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ORIGINAL ARTICLE

Biological control of *Frankliniella occidentalis* on greenhouse bell pepper using *Beauveria bassiana* in combination with soil cover practices

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Abstract

The excessive use of chemical products to control thrips and the tomato spotted wilt virus (TSWV) is not only harmful to human health, the environment, and biodiversity, but also the resistance these generate in insects turns them inefficient in the long run. Consequently, to achieve sustainable and residue-free production, control alternatives must be explored. This work proposes the use of *Beauveria bassiana* (BB) in combination with inter-row cover (IC) to reduce the population of thrips and the incidence of TSWV on bell pepper. For this purpose, a trial was carried out in a bell pepper greenhouse, consisting of four randomly distributed treatments with four repetitions of 66 plants each. The treatments assayed were: T (without BB inoculation or IC), TC (without BB inoculation and with IC), B (inoculated with BB), and BC (inoculated with BB and IC). The *B. bassiana* CEP147 strain was used based on its effectiveness in previous laboratory tests. After detecting one thrips per flower, five foliar spray applications were made at weekly intervals. The trial lasted 4 months. During this time, the number of thrips in the three central plants of each repetition, the presence of symptoms compatible with TSWV, as well as the number of fruits, and their weight, length, width and health were monitored weekly. Between the fourth and sixth weeks after the last application, a significant reduction in the population of total thrips (nymphs + + adults) was observed in both treatments B and BC compared to T and TC. In addition, plants with symptoms compatible with TSWV were very scarce, and the fruits showed significant differences in their quality parameters, producing the longest and heaviest in the BC treatment. The results showed that combining biological and cultural control makes sustainable pepper production possible.

Keywords: biocontrol, *Capsicum annum*, entomopathogenic fungi, TSWV, western flower thrips

Introduction

Bell pepper (*Capsicum annum* L.) is one of the most relevant greenhouse crops produced in Argentina (Molina 2017). However, its yield is mainly affected by pests such as the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), which causes direct damage during feeding and oviposition and indirect damage by acting as the main vector of tomato spotted wilt virus (TSWV) (Riley *et al.* 2011). TSWV and *F. occidentalis* were recorded in Argentina in 1995 (Dal Bó *et al.* 1995; De Santis 1995). Since then, the economic losses have been considerable, not only in pepper but also in tomato, lettuce, artichoke, celery, and different ornamental species (Dal Bó 2011).

The ability to transmit plant viruses combined with their high reproductive rate, cryptic behavior, broad host range and resistance to insecticides, make western flower thrips a challenging pest to manage (Reitz 2009). Currently, thrips control is based mainly on systematic applications of chemical insecticides, but considering the harmful effects of their excessive use on the environment, ecosystem biodiversity and human health, it is necessary to explore eco-friendly alternatives for integrated pest management. An alternative to agrochemicals is the use of entomopathogenic fungi. These natural biocontrol agents have an extremely high reproductive capacity and a very short generation time. They are sometimes highly specific in their actions, parasitizing only the host with which they have co-evolved. In addition, they have saprophytic phases in which they can survive without the host and remain in the environment until it reappears (Kendrick 2000). Their use does not allow the development of resistance by target organisms and their application is compatible with other biological control agents, some fungicides and many other types of pesticides. They do not require pre-harvest intervals and it is feasible to carry out controls over a long period of time (Goettel *et al.* 1990; Goettel and Hajek 2001; Vestergaard *et al.* 2003; Wraight *et al.* 2007).

Within the entomopathogenic fungi, *Beauveria bassiana* (Vals. – Criv.) Vuillemin (Hypocreales: Cordycipitaceae) is one of the most widely used for pest control worldwide due to its wide host range, easy mass multiplication, mode of application, and compatibility with certain synthetic fungicides and natural enemies of pests (Jacobson *et al.* 2001; Wraight *et al.* 2007; Gao *et al.* 2012; Wu *et al.* 2014, 2015; Lee *et al.* 2017). It is a cosmopolitan, haploid, mitosporic, and saprophytic organism that inhabits various environments such as soil, bark, and foliage of trees. It can establish itself as an endophyte in several cultivated plants, acting as a growth-promoting agent and conferring resistance to pests and diseases (Vega 2018). Recently, some publications have shown that the establishment of *B. bassiana* as an endophyte in pepper improved several plant growth parameters and had a controlling effect against the insect pest *Myzus persicae* (Hemiptera: Aphididae) and the fungal disease caused by *Botrytis cinerea* Pers. (Helotiales: Sclerotiniaceae) (Jaber and Araj 2018; Allegrucci *et al.* 2020; Barra-Bucarei *et al.* 2020).

To date, several authors have evaluated the biocontrol effect of *B. bassiana* against *F. occidentalis* both *in vitro* and in field trials (Jacobson *et al.* 2001; Ugine *et al.* 2006; Gouli *et al.* 2008, 2009; Bustillo Pardey *et al.* 2009; Mukawa *et al.* 2011; Villalobos Moya *et al.* 2011; Skinner *et al.* 2012; Saito and Brownbridge 2016; Wraight *et al.* 2016; Wu *et al.* 2016, Lee *et al.* 2017; Zhang *et al.* 2019; Zhang *et al.* 2021; Davari *et al.* 2021). However, as far as we know, the combined effect of the entomopathogen with cultural practices such as soil cover has not yet been evaluated.

Covers are classified as natural or synthetic. Natural materials include straw, compost, peat moss, bark chips, sawdust, etc. In contrast, synthetic materials include plastics of several colors and aluminum (Weintraub and Berlinger 2004). These materials are used to maintain soil temperature, suppress weeds, promote plant growth, and reduce the chance of insect attack (Bégin *et al.* 2001; Díaz-Pérez 2010). Plastic covers can have an impact on pest populations, especially in the control of thrips (Greenough and Black 1990; Brown and Brown 1992; Abou-Jawdah *et al.* 2000; Riley and Pappu 2000; Stavisky *et al.* 2002; Momol *et al.* 2004; Salas 2004; Díaz-Pérez 2010; Razzak and Seal 2017). Plastic mulches with a specific color and reflectance properties can deter insects by influencing their vision and locomotory behavior (Summers *et al.* 2010; Tyler-Julian *et al.* 2018). In addition, white and silvery plastic mulch reduced thrips injury and delayed virus epidemics contributing to improved crop production (Vos *et al.* 1995). In our country, plastic covers are widely used in solanaceous crops to improve moisture maintenance and prevent waterlogging.

Within this context, this study aimed to evaluate the effect of *B. bassiana* alone or in combination with soil cover on *F. occidentalis* population control on bell pepper cultured under greenhouse conditions.

Materials and Methods

Crop sampling

To identify the thrips species present in the study site, insects were collected from a 5-month-old pepper crop planted in the same greenhouse where the trial was consequently carried out. Adult specimens were collected from flowers and young shoots from the center and the edges of the greenhouse, placed in plastic containers, fixed in 70% ethanol and transported to the laboratory for assembly and identification. The preparations were observed and photographed under a Nikon YS2-H optical microscope equipped with a Nikon D40 digital camera to carry out the morphological identification using the keys of Cluever and Smith (2017).

Insect rearing

Bell pepper flowers with the presence of thrips were cut from crops in the same greenhouse where the application of *B. bassiana* was subsequently carried out. After morphological identification, using the taxonomic keys of Cluever and Smith (2017), adults of *F. occidentalis* were used to initiate the colony. Insects were sexed and placed in 750 cm^3 plastic containers with lids provided with ventilation through a perforation covered with filter paper, and conditioned with sweet beans (*Phaseolus vulgaris* L.) for feeding. Rearing containers were maintained in a growth chamber at 25 ± 1°C, 65 ± 5% relative humidity, and a L16 : D8 photoperiod at the Centro de Investigaciones de Fitopatología (Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires, Argentina).

Fungal strains

Highly virulent isolates of *B. bassiana* s.l. previously evaluated by Toledo *et al.* (2007) against planthopper and leafhopper pests of maize crops were used. Isolates namely BbCEP147 and BbCEP189 were obtained from infected adults of *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) and *Oliarus dimidiatus* Berg (Hemiptera: Cixiidae) in Tucumán and Buenos Aires provinces (Argentina), respectively, and were preserved in the mycological collection of the USDA-Agricultural Research Service Collection of Entomopathogenic Fungal Cultures-ARSEF (Ithaca, NY, USA) under accession numbers ARSEF 8372 (BbCEP147) and ARSEF 7776 (BbCEP189). In addition to their morphological identification, their identity was confirmed by their internal transcribed spacer (ITS) sequences available at the GenBank database (www.ncbi.nlm.nih.gov) under accession numbers KF308683.1 (BbCEP147) and KT952326.1 (BbCEP189) (Toledo *et al.* 2019).

Pathogenicity assays under laboratory conditions

In vitro pathogenicity assays were performed to select the most virulent strain to be evaluated in the field. The methodology described by Toledo *et al.* (2007) was adapted for these assays. Briefly, adult insects were placed in groups of 30 in glass bottles (200 ml capacity) with plastic lids perforated and sealed with filter paper to allow air diffusion. Bottles were provided with sweet beans for feeding. Both *B. bassiana* strains (BbCEP147 and BbCEP189) were cultured on malt extract agar 2% (MEA2%) for 10 days at 26°C in darkness. Conidia were harvested and suspended in 0.01% (vol/vol) Tween 20 (Biopack®, Buenos Aires, Argentina). Conidial suspensions were standardized to 1×10^8 conidia · ml^{-1}, and their viability was estimated according to the methodology described by Inglis *et al.* (2012). Three hundred μl per group of 30 insects were inoculated using a professional airbrush (model 180, nozzle diameter 0.25–0.3 mm, fluid cup capacity 9 ml). Three replicates and one control of 30 insects each were made for each fungal strain. Controls were sprayed with 300 μl of 0.01% (vol/vol) Tween 20. Treated and control insects were maintained at 25 ± 1°C, 80% RH (relative humidity), and L16 : D8 photoperiod. Insects were checked every 24 h up to 7 days. Dead insects were removed daily, surface sterilized in 70% ethanol for a few seconds, washed in sterile distilled water, and placed in 0.5% sodium hypochlorite for 1 min. Finally, they were rinsed in distilled water, placed on Petri dishes with filter paper moistened with sterile distilled water, and incubated at 25°C for 3–5 days. Only those insects showing external mycelia were considered dead due to fungal infection. After 7 days, the cumulative mortality was recorded and median survival time (MST) was calculated. Tests were performed twice.

Greenhouse trial

Based on the results of the *in vitro* pathogenicity test, the BbCEP147 strain was selected and tested for efficacy against thrips on bell pepper under greenhouse conditions. The trial was conducted in Florencio Varela, Buenos Aires, Argentina (34°51'56.88"S – 58°16' 58.45"W). The greenhouse (20 m wide, 50 m long and 3.7 m high) consisted of four modules of a wooden structure and a plastic cover, with roof ventilation. It was covered around its entire perimeter with an anti-aphid mesh, a common technique used as a physical barrier. Before transplanting, each plantation ridge was covered with a 50 μm thick black plastic mulch. Pepper seedlings (Almuden variety, Syngenta, Buenos Aires, Argentina) were transplanted into equidistant perforations made in black plastic mulch on October 30, 2019 when peppers had four leaves. The planting density was 1.33 plants per m^2, with a total of 1,333 plants in the greenhouse. The irrigation system consisted of two self-compensating drip hoses, with drippers every 0.10 m. The trial consisted of four randomly distributed treatments with four replicates each. Each replicate (13.5 m^2) was comprised of 66 plants, without previous treatment to prevent pests or diseases and was not circumscribed with any mesh. The treatments assayed were: T (plants without BbCEP147 inoculation or inter-row cover), TC (plants without BbCEP147 inoculation and with inter-row cover), B (plants inoculated with BbCEP147) and BC (plants inoculated with BbCEP147 and inter-row cover). The inter-rows were covered with a 150 μm thick white nylon for the treatments that included soil cover. The rest of the inter-rows without cover were kept free of weeds by hand hoeing. For treatments that contained BbCEP147, a suspension of 1×10^8 conidia · ml^{-1} in 0.01% (vol/vol) Tween 20 was inoculated by foliar aspersion using a 1.5 L pressure hand sprayer (Stihl Sg1L) uncovering full plant coverage at a rate of 200 l · ha^{-1}. The suspension was prepared by harvesting fungal conidia from cultures grown on Sabouraud dextrose agar plus 1% yeast extract (SDAY1%) for 15 days at 26°C in darkness. The first fungal application (November 20, 2019) was carried out when the monitoring, made at weekly intervals, showed one thrips per flower. The four remaining applications were made at intervals of approximately 1 week on December 9, 13, 20 and 27, 2019. The inoculum applications were made within 2 h of being prepared, between 6.00 and 7.00 p.m. to avoid the incidence of solar radiation and to coincide with an increase in humidity (Jacobson *et al.* 2001; Bustillo Pardey 2009). The trial lasted 4 months (November 20, 2019 to March 27, 2020) and during this time, the number of thrips (adults and nymphs), the presence of plants with TSWV symptoms, the number of fruits and their weight, length, width and health were weekly monitored. Dead thrips found on the treated plants were placed individually in Eppendorf tubes and transported to the laboratory to be deposited in humid chambers to verify fungal infection. The plants sampled were always the three central ones, ensuring that the surrounding plants constituted a barrier that would minimize the displacement of the thrips between the different treatments. In the pre-flowering stage, the entire plant was sampled, while from the anthesis period, the sampling of three open flowers per plant was incorporated. Throughout the trial, the temperature and humidity inside the greenhouse were recorded using a data logger (FlareSense).

Data analysis

After checking the assumptions of normality and homogeneity of variance, differences between the percentages of *in vitro* mortality caused by both fungal strains were statistically analyzed through an ANOVA followed by Tukey's test for pairwise comparisons (α = 0.05). Insect survival was estimated using the Kaplan-Meier method and the curves for both fungal strains were compared using the log-rank chi-square test ($p \leq 0.05$). For the greenhouse trial, the number of thrips (adults and nymphs) recorded in the different treatments during the different sampling dates was analyzed using Generalized Linear Models (GLM), and Fisher's Least Significant Differences (LSD) test for pairwise comparisons (α = 0.05). Adults and nymphs were analyzed separately. Sampling dates were incorporated into the analysis as a covariate. For this purpose, each sampling date was considered to be a sampling day after the first sampling. Thus, the first sampling (November 25, 2019) was considered day 1, the following day 14, the following day 18, and in the same manner until day 122, which corresponded to the last sampling date and the end of the trial (March 27, 2020). The analysis was carried out using the lme4 function of the nlme package of the R Statistical Software, through an interface implemented by the InfoStat Software (Di Rienzo *et al.* 2020). Also, the Kruskal-Wallis test (α = 0.05) was used to analyze the differences between the quantity, size and weight of the fruits harvested from February 7, 2020 to March 4, 2020 in the different treatments, while the method described by Conover (1999) was used to separate the medians. The incidence of TSWV in plants and the presence/absence of anomalies in the fruits caused by viruses were analyzed through contingency tables using the maximum likelihood ratio (G^2) as a statistical test. All analyses were performed using InfoStat version 2020 software (Di Rienzo *et al.* 2020)

Results

Crop sampling

All the specimens collected on pepper crops (n = 50) were identified as *F. occidentalis.*

Pathogenicity assays under laboratory conditions

The viability recorded for strains BbCEP147 and BbCEP189 was 98.3% and 99%, respectively. The difference between the percentages of cumulative mortality of *F. occidentalis* caused by both fungal strains was statistically significant (F = 8.65, df = 1, p = 0.0148). The BbCEP147 strain registered higher mortality (81.58%) than the BbCEP189 strain (48.39%). Likewise, the survival curves estimated by the Kaplan-Meier method differed significantly between both fungal strains (χ^2 = 17.5, p = 0.000029). The mean survival time recorded for the BbCEP147 strain was 4.3 ± 0.18 days, while the BbCEP189 strain failed to eliminate 50% of the thrips during the evaluation period. Only 18.42% of the thrips inoculated with BbCEP147 survived after 7 days (Fig. 1).

Greenhouse trial

The results obtained after application of BbCEP147 showed significant differences in the number of thrips, both nymphs (F = 25.97, df = 3, p < 0.0001) and adults (F = 7.12, df = 3, p = 0.0001), and between treatments in the period from November 25, 2019 to March 27, 2020. All nymphs' treatments differed from the control (T), registering the lowest number of insects in treatment B. In the case of adults, only treatment BC differed from the rest, registering the lowest number of thrips throughout the trial (Table 1).

When analyzing the number of thrips present on the different sampling dates, significant differences were also observed, both for nymphs (F = 257.32,

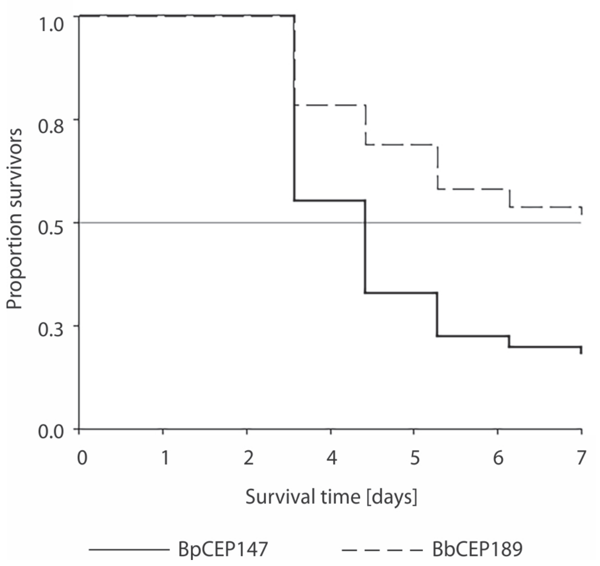

Fig. 1. Survival curves of *Frankliniella occidentalis* after inoculation with two strains of *Beauveria bassiana* (BbCEP147 and BbCEP189) under laboratory conditions

Table 1. Average number of thrips sampled in each treatment from November 25, 2019 to March 27, 2020

Treatment	Thrips nymphs	Thrips adults
T	21.83 ± 0.56 a	16.29 ± 0.49 a
TC	17.24 ± 0.5 bc	17.04 ± 0.5 a
B	15.71 ± 0.48 c	16.31 ± 0.49 a
BC	17.85 ± 0.51 b	14.06 ± 0.45 b

Values are given as mean ± SE. T – plants without BbCEP147 inoculation or inter-row cover, TC – plants without BbCEP147 inoculation and with inter-row cover, B – plants inoculated with BbCEP147 and without inter-row cover, BC – plants inoculated with BbCEP147 and with inter-row cover

$df = 1$, $p < 0.0001$) and for adults ($F = 8.55$, $df = 1$, $p = 0.0038$). Between the fourth and sixth weeks after the last application, a greater reduction in the population of total thrips (nymphs + adults) was observed in both treatments B and BC compared to T and TC. Four weeks after the last application (January 24, 2020) the number of thrips in treatment B was reduced by 43% compared to T and 23.03% compared to TC, while in treatment BC the reduction was 66% with respect to T and 53.95% with respect to TC. Five weeks after the last application (January 31, 2020), treatment B showed a reduction of 8.74% in the number of thrips sampled compared to T and 11.74% compared to TC, while the reduction in BC was 23.70% compared to T and 26.29% compared to TC. Finally, in the sixth week post-application of the mycoinsecticide (February 7, 2020), the reduction in the thrips population of B was 30% compared to T and 29.11% compared to TC, while in BC it was 27% compared to both T and TC. These results show that the greatest pest reduction occurred 4 weeks post-application of BbCEP147 in the BC treatment. In addition, Figure 2 shows how, since the last application of the entomopathogen, the thrips population began to stabilize, remaining below the maximum levels reached in the sampling on December 27, 2019. Death by fungal infection was confirmed in 33.3% and 25% of the insects collected from B and BC treatments, respectively. On the other hand, throughout the trial, inter-row cover significantly reduced the number of thrips nymphs without altering the number of recorded adults (Table 1).

The incidence of TSWV during the trial was very low. Only 2.1% of the sampled plants showed viral symptoms attributed to this pathology (22 diseased plants out of 1,056 sampled plants). However, significant differences were observed between treatments ($G^2 = 16.57$, $df = 3$, $p = 0.0009$). The least affected plants were those sampled in the BC treatment (4.55%) and the most affected were those corresponding to the TC treatment (63.64%). The affected plants in treatments T and B were 18.18% and 13.64%, respectively. The number of thrips recorded throughout all the sampling dates showed no correlation with the recorded values of temperature ($r = 0.32$, $p = 0.162$) or humidity ($r = 0.15$, $p = 0.505$). The average temperature and relative humidity recorded on the day of the first application of *B. bassiana* were 28.6°C and 50%, respectively. In addition, the second application showed values of 26.8°C and 50%, and the third 19.5°C and 38%, respectively. The values for the fourth application were 21.7°C and 72%, and for the last application 17.7°C and 83%, respectively. Average temperatures and relative humidity of 22.8°C and 68.8% were recorded during the application period.

No significant differences were recorded with respect to the number of fruits harvested from the different treatments ($H = 4.28$, $df = 3$, $p = 0.23$), nor with respect to their width ($H = 4.8$, $df = 3$, $p = 0.19$). However, statistically significant differences were observed for length ($H = 9.57$, $df = 3$, $p = 0.02$) and weight ($H = 9.81$, $df = 3$, $p = 0.02$). Fruits slightly longer and heavier than both controls (T and TC), were obtained in the BC treatment (Table 2).

Regarding the presence of anomalies in the fruits, significant differences were observed in TSWV symptoms ($G^2 = 17.66$, $df = 3$, $p = 0.0005$). The least affected fruits were those harvested in the BC treatment (10.94%) and the most affected were those corresponding to the TC treatment (37.04%).

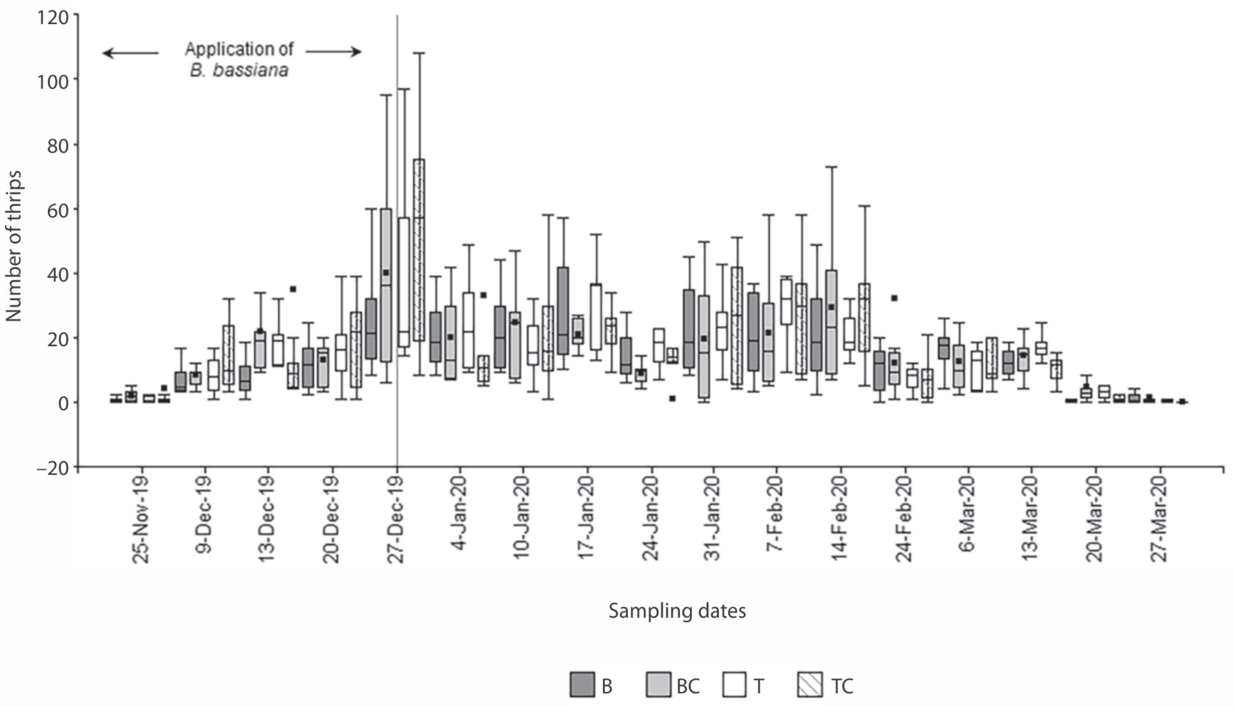

Fig. 2. Box-and-whisker plot showing the mean ± SE of the total number of thrips (nymphs + adults) sampled in the different treatments throughout the trial. T – plants without BbCEP147 inoculation or inter-row cover, TC – plants without BbCEP147 inoculation and with inter-row cover, B – plants inoculated with BbCEP147 and without inter-row cover, BC – plants inoculated with BbCEP147 and with inter-row cover

Table 2. Quantity, size and weight of fruits harvested from the different treatments applied to the bell pepper crop under greenhouse conditions

Treatment	Number of fruits	Length [cm]	Width [cm]	Weight [g]
T	19 ± 13.83 a	11.5 ± 1.49 ab	8.2 ± 1.03 a	211 ± 51.98 a
TC	16 ± 6.3 a	11.2 ± 1.3 a	8 ± 0.88 a	210.5 ± 47.2 a
B	18 ± 6.75 a	11.7 ± 1.38 bc	8 ± 0.94 a	219 ± 89.8 ab
BC	15 ± 1.82 a	11.8 ± 1.51 c	8.5 ± 0.82 a	222 ± 60.1 b

Values are given as mean ± SE. T – plants without BbCEP147 inoculation or inter-row cover, TC – plants without BbCEP147 inoculation and with inter-row cover, B – plants inoculated with BbCEP147 and without inter-row cover, BC – plants inoculated with BbCEP147 and with inter-row cover. Different letters in the same column denote significant differences between treatments according to the Kruskal-Wallis analysis followed by the comparison of medians according to Conover (1999) ($p < 0.05$)

Discussion

Concerning the morphological identification of thrips collected in the field, our results coincide with those reported by Saini *et al.* (2000) and Carrizo and Benítez (2002). These authors mention *F. occidentalis* as the dominant species in pepper crops in greenhouses in the study area.

Regarding the evaluation of *B. bassiana* biocontrol against *F. occidentalis*, after the results of *in vitro* tests, we selected the BbCEP147 strain as the most virulent against the pest. Values of viability and mortality higher than 90 and 80%, respectively, should be assured before the selection of the fungal strain to achieve good efficacy, stability, and permanence in the field (González-García *et al.* 1993; Marín *et al.* 2000). In this sense, our results are in agreement with those reported by Toledo *et al.* (2007) who selected the same strain as the most effective against Delphacidae and Cicadellidae corn pests. However, in the present work, it was shown that this strain was more effective against *F. occidentalis* than against the Hemiptera previously evaluated. Likewise, the mortality (81.58%) and the mean survival time of the pest (4.3 days) were similar to those observed by Wu *et al.* (2014), who recorded a maximum mortality of 96% and a minimum mean survival time of 4 days after testing 28 isolates of *B. bassiana* against *F. occidentalis* under laboratory conditions.

Regarding the evolution of the thrips population throughout the trial, we observed significant differences between the treatments and the sampling dates. In this respect, between the fourth and sixth weeks after the last application, the thrips population in treatments B and BC was significantly smaller than in treatments T and TC. Our results agree with those recorded by Mendoza Ulloa and Toledo Marquez (2019), who made three applications of *B. bassiana* at 7-day intervals for controlling *F. occidentalis* in cucumber crops (*Cucumis sativus* L.). These authors recorded the highest reduction in the thrips population (66%) 1 week after the last application. In comparison, we obtained a similar reduction (43–66%) 4 weeks after the last application for B and BC, respectively. These results demonstrate the need to carry out more than one application at weekly intervals to achieve the establishment of the entomopathogen in the crop. Likewise, another point to consider is the density of the pest at the time of application. With respect to pest density, our results were similar to those reported by Ludwig and Oetting (2002). They established that when thrips populations were high, the application of *B. bassiana* resulted in higher pest reduction than when thrips densities were lower. In our trial, the first application of the entomopathogen was carried out with a pest pressure of approximately one thrips per flower. The fungus recorded no effect until after the last application, when the pest density reached its maximum levels, with an average of 36 thrips per flower. When the number of thrips recorded from treatments TC and T was compared, we observed that inter-row cover significantly reduced the number of nymphs (17.24 ± 0.5) compared to the control (21.83 ± 0.6), and did not affect the number of adults recorded. However, inter-row cover significantly affected both nymphs and adults when used in combination with *B. bassiana*. Similar results were observed when UV-reflective mulches were combined with acibenzolar-S-methyl and insecticides (Momol *et al.* 2004) or combined with companion plants and kaolin (Tyler-Julian *et al.* 2018) on tomato.

According to the bibliography, the optimum temperatures and relative humidity for *B. bassiana* mycelium growth, sporulation and conidia germination are 25–32°C and above 92%, respectively (Walstad *et al.* 1970; Fargues *et al.* 1992). In the present work, the BbCEP147 strain exerted significant control of the pest under lower temperature (22.8°C) and humidity (68.8%) conditions, evidencing that not all strains have the same requirements and raising the need for previous laboratory tests to select strains capable of developing under conditions that are not optimal.

On the other hand, the incidence of TSWV recorded during the trial was very low since only 2.1% of the sampled plants presented symptoms compatible with this pathology. Our results contrast those reported by Ferrand *et al.* (2015), who recorded 100% losses caused by this disease in pepper crops in the study area. Given the difference in incidence, the causes cannot be established, but we can mention that the least affected plants were those corresponding to the BC treatment. A lower disease incidence in this treatment could be due to the reduction in the thrips population by the enhanced effect of *B. bassiana* and inter-row cover. Similarly, the results obtained by Greenough *et al.* (1990) and Stavisky *et al.* (2002) agree in establishing a reduction in the incidence of TSWV in association with a reduction in the thrips population when inter-row cover is used. Our results also show a positive effect of using *B. bassiana* in combination with inter-row cover concerning quality of the fruits, since in this treatment the longest and heaviest fruits were obtained. These results agree with those reporting that plastic covers alter the microclimate of the crop by changing the energy balance of the soil (Liakatas *et al.* 1986; Tarara 2000), resulting in changes in temperature that can positively affect plant growth and yield (Cooper 1973; Díaz-Pérez and Batal 2002; Ibarra-Jiménez *et al.* 2006; Lamont 2005; Díaz-Pérez 2010). Likewise, the fact of obtaining the longest and heaviest fruits in the treatments with the application of *B. bassiana* shows the possibility of the establishment of the BbCEP147 strain as an endophyte and its effect on promoting plant growth, as was recently recorded by Allegrucci *et al.* (2020), Barra-Bucarei *et al.* (2020) and Shaalan *et al.* (2021) when applying this fungal species on pepper, tomato and cucumber crops, respectively. However, future studies are needed to confirm the establishment of this strain as an endophyte and its effect in promoting bell pepper growth under greenhouse conditions.

In conclusion, we provide evidence that sustainable bell pepper production is made possible by combining biological and cultural control.

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