

Biological control of late leaf rust disease [*Pucciniastrum americanum* (Farl.) Arthur] in raspberry (*Rubus idaeus* L.) using two biological products: *Bacillus subtilis* (Fungizard®) and *Larrea tridentata* botanic extract (CleanCrop®) under greenhouse conditions

Control biológico de la roya tardía de la hoja [Pucciniastrum americanum (Farl.) Arthur] en frambuesa (Rubus idaeus L.) a través de dos productos biológicos: Bacillus subtilis (Fungizard®) y extracto botánico de Larrea tridentata (CleanCrop®) bajo condiciones de malla sombra

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ABSTRACT

This study aimed to evaluate the suitability of native *Trichoderma* strains for the biological control of Late Leaf Rust Disease (LLRD) in an organic *Rubus idaeus* plantation under greenhouse conditions. Four treatments were evaluated: T1) *Trichoderma* sp. strain Clombta; T2) *Trichoderma* sp. strain Chlorolota; T3) Co-application of both *Trichoderma* strains (Clombta + Chlorolota) and T4) Control, which consisted in the application of two biological products: *Bacillus subtilis* (Fungizard®) and *Larrea tridentata* botanic extract (CleanCrop®)]. Disease severity, disease severity index (DSI) and Area Under the Disease Progress Curve (AUDPC) were evaluated. The Co-application of *Trichoderma* sp. strain Chlorolota and *Trichoderma* sp. Clombta (from 43,44 to 35,73%) reduce the LLRD severity at the same level than the Control (from 44,61 to 34,33%). For DSI, *Trichoderma* sp. strain Chlorolota (64,13) and the Co-application (61,11) showed similar values than those from the Control (59,84). Co-application of both *Trichoderma* strains showed the lowest AUDPC (71,2), at the same level that the Control (68,7). However, *Trichoderma* sp. Chlorolota (72,1) achieved the same AUDPC that the Co-application. The use of *Trichoderma* sp. Chlorolota or its Co-application with *Trichoderma* sp. strain Clombta was able to reduce the LLRD in *R. idaeus*.

Key words: berries, incidence, organic production, foliar application, severity.

RESUMEN

El objetivo del presente estudio fue evaluar la idoneidad de cepas nativas de *Trichoderma* para el control biológico de la roya tardía de la hoja (RTH), en una plantación orgánica de *Rubus idaeus*, bajo condiciones de malla sombra. Se evaluaron cuatro tratamientos: T1) *Trichoderma* sp. cepa Clombta; T2) *Trichoderma* sp. cepa Chlorolota; T3) Co-aplicación de ambas cepas de *Trichoderma* (Clombta + Chlorolota) y T4) Control, el cual consistió en la aplicación de dos productos biológicos: *Bacillus subtilis* (Fungizard®) y extracto botánico de *Larrea tridentata* (CleanCrop®)]. Se evaluó la severidad de la enfermedad, el índice de severidad de la enfermedad (IS) y el área bajo la curva de progreso de la enfermedad (ABCPE). La co-aplicación de *Trichoderma* sp. cepas Chlorolota y Clombta (de 43,44 a 35,73%) redujo la severidad de RTH al mismo nivel que el Control (de 44,61 a 34,33%). Para el IS, *Trichoderma* sp. cepa Chlorolota (64,13) y la Co-aplicación (61,11) mostraron valores similares al Control (59,84). La co-aplicación de ambas cepas de *Trichoderma* obtuvo el menor ABCPE (71,2), al mismo nivel que el Control (68,7). Sin embargo, *Trichoderma* sp. Chlorolota (72,1) logró el mismo ABCPE que la Co-aplicación. El uso de *Trichoderma* sp. Chlorolota o su Co-aplicación con la cepa Clombta fueron capaces de reducir la RTH en *R. idaeus*.

Palabra clave: frutillas, incidencia, producción orgánica, aplicación foliar, severidad.

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Introduction

Red raspberry (*Rubus idaeus* L.) is cultivated in all continents, mainly North America (U.S.A. and México) and Eastern Europe. The world production is in continuous development, with a world production of 543,421 t year⁻¹. Red raspberry and other berries are appreciated by their high sensory value and nutritional compounds, since they are rich in phenolic compounds such as anthocyanins, ellagic acid and vitamin C (Giovanelli *et al.*, 2014).

Red raspberry production around the world is affected by many diseases, including white root rot (*Vararia* spp.), anthracnose (*Elsinoe veneta*, *Colletotrichum acutatum*, *Alternaria* spp.), spur blight (*Didymella appianata*), crown gall (*Agrobacterium tumefaciens*), verticilliosis (*Verticillium dahliae*), gray mold (*Botrytis cinerea*) and late leaf rust disease (*Pucciniastrum americanum*) (Pascoe *et al.*, 1984; Shioy *et al.*, 2010; Comeau *et al.*, 2012). This last disease is also called autumn rust, late raspberry rust and yellow late rust. This disease attacking cultivated red and purple raspberries and wild red genotypes (Nelson, 2011).

The main symptoms of leaf disease (LLRD) include small spots, which are initially yellow and turn brown in senescence. Highly susceptible crops are often reduced to leafless canes, where small, light yellow pustules appear on the underside of the infected leaves and light yellow powdery masses damage the fruit and cause early ripening and decay preventing it from being marketed (Nelson, 2011). The principal causal agents are *P. americanum* and *Pucciniastrum arcticum* (Lagerh.) Tranz. (Rebollar-Alviter *et al.*, 2001); according to Converse (1966) both fungus will not cross-infect their respective *Rubus* host. *Pucciniastrum americanum* infects canes, leaves, petioles and fruits at all stages of development, this situation that makes this uredinal the principal disease in *R. idaeus* production (Rebollar-Alviter *et al.*, 2001).

The control of LLRD in raspberry cultivation has been carried out by the use of chemical fungicides, principally by cupric molecules, which are costly and pollute the environment, creating risks to human health and affect the quality of the exportable fruit (Masson *et al.*, 2013). Actually, in Mexico the production of *R. idaeus* are in organic systems under screen- and greenhouse condition, this situation makes important the search of biological alternatives to control of LLRD. Several alternatives

have been sought to contribute in the control of leaf rust diseases, such as plant extracts, mineral oils (olive, citronella and green tea) and microorganism including bacteria an antagonist fungi (Borges-Pereira *et al.*, 2012; Shabana *et al.*, 2017).

The use of *Trichoderma* sp. to control fungal pathogens in plants is an alternative to explore against LLRD in raspberry plants. *Trichoderma* species are considerable commercial importance due to their ability to suppress many foliar plants pathogenic fungi such as *Botrytis cinerea*, *Phyllactinia corylea*, *Cladosporium fulvum*, amount others (Sawant, 2014; Prabhakaran *et al.*, 2015). The proposed mechanisms to explain the biocontrol ability of *Trichoderma* species are antibiosis, cell lysis, competition for nutrients and space, siderophore production and mycoparasitism (Ragi *et al.*, 2013). Therefore, the objective of this present study was to evaluate suitability of native *Trichoderma* strain for the biological control of late leaf rust disease in *R. idaeus* production under greenhouse conditions.

Materials and Methods

Fungal source

Two *Trichoderma* strains used in the present work were isolated using the soil particle washing technique; strain *Trichoderma* sp. "Clombta" was isolated from rhizospheric soil of *Cucumis melo* L. sampled in Armeria, Colima, Mexico (Location: 19° 01' 26.2 N", 103° 58' 24.1" W); and strain *Trichoderma* sp. "Chlorolota" was isolated from the rhizosphere of *Musa* sp. sampled in Coahuayana de Hidalgo, Michoacán, Mexico (Location: 18° 26' 02.1 N", 103° 04' 03.9" W). Both strains was maintained and reactivated in Potato Dextrose Agar (PDA) added with yeast (1%) and chloramphenicol (100 ppm), under laboratory conditions (25±1 °C, 75% relative humidity (RH) and 12:12 light: darkness) during the study.

Conidia massive production

Fungal strains were cultivated under solid state fermentation using rice grains. Previous sterilization the rice grains was washed with purified water three times and hydrated with a solution of chloramphenicol dissolved in methanol and diluted in water at 100 ppm. Then, 200 g of washed rice grains were placed in polyethylene plastic bags

(2 L of capacity) and autoclaved during 45 min at 120 °C and 15 psi, autoclaving was repeated three times in intervals of 12 hrs. Sterile rice bags were inoculated with 10 mL of a conidia suspension of *Trichoderma* strain at a concentration of 1×10^6 conidia mL⁻¹. Inoculated rice bags were deposited in an incubation room at 25 °C, 80% of RH, and 8:16 h L/D (artificial white light at 80 W). After the incubation time, the conidia were harvested from rice grains by centrifugation. Each rice bag was washed twice with 500 mL of distilled water with Tween 80® (0.1% v/v). Washing liquid and rice were filtered in sieves of 200 and 40 mesh. Filtered liquid (conidia) was centrifuged at $2200 \times g$ for 20 min and dried in darkness in a Class II Biosafety Cabinet (NU-425, NUAIRE, Minnesota, USA) for five days at 25 °C (Lezama-Gutiérrez *et al.*, 2012). Harvested conidia were maintained in darkness at 4 °C until utilized in the screenhouse trials.

Foliar *Trichoderma* application on *Rubus idaeus* plants under screenhouse conditions

Trichoderma conidia powder was applied in the mornings (before 7:00 hrs.) to avoid solar radiation, sprays were performed using a backpack sprayer pump (HYD20L-Ecomaqx®, Guadalajara, Jalisco, Mexico), which was calibrated at 1.5 L min⁻¹ and 110° of amplitude. Previous to the application, conidia powder was diluted and mixed in clean water added with soybean oil at 1% (v/v) as an organic surfactant, avoiding chemical compounds. Applications were made twice per week (Wednesday and Saturday) during 1,5 months. During that period, a two-sample monitoring session was carried out before the conidia applications. Applications were made during February and March of 2016; in that period, climatic variables such as temperature (Figure 1A and 1B) and relative humidity (Figure 1C) were monitored through a weather station (No. 766560, situated at latitude: 19,71°, length: -103,46° and altitude: 1507 m).

Trichoderma Treatments

Furrows of the experimental plot consisted of a distance of 44,1 m in length and 2,4 m of spacing between them, with a distance between plants of 0,5 m and with 93 plants per furrow. The evaluated treatments were: T1) the application of *Trichoderma* sp. strain Clombta (1×10^{13} conidias ha⁻¹); T2) the application of *Trichoderma* sp. strain Chlorolota

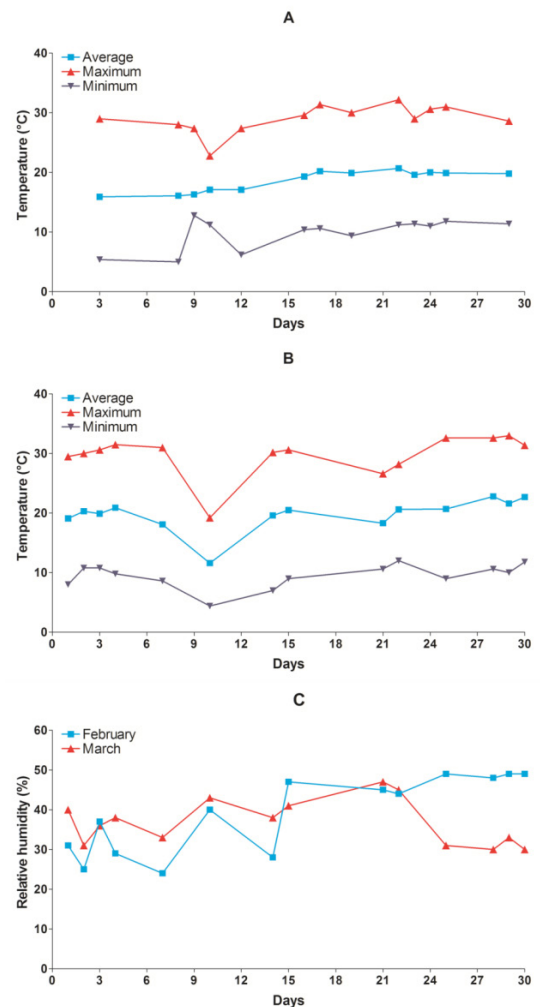


Figure 1. Temperatures in February (A), March (B), and relative humidity (C) values during the experiment.

(1×10^{13} conidias ha⁻¹); T3) the application of both *Trichoderma* strains (Clombta- Chlorolota, referred to as Co-application, at 1×10^{13} conidias ha⁻¹) and T4) Control, which consisted in the application of a mix of three commercial organic products: *Bacillus subtilis* (Fungizard®, 750 mL ha⁻¹) as an organic fungicide, *Larrea tridentata* as a botanic extract (CleanCrop®, 500 mL ha⁻¹) and a natural agricultural adjuvant derived from pine (Eco-Film®, 100 mL ha⁻¹). This used treatment as a control is the main method used by growers to manage LLRD. The experiment was carried out in an organic plantation; therefore, it was not possible to use a positive control (chemical fungicide) and a negative control (sterile water).

Development of the diagrammatic scale

Diagrammatic scales are adequate tools for epidemiology of plant diseases, the use of diagrammatic scale reduce the subjectivity of the visual estimation of disease severity, while their use is conventional to be easy and fast for wide range of conditions producing concrete, precise and reproducible results (Angelotti *et al.*, 2008; Michereff *et al.*, 2009). For developing the scale, 25 leaves of *R. idaeus* with different levels of late leaf rust ranging from minimum to maximum severity were collected in an organic orchard from "Berries Paradise S.A.P.I. de C.V." situated in Tuxpan, Jalisco, Mexico (Location: 19°53'15" N y 103°40'93" W). Leaves with other symptoms were not included in the analysis. Collected leaves were photographed with a digital camera (Sony® Cyber-shot Dsc-w290) at the same distance from the camera lens to the leaves (25 cm) and the images were used for assessing the percentage of leaf area effected by the late lead rust disease using the software ImagenJ® (Rasband, 2014). Figure 2 shows the diagrammatic scale with six severity levels ranging from 0 to 50 percentage of damage.

Response variables

Late leaf rust disease severity was evaluated using the diagrammatic severity scale (Figure 2); an initial evaluation was made before to apply the treatments. The monitoring was made regularly at intervals of four days. The Disease Severity Index (DSI) was calculated using the disease severity data obtained through the diagrammatic severity scale (Figure 2) and the following equation $IS = [\sum(n \times b) / (N \times B)] \times 100$, where n is the severity level, b is frequency of each level, N is the highest level of severity, and B is the total tested plants (Merchán-Gaitán *et al.*, 2014). Finally, the area under the disease progress curve (AUDPC) was calculated, the AUDPC is a useful quantitative summary of disease intensity over time, for comparison across years, locations, or management tactics; therefore, AUDPC was calculated using the equation 1 described by Shaner and Finney (1977), where Y_i is the disease level at time t_i and $t_{(i+1)} - t_i$ is the time (days) between two diseases scores.

$$AUDPC = \sum_{i=1}^n [(Y_{i+1} + Y_i)] [(t_{i+1} - t_i)] \quad \text{Equation 1}$$

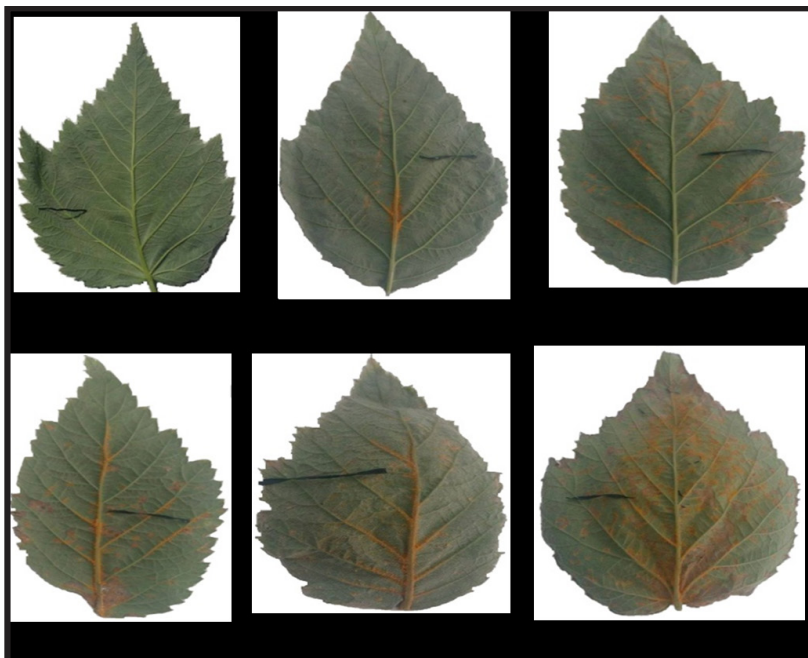


Figure 2. Diagrammatic scale of late leaf rust (*Pucciniastrum americanum*) in *Rubus idaeus* showing increasing percentage of affected leaf area-severity. The black line indicates the used scale (1 cm).

Experimental design and data analysis

A randomized complete block design with four treatments and three replicates was established. Each replicate consisted of three plants (experimental unit). The experiment was replicated twice at the same time in two different greenhouses located in the same experimental site. The severity of late leaf rusts, DSI and the AUDPC were analyzed by an analysis of variance and a multiple comparison test using the Least Significant Difference ($\alpha=0.05$), prior to a homoscedasticity test (Levene test $\alpha=0.05$). All tests were performed using StatGraphics Plus software.

Results

Late leaf rust severity

Table 1 shows the late leaf rust severity across the evaluation time (24 days). In the initial evaluation after the treatments applications, a significant difference ($F=6.41$, $P=0.0017$) in the late leaf rust severity was found between the treatments; application of *Trichoderma* sp. strain Clombta achieved the highest severity with 49.55%; while the application of *Trichoderma* sp. strain Chlorolota (46.05%) and the co-application (43.44%) of both strains showed the same lowest percentage of severity in comparison to the control (44.61%). At three days after application of the treatments, the co-application and the control showed the lowest severity with 43.17 and 41.78%,

respectively, while the single *Trichoderma* strains achieved the highest severity (*Trichoderma* sp. strain Clombta=49.55% and *Trichoderma* sp. strain Chlorolota=46.05%).

During the evaluations at 7, 10, 14 and 17 days, a significant difference between treatments was found. In these evaluations the lowest severity was found in the control and in the treatments: *Trichoderma* sp. strain Chlorolota and the co-application; in the control the severity ranged from 39.83% (7 days) to 44.77% (10 days), while in *Trichoderma* sp. strain Chlorolota and the co-application the severity ranged from 41.39% (7 days) to 46.16% (10 days) and from 41.44% (7 days) to 46.44% (10 days), respectively. By other hand, the treatment *Trichoderma* sp. strain Clombta achieved the highest severity, which ranged between 46.67 (17 days) and 49.00% (14 days). However, in the last two evaluations at 21 ($F=16.29$, $P=0.00001$) and 24 days ($F=11.26$, $P=0.00001$), the co-application and the control showed the lowest severity percentage of late leaf rust disease. At 21 days the co-application and the control achieved 38.61 and 38.39% of severity, respectively; while at the end of evaluation (24 days), the co-application and the control obtained 35.72 and 34.33% of severity. Regarding to the blocks of the experimental design, there were not found significant differences ($P>0.05$) during the first 17th days. However, in the last evaluation (21 days, $P=0.00001$ and 24 days, $P=0.00001$) the blocks showed significant differences, this differences could be attributed to the microclimate under the screenhouse conditions.

Table 1. Late leaf rust (*Pucciniastrum americanum*) severity (%) in *Rubus idaeus* under the application of *Trichoderma* strains.

Treatments	Evaluation period (days)							
	1	3	7	10	14	17	21	24
<i>Tri.</i> sp. Clombta (T1)	49.55 a	48.33 a	47.44 a	48.94 a	49.00 a	46.67 a	46.28 a	42.67 a
<i>Tri.</i> sp. Chlorolota (T2)	46.05 b	45.22 ab	41.39 b	46.16 b	43.83 b	41.83 b	43.55 a	39.78 a
Co-application (T3)	43.44 b	43.17 b	41.44 b	46.44 b	44.11 b	41.72 b	38.61 b	35.72 b
Control (T4)	44.61 b	41.78 b	39.83 b	44.77 b	42.27 b	40.05 b	38.39 b	34.33 b
<i>F</i> -test	6.41	5.54	6.33	4.06	8.10	13.87	16.29	11.26
<i>P</i> -value	0.0017	0.0038	0.0019	0.0156	0.0004	0.00001	0.00001	0.00001
Blocks								
B1	45.20	43.79	40.79	46.33	44.33	41.87	44.16 a	40.75 a
B2	45.37	44.79	4.20	46.16	44.45	42.16	40.08 b	36.58 b
B3	47.17	45.29	43.58	47.25	45.62	43.67	40.87 b	37.04 b
<i>F</i> -test	1.43	0.53	1.71	0.61	0.65	2.10	6.81	5.40
<i>P</i> -value	0.2540	0.5932	0.1974	0.5492	0.5306	0.1396	0.0037	0.0099

Disease severity index values in each column with different letters are significantly different from each another (DMS, $\alpha=0.05$, $n=3$).

Disease severity index

There were significant differences in the DSI of the late leaf rust during the evaluation time. In the first three evaluations, the application of *Trichoderma* strain Clombta did not reduce the DSI in the treated plants and achieved the highest values with 80.42 ($F=6.02$, $P=0.0025$), 81.11 ($F=10.85$, $P=0.00001$) and 81.98 ($F=23.13$, $P=0.00001$) at 1, 3 and 7 days, respectively (Figure 3). While *Trichoderma* strain Chlorolota (DSI ranged from 75.45 to 71.74), the co-application (DSI ranged from 73.49 to 72.77) and the control (DSI ranged from 71.64 to 70.00) reduced in a few amount the DSI, achieving significantly the lowest values in comparison to *Trichoderma* strain Clombta (Figure 3). At the fourth evaluation (10 days) a slight increase of the DSI in the treatments *Trichoderma* strain Clombta, co-application and the control was found, when was compared with the first evaluation; while *Trichoderma* sp. strain Chlorolota maintained its DSI. This behavior may be related with the decrease of the maximum temperature and the increase of the minimum temperature in the evaluation period (9th and 10th of February, Figure 1A), in addition, could be related to the increase of the relative humidity at the same date (Figure 1C).

During the 5th (14 days) and 6th (17 days) evaluation (Figure 3), the treatments co-application (14 days=70.05 and 17 days=68.13) and the control

(14 days=68.51 and days=67.10) achieved the lowest DSI [14 days: $F=41.30$, $P=0.00001$ and 17 days: $F=42.53$, $P=0.00001$). However, *Trichoderma* sp. strain Chlorolota (14 days=72.32 and days=70.04) statistically showed the same DSI in comparison to the co-application. By other hand, *Trichoderma* sp. strain Clombta achieved the highest values (14 days=81.75 and 17 days=79.28). At the penultimate evaluation (21 days), the treatments *Trichoderma* sp. strain Chlorolota (68.14) and the co-application (66.19) achieved statistically ($F=15.29$, $P=0.00001$) the same DSI in comparison to the control (63.96); however, the *Trichoderma* sp. strain Clombta obtained the highest value (77.72). Finally, in the evaluation at 24 days, the highest ($F=13.35$, $P=0.00001$) DSI was found in the treatment of *Trichoderma* sp. strain Clombta (76.03) as happened throughout the experiment; while the treatments *Trichoderma* sp. strain Chlorolota, co-application and control achieved low values of DSI with 64.13, 61.11 and 59.84 respectively (Figure 3). Regarding to the blocks of the experimental design, only two evaluations were found to be significantly different (21 days: $F=6.81$, $P=0.0037$ and 24 days: $F=5.40$, $P=0.0099$). These differences could be attributed to other environmental variables (intensity of light, evapotranspiration, humidity and other facts in to the screenhouse etc.) that were no taken into consideration for the present study.

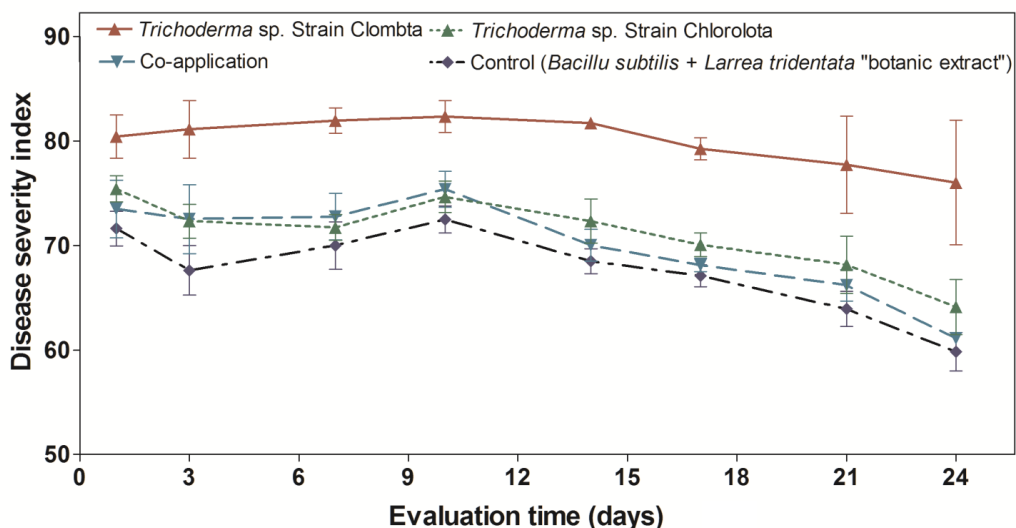


Figure 3. Disease severity index of late leaf rust in *Rubus idaeus* under the application of *Trichoderma* strains as biological control. Each point represents the average (\pm SEM) of at least three replications.

Area under the disease progress curve

The AUDPC was estimated with a trapezoidal method (equation 1), which discretize the time variable and calculate the mean disease intensity between each pair of adjacent time points. The result indicates that the control (a mix of *Bacillus subtilis* and *Larrea tridentata* “botanic extract”) achieved the lowest AUDPC with 68.7 (Figure 4); however, this value was statistically equal to the co-application of both strains (Clombta and Chlorolota) of *Trichoderma* sp. with a value of 71.2. The application of *Trichoderma* sp. strain Chlorolota (72.1) achieved statistically the same AUDPC with the co-application treatment, but it was different to the control. Otherwise, the application of *Trichoderma* sp. strain Clombta achieved the highest AUDPC value ($F=26.87$, $P=0.00001$) with 80.6.

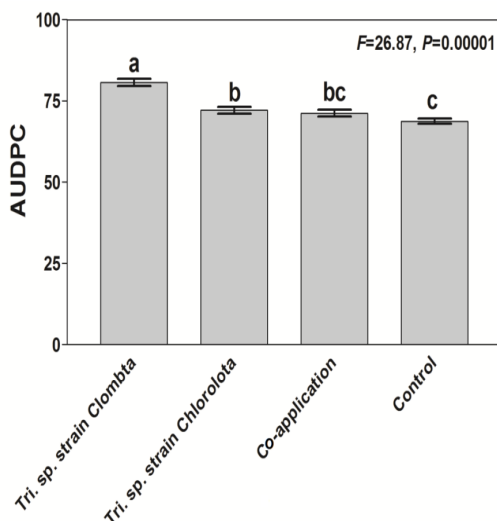


Figure 4. The total area under the disease progress curve (AUDPC) of late leaf rust (*Pucciniastrum americanum*) in *Rubus idaeus* under the application of *Trichoderma* strains as biological control. Means (\pm standard error) with different letters are significantly different from each another (MSD, $\alpha=0.05$, $n=3$).

Discussion

Late leaf rust disease is one of the most important plant diseases in the organic and conventional production of raspberry under greenhouse conditions. There is little information about this disease in *R. idaeus* production, according to Rebollar-Alviter *et al.* (2001); the causal agent of LLRD on *R. idaeus* in Mexican production is *Pucciniastrum americanum*. The epidemiology of LLRD was

described by Rebollar-Alviter *et al.* (2003), the authors suggested that the severity of LLRD is different according to the plant strata; basal (10-55%) and middle (2-60%) strata are more prone to the rust. Although in this study we did not evaluate the severity by strata, the severities found were less than 50% in all treatments, this can be due to the effect of the application of biological products (*Trichoderma* sp.).

In non-organic plantations of raspberry, the control of LLRD is based only on the application of chemical fungicides, in a previous study, Rebollar-Alviter *et al.* (2003) reported severities of LLRD in raspberry with values less than 20%, unlike of this study, the authors evaluated chemical fungicides to control LLRD; treatments with lower (<5.0%) severity were: Tebuconazole-copper, Triadimefon-anilazine and paraffinic oil, the latter is considered an agroecological insecticide; however, it is a byproduct of petroleum distillation. These severity values reported by Rebollar-Alviter *et al.* (2003) are low if we compared with that obtained in this study with the application of *Trichoderma* sp. strains. In addition, Rebollar-Alviter *et al.* (2003) reported less cumulative incidence for Triadimefon-anilazine (<140%) and for paraffin oil (<150%). We cannot make comparisons with the results of the present study, because different methodologies were used to determine the severity indexes.

For biological control of LLRD in other economically important crops is based principally on the use of botanic extracts; recently, Shabana *et al.* (2016) reported the inhibition of spore germination of *Puccinia triticina*, causal agent of the leaf rust disease in wheat, by the effect of plant extracts such as garlic (93.71%), clove (98.86%), white cedar (97.76%), Brazilian pepper (97.12%), neem (98.99%) among other extracts. In addition, the efficacy of plant extract on the leaf rust severity was evaluated, a substantial efficacy of 40.41% was found, when it was applied white cedar (Shabana *et al.*, 2017). In other study, Reiss and Jorgensen (2016) reported that the *Bacillus subtilis* strain QST713 is effective to reduce the severity of the yellow rust (*Puccinia striiformis* f. sp. *tritici*) in wheat, providing up to 60% of control.

Regarding to the AUDPC, Rebollar-Alviter *et al.* (2001) reported a higher value in three different strata of the raspberry plant, values were 121.81, 131.07 and 94.3 for the basal, medium and high strata, respectively, it should be mentioned that in such study, no control methods were evaluated, it was only the

epidemiological description of rust. In another study, Rebollar-Alviter *et al.* (2003) reported an adjusted AUDPC, the values were lower than those of the present study (between 2,8 and 19,51), and this is due to the evaluated products, which were chemical fungicides such as Tebuconazol-copper (2,85) and the paraffin oil (3,29). More recently, Reiss and Jorgensen (2016) reported that the application of *B. subtilis* (Serenade®, 2 L ha⁻¹) reduce the AUDPC at the same level that the application of prothioconazole (200 g of i. a. ha⁻¹), with values of 550 and 150 approximately.

In other phytopathogens and crops, the effectiveness of *Trichoderma* sp. to reduce SI and severity of plant diseases is well known. In this sense Yao *et al.* (2016) reported that *Trichoderma* sp. strain HNA14 reduces the SI of the late blight of the potato (*Phytophthora infestans*), in addition to increasing the fresh and dry weight of the plants.

The foliar application (phyllosphere and the fructosphere) of *Trichoderma* is exposed to the harsh gaseous atmosphere and faces sharp fluctuations in temperature and moist on leaf surface, vapor water pressure deficit, gases, air pollutants, wind, radiation, etc. These factors make the foliar environment very different from the soil environment and the epiphytic microbial populations differ substantially from the rhizosphere populations (Sawant, 2014). The spores of *Trichoderma* in the phyllosphere competes principally with bacteria and yeasts, these populations keep changing as a result of fluctuations in the physical, chemical and nutritional environment of the phyllosphere (Sylla *et*

al., 2013). However, the effectiveness of *Trichoderma* to reduce foliar plant pathogens has been demonstrated, i. e. Co-application of *Trichoderma harzianum* and *Trichoderma viride* was able to reduce severity damage of head blight (head scab) in wheat, when the AUDPC were 305,7 in the *Trichoderma* Co-application, while in the control (unsprayed plants) were 1047,5 (Panwar *et al.*, 2014). This study sets the background for future related works in order to test the studied *Trichoderma* strains (Clombta and Chlorolota), as well as to elaborate and evaluate the formulated products (liquids or wettable powders) of these native strains.

Conclusion

The co-application of two native *Trichoderma* strains (Clombta and Chlorolota) reduced the severity of the LLRD in *R. idaeus* at the same level that the application of two biological commercial products (Control: Fungizard®=*B. subtilis* and CleanCrop®=*L. tridentata* botanic extract). *Trichoderma* sp. Chlorolota and its co-application with *Trichoderma* sp. Clombta were able to reduce the severity index of LLRD at the same level that the Control. The Co-application of the two native *Trichoderma* strains and the Control achieved significantly the same and the lowest AUDPC of LLRD in *R. idaeus*. The foliar application of *Trichoderma* sp. Chlorolota or its co-application with *Trichoderma* sp. Clombta is an alternative to management of LLRD in the organic production of raspberry in greenhouse condition.

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