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Biological control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) by Entomopathogenic fungi and their side effects on natural enemies

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Abstract

Background: The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is an insect pest species responsible for damages of citrus fruit quality and tree health. This insect is a vector of bacteria '*Candidatus* Liberibacter' spp. a putative causal agent of citrus greening disease known as Huanglongbing (HLB), considered one of the most destructive diseases of citrus orchards worldwide. Disease management is mainly based on vector control using pesticides which can affect natural enemies that play an important role in pest control. The entomopathogenic fungi (EPF) *Beauveria bassiana* (2067 and 2121) and *Metarhizium anisopliae* (2411) were achieved by applying a suspension of 1×10^7 spores against *D. citri* nymphs and artificially infected the citrus seedlings under controlled and semi-field conditions. Also, the activity of these EPFs on the coccinellid and lacewing predators was evaluated. The effects of the main chemicals used in citrus plantations on the selected fungi for possible combined use was also investigated.

Results: The obtained results showed that under controlled conditions, the percentage of insect mortality produced by EPF varied between 82.8 and 85.9%. Under semi-field conditions, the infection and mortality of *D. citri* caused by the *M. anisopliae* 2411 strain was significantly higher than that of *B. bassiana* 2067, 78.9 and 51%, respectively. Non-significant effect of EPF on the natural enemies of *D. citri* was observed, suggesting that EPF and predators had compatible effects.

Conclusion: This study clearly demonstrated that EPF were able to kill *D. citri* and can be explored as a promissory biocontrol candidate. Simultaneous use in an integrated pest management program could be possible by applying EPF combined with pesticides.

Keywords: Citrus, Huanglongbing, Diaphorina citri, Beauveria bassiana, Metarhizium anisopliae, Predators, Pesticides

Background

Citrus production in Uruguay has an important social and economic impact, 14107 ha of citrus have been planted, producing 242645 tons of fruits. About 43% of the production was exported, 33% was for internal consumption as fresh fruit and 22% was industrialized (DIEA 2019).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a sap-sucking insect pest species, responsible for direct or indirect damages of citrus fruit quality and tree health. The insect feeds on sap sucking of younger leaves or buds and can inject toxins that produce deformations that can cause the death of the apical bud. They produce honeydew that allows the development of sooty mold in plants. Moreover, *D. citri* is a vector of bacteria '*Candidatus* Liberibacter' spp., a putative causative agent of citrus greening disease, known also as Huanglongbing (HLB), considered one of

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the most destructive diseases of citrus worldwide (Bové 2006). The presence of the vector has been reported (Bernal 1991), but the HLB has not been found so far. However, the disease has been reported in the bordering countries of Brazil and Argentina (Lopes et al. 2013), denoting an imminent infection risk for Uruguay. Disease management is mainly based on the production of healthy plants, the eradication of diseased plants and vector control (Orduño-Cruz et al. 2015). This pest is the most often controlled by an excessive use of synthetic pesticides, which may increase the probability of developing resistance to pesticides (Tian et al. 2018; Naeem et al. 2019). On the other hand, it can affect natural enemies that play an important role in the pest's control. In addition, pesticide residues can remain in food causing a risk to food safety. International regulations are now increasingly restrictive with the maximum levels of pesticide residues allowed. The combined use of chemical pesticides and biological control emerges as an interesting strategy for sustainable management of *D. citri* (Qureshi et al. 2014). Natural enemies of *D. citri* include predators such as coccinellids (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), syrphids (Diptera: Syrphidae) and spiders (Arachnida: Araneae), and the parasitoids such as *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* Shafee, Alam & Argarwal (Hymenoptera: Encyrtidae) and the EPF such as *Isaria fumosorosea* Wize, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams, *Beauveria bassiana* (Bals. –Criv.) Vuill and *Hirsutella citriformis* Speare (Lezama-Gutiérrez et al. 2012). *Chrysoperla externa* Shneider (Neuroptera: Chrysopidae) and *Harmonia axyridis* Palla (Coleoptera: Coccinellidae) are the main predatory species found on the citrus plantations of Uruguay (Pechi et al. 2016). EPF are extensively used as a biocontrol agent, and they act by contact and do not need to be ingested to infect their hosts. Their spores can be dispersed by biotic or abiotic factors, as horizontal transmission is possible and epizootics in the field can occur (Conceschi et al. 2016).

Hence, the aim of this work was to evaluate the potential of the EPF: *B. bassiana* and *M. anisopliae* isolates against *D. citri* biocontrol in citrus plantations and also to evaluate the effect of EPF on the coccinellid and lacewing predators and in turn to investigate the effect of the main chemicals used in citrus plantations on the selected fungi for possible combined use.

Methods

Evaluation of *D. citri* fungal infection in citrus seedlings under controlled conditions

Two strains of *B. bassiana* (2067 and 2121) and one of *M. anisopliae* (2411) were evaluated. The virulence of each fungal strain on *D. citri* was performed, using a suspension of 1×10^7 viable conidia/ml 0.02% (v/v) Tween 80. Application of fungal suspension was carried out on 7-month-old citrus seedlings, artificially infected with different nymphal instars of *D. citri*. A total of 2132 individuals were obtained from the mass rearing of the Instituto Nacional de Investigación Agropecuaria (INIA) Salto Grande. Twenty-four pots, with 3 seedlings each, were used. For each selected fungal strain, 30 ml of the conidial suspension was sprayed on the foliar surface of seedlings in each pot. Negative controls were sprayed only with 0.02% (v/v) Tween 80. Six replicates (pots) were done per treatment. Each pot was covered with fine mesh that prevents the movement of insects from one pot to another. Plants were incubated at 25 ± 2 °C for 5 days and then buds of treated and control seedlings were cut and investigated with a stereomicroscope. Living and dead individuals of nymphs were counted, and the presence of mycelium emerging from insects was recorded. Dead insects without evident presence of mycelium were transferred to individual moist chambers to determine if fungal infection had occurred. Insects were individually placed to avoid fungal transmission between samples and the overestimation of infection, as suggested by Hesketh et al. (2010). The percentage of insect mortality caused by fungi was calculated as the number of dead insects with the EPF/total of treated insects × 100.

To evaluate if there are significant differences between insect mortalities, data were transformed as arcsen$\sqrt{x}$ and Tukey's test was performed considering $\alpha = 0.05$.

Evaluation of insect infection under semi-field conditions

To evaluate the insect infection under field conditions, a similar assay as mentioned before under controlled conditions was carried out. Seven-month-old citrus seedlings were artificially infected by nymphs of *D. citri* at different stages of development. A total of 1657 individuals were obtained from the mass rearing of INIA Salto Grande. For each fungus strain, 10 pots containing 3 seedlings each were inoculated. Surface of seedling leaves was sprayed by 30 ml suspension of 1×10^7 viable conidia/ml 0.02% (v/v) Tween 80. The control was performed by applying 0.02% (v/v) Tween 80 (10 pots with 3 infected seedlings each). Each pot was covered by a fine mesh to prevent exit of insects. All pots were placed randomly under the trees canopy interspersed inside a citrus plantation. After 5 days, the effect of the inoculation with EPF on *D. citri* infection was evaluated.

All living and dead individuals were counted by using a stereomicroscope. Dead insects were placed in a humid chamber to analyze fungal infection. Percentage of insect mortality caused by the fungi was calculated as the number of dead insects with EPF evidence/total of treated insects. To determine if there were significant differences in the mortality of *D. citri* between treatments, data were transformed as arcsen√x and Tukey's test was performed considering $\alpha = 0.05$.

Effect of temperature on EPF

Since the highest infection occurs in summer, where the temperature is highly variable, the conidial viability at different temperatures were evaluated. A conidial suspension of 1×10^3 conidia/ml 0.02% (v/v) Tween 80 was inoculated on potato dextrose agar (PDA) culture medium (Oxoid^{TM}) and incubated at 15, 20, 25, 30, and 35 °C for 20 h in the dark. Five replicates were tested, and 200 spores were counted per plate. Viability of spores was calculated as the germinated conidia/total conidia × 100. A spore was considered viable, if the germinated tube reached ¾ the size of the spore. To evaluate if there were significant differences in spore germination at different temperatures, the transformation of the data was done as arcsen√x and Kruskal-Wallis test was performed considering $\alpha = 0.05$.

Evaluation of EPF incidence on the predators of *D. citri*

Breeding units of *C. externa* and *H. axyridis* were established in the Laboratorio de Entomología, Facultad de Agronomía-Universidad de la República, to supply individuals for the assays.

Effect of EPF on *H. axyridis*

Suspensions of spores of *B. bassiana* 2067 or *M. anisopliae* 2411 of 1×10^7 viable conidia/ml 0.02% (v/v) Tween 80 were applied on the insects. The control was carried out by applying only 0.02% (v/v) Tween 80. The individuals were placed separately in cages provided with specific food. The larvae were placed individually to avoid cannibalism. The bioassay was kept for 7 days under controlled conditions of temperature (24 ± 2 °C). Three replicates of each assay were performed. A total of 207 individuals of *H. axyridis* were analyzed, 41 larvae and 166 adults. The insects that died in the trial were placed in a humid chamber to observe fungal infection. If fungal mycelium was present, microscopic observation was performed to confirm the presence of EPF. To determine if there were significant differences in the mortality rates of *H. axyridis* between treatments, data were transformed as arcsen√x and Dunn's test was performed considering $\alpha = 0.05$

Effect of EPF on *C. externa*

Cages of *C. externa* larvae, with one individual per cage, were performed to avoid cannibalism. On the other hand, groups of adult individuals were placed in cages. In each cage, a wet cotton and yellow paper with eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) as food was placed. Suspensions of 1×10^7 viable conidia/ml 0.02% (v/v) Tween 80 of *B. bassiana* and *M. anisopliae* were applied on the larvae and adult individuals of *C. externa*. In control cages, only 0.02% (v/v) Tween 80 was applied on the insects. The bioassay was kept for 7 days under controlled conditions of temperature (24 ± 2 °C). Three replicates of each assay were performed. A total of 321 individuals of *C. externa* were analyzed, 183 larvae and 138 adults. Dead or alive individuals were counted daily, and dead insects were placed in a humid chamber to corroborate the fungal infection. To determine if there were significant differences in the mortality rates of *C. externa* between treatments, data were transformed as arcsen√x and a Dunn's test was performed considering $\alpha = 0.05$.

Effect of pesticides on selected EPF

The effect of copper oxychloride, abamectin, and mineral oil products, the most frequently used in citrus plantations, on conidial germination and mycelial growth, was evaluated. Three concentrations (350, 500, and 650), (1.75, 2.50, and 3.25), and (12.25, 17.50, and 22.75) for Abamectin (μl/l), copper oxychloride (g/l), and mineral oil (ml/l), respectively, were used, based on the concentration usually applied in the field and additionally + a concentration 30% high and 30% low.

Both copper oxychloride and mineral oil were incorporated into the PDA culture medium before autoclaving. Since abamectin is a very volatile compound, it was incorporated by filter-sterilization into the medium after the sterilization process. To determine the viability of the conidia in presence of copper oxychloride and abamectin, conidia germination was evaluated (germinated conidia/total conidia × 100). Viability with mineral oil was determined by means of colonies quantification after 3 days of incubation. The effect of pesticides on conidial germination was determined by plating 200 μl of a suspension of 1×10^3 conidia/ml 0.02% (v/v) Tween 80, onto 9-cm Petri dish diameter with 2% PDA culture medium (Oxoid^{TM}), which containing pesticides in the concentrations was indicated. Plates were incubated at 25 °C for 24 h in the dark. Four replicates were performed and 200 spores per plate were counted.

The effect of pesticides on mycelial growth was evaluated by placing a 0.7-cm disc with a mycelium 4 days old onto 9-cm Petri dish diameter with PDA solid medium (Oxoid^{TM}) containing pesticides in the concentrations

Table 1 Percentage of *Diaphorina citri* mortality under controlled conditions with different EPF

Treatments	Mortality (%)
Control	20.8 ± 8.7 **a**
***Beauveria bassiana* 2067**	85.9 ± 3.8 **b**
***B. bassiana* 2121**	84.8 ± 5.2 **b**
***Metarhizium anisopliae* 2411**	82.8 ± 10 **b**

Averages followed by different letters differ significantly ($p < 0.05$)

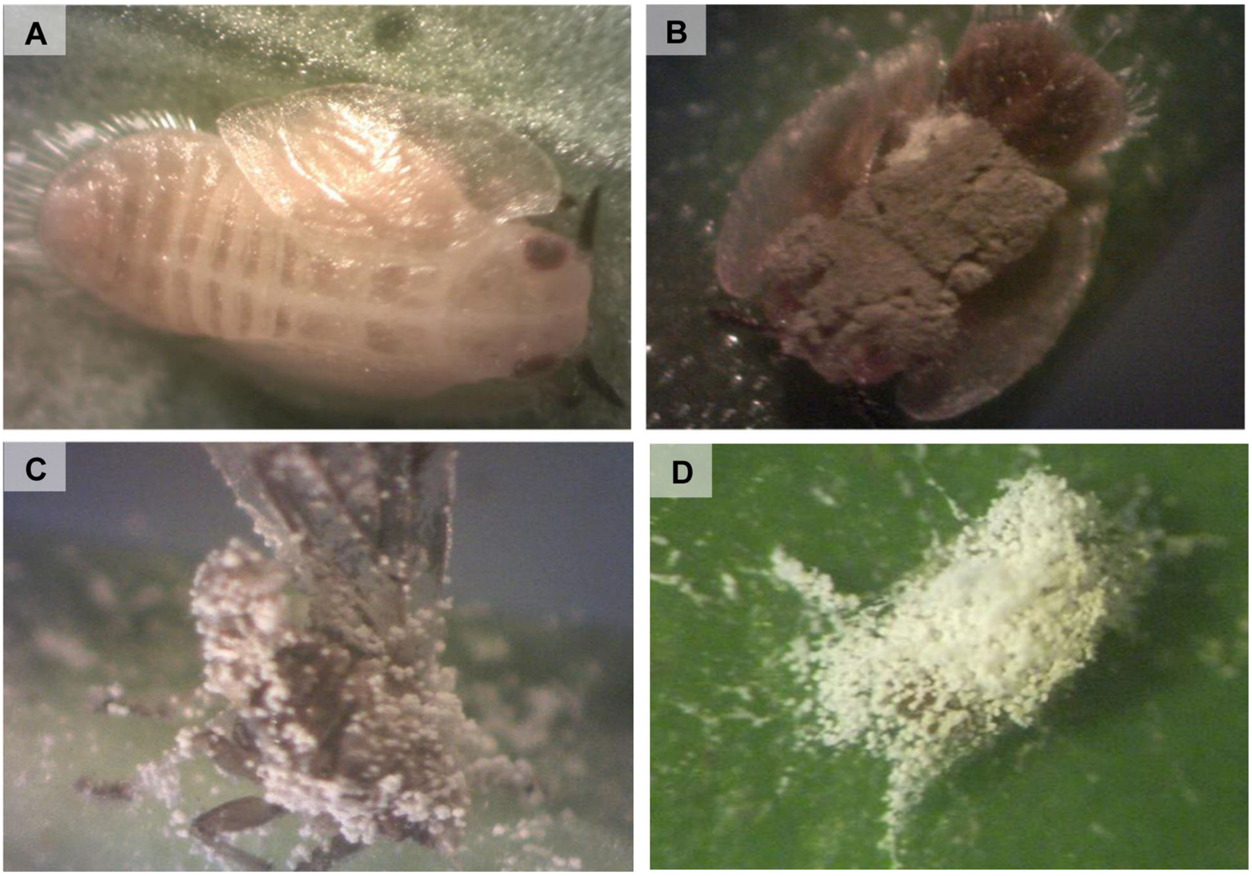

Fig. 1 Fungal infection of *Diaphorina citri*. **a** Control nymph of *Diaphorina citri*. **b** Nymph with *Metarhizium anisopliae* 2411. **c** Adult with *Beauveria bassiana* 2067. **d** Nymph with *B. bassiana* 2067

indicated. Inoculated media were incubated at 25 °C for 8 days, and the diameter of the colony was daily recorded. Four replicates of each assay were performed. Kruskal-Wallis Test (α = 0.05) was used to evaluate the effect of pesticides on conidial germination and mycelial growth.

Results

Under controlled conditions, the percentage of insect mortality produced after a single application of the EPF that evaluated on the 5th day and varied between 82.8 and 85.9% compared to 20.8% of mortality in the controls (Table 1).

Significant differences ($p < 0.05$) were found in insect mortality between the control and treatments of EPF. In contrast, non-significant difference was observed among the different fungal strains. The results indicated that the 3 fungi assayed showed biocontrol activity against *D. citri* in controlled conditions. The performance of the two strains of *B. bassiana* evaluated under controlled conditions was very similar. The strain (*B. bassiana* 2067) was selected for further assays. Under semi-field conditions, significant differences ($p < 0.05$) in insect mortality were observed between the control and the treatments with EPF. At assessment moment, development of the EPF was observed on *D. citri* nymphs and adults (Fig. 1). The infection and mortality of *D. citri* caused by the *M. anisopliae* 2411 strain was significantly higher than that of *B. bassiana* 2067, 78.9, and 51%, respectively (Table 2).

In all cases, an effect of temperature on the viability of the spores was observed. Significant differences ($p < 0.05$) were found in the viability of *B. bassiana* and *M. anisopliae* strains. *B. bassiana* 2067 had above 80% of viability between 15 and 30 °C (Table 3).

Table 2 Percentage of *Diaphorina citri* mortality with different treatments under semi-field conditions

Treatments	Mortality (%)
Control	12.3 ± 10.4 **a**
***Beauveria bassiana* 2067**	51.0 ± 17.2 **c**
***Metarhizium anisopliae* 2411**	78.9 ± 11.2 **b**

Averages followed by different letters differ significantly ($p < 0.05$)

Table 3 Spores viability (germination percentage) at different temperatures

Strain	Temperature (°C)				
	15	20	25	30	35
***Beauveria bassiana* 2067**	80.7 ± 6.7 **a**	97.5 ± 2.2 **b**	98.4 ± 1.1 **b**	99.3 ± 0.7 **b**	0.3 ± 0.7 **c**
***B. bassiana* 2121**	93.2 ± 2.1 **a**	92.9 ± 6.3 **a**	99.5 ± 0.6 **b**	99.8 ± 0.4 **b**	0.0 ± 0.0 **c**
***Metarhizium anisopliae* 2411**	0.0 ± 0.0 **a**	86.3 ± 5.5 **bc**	92.1 ± 3.1 **b**	75.5 ± 2.8 **c**	0.3 ± 0.6 **a**

Averages followed by different letters differ significantly ($p < 0.05$)

M. anisopliae spores, however, were not able to germinate at 15 °C, and their viability was reduced considerably at 30 °C. The spores of all strains evaluated were not viable at 35 °C.

Both strains of EPF (*B. bassiana* and *M. anisopliae*) had very low virulence against the predators. Non-significant effect of EPF on *H. axyridis* was observed. Fungi were able to infect adults, but the mortality rate was low. No effect of the fungi was observed on *H. axyridis* larvae when inoculated with *B. bassiana* or *M. anisopliae*, and it was even observed that they completed their cycle reaching the adult stage (Fig. 2). Although both fungi were pathogenic against *C. externa*, their virulence was low. Adult insects were more susceptible to *M. anisopliae* infection than larvae (Fig. 3).

All conventional pesticides showed a fungistatic effect on both fungi decreasing the viability of spores. Copper oxychloride affected the viability of the *B. bassiana* and *M. anisopliae* spores (Table 4). However, at the lowest concentration of copper, non-significant differences were observed in the viability (germination) of the conidia with respect to the control for either of the two strains.

Abamectin caused a decrease in spore germination of *M. anisopliae* and *B. bassiana* (Table 5).

The 3 concentrations showed significant differences in the viability of the *M. anisopliae* spores with respect to the control. Although the viability of *B. bassiana* spores remained above 90% in all treatments, the highest concentration of abamectin slightly decreased the viability of the spores showing, significant differences than the control. At any concentration of mineral oil, non-significant differences on spore viability, between control and *M. anisopliae* treatments (p = 0.959 > 0.05) or *B. bassiana* (p = 0.119 > 0.05), were observed (Table 6).

As shown in Figs. 4 and 5, all pesticides affected fungal growth, showing significant differences between the control and all treatments for *B. bassiana* and *M. anisopliae*. In both cases, copper oxychloride produced the greatest reduction in growth, while mineral oil had a low effect on mycelial growth. On the other hand, it was observed that abamectin significantly affected the growth of *B. bassiana* and *M. anisopliae*.

Discussion

Conidial formulations of *B. bassiana* and *M. anisopliae* were able to infect and kill *D. citri* with similar virulence under controlled conditions. However, *M. anisopliae* conidia were more virulent under semi-field conditions.

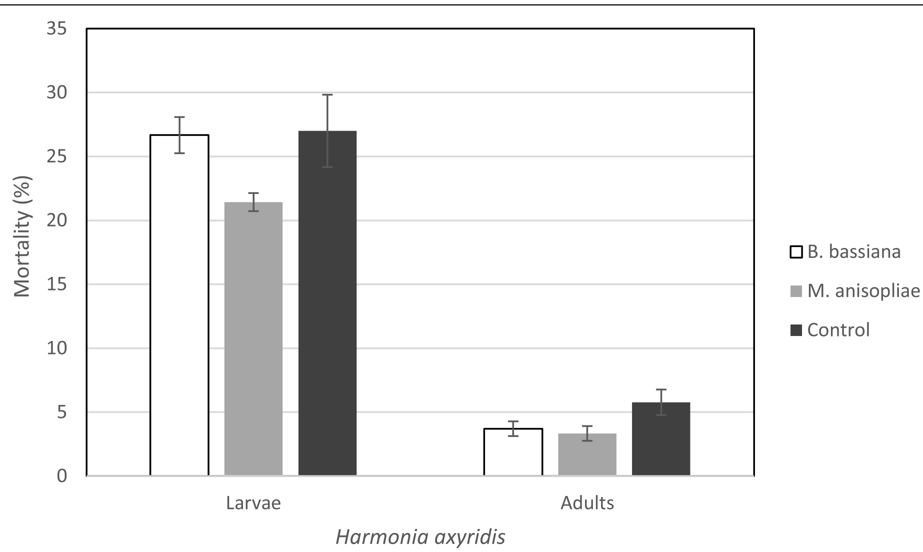

Fig. 2 Mortality percentage of larvae and adults of *Harmonia axyridis* with entomopathogenic fungi

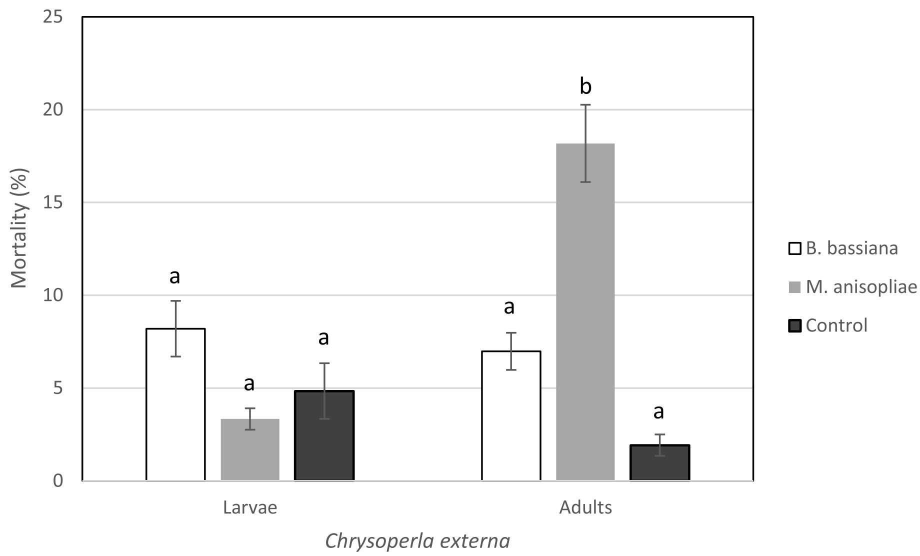

Fig. 3 Mortality percentage of larvae and adults of *Cycloneda externa* with entomopathogenic fungi

The highest virulence of *M. anisopliae* may be due to its pigmented conidia that offer its UV resistance or to the favorable environmental conditions for its development during the assay. In any case, both species showed high mortality rates of *D. citri* in a short time under laboratory and semi-field conditions, so that either one or both species could be used. To protect the conidia from the negative effects of UV radiation and dryness, a formulation could probably allow obtaining more successful results.

Lezama-Gutiérrez et al. (2012) and Ausique et al. (2017) reported high mortality rates of *D. citri* nymphs and adults under field conditions, using *M. anisopliae* and *B. bassiana*, and demonstrating their potential as a biological control agent. Although this study evaluated the direct effects (mortality) on *D. citri* individuals, indirect effects such as decreasing feeding activity could also occur, as reported by Avery et al. (2011). They suggested that when *I. fumosorosea* decreases the feeding activity of *D. citri*, adults may reduce the ability of HLB transmission. Obtained results showed that the spray application method was suitable, as found by Majeed et al. (2017). This could be due to the behavior of *D. citri* nymphs that aggregate in young shoots and have little movement in the plant. Consequently, nymphs become a good target for fungal conidia impact. Gregarious behavior can also facilitate the transmission of spores among individuals improving new infection events (Conceschi et al. 2016).

Both fungal species showed a high viability of the conidia between 20 and 30 °C, which could lead to a successful performance in nature. In this temperature range, infections with *D. citri* occurred mostly in the field. In addition, it is very important to consider the interaction of EPF and the predators of *D. citri* in order to minimize the impact on non-target species and maximize the mortality of *D. citri.* The obtained results showed that both strains of EPF (*B. bassiana* and *M. anisopliae*) had very low virulence against the natural predators of *D. citri*.

Similarly, several studies about the interactions between EPF and other natural enemies (parasitoids and predators) have been performed worldwide (Roy and Pell 2000; Bayissa et al. 2016). In particular, the interactions between coccinellid and lacewing species and the EPF have been studied. In the case of coccinellids, the most susceptible stage of infection by *B. bassiana* was the 1st larval instar, being mortality in the other

Table 4 Viability (germination percentage) of the spores according to the different treatments with copper oxychloride

Fungal species	Copper oxychloride concentration (g/l)			
	0	1.75	2.50	3.25
***Beauveria bassiana* 2067**	99.3 ± 0.6 **a**	98.8 ± 1.1 **a**	0.0 ± 0.0 **b**	0.0 ± 0.0 **b**
***Metarhizium anisopliae* 2411**	90.3 ± 2.2 **a**	88.9 ± 4.8 **a**	0.0 ± 0.0 **b**	0.0 ± 0.0 **b**

Averages followed by different letters in a row differ significantly ($p < 0.05$)

Table 5 Viability (germination percentage) of the spores according to the different treatments with abamectin

Fungal species	Abamectin concentration (μl/l)			
	0	350	500	650
***Beauveria bassiana* 2067**	99.2 ± 1.2 **a**	97.6 ± 0.4 **a**	96.9 ± 1.4 **a**	91.5 ± 1.6 **b**
***Metarhizium anisopliae* 2411**	96.3±0.6 **a**	64.9 ± 6.5 **b**	35.0 ± 5.7 **c**	23.8 ± 3.3 **c**

Averages followed by different letters in a row differ significantly ($p < 0.05$)

Table 6 Viability (colonies number) of the spores according to the different treatments with mineral oil

Fungal species	Mineral oil concentration (ml/l)			
	0	**12.25**	**17.5**	**22.75**
***Beauveria bassiana* 2067**	227 ± 10	236 ± 10	239 ± 11	217 ± 16
***Metarhizium anisopliae* 2411**	142 ± 16	143 ± 16	138 ± 17	141 ± 13

stages very low (Scorsetti et al. 2017). In the case of lacewings, Castro López and Martínez Osorio (2019) observed that the 1st instar *C. externa* of larvae were more susceptible to infection with *B. bassiana* and *M. anisopliae*, and the infection was very low in the other stages of development. The mean mortality rates observed were under 15 and 10% for *B. bassiana* and *M. anisopliae*, respectively. The results suggest that EPF and predators were compatible and that the simultaneous use of these biological control agents in an integrated pest management program is possible for *D. citri.*

The use of selected insecticides and fungicides for their application during the pre-harvest in citrus trees should be considered to determine the moment of application of the EPF. In Uruguay, copper oxychloride, mineral oil, and abamectin are applied for either orange or tangerine. In this study, an effect of these conventional pesticides on the development of fungi was observed; therefore, fungi and pesticides should be applied at different times, except mineral oil, which can be applied together with EPF.

Several EPF have been investigated for the control of *D. citri*. Among them, *I. fumosorosea* and *B. bassiana* have received the highest interest, with commercial products registered or in process, at several countries of the world (Gandarilla-Pacheco et al. 2013; Ausique et al. 2017).

The efficacy of certain fungal species against nymphs and adults of the Asian citrus psyllids has been reported. However, most of these studies were performed under laboratory conditions or only reported scattered results from field trials (Avery et al. 2011; Lezama-Gutiérrez et al. 2012; Stauderman et al. 2012).

Since in the present study, the successful use of *B. bassiana* 2067 and *M. anisopliae* 2411 to induce high levels of mortality of *D. citri* nymphs and adults under laboratory and semi-field conditions have been shown, it could be consider that it constitute an advance for the friendly environmental control of HLB.

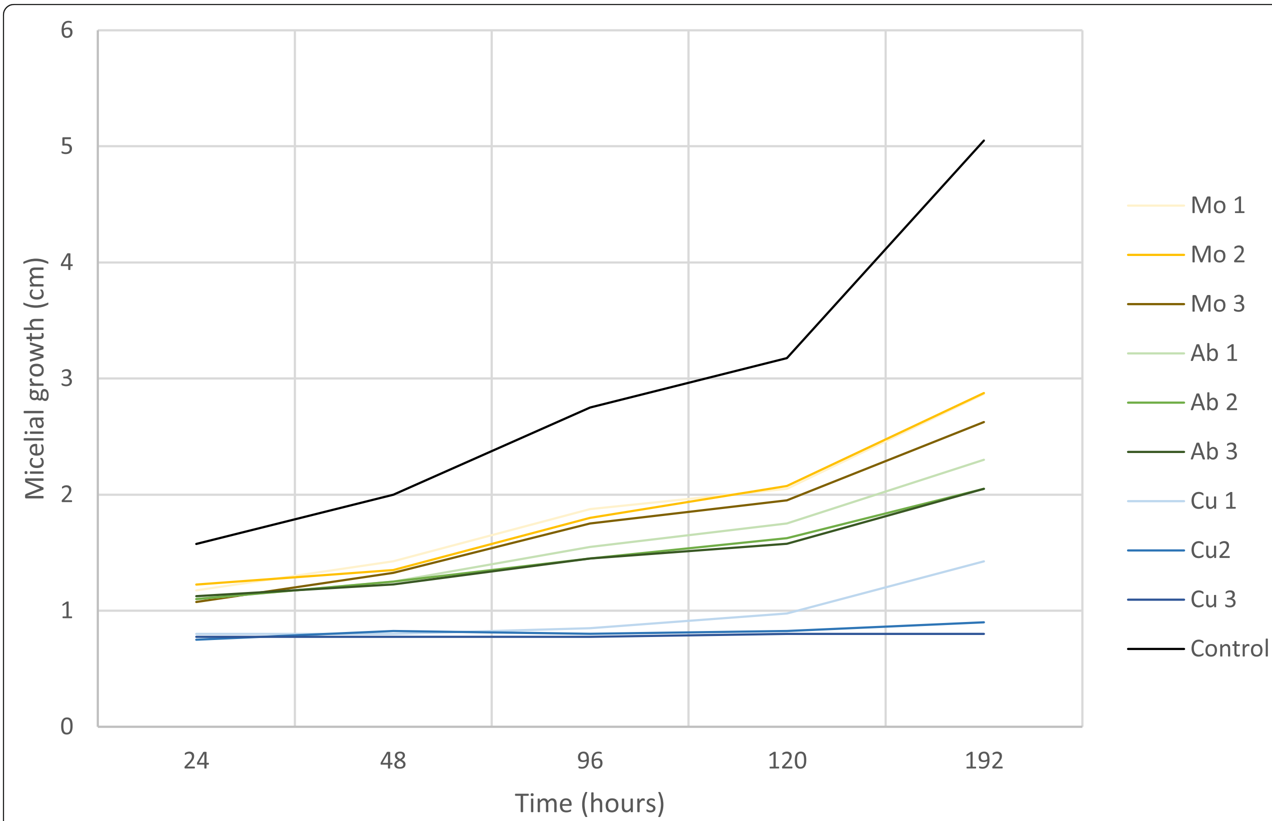

Fig. 4 Growth of *Beauveria bassiana* 2067 in media containing different concentrations of pesticides (Mo: mineral oil, Ab: abamectin, Cu: copper oxychloride). Mo1: 12.25 ml/l, Mo2: 17.5 ml/l, Mo3: 22.75 ml/l; Ab1: 350 μl/l, Ab2: 500 μl/l, Ab3: 650 μl/l; Cu1: 1.75 g/l, Cu2: 2.50 g/l, Cu3: 3.25 g/l

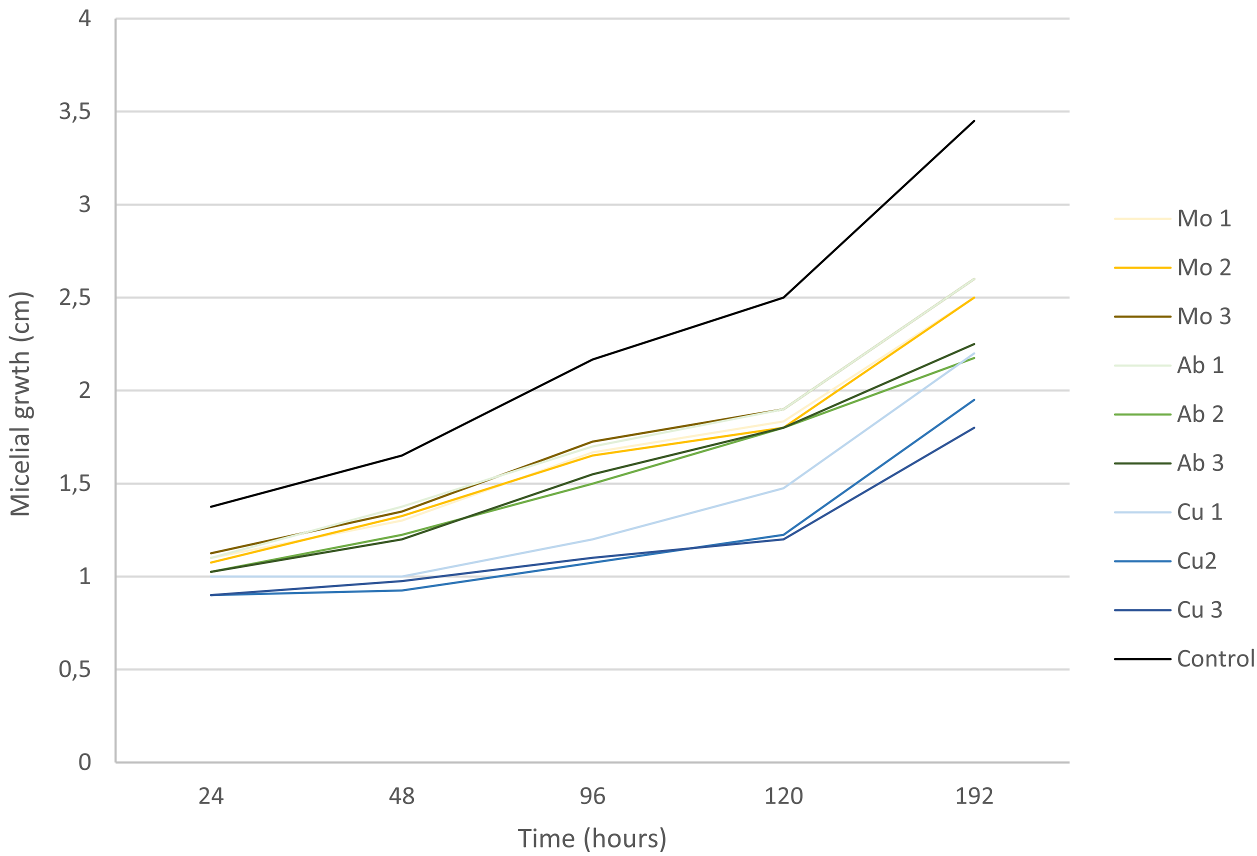

Fig. 5 Growth of *Metarhizium anisopliae* 2411 in media containing different concentrations of pesticides (Mo: mineral oil, Ab: abamectin, Cu: copper oxychloride). Mo1: 12.25 ml/l, Mo2: 17.5 ml/l, Mo3: 22.75 ml/l; Ab1: 350 μl/l, Ab2: 500 μl/l, Ab3: 650 μl/l; Cu1: 1.75 g/l, Cu2: 2.50 g/l, Cu3: 3.25 g/l

Conclusion

The strains of EPF *B. bassiana* (2067, 2121) and *M. anisopliae* (2411) showed promising results for their application as a bio-control agent against *D. citri.* Both strains of fungi showed also a very low virulence against the predators coccinellids and lacewings, and consequently, they could be used together in an integrated pest management program.

Abbreviations

HLB: Huanglongbing; EPF: Entomopathogenic fungi; PDA: Potato dextrose agar

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Authors' contributions

ABC and ST conceived the research. ABC, EP, and ST conducted the experiments. ABC, EP, LB, and ST contributed to the materials. ABC, LB, and ST wrote the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The data used and analyzed during this study is available from the corresponding author on request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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