasps were selected. Sweet alyssum (*Lobularia maritima* (L) Desv.*,* Cruciferae), borage (*Borago officinalis* L Boraginaceae), moricandia (*Moricandia* sp, Brassicaceae), coriander (*Coriandrum sativum* L, Apiaceae) red clover (*Trifolium pratense* L, Fabaceae*)* and white clover (*Trifolium repens* L, Fabaceae) (Begum et al., 2006; Bell et al., 2006; Berndt and Wratten, 2005; Lavandero I et al., 2006; Rebek et al., 2006; Vattala et al., 2006; Wäckers, 2004; Wade and Wratten, 2007; Witting-Bissinger et al., 2008) were sown in plastic bags of 1. 5 Kg capacity, in natural soil with sand and organic matter (2:1:1 in proportion). Plants were fertilized with N:P:K 10-20-20 (Abocol®) during sowing (between March, 2009 and

December, 2010) and 30 days after sowing with Tottal®, a foliar fertilizer with macro and micronutrients imported by Stoller de Colombia S.A. The plants were kept under greenhouse conditions until used for experiments.

2.3 Adult emergence and longevity of three *Trichogramma* wasps under different conditions:

To compare the fraction of adult emergence and longevity of the different species of *Trichogramma* under different climatic conditions, two factorial experiment was set up. One factor being the *Trichogramma* species (*T. atopovirilia, T. exiguum* and *T. pretiosum*) and the other factor the following three climatic conditions: field conditions (with maximum temperature of 30^0C and minimum of 10^0C; relative humidity of ± 70%), greenhouse conditions (with maximum temperature of 32^0C and minimum of $9{,}2^0$C and relative humidity of ± 80%) and laboratory conditions (with maximum temperature of 22^0C and minimum of 19^0C, and relative humidity of ± 60%).

A cardboard with *S. cerealella* parasitized eggs was cut into small segments with approximately 33 ± 10.57 (mean± SE; max= 56; min= 16; n= 90) parasitized eggs for each of the *Trichogramma* species. For each segment the exact number of eggs was counted and then placed it into separate glass test tubes until adult wasps emerged. Adult wasps were fed with a honey – water solution impregnated in a cotton ball placed at the opening of the test tube. Cotton balls were changed daily. Each test tube was taken as the experimental unit and 10 replicates were established for each of the nine treatment combinations. At intervals of 24 (+/- 1) hrs test tubes were tapped gently to encourage insect activity and to count the number of living adult wasps. Percentage of adult emergence from the eggs as well as adult longevity could be recorded this way. Daily observations of each test tube continued until no living wasps were recorded. Data on the mean number of days survived and % emergence were analyzed by two-way ANOVA. To determine differences in sex ratio between species at the end of the experiment all the individuals in the tubes were sexed and the sex ratio of each species, expressed as the % of females [(no. of females/(no. of females + no. of males) x 100], was calculated (Maceda et al., 2003); the data were analyzed with one way ANOVA.

2.4 Effectiveness of different *Trichogramma* species for the control of *S. frugiperda* and *C. decolora:*

The parasitism rate of *T. atopovirilia, T. exiguum* and *T. pretiosum* on eggs of the noctuids *S. frugiperda* and *C. decolora* was evaluated in the laboratory at 21 +/- 1°C in petri dishes (10 cm in diameter). Additionally, the parasitism rate of the three species on eggs of *S. frugiperda* was tested in microcosms on *Physalis peruviana* plants.

To evaluate the parasitism rate, of the three parasitoids species, a piece of paper towel with 25 eggs of *S. frugiperda* was placed in a petri dish and released two females and one male wasp from each species. This set-up was repeated 7 times for each parasitoid species.

At intervals of 48 hours, the paper towel with the host eggs was replaced by a new one and taken to an incubator (25°C) to accelerate development. After 2 days in the incubator the number of parasitized eggs (which turned black) was counted. New paper towel pieces with eggs were placed every second day until all adult wasps died. Data were analyzed with a repeated measure ANOVA.

The effectiveness of *T. atopovirilia, T. exiguum,* and *T. pretiosum* using *S. frugiperda* eggs on *Physalis* plants in microcosms were also evaluated. Each microcosm consisted of one *Physalis* seedling with 6 to 8 true leaves planted in natural soil in 12 oz plastic cups and covered by another 12 oz plastic cup with a veil bottom, which prevented water condensation in the microcosm. On each plant, 100 *S. frugiperda* eggs were placed on the upper leaves with a brush. 10 adult wasps were liberated inside each microcosm and 10 microcosms were used as replicates for each *Trichogramma* species. A control treatment was established using the same setup but without the release of any parasitoid species. The parasitized eggs of *S. frugiperda* eggs were counted 7 days after liberating the parasitoids. In addition and in order to corroborate the effect of the parasitoids the number of live larvae of *S. frugiperda* inside each microcosm as well as the percentage damage caused by hatched *S. frugiperda* larvae on the plant were estimated. Data were analyzed with a one way ANOVA.

Since *C. decolora* larvae normally feed on reproductive structures of *Physalis* plants, the experimental set up to test the parasitism effectiveness of the *Trichogramma* species on *C. decolora* eggs had to be changed by using a reproductive plant structure (flower buds or fruits) and leaves which were placed in petri dishes. Inside the experimental unit 10 eggs of *C. decolora* were placed with a brush on the leaves subtending a reproductive structure, as have been observed in the field. 10 adult wasps were liberated inside each experimental unit and 10 experimental units were used as replicates for each *Trichogramma* species. The parasitism rate of the three species was evaluated 7 days after the wasps were liberated, as described above for *S. frugiperda.* Data of parasitism rates (%) were analyzed by one way ANOVA.

2.5 Effect of food source on survival and parasitism rate of *T. atopovirilia:*

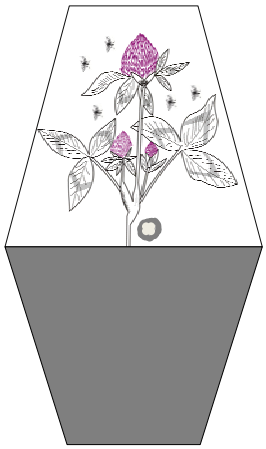
Based on our results (see below) *T. atopovirilia* was chosen as a promising species to control *S. frugiperda* and *C. decolora*. Although it is well known that food resources are important in the development of different parasitoids species (Sood and Pajni, 2006; Steppuhn and Wäckers, 2004; Vattala et al., 2006; Wackers, 2001; Wanner et al., 2006) there was no information regarding the importance of food resource on *T. atopovirilia* development or parasitism rate. To evaluate how sugar containing food source affect the biological control potential of *T. atopovirilia* a honey-based diet was used; 150 *S. cerealella* eggs were placed to cardboard in a petri dish and then released 20 adult *T. atopovirilia* wasps into it. Three treatments were established: honey based diet (2.5% agar, 77.5% water 20% honey), water diet (2.5% agar 97.5% water) and control (without any food source) (García and Piqueras, 1985). There was decided to work with solid diet given the small size of the wasps, which easily drown in liquid diets (García and Piqueras, 1985).0.125 cm^3 of diet were placed in each petri dish. Every 2 days the numbers of live insects were counted and the cardboard with the host eggs was replaced. The eggs that were removed were incubated at 25^0C and the number of parasitized eggs was counted. The proportion of parasitized eggs per each cardboard (= total parasitized eggs/ total available eggs) was arcsin square root transformed to increase homogeneity of variance and normality of residuals before performing a repeated measure ANOVA.

2.6 Effect of different flowering plants on the longevity and parasitism rate of *T. atopovirilia:*

2.6.1 No-choice experiment:

Flowers and inflorescences of *Physalis*, sweet alyssum, borage, moricandia, coriander, red clover and white clover were collected and sealed in inverted 12 oz. plastic cups with a veil bottom. Each flower / inflorescence was placed in a cup and their peduncle was inserted through the central hole of the lid that closed the cup. The cups were closed tightly and inverted (with the peduncles pointing out and down) and placed on top of another cup filled with water to guarantee water supply to the flowers/inflorescences (Fig 2-1). A control treatment was established using an empty setup. Excised flowers were used to make their manipulation easier during the experiment. Wade and Wratten (2007) showed that the use of excised or intact inflorescences does not affect parasitoid longevity.

Figure 2-1: Experimental set up to test the influence of flowering plants on the parasitism rate of *T. atopovirilia* (Experiment 2.6.1). Flowering plants were collected and placed in 12 oz. plastic cups. The grey circle with white points at the bottom of the plant represents the 50 eggs of *S. frugiperda* or *S. cerealella* deposited on the plants and the wasps in the cup represent the 10 adults wasps released in the cup.



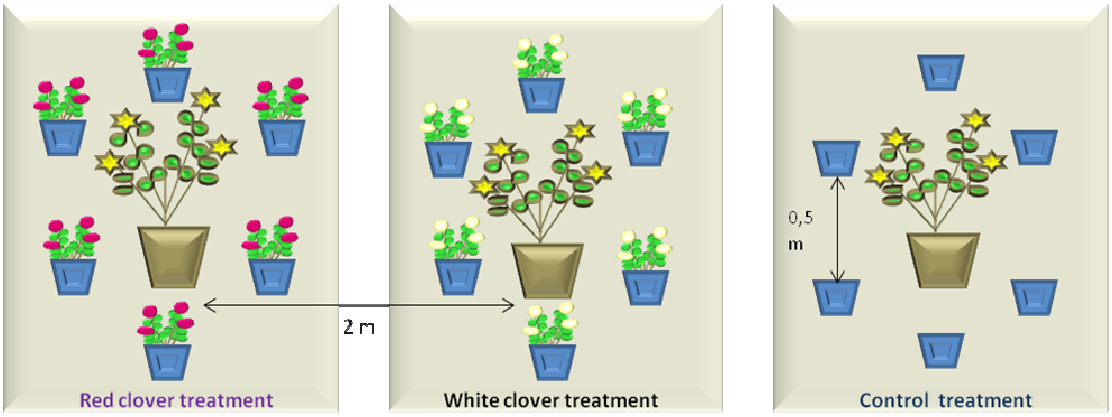
This set-up was replicated ten times for each plant species and for the control treatment. 50 eggs of *S. frugiperda* were placed on small paper towel pieces near the inflorescence of each species and 10 *T. atopovirilia* adults were liberated in each experimental unit. The paper towels with the 50 eggs of *Spodoptera* were removed and replaced every 2 days until all wasps died. The removed eggs were incubated at 25°C and the number of parasitized eggs and larvae of *S. frugiperda* was counted every 2 days; any recently hatched larvae were removed. Ten replicates per treatment were established. The proportion of parasitized eggs per each cardboard (= total parasitized eggs/ total available eggs) was arcsin square root transformed to increase homogeneity of variance and normality of residuals before performing a repeated measure ANOVA. The number of hatched larvae was analyzed with a repeated measure ANOVA. This experiment was repeated using *S. cerealella* eggs, with the purpose to investigate whether rate of parasitism depended on the host species. The results provided by this experiment demonstrated that the parasitism behavior of *T. atopovirilia* is very similar with both host species (for *S. frugiperda* $F_{7, 48}$ = 19,089 $p < 0.001$); for *S. cerealella* $F_{7, 71}$ =7.6166 $p < 0.001$). Thus, for the remaining experiments only *S. cerealella* eggs were used because they were commercially available in high numbers.

2.6.2 Multiple-choice experiment:

To evaluate the effect of the presence of multiple flowering plants on the oviposition choice *of T. atopovirilia*, an experimental plot in an open greenhouse was established at the Universidad Nacional de Colombia, Bogotá. The experiment took place from September to December 2010 in a plot of approximately 6 x 26 m covered and surrounded by greenhouse plastic. Greenhouse plastic curtains were not closed to permit the movement of flying organisms to and from the experimental plot. 3 month old *Physalis* plants that had been grown in the greenhouse into the ground were transplanted, with 2 m between plants. All plants were developing their first fruits. 6 plastic cups with inflorescences of one flowering species were placed in a 0.5 m radius circle around each plant (Fig 2-2). Eight replicates of each flower treatment were established in a completely randomized design including a control treatment *(Physalis* plants surrounded by 6 water cups and no flowers). On each *Physalis* plant, a paper towel with approximately 64.28 ± 14. 62 (mean ± SE, max = 186, min = 30, n = 220) *S. cerealella* eggs were placed in the middle third of the plant. The parasitoids were released by putting 0, 25 cm^2 squares of cardboard with *S. cerealella*

eggs visibly parasitized by *T. atopovirilia* between two *Physalis* plants. “Control” wasps were kept in eppendorf tubes with sugar diet, to monitor the approximate time in which the population died. The paper towels with the eggs of *S. cerealella* were removed and replaced every 2 days until all wasps in the eppendorf died. At the beginning of the experiment approximately five inflorescences of each flowering species were placed in each cup surrounding the focal plants. Every day the cups were refilled with water and 5 extra inflorescences added in order to make fresh resource available without removing the previous flowers, which could harbor the parasitoids. Longevity of *T. atopovirilia* in each experimental unit was calculated by analyzing the number of days until parasitized eggs were observed in each treatment. Longevity data were analyzed with a one way ANOVA. After collection, the eggs were incubated at 25^0C and the number of parasitized eggs was counted every 2 days. The proportion of parasitized eggs (= total parasitized eggs/ total available eggs) were arcsin square root transformed to increase homogeneity of variance and normality of residuals before performing a repeated measure ANOVA.

Figure 2-2: Example of the experimental area used in the multiple choice experiment to test oviposition rate of *Trichogramma atopovirilia* on *Sitotroga cerealella* eggs placed on *Physalis peruviana* under different treatments of surrounding flowering plants (Experiment 2.6.2). Approximately 65 *S. cerealella* eggs were put on each central *Physalis* plant. Surrounding each plant we placed 6 cups containing inflorescences of one species of flowering plant.



2.7 Effect of different flowering plants on the parasitism rate of *T. atopovirilia* under field conditions:

In a commercial *P. peruviana* field of 14, 243 m^2 at 2569 m.a.s.l. located in the municipality of Tabio, Cundinamarca, Colombia (latitude 4° 59' 60.00", longitude -74° 4' 58.80; average temperature of 14°C) was established a experiment which took place from January to February 2011. The plants were ten months old, in full production and sown 2 m apart. The field had a natural high incidence of grass (*Pennisetum clandestinum*), red , and white clover as well as other plants, which are naturally present in *Physalis* fields (Plaza and Pedraza, 2007).

Taking advantage of the natural variation in the abundance of the different species in the field, plots were chosen and were dominated by 1) red clover, 2) white clover, 3) a mixture of species (hereinafter mix cover) and 4) grasses which did not present any flowering plants (control). These different plot types were designated treatments (Fig 2-3). Ten plots (6m x 6m) per treatment, each with 9 plants (3 x 3 plants) of *Physalis* were selected. The plots were separated from each other by at least 8m and the central plant of each plot was selected as the focal plant. The percentage of groundcover in each treatment was calculated by estimating the percentage of cover of each flowering plant in two square meters in each plot (Table 2-1).

Commercially, in Colombia, 50 square inch / hectare of *Trichogramma* spp are released for the management of Lepidopteran pests (Perkins, 2011). Based on this the total area of the crop was divided in 60 equal subplots, in each of these, one square inch of cardboard with *S. cerealella* eggs visibly parasitized by *T. atopovirilia* was released. Simultaneously 144 ± 25.05 (mean ± SE, max = 200, min = 40, n = 180) eggs of *S. cerealella* were glued on paper towels in the middle third of the focal plant, on the midrib of a leaves. The paper towels with the eggs of *S. cerealella* were removed and replaced every 2 days until did not observe further parasitized eggs (approximately 10 days after released the wasps). The eggs collected were incubated at 25°C and the number of parasitized eggs was counted. Data on the total proportion of parasitized eggs per plot (= total parasitized eggs/ total available eggs in that plot) were analyzed with a one way ANOVA.

Figure 2-3: Natural groundcover found between *Physalis* plants. This image represents the typically cover in the “mixed groundcover” treatment, with the presence of several species of flowering plants such as red and white clover (Picture K. Poveda).

2.8 Effect of different flowering plants on the oviposition of *S. frugiperda*:

Since the provision of floral resources could also have a positive effect on the performance of the pest, the effect of different flowering plants on the oviposition rate of *S. frugiperda* adults was tested. Inflorescences of *Physalis*, sweet alyssum, borage, moricandia, red clover and white clover were collected and placed them in plastic cups with lids as previously described. Each cup was located in a glass jar (3.8 l) covered with bridal veil (Fig 2-4). Each flowering species was taken as a treatment and a control treatment was established using the same setup but without flowering plants. One replicate per flowering species was set up every week with a total of 10 replicates in a random block design. Two females and two males of *S. frugiperda,* that had emerged less than 24 hours earlier, were released into each experimental treatment. The experiment was conducted until all adult *S. frugiperda* died. Data on total number of eggs oviposited were analyzed with a non

parametric Kruskal – Wallis ANOVA. All statistics analyses were performed with the program Statistica 6 (StatSoft. 2003).

Figure 2-4: Experimental set up to test oviposition rate of *S. frugiperda* on flowering plants (Experiment 2.8). Cut flowers were placed in 12 oz cups. Each cup was located in a glass jar (3.8 l). The moths represent the two males and the two females that were released in each jar.

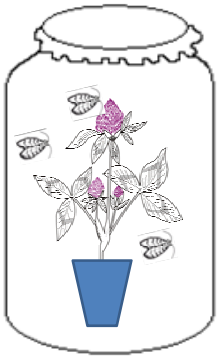


Table 2-1: Maximum, minimum and average percentage cover ± standard error of each plant species present in the different treatment plots (mixed cover, white clover, red clover and control) in the *Physalis peruviana* field where the experiment took place.

Family	**Plant species**	**Mixed cover**			**White Clover**			**Red clover**			**Control**		
		Max cover	Min cover	Mean ± SE	Max cover	Min cover	Mean ± SE	Max cover	Min cover	Mean ± SE	Max cover	Min cover	Mean ± SE
ASTERACEAE	*Taraxacum officinale* F.H. Wigg	20	0	5.5 ±6.43	10	0	3.0 ± 3.49	5	0	1.81 ± 2.52	0	0	0 ± 0
	Sonchus asper L. Hill.	10	5	8.0 ± 2.58	10	0	4.0 ± 3.16	10	0	3.18 ± 3.37	0	0	0 ± 0
FABACEAE	*Trifolium pratense* L	30	5	15.0 ± 9.42	20	0	8.5 ± 7.09	60	40	50.0 ± 7.74	10	0	1.10 ± 3.3
	Trifolium repens L	20	5	13.5 ± 5.79	60	40	48.0 ± 7.88	20	0	10,9 ± 5.39	10	0	1.10 ± 3.3
MALVACEAE	*Fuertesimalva limensis* (L.) Fryxell	30	0	12.5 ± 9.78	20	0	3.0 ± 6.74	10	0	3.18 ± 4.62	0	0	0 ± 0
OXALIDACEAE	*Oxalis corniculata* L	5	0	3.0 ± 2.58	10	0	4.0 ± 3.94	10	0	4.09 ± 3.01	5	0	1.10 ± 2.2
POLYGONACEAE	*Polygonum segetum* Kunth	60	10	30.5 ± 16.7	20	0	11.0 ± 7.37	20	0	9.09 ± 5.39	30	0	13.3 ± 10
POACEAE	*Pennisetum clandestinum*	20	0	7.5 ± 6.34	30	10	15.0 ± 8.49	20	10	13.18 ± 4.62	20	0	2.20 ± 6.6
	Lolium sp	10	0	4.5 ± 4.97	15	0	5.0 ± 7.07	25	0	4.54 ± 7.56	10	0	1.10 ± 3.3

3. RESULTS

3.1 Adult emergence and longevity of three *Trichogramma* wasps under different climatic conditions:

The emergence rate of the parasitoid wasps from the parasitized *Sitotroga cerealella* eggs differed between the three parasitoid species. The environmental conditions or the interaction between species and environment did not have any effect (Table 3-1). Adult longevity was affected by species identity and environmental conditions, but not by the interaction of both (Table 3-1). Taken together was observed that *T. exiguum* had the highest percentage of emergence from the eggs and adult survival rate over time, in comparison to *T. pretiosum* which had the lowest emergence and survival rates (Fig 3-1 a, b). The longevity of all species was higher under laboratory conditions than under field conditions (Table 3-1, Fig 3-1 c). The sex ratio of all *Trichogramma* species was approximately 3:1 and did not vary between different species ($F_{2,81}$=0.39, p= 0.67).

Table 3-1: *F* and p- values for ANOVA's testing the effect of parasitoid species (species), environmental conditions (treatment) and their interactions on adult emergence and survival of three Trichogramma egg parasitoids.

	Emergence of adults (%)	**Average number of days that adults survived**
Species	$F_{2,81}$=15.64, p< 0.001	$F_{2,81}$= 6.18, p < 0.001
Climate conditions	$F_{2,81}$=0.10 p=0.90	$F_{2,81}$=4.19, p<0.001
Species*Climate	$F_{4,81}$ =2.14 p=0.082	$F_{4,81}$=0.22, p=0.92

3.2 Effectiveness of different *Trichogramma* species for the control of *S. frugiperda and C. decolora:*

In petri dishes *S. frugiperda* eggs were parasitized at a higher rate by *T. atopovirilia* and *T. pretiosum* than by *T. exiguum* ($F_{2, 27}$ =9.4996; p = 0.00075, Fig 3-2). Similarly, in the experiment on *Physalis* plants the number of parasitized eggs in the treatment with *T. exiguum* is the lowest , while the *T. atopovirilia* treatment showed the highest values of parasitized eggs ($F_{3,36}$ =6.7622, p = 0.00098, Fig 3-3 a). A similar pattern was seen when looking at the number of *S. frugiperda* larvae and percentage plant damage. It was observed that the presence of *T. atopovirilia* reduced the presence of hatched larvae, while *T. exiguum* did not differ from the control treatment ($F_{3,36}$ = 4,4255, p = 0.0095, Fig 3-3 b).

Also *T. atopovirilia* and *T. pretiosum* had the lowest values for the percent damage, while *T. exiguum* reported equal values to the control treatment ($F_{3,36}$= 4,7197, p = 0.00705, Fig 3-3 c).

For the host species *C. decolora,* the proportion of parasitized eggs was greatest in the *T. pretiosum* treatment, followed by the treatments with the species *T. atopovirilia* and *T. exiguum* ($F_{2,27}$ = 48. 750, p < 0.001, Fig 3-4).

Figure 3-1: Effect of parasitoid species (*T. atopovirilia*, *T. exiguum and T. pretiosum*) on the mean ± SE percentage of emergence (a) mean ±SE adult survival (b).Effect of the three environmental conditions (Field, Greenhouse and Laboratory) on the mean ±SE adult survival of the three parasitoid species (c). Different letters indicate significant differences (Tukey HSD $p < 0.05$)**.**

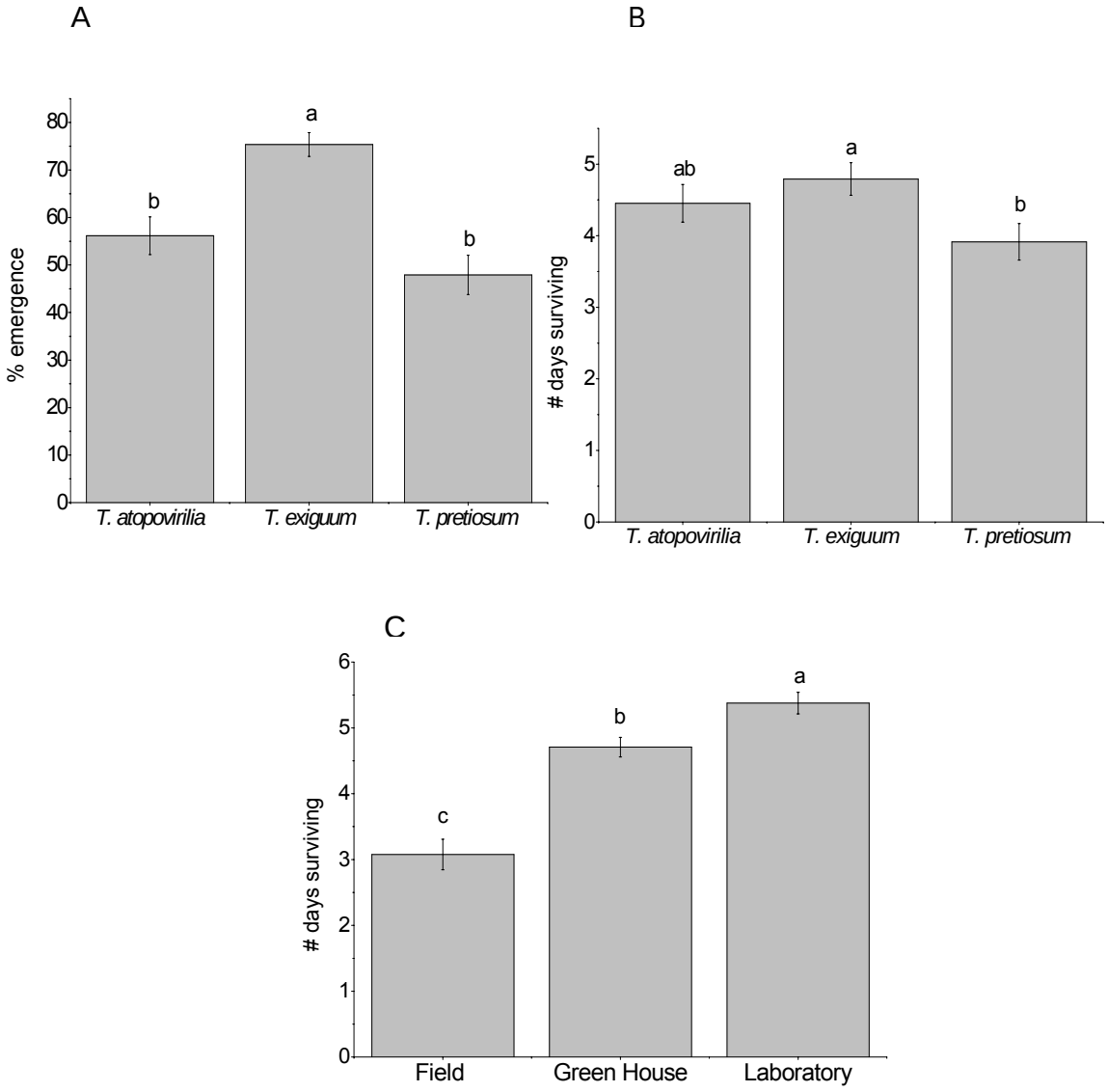
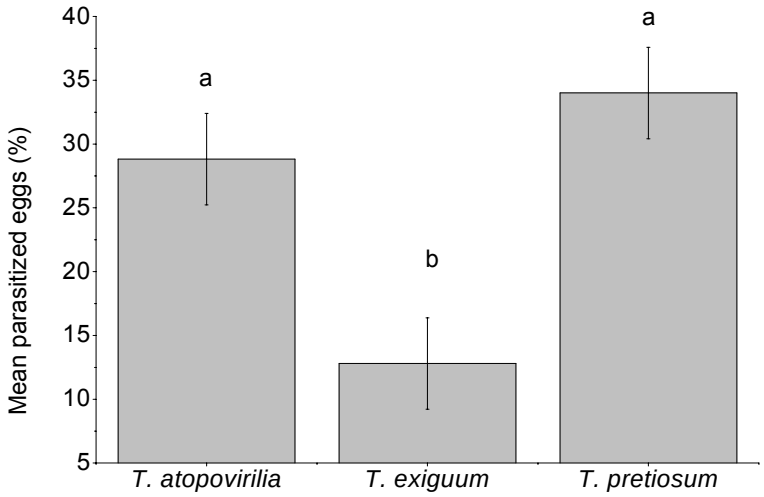


Figure 3-2: Parasitation percentages ± SE of the three *Trichogramma* species on *S.frugiperda* in petri dishes. Different letters indicate significant differences (Tukey HSD p< 0.05)



3.3 Effect of food source on survival and parasitism rate of *T. atopovirilia*:

When analyzing the effect of different food sources on survival and parasitism rate of *T. atopovirilia* it was observed that the honey diet increased the survival rate of the parasitoid species, compared with the control and water diet treatments ($F_{2, 27}$= 125. 91 p < 0.001 Fig 3-5 a). saw a similar pattern when looking at the proportion of parasitized eggs, which was largest with the honey diet ($F_{2, 26}$ = 74. 454, p < 0.001, Fig 3-5 b).

Figure 3-3: Parasitation percentages ± SE of the three *Trichogramma* species on *S. frugiperda* (a), number of larvae of *S. frugiperda* (b), and larval damage on *Physalis* plants (c). Different letters indicate significant differences (Tukey HSD $p < 0.05$)

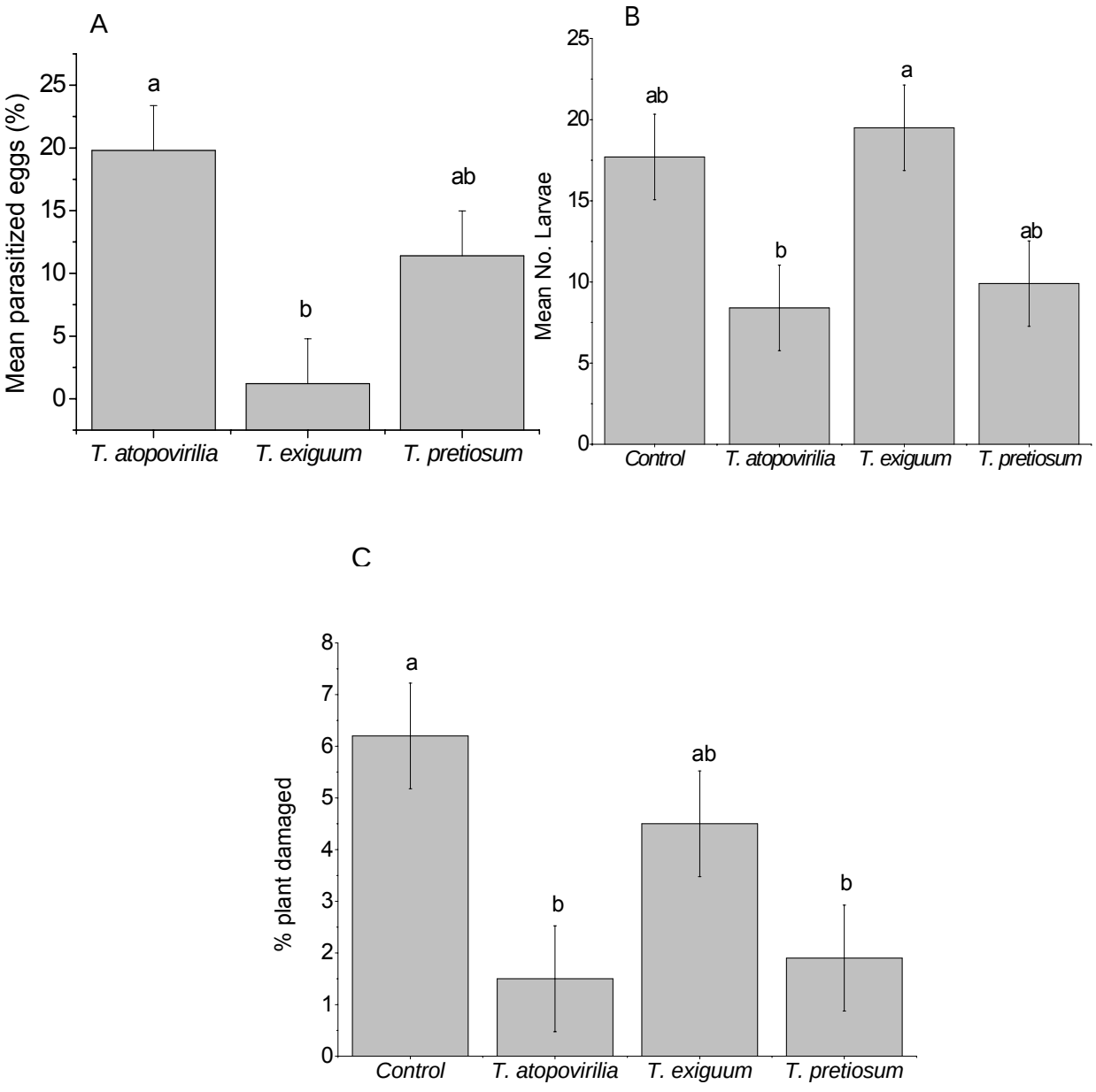
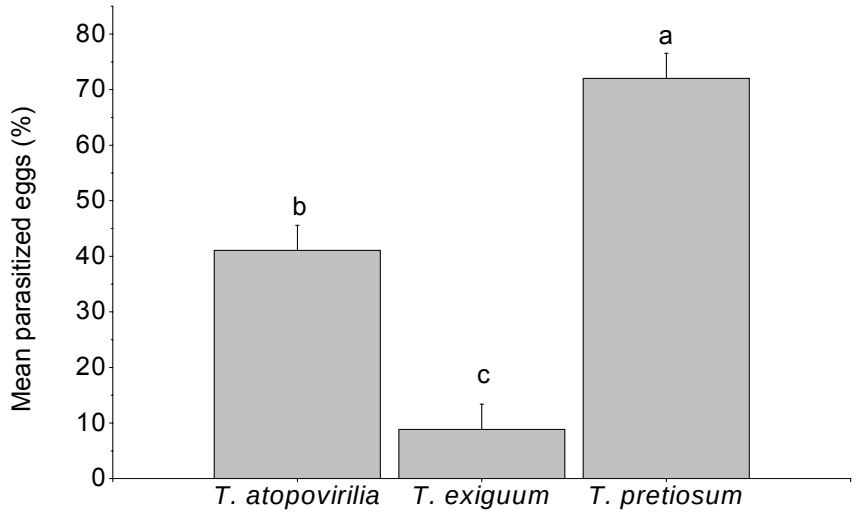


Figure 3-4: Parasitation percentage ± SE of the three *Trichogramma* species on *C. decolora* eggs parasitized. Different letters indicate significant differences (Tukey HSD p < 0.05)



3.4 Effect of different flowering plants on the longevity and parasitism rate of *T. atopovirilia:*

3.4.1 No-choice experiment:

When analyzing the effect flowering plant species on the proportion of parasitized eggs of *S. frugiperda* and *S. cerealella* (Fig 3-6 a) it was observed that the presence of *Trifolium pratense* (red clover) increased the parasitism capacity of the wasp compared with the control treatment, while none of the other species used in the experiment influenced the parasitoid activity (for *S. frugiperda* $F_{7, 48}$ = 19,089 p < 0.001); (for *S. cerealella* $F_{7, 71}$ =7,6166 p < 0.001; Fig 3-6 a). The previous data were confirmed looking at the number of hatched larvae of *S. frugiperda.* There were more *S. frugiperda* larvae in the control than in treatments where we liberated the wasps parasitoids in presence of red clover and borage ($F_{7, 48}$ = 7. 6837 p < 0.001; Fig 3-6 b).

Figure 3-5: Effect of food source on the mean ± SE adult survival of *T. atopovirilia* (a) and mean ±SE of proportion of *S. Cerealella* eggs parasitized (b). Different letters indicate significant differences (Tukey HSD $p < 0.05$)

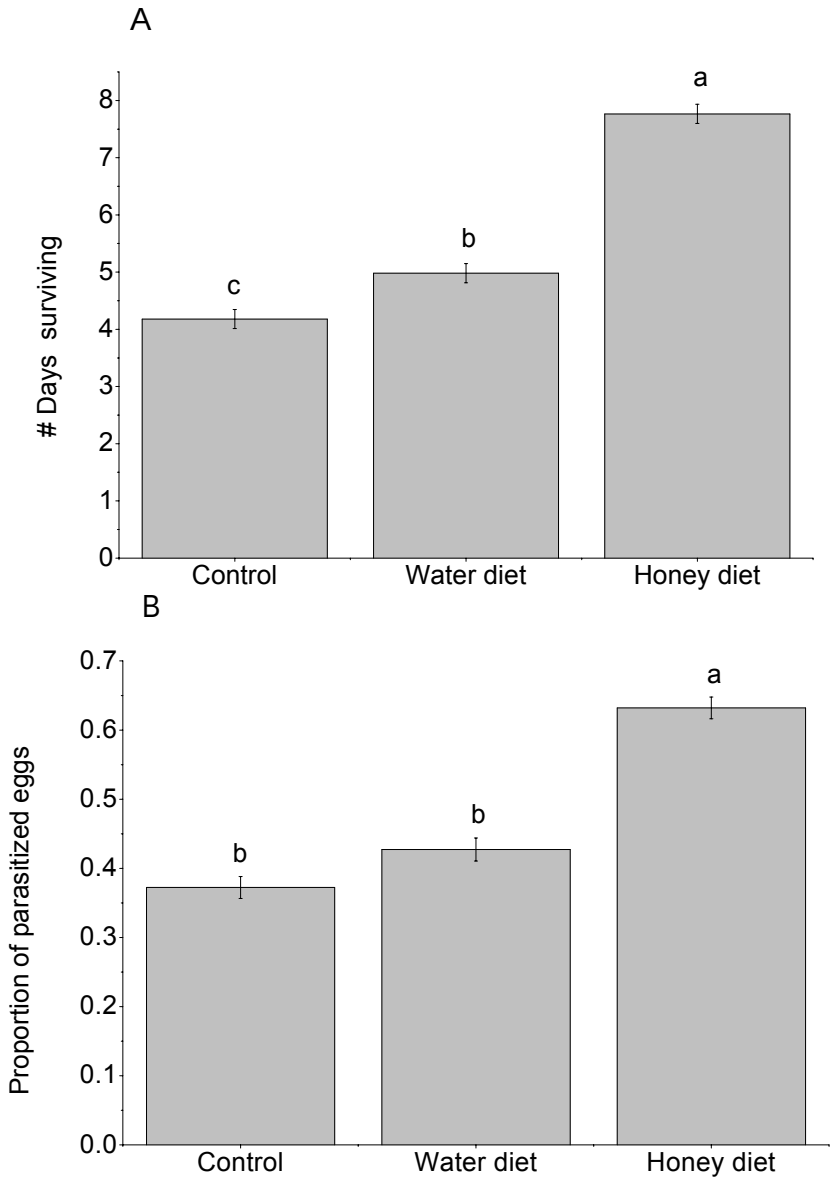
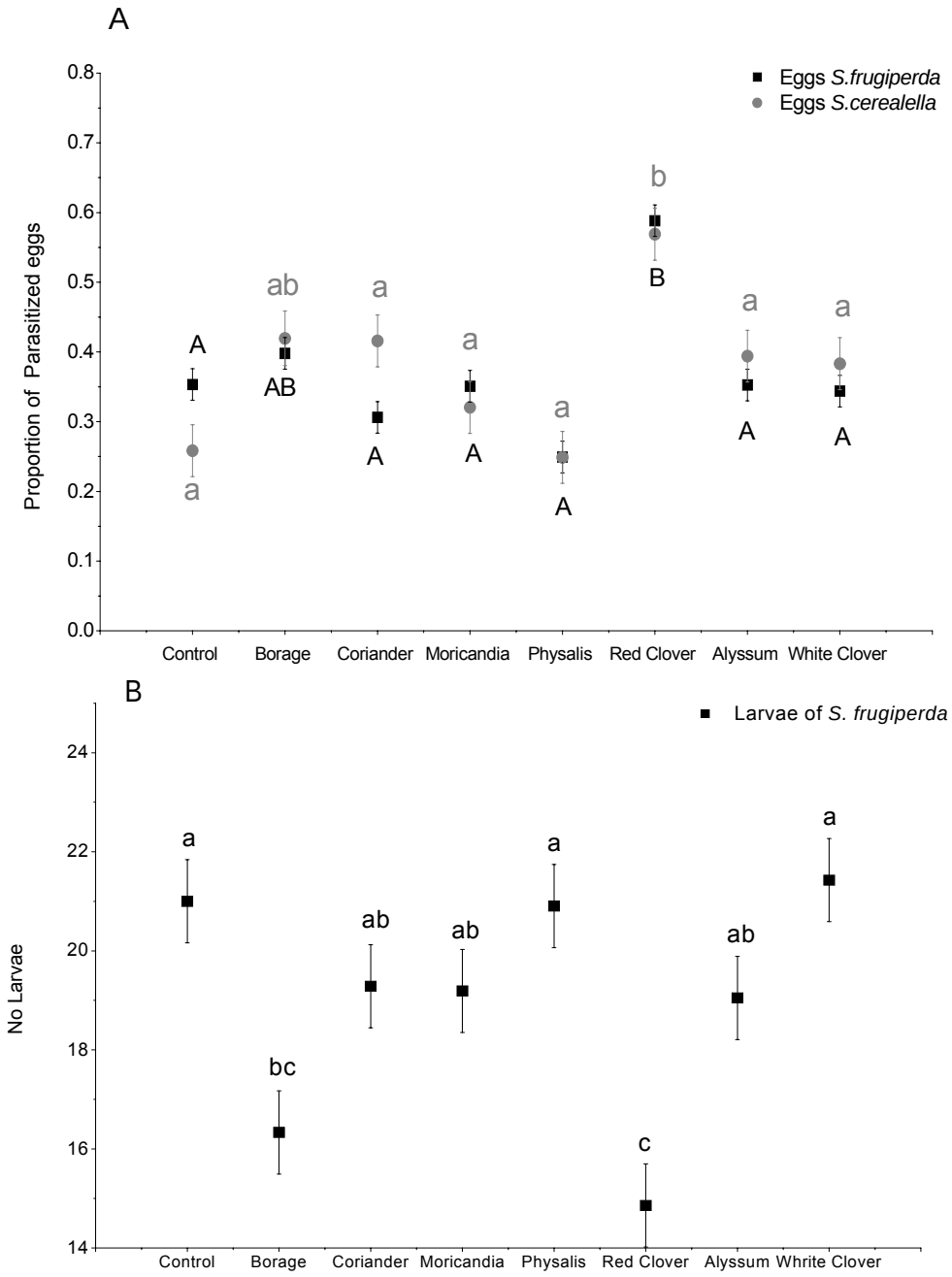


Figure 3-6: Effect of the presence of the flowering plants borage, coriander, moricandia, sweet alyssum, red clover and white clover on the parasitism rate of *T. atopovirilia.* Mean ± SE of parasitized eggs of *S. frugiperda* (■) and *S. cerealella* (●) (a).Mean ± SE of the number of hatched larvae of *S. frugiperda* (b). Different letters indicate significant differences (Tukey HSD P< 0.05)



3.4.2 Multiple-choice experiment:

In the multiple choice experiment, *T. atopovirilia* oviposition differed among treatments ($F_{6,49}$ =3.4390 p= 0.0065). The proportion of *S. cerealella* eggs parasitized by *T. atopovirilia* was higher in *Physalis* plants surrounded by red clover, white clover, borage and sweet alyssum inflorescences than in moricandia and control treatments (Fig 3-7a). When the longevity of adult's wasps, measured as the number of days a parasitism event was recorded, significant differences between treatments were observed ($F_{6, 49}$ = 3.1033, p = 0.01176), where red clover and alyssum favored the persistence of the parasitoid specie (Fig 3-7 b).

3.5 Effect of different flowering plants on the parasitism rate of *T. atopovirilia* under field conditions:

When the effect of different types of ground cover on the oviposition of *T. atopovirilia* under field conditions was evaluated, it was found that the parasitism rate was higher in the treatment with red clover groundcover than in the mixed cover and control treatments ($F_{3, 37}$ = 7. 0721, p = 0.00071) (Fig 3-8). Treatments with white clover and mixed cover did not differ from the control.

3.6 Evaluation of different flowering plants on the oviposition of *S. frugiperda:*

When the effect of the different flowering plants on the oviposition of S*. frugiperda* was analyzed, it was observed that in the treatments with red clover, borage and *Physalis* the total number of eggs oviposited (Kruskal Wallis Test H (6, N=75) = 39.46 p < 0.001) were significantly higher than in the control treatment (Fig 3-9).

Figure 3-7: Effect of the presence of the flowering plants moricandia, borage, sweet alyssum, white clover, red clover and *Physalis* on oviposition choice and longevity of *T. atopovirilia* under greenhouse conditions. a) Mean ± SE proportion of parasitized eggs of *S. cerealella.* b) Mean ± SE of adult wasps survival. Different letters indicate significant differences (Tukey HSD P< 0.05)

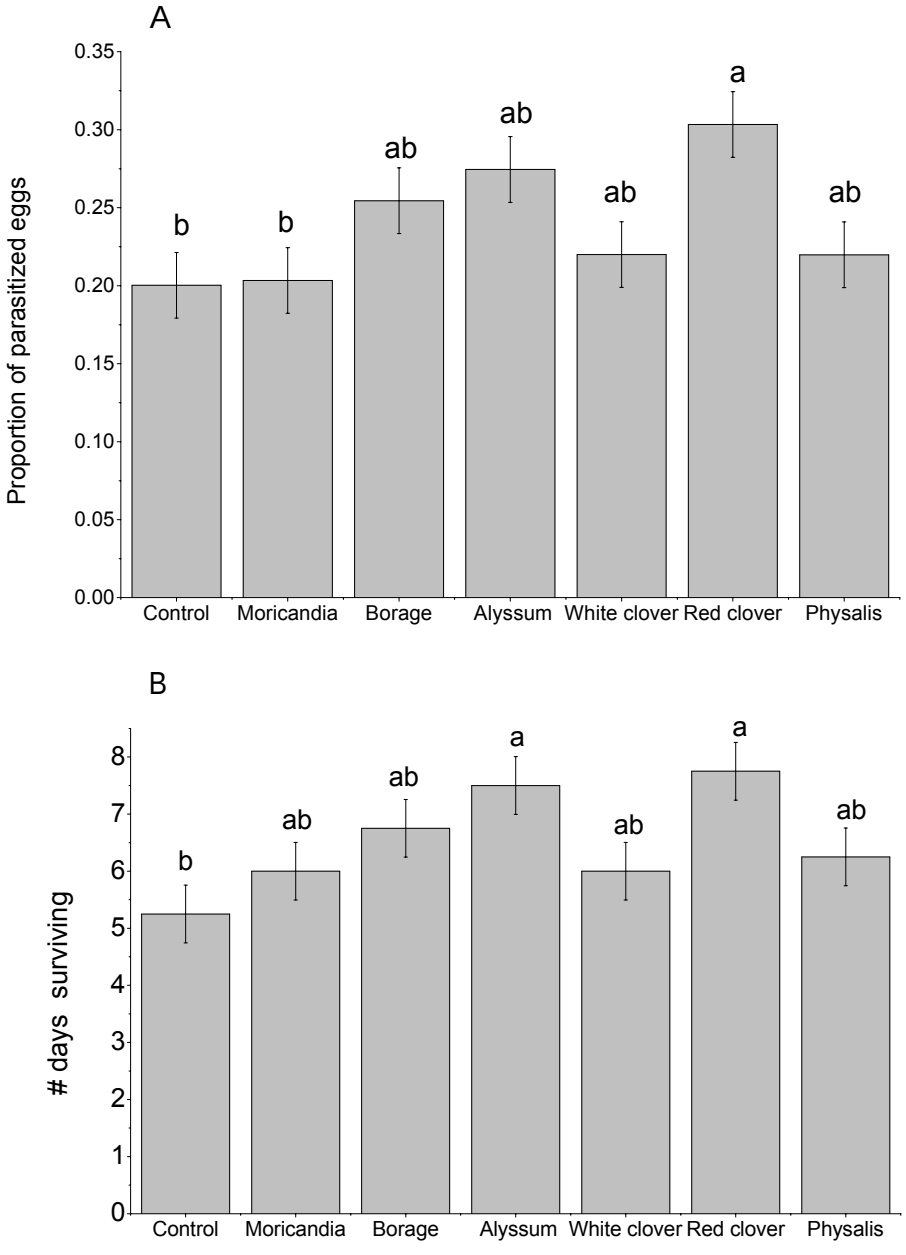


Figure 3-8: Effect of the presence of different type of cover on the oviposition behavior of *T. atopovirilia* under field conditions. Mean ± SE of parasitized eggs of *S. cerealella.* Different letters indicate significant differences (Tukey HSD P< 0.05)

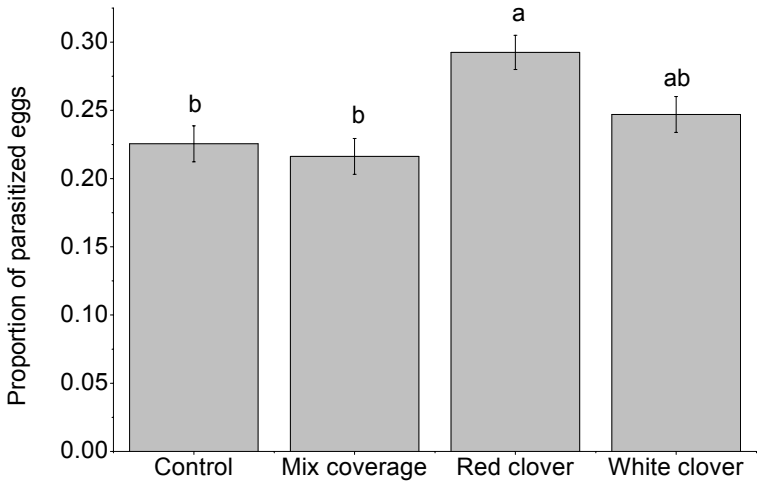
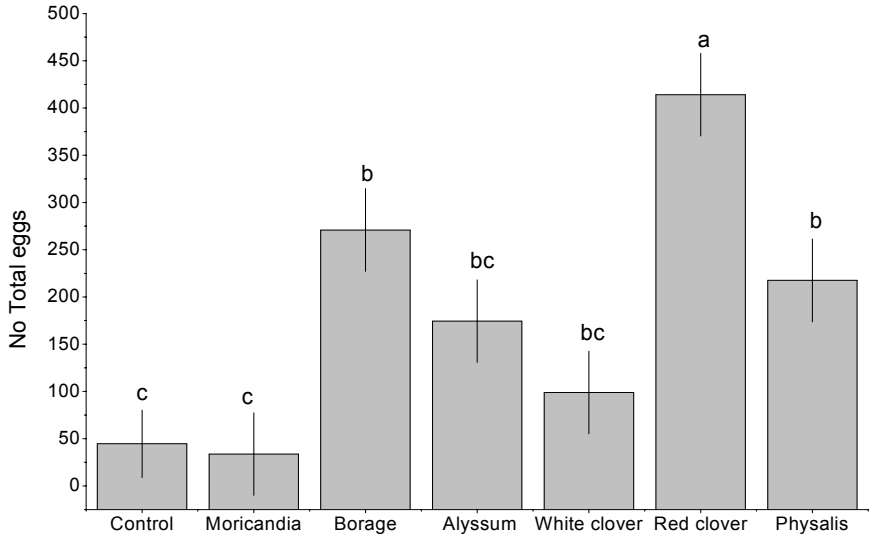


Figure 3-9: Effects of the presence of the flowering plants borage, moricandia, sweet alyssum, white clover, red clover and *Physalis* on the oviposition by *S. frugiperda*. Mean ± SE total number of *S. frugiperda* eggs. Different letters indicate significant differences (Multiple comparisons Kruskal – Wallis Test)



4. DISCUSSION:

The success of biological control practices is determined largely by the longevity and reproductive success of agents like parasitoids (Begum et al., 2006). The results show that the most promising commercially available *Trichogramma* species to control *S. frugiperda* is *T. atopovirilia* and for *C. decolora* is *T. pretiosum*. These parasitoid species adapted well to the environmental conditions of *Physalis* crops, and parasitized the noctuid pests. When evaluating the importance of floral resources for the longevity and parasitism rates of *T. atopovirilia* results demonstrate that the introduction or conservation of flowering plants, specifically red clover in *Physalis* productive systems, can promote the presence as well as the longevity and the parasitism capacity of this species.

Although *T. exiguum* has the highest emergence rate and longevity, its ability to parasitize *S. frugiperda* and *C. decolora* eggs is very low, while *T. atopovirilia* and *T. pretiosum* showed a high parasitism rate of the two host species, and a good adaptability to the environmental conditions. The emergence rate of the parasitoid wasps differed among species, but was not influenced by the environmental conditions. This is in accordance with results from Bueno, et al (2009), who showed that the temperature does not affect the emergence of *T. pretiosum.* However, it is known that temperature is one of the main environmental factors controlling the development of the parasitoids (Haile et al., 2002; Hance et al., 2007; Prasad et al., 2002). The present study shows that the longevity of the different *Trichogramma* species varied between the three environmental conditions. In the laboratory the three parasitoid species lived longer than under field and green house conditions. Although variations in relative humidity (Kalyebi et al., 2005) throughout the day as well as differences in the amount of time parasitoids were exposed to high and low temperatures (Bueno et al., 2009; Hance et al., 2007; Kalyebi et al., 2005) could be a cause for this difference, the data do not permit make any conclusions about the mechanisms behind a variation of longevity under different environmental conditions.

Based on the results on the longevity and emergence rate under different environmental conditions and the parasitism rate of the three parasitoids species on *S. frugiperda* and *C. decolora*, the species *T. exiguum* was discarded due to its low capacity to parasitize both hosts, while *T. atopovirilia* and *T. pretiosum* showed to be promising species for the management of *S.frugiperda* and *C. decolora* respectively. Based on these findings *T. atopovirilia* was chosen as model species to evaluate the effect of the provision of sugar and floral resources on longevity and parasitism rate of the parasitoid. The results showed that the provision of sugar resources, in the form of honey-based diet, increases the longevity and parasitism rate of *T. atopovirilia*. Similar results have been reported in other studies where it has been shown that adult hymenopteran parasitoids benefit from carbohydrate food resources (Jervis et al., 2001; Sood and Pajni, 2006).

A natural way of increasing food resources to parasitoids is by the provision of flowers as a nectar source. This study showed that in the presence of red clover *T. atopovirilia* had an increased parasitism rate of *S. frugiperda* and *S. cerealella* eggs and a greater longevity in no choice experiments. This is in accordance with studies on other parasitoid species that were exposed to flowering resources. For example Berndt and Wratten (2005) found that alyssum flowers (*Lobularia maritima*) increased the longevity and fecundity in *Dolichogenidea tasmanica* (Hymenoptera: Braconidae). Witting Bissinger, et al (2008) concluded that provisioning *T. exiguum* with honey or buckwheat flowers resulted in greater longevity of females than water alone. Fadamiro and Chen (2005) also obtained an increased lifespan of *Pseudodacteon tricuspis* (Diptera: Phoridae), a parasitoid of *Solenopsis* spp when they evaluated the effect of aphid honeydew or buckwheat floral nectar. In contrast to these investigations, laboratory results showed that borage and alyssum, which increased parasitoid performance in previous studies, did not have any effect on *T. atopovirilia*. This supports the idea that in order to choose a flowering species which effectively increases parasitoid performance data on a case by case basis have to be gathered (Barbosa and Benrey, 1998).

The results of this study showed that red clover had a positive effect on the longevity and parasitism rate of *T. atopovirilia*; Vattala, et al (2006) demostrated that this flowering plant is inaccesible to *Microctonus hyperodae* (Hymenoptera: Braconidae) as a food source. Their argument is that the small opening and depth of the corolla of red clover flowers,

together with the morphology of the head of the parasitoid (its large size) impede the parasitoid´s access to the floral resource. Ussualy most of the nectariferous tissue of the red clover are located on the abaxial side of the flower. However, a small amount occurs near the base of the free stamen on the adaxial side (Teuber et al., 1980), where only very small insects, such as species of the genus *Trichogramma*, can access and exploit the resource. Wäckers (2004) demostrated that *Trifolium pratense* provided accesible nectar for the parasitoids *Heterospilus prosodopis* and *Cotesia glomerata* (Hymenoptera: Braconidae), while *Pimpla turionellae* (Hymenoptera: Ichneumonidae) could not access to this floral resource, probably because of size its. This point underlines the fact that studies showing flower inaccesibility do not necessarily hold true for smaller parasitoids that may be able to reach concealed nectaries not accessible to other parasitoids (Baggen et al., 1999).

Although the experiments under laboratory conditions provided interesting results on the importance of flowering plants for parasitoids, they do not necessarily correspond to insect food use under field conditions (Witting-Bissinger et al., 2008). For this reason no-choice experiments were complemented with choice experiments and field experiments which confirmed previous findings. Under open greenhouse conditions plots with red clover had a high parasitism rate and longevity of *T. atopovirilia*. Although red clover plots were the only treatment statistically different from control plots, treatments with alyssum, borage, white clover and *Physalis* were intermediate, and did not differ significantly from red clover plots. This difference from the outcome of the no choice experiments, were all other treatments were different from the red clover, could be because parasitoids were allowed to move freely in the open greenhouse. This would be even more evident under field conditions were interactions with different organisms could obscure the patterns found under laboratory and greenhouse conditions. However, similar to multiple-choice experiments, under field conditions red clover plots were still more attractive to the parasitoid species as reflected by an increased parasitism rate in this treatment.

Many studies ignore that flowering plants can have a positive effect on the performance of pest species (Landis et al., 2000). This was however the case in our study, where *S. frugiperda* oviposited more on red clover plants compared with the other species tested. In previous studies that have faced this problem (Araj et al., 2009; Lavandero et al., 2005; Lavandero I et al., 2006) researchers select a flowering plant where the parasitoids can benefit but the pest cannot. However, any plant besides red clover, increased the

performance of *T. atopovirilia.* One potential benefit of using a plant for parasitoid feeding that is also attractive to the host (red clover in this case) is the combination of flowering plants for parasitoids with a trap plant for herbivores. Data from our own group (Martinez et al, unpublished data and Gomez et al, unpublished data) confirm that red clover seems to be an ideal trap plant for *S. frugiperda* and *C. decolora* in *Physalis* fields. Taking into account that it also seems to be attractive to parasitoids and increase their performance, red clover may be a candidate plant to use as a dead-end trapcrop. Dead- end trap crops have been described as plants that are highly attractive to insects but on which they or their offspring cannot survive (Shelton and Badenes-Perez, 2006; Shelton and Nault, 2004). Although it was not the goal of this study we suggest that using red clover as groundcover could significantly increase the oviposition, and presumably away from the main crop and at the same time increase the parasitism rate of the eggs on those plants controlling the pest more effectively. This would be similar to findings of Virk, et al (2004) who demonstrated that a sorghum trap crop used to manage cotton bollworm, *Helicoverpa armigera*, also increases rates of parasitism by *Trichogramma chilonis* and decreases damage by the pest by 10.99%. An additional advantage of using red clover in *Physalis* fields would be their natural presence under the current management regime. Although not native to the Americas, red clover is a naturalized species (Fergus and Hollowell, 1960) and has been used as cover crop (Cordoba, 2001; den Hollander et al., 2007; Sanchez, 1986) and fodder for cattle (Rocha, 2010) in the high Andean region. The presence of red clover patches in *Physalis* fields is ubiquitous (Plaza and Pedraza, 2007) so its use by the farmers could be easily adopted.

Given that most of the growers use soil cover between rows of *Physalis* plants, management strategies aiming at increasing the presence of red clover would not be very different from current management practices. However in order to propose this kind of management practices further field studies would need to be conducted with natural populations of noctuids. Given that the presence of red clover could potentially exacerbate the pest problem in the field studies quantifying pest pressure in fields with high densities of red clover in comparison to absence of red clover would be needed. Comparing this data with field studies were parasitoids are released would permit to identify if the combination of this two strategies would be an economically viable option for *Physalis* growers in the Andes.

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