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Biological Control of *Hemileia vastatrix* (Berk & Broome) using *Lecanicillium* spp in Coffee (*Coffea arábica* L) Cultivation

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Abstract **Original Research Article**

The objective of this research was to develop biological management options for coffee rust by evaluating native isolates of *Lecanicillium* spp. Under field conditions. The inoculum was obtained from hyper parasitized rust pustules and purified in potato dextrose agar culture medium and then massively multiplied for flooding applications in the field. Eight treatments were evaluated, six native isolates of *Lecanicilium* and two controls, one relative and one absolute. The trial was established in a randomized complete block experimental design with three replicates. The total area of the trial was 1 ha. Each subplot was of an approximate area of 100 m^2 containing 200 plants, in which 25 plants were sampled per treatment, in each plant 2 bandolas were sampled. The applications and data collection were carried out every 15 days where the variables of rust incidence, parasitism of *Lecanicillium* spp on rust and rust pustules parasitized by *Lecanicillium* were quantified. Variables were analyzed using the statistical analysis program SAS version 9.1, other variables were analyzed using the statistical program Infostat Version 2016e. Data were analyzed according to the design used (BCA) by analysis of variance (ANOVA) with a significance level of $p \leq 0.005$ when applicable, separation of means was used by Tukey's test (p=0.05). All native isolates of *Lecanicillium* spp caused parasitism on uredospore's achieving a decrease of the disease from45.87% to 18.41% in the evaluation period. The San Ramón isolate was the most promising for rust reduction during the 6 months of the study.

Keywords: Hyperparasite, biological control, uredospore's, parasitism.

INTRODUCTION

Hyper parasitic fungi are widely distributed in the environment in diverse ecosystems, where they play an important role in regulating pathogen populations (Infante *et al*., 2009).

Lecanicillium is considered a natural biological controller of plant pathogens and has a wide host range, including *Hemileia vastatrix* (Romero *et al*., 2023). It has been reported that coffee agricultural fields host high densities of *Lecanicillium* spp. compared to different ecosystems within the same region, such as forests and pastures, so they are frequently isolated from soil samples (Romero., 2020).

Coffee cultivation is limited by several factors that affect its production, among these factors are mainly soil and climatic conditions, as well as pest and disease problems; mainly rust (*H. vastatrix*). Worldwide, this disease is considered to be the most destructive of coffee plants because it causes the premature fall of leaves if there are severe infestations, leading to the reduction of photosynthetic capacity, as well as the weakening of diseased trees, thus predisposing them to the attack of other diseases; high severity can cause the regressive death of branches and even the death of the trees (Guharay, 2000). The fungus *Lecanicillium* spp. is one of the most common hyperparasites of coffee rust; it occurs naturally in coffee plantations and could be a good candidate for the biological control of coffee rust (Leguizamón *et al*., 1989).

The success of a biological pest control program based on biocontrol fungi depends on the production of inoculum for field applications, the selection of strains or isolates with high virulence, good growth, sporulation, and resistance to adverse environmental conditions (García *et al*., 2015), (Castillo-Arévalo, 2023). In the search for biological control agents, one of the basic strategies is the initial exploration of native natural antagonists, before

introducing exotic agents, since native strains are adapted to the environmental conditions of the area; unlike the strains of transient microorganisms, which due to their lack of adaptability may be ineffective in different agroecosystems (Castillo-Arévalo, 2021).

Nicaraguan farmers face many phytosanitary problems, according to De Araujo *et al*., (2015) and (Castillo-Arévalo, 2022) who make irrational use of pesticides, this has increased phytosanitary problems, among which environmental contamination, public health and pest resistance stand out, added to the interaction with environmental factors that are capable of accelerating losses due to pathogen reproduction. These effects have motivated the search for more environmentally compatible strategies to regulate these pathogens. Native isolates of *Lecanicillium* have been the most investigated recently (Rezende *et al*., 2015). Given the importance of coffee rust disease, the purpose of the present study is to contribute to the development of new alternatives for biological management of the disease using native isolates of *Lecanicillium* spp, under field conditions.

Among the biological alternatives to combat plant pathogens, fungi of the genus *Trichoderma* spp. and *Lecanicillium* spp. stand out (Romero *et al.*, 2023) and (Castillo-Arévalo, 2022).

MATERIAL AND METHODS

The study was conducted at La Hermandad farm, located in the municipality of San Ramón in the department of Matagalpa between the coordinates 12°57' north latitude and 85°48' west longitude with an altitude of 1056 meters above sea level, with rainfall ranging between 2,000 and 2,400 mm characterized by a good distribution throughout the year, the average temperature ranges between 22°C and 26°C. The study includes experimental research methods, where the parasitic capacity of 6 native isolates of *Lecanicillium* spp. on *H. vastatrix*, which causes coffee rust, was tested under field conditions through flooding applications to determine the behavior of rust incidence in 6 months of study. The management of the coffee plantation where the field phase was established was traditional (non-chemical).

For the collection of samples of *Lecanicillium* spp, three farms were selected in the department of Jinotega and one in the department of Matagalpa, where the samples were obtained, with a minimum of 50 leaves per sample. The variety of coffee trees where the samples were collected were Caturra and yellow Catuaí, with an age of approximately 10 years after the plantation was established (Table 1).

Table 1: Sample localities for *Lecanicillium* spp, in the period 2017-2018

Isolated	Geographical area	Farms	Crop control
Jinotega	Jinotega	Santa Martha	Traditional
Mankotal	Jinotega	Santa Elena	Traditional
Majada	Matagalpa	Santa Emilia	Conventional
Gotera	Matagalpa	Santa Emilia	Conventional
Granadillo	Matagalpa	Santa Emilia	Conventional
San Ramón	Jinotega	Linda vista	Conventional

Eight treatments were evaluated corresponding to the six isolates of *Lecanicillium* sp, a relative control consisting of copper sulfate pentahydrate at a dose of 1 ounce of product / 20 liters and an absolute control that was sprayed with water (Table 2).

Table 2

Control	Description
1. *Lecanicillium* spp	Isolated Jinotega
2. *Lecanicillium* spp	Isolated Mankotal
3. *Lecanicillium* spp	Isolated Majada
4. *Lecanicillium* spp	Isolated La gotera
5. *Lecanicillium* spp	Isolated EL Granadillo
6. *Lecanicillium* spp	Isolated San Ramón
7. cobre	Relative control (copper pentahydrate)
8. agua	Absolute control

The experiment was set up in a randomized complete block experimental design with three replications.

The total area of the trial was 1 ha. Each subplot had an area of approximately 100 m^2 and contained about 200 plants. The coffee trees were established under permanent shade and were managed in a traditional manner. The applications and data collection were carried out every 15 days where the corresponding variables were quantified. Foliar applications were made in the morning hours every 15 days, directed to the

underside of the leaves to achieve a good effectiveness of the hyperparasite on the rust pustules, since the mode of action of *Lecanicillium* spp. is contact. For the application, fungus was used in rice substrate, approximately 200 grams containing a concentration of $5x10^7$ conidia, for the application a premix was made by manually rubbing the substrate to detach the conidia, from the premix the final mixture was prepared, which was applied with a backpack pump of 20 liters, properly calibrated.

For data collection, five sampling sites were selected, composed of five plants each, for a total of 25 plants per plot. The number of leaves with rust and the number of pustules parasitized with *Lecanicillium* spp. were quantified in each band. Sampling was carried out every 15 days, starting 15 days after the first application.

The percentages of rust incidence and percentage of parasitism were calculated as follows (Monzón 1992).

$$\textbf{\textit{Incidence of coffee rust}}\ (\%) = \frac{\textbf{\textit{Number of leaves with coffee rust per branch}}}{\textbf{\textit{total number of leaves sampled per branch}}} \textbf{x100} \quad \ldots\ldots\ldots\ldots \textit{Equation 1}$$

$$\textbf{\textit{Parasitism incidence}}\ (\%) = \frac{\textbf{\textit{Number of leaves with rust parasitized by branch}}}{\textbf{\textit{Total number of leaves with rust by branch}}} \textbf{x100} \quad \ldots\ldots\ldots\ldots \textit{Equation 2}$$

$$\textbf{\textit{Parasitism on pustules}}\ (\%) = \frac{\textbf{\textit{Number of parasitized pustules on leaves}}}{\textbf{\textit{Total number of pustules}}} \textbf{x100} \quad \ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation 3}$$

The variables evaluated in this study were rust incidence, parasitism of *Lecanicillium* spp on leaf rust, as well as the number of parasitized pustules. The data obtained were organized in a database using Microsoft Excel software and then some variables were analyzed using the statistical analysis program SAS version 9.1, other variables were analyzed using the statistical program Infostat Version 2016. Data were analyzed according to the design used (BCA) by analysis of variance (ANDEVA) with a significance level of $p \leq 0.05$ when applicable, separation of means was used by Tukey's test ($p=0.05$).

The effect of native isolates of *Lecanicillium* sp on rust incidence was varied. Prior to the applications in the study, a preliminary sampling was carried out to determine the percentage of incidence in the area where the trial was established, and an incidence of 57.18% was found. The disease was present during the entire period of the trial. The analysis of variance performed indicated that there were statistical differences between sampling dates in rust incidence ($p=0.0001$), indicating that the effectiveness of the application of *Lecanicillium* spp isolates decreased the disease over time (Figure 1).

RESULTS

Coffee Rust Incidence

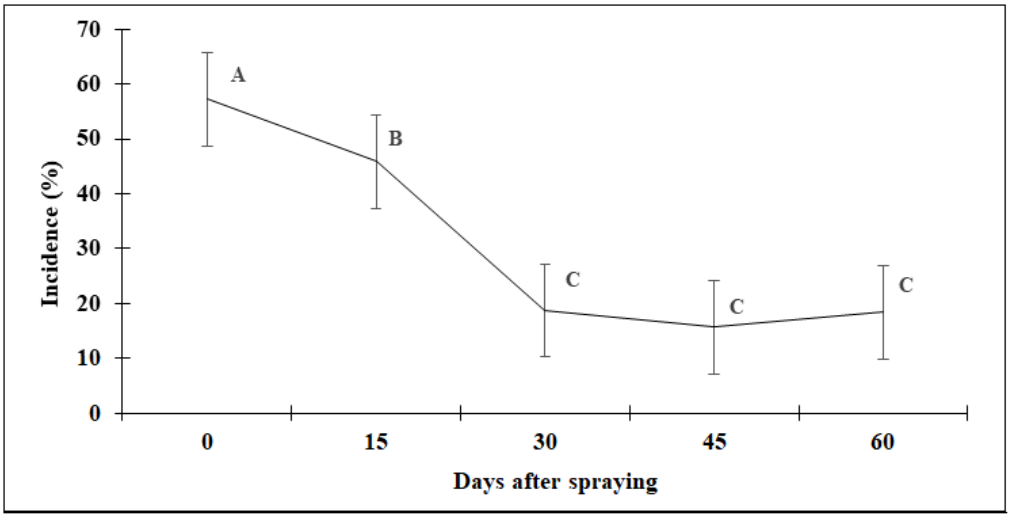

Figure 1: Incidence of rust on five dates on shaded coffee trees. Lines at the points indicate the standard error

The incidence of the disease had a decreasing behavior as the applications of the treatments were carried out. The highest incidence was 57.18% and was recorded before the applications, followed by the incidence recorded 15 days after the first application. After the second application the incidence was 45.87%, and in the subsequent samplings a similar behavior was maintained in the following applications. In the sampling carried out 60 days after the first application, the incidence was 18.41%, registering a slight increase with respect to the previous sampling, perhaps favored by the higher rainfall recorded at that time, in addition to the age of the coffee tree and the type of traditional management given to the plantation influenced the high incidence observed in general (Figure 1).

Parasitism of *Lecanicillium* spp isolates on *H. vastatrix* under field conditions.

Parasitism of native isolates of *Lecanicillium* spp on rust ranged from 21.47% to 11.54%. The San Ramon isolate presented the highest degree of parasitism. The analysis of variance performed indicates that there were statistical differences for the sampling dates on the parasitism of *Lecanicillium* sp on *H. vastatrix* (p=0.0001); it also indicates that there are statistical differences between treatments (p=0.0033), which indicates that the effectiveness of the application of native isolates of *Lecanicillium* spp significantly decreased the disease over time as the applications were made, also indicating that the treatments have different levels of parasitism on the disease. All isolates caused parasitism and were pathogenic to rust, but with different percentages of parasitism, they had different degrees of disease control. The San Ramón isolate presented the highest degree of parasitism of 21.47 % on leaves with rust; the other isolates were statistically similar. The treatment with copper pentahydrate (relative control) showed the lowest parasitism of 11.54% (Figure 2).

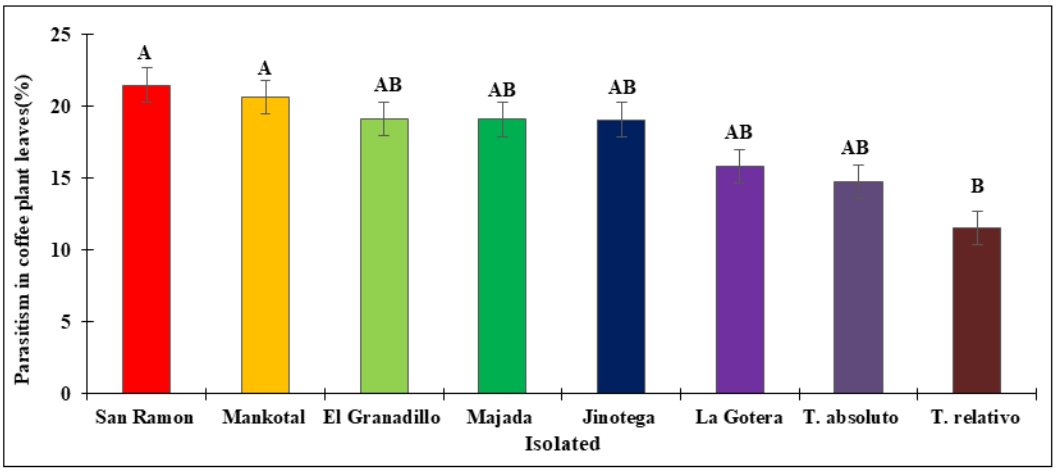

Figure 2: Percentage parasitism of *Lecancillium* spp. isolates on leaves. Lines above the bars indicate the standard error

Parasitism of native isolates of *Lecanicillium* spp. on *H. vastatrix* on different dates

In this study, the results on the behavior of parasitism percentages on leaves showed a decrease of 22.19% on the second evaluation date. After the second application, parasitism decreased to 13.45%; this decreasing behavior was observed on the first three dates; however, at 60 days there was an increase in the disease, which shows that as the incidence of the disease increases, parasitism on leaves also increases (Figure 3).

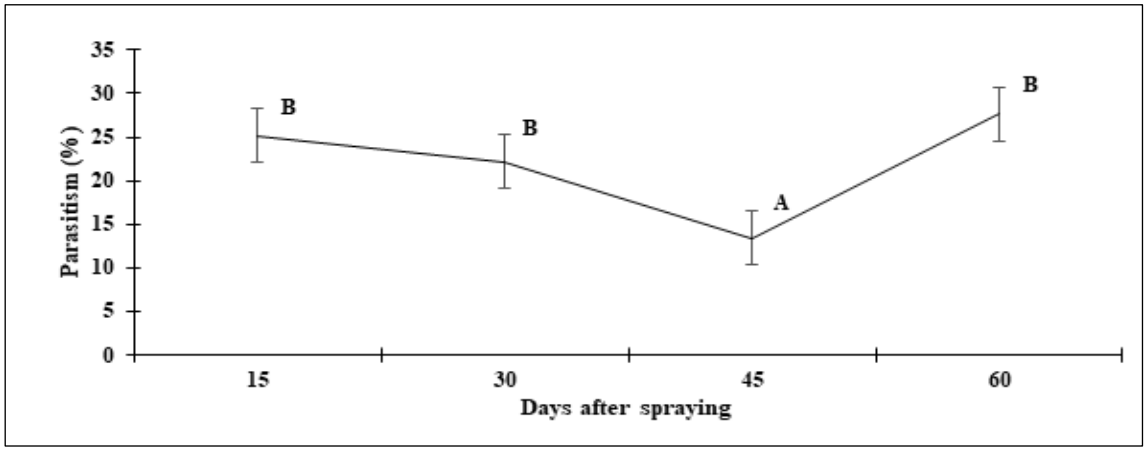

Figure 3: Incidence of parasitized pustules under field conditions by applications of native isolates of *Lecanicillium* spp with sampling and applications every 15 days. Dotted lines indicate standard error

Parasitism of *Lecanicilium* spp. isolates on pustules of *H. vastatrix*

The treatments that presented the highest parasitism were the San Ramon and Majada isolates with 15.83% and 14.69%, respectively, while the chemical treatment (copper pentahydrate) presented the lowest percentage of parasitism of 8.24%. The other treatments presented intermediate parasitism values ranging from 14.23% to 9.7% (Figure 4). The analysis of variance performed indicates that there were statistical differences for the sampling dates for parasitism on rust pustules (p: 0.0001); it also indicates that there are significant

differences among treatments (p:0.0029), indicating that the effect of isolates on incidence does not depend on the evaluation date (Figure 5).

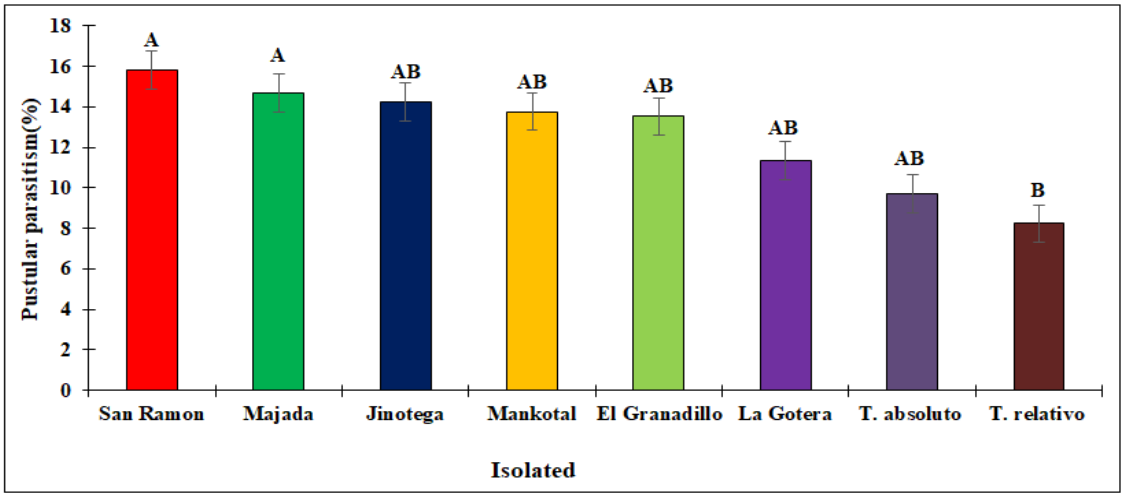

Figure 4: Parasitism of native isolates of *Lecanicillium* sp on *H. vastatrix* in pustules on different dates

Parasitism of *Lecanicilium* spp. isolates on pustules of *H. vastatrix* on different dates

Parasitism at 15 days after the first application was 24.5%. In the following dates the percentage of parasitism was reduced to 7.89%, which indicates that it had a downward behavior (Figure 5), however, at 60 days there was a slight increase possibly due to environmental conditions that favored the disease. parasitism presented a similar behavior to those found in leaf parasitism, as well as rust incidence on the dates evaluated Vargas, (2017) found this same phenomenon in applications of *Lecanicillium lecanii* in different concentrations in field conditions.

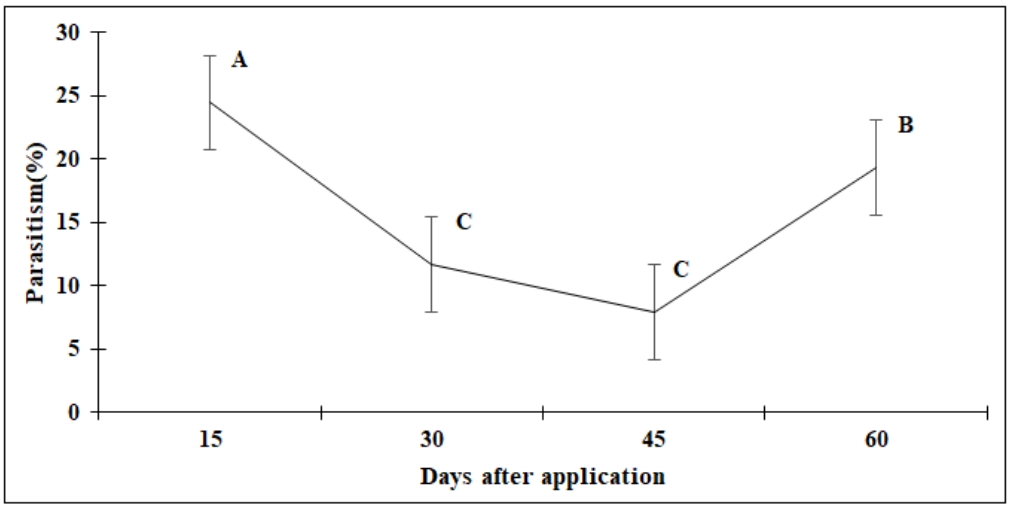

Figure 5: Parasitism of *Lecancillium* spp. isolates on pustules on different dates. Dotted lines indicate standard error

DISCUSSION

The effect of native isolates of *Lecanicillium* spp. on the incidence of rust in field conditions on the disease was significantly reduced from 57.18% to 18.41%, which corresponds to 45 days after the first application. In our study on the last sampling date there was a slight increase, possibly due to the traditional coffee management (non-chemical), these conditions benefit the establishment of the hyperparasite; the above shows that when a greater biological diversity is promoted, a greater ecological network is increased, which allows a balance between the different species (Jackson *et al.*, 2012). Vandermeer *et al.*, (2009) observed in a study a weak but statistically significant effect of hyper parasitic control of coffee rust at two different scales: in a plot of 45 ha and at a scale of approximately 10 m. in this study the indirect effect of anticoccidial mutualism was evaluated, where *L. lecanii*

was a coccidal parasite (Figure 1). Similar results were obtained by Vargas (2017) where he observed a decrease in rust incidence in evaluations following flooded field applications by *Lecanicillium lecanii*.

The percentage of parasitism of native isolates of *Lecanicillium* spp on *H. vastatrix* under field conditions was varied. The percentages of parasitism are closely related to disease incidence. The San Ramon isolate presented the highest levels of parasitism while the relative control presented the lowest percentage of parasitism as expected. It is important to note that *Lecanicillium* sp establishes itself on rust pustules, being an obligate hyperparasite, so the more rust present, the more likely it is to establish itself in the field (Monzón, 1992).

Parasitism of native isolates of *Lecanicillium* spp. on *H. vastatrix* on different dates had a downward behavior like the behavior of rust incidence, except that the values for this variable were lower due to the number of samples with which this variable was measured. The lowest percentage of parasitism was observed 45 days after the first application with 13.45% (Figure 3).

The San Ramón, Majada and Jinotega isolates presented average percentages higher than 14% of parasitism in pustules, the rest of the isolates remained below this value, including the two controls (Figure 4). In general, the average parasitism on pustules did not exceed 12.7%. Canjura *et al*., (2002) obtained similar results when they applied *Lecanicillium* to coffee plants established in pots, although they found no significant differences with respect to the control, the degree of parasitism remained at 10.5% in that study. Likewise, studies carried out by Monzon (2001) found similar results, where parasitism did not exceed 14% in parasitized pustules.

The highest percentages of parasitized pustules were found on the first and last dates, corresponding to 15 and 60 days after the application of *Lecanicillium* spp, due to the high incidence of rust on the first date (Figure 5). Similarly, at 60 days parasitism is increased by a slight increase in rust incidence. In this study it was observed that when *Lecanicillium* spp. were applied with high rust incidence, premature leaf drop occurred. The same phenomenon occurred in a study by Monzón (1992). Leguizamón *et al*., (1989) stated that leaves parasitized by this fungus often show necrosis and fall faster than those not parasitized and that some metabolites are produced because of the interaction of *H. vastatrix* and *Lecanicilium lecanii*, which causes leaf necrosis. Santiesteban *et al*., (2004) corroborate what was described by Leguizamón and collaborators where they affirm through a study that the fungus *Lecanicillium lecanii* (Verticillium) produces metabolites in submerged culture among which are proteases, lipases and antibiotics called Verticillium. It is important to emphasize that to the extent that the isolates present higher percentages of parasitism on rust pustules, the greater the potential for disease control, since the effect of *Lecanicillium* spp. on the disease is based mainly on the reduction of inoculum in subsequent epidemiological cycles.

CONCLUSION

Under field conditions, all native isolates of *Lecanicillium* spp. caused parasitism on uredospore's, achieving a reduction of the disease during the evaluation period. The San Ramón isolate was the most promising for rust reduction. The effect of *Lecanicillium* to reduce the inoculum on coffee rust is effective and it is important to be considered for future research where the use of *Lecanicillium* spp. is integrated as a tool within integrated pest management to reduce inoculum for subsequent cycles of the disease.

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