Investigation note

Effectiveness of fungicides and *Trichorderma* spp. for the control of *Lasiodiplodia* spp. in 'Persian' lemon orchards in Veracruz

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Abstract

The descending death of 'Persian' lemon (*Citrus latifolia* Tan.) caused by *Lasiodiplodia* spp., is a high-importance disease. The intensity of lemon pruning increases the susceptibility and annual loss of up to 35% of trees, resulting in 60% reduction in production. During 2016 and 2017, in the municipality of Papantla, Veracruz it was assessed the effectiveness of chemical and biological control against *Lasiodiplodia* spp., after pruning. A complete block design was performed at random; five treatments were applied: methyl thiophanate (1 g L^{-1}), thiabendazole (2.5 g L^{-1}), chlorothalonil (3 g L^{-1}), mancozeb (4 g L^{-1}), *Trichoderma harzianum* + *Trichoderma viridae* (20 ml L^{-1}) and a witness (without application). Two sprays were made aimed at trunk, branches and foliage up to drip point. The incidence and severity of the disease was evaluated every seven days after the first application. With the data, epidemiological models were adjusted and descriptive parameters such as: initial intensity, rate of increase and area under the disease progress curve were calculated. Methyl thiophanate fungicide was most effective in disease control, followed by thiabendazole.

Keywords: *Lasiodiplodia* spp., descending death, disease control.

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The lemon 'Persian' (*Citrus latifolia* Tan.) belongs to the family Rutaceae in Mexico the area with the highest production of this fruit is located in the Gulf (Mendoza-Tornez *et al*., 2016). Factors that reduce 'Persian' lemon production include various diseases, including sadness virus, exocortis, citrus greening disease, anthracnosis, descending death, gummosis among others. Gummosis damage in citrus fruits is caused by *Phythopthora* spp. For which the application of fungicides such as fosetyl-Al and metalaxyl is recommended (Jadeja *et al*., 2000; Raina, 2012; Acosta-Pérez *et al*., 2014).

However, in some cases, these applications do not have satisfactory results when controlling gummosis, because *Lasiodiplodia* spp., also induce gummosis in affected areas and are generally confused with *Phytophthora* spp. This situation occurs in the production areas of Veracruz, where production in 'Persian' lemon trees is reduced. In 2019 *L. pseudotheobromae*, *L. theobromae*, *L. brasiliense, L. subglobosa*, *L. citricola* and *L. iraniensis* were reported as the causal agents of descending death and canker in commercial 'Persian' lemon orchards in Puebla and Veracruz (Bautista-Cruz *et al*., 2019).

In 2019 in Morelos, it was reported to *L. citricola, L. theobromae and L. pseudotheobromae* affecting 'Persian' lemon (Valley- de la Paz *et al.*, 2019a). Also, Polanco-Florian *et al*. (2019) reported to *L. theobromae*, *Fomitopsis meliae* and *Eutrypella citricola* as causing the descending death in sweet orange from Nuevo Leon and Tamaulipas.

Fungi of the genus *Lasiodiplodia* are nonspecific phytopathogens, can survive as endophytes and as saprophytes under abiotic stress conditions. They develop in subtropical and tropical regions where they affect more than 1 100 trees, causing diseases such as descending death, gummosis and peduncle rot (McDonald *et al*., 2011; Al-Sadi *et al*., 2013; Coutinho *et al*., 2017). Also, *Lasiodiplodia* spp., has been reported inducing symptoms of descending death, gummosis and canker in a wide range of citrus species (Al-Sadi *et al*., 2013; Coutinho *et al*., 2017).

Lasiodiplodia spp., causes descending death and gummosis, characterized by necrosis of tree bark and wood, as well as canker on stems in 'Persian' lemon orchards reported in the state of Veracruz, in the region of Martínez de la Torre, Tlapacoyan and Papantla and in the state of Puebla in Acateno and Hueytamalco (Bautista-Cruz *et al*., 2019). These phytopathogens fungi decrease citrus productivity 60% and annual loss of trees can reach up to 35%. Some studies reported the effectiveness of fungicides such as methyl thiophanate for the control of the descending death of mango orchards caused by *Lasiodiplodia* spp. (Khanzada *et al*., 2005; Shahbaz *et al*., 2009; Naqvi *et al*., 2015).

Other studies reported the effectiveness of *Trichoderma* for the control of fungal diseases in citrus fruits (García *et al.*, 2011; El- Mohamedy *et al*., 2012). In the case of mango, it has been reported that the main access point of *Lasiodiplodia* spp., is through pruning wounds, damage caused by insects, branches broken by wind or oversupply of fruit (Sakadilis *et al*., 2011). In the crop of 'Persian' lemon intense pruning is carried out as part of the management of the crop, and this favors the incidence of gummosis, so it is necessary to protect the trees immediately after pruning. For this reason, the objective of this research was to evaluate the effectiveness of chemicals and biological products, after pruning, for the management of descending death in commercial 'Persian' lemon orchards.

It was selected a 'Persian' lemon orchard grafted in *Citrus volkameriana*, in the ejido of Insurgente Socialista, in the municipality of Papantla, state of Veracruz, Mexico and where previously six species of *Lasiodiplodia* were identified (Bautista-Cruz *et al*., 2019), lemon trees were eight years old and had planting distance of 5 x 5 m. In the selected orchard, during 2016 and 2017, the effectiveness of the products methyl thiophanate, thiabendazole, chlorothalonil, mancozeb was evaluated (Table 1), a product based on *Trichoderma harzianum* + *Trichoderma viridae* at a concentration of 8 x 10^8 conidia ml^{-1} and finally a control, where only water was applied, which gave a total of six treatments established in the field under a random full blocks design.

Table 1. Chemicals for the control of *Lasiodiplodia* spp. in 'Persian' lemon.

Active Ingredient	Mode of action	IA (%)	Dosage (g L^{-1})
Methyl thiophanate	Systemic	70	1
Thiabendazole	Systemic	60	2.5
Maconzeb	Contact	80	3
Chlorothalonil	Contact	75	4

Each treatment had six trees and from each tree two branches were pruned with an average diameter of 3.5 cm, leaving a final length of 20 cm and that were located at each cardinal point, giving a total of 48 sampling units.

After pruning the branches, treatments with an SR 420 motorized sprinkler (Sthil, Mexico) were applied to each lemon tree up to drip point to ensure product coverage. A second application was made 20 days after the first application. Seven weekly evaluations were conducted, which began after seven days of the first application of the treatments.

The variable response was the length of lesions of *Lasiodiplodia* on pruned branches. The measures obtained were used to characterize the severity of the disease. The evaluation data made adjustments to epidemiological models that described the development of the disease over time. With the help of the SAS version 9.2 statistical package (SAS, 2008), epidemiological models (Exponential, Monomolecular, Logistics and Gompertz) were analyzed.

The selection of the disease descriptor model was with the coefficient of determination (R^2). In addition, epidemic parameters such as initial severity of disease (Y_0), disease increase rate (% day) and area under the disease progression curve (ABCPE) (%-day) were determined using the trapezoidal integration method (Campbell and Madden, 1990).

To determine the effect of the treatments and compare each of the epidemiological parameters, a variance analysis was performed (α= 0.05). In addition, a comparison of means was performed using the significant minimum difference test (Fisher, α= 0.05). The SAS version 9.2 statistical package was used for all analyses. The treatments used slowed the progression of symptoms of descending death, while the witness trees presented an obvious development of the disease. The model that best described the epidemic was logistics ($y=1/ (1+ ((1-y_0)/y_0)*ex (-rL*t))$ (Table 2).

Table 2. Effect of treatments on the descriptive parameters of the development of *Lasiodiplodia* spp. in 'Persian' lemon.

Treatment	Y_0	rL	ABCPE
Methyl thiophanate	0.03008 B	0.04536 F	514.90461 F
Thiabendazole	0.030541 A	0.049764 E	4380.19606 E
Trichoderma spp.	0.022177 C	0.078434 D	15374.4392 D
Maconzeb	0.00871 D	0.114338 C	59421.3886 B
Chlorothalonil	0.00137 E	0.164006 B	37380.0589 C
Witness	0.000757 F	0.223954 A	336936.28 A

Y_0= initial intensity of the disease; rL= rate of increase of the disease (% day); ABCPE= area under the disease progress curve (%-day).

It was observed that in all treatments the initial intensity of the disease (Y_0) had some variations, indicating a lower initial intensity in the witness. This situation could have been due to the natural conditions under which the experiment was established, due in the field the variation of the inoculum could be due to the spatial distribution of the trees evaluated, in addition in this study there was no direct inoculation of *Lasiodiplodia* spp., but was waited for the infection to occur due to natural conditions considering the principle of contagion through inoculum in the soil and surrounding branches that had symptoms and spores that increased the initial intensity of the disease. However, with treatment applications the initial intensity of inoculum may be reduced to generate less development of the epidemic.

By performing a second application at 20 days, the reduction of the inoculum generated after the start of the experiment can be guaranteed, and this has a direct effect on the rate of increase (rL). Considering this, it can be indicated that the best product to control *Lasiodioplodia* spp., it is methyl thiophanate, as it reduced rL (0.04536%-day) and was observed a lower ABCPE (514.90461%-day) compared to other treatments which, as an integrative parameter in the description of disease behavior, provided a better understanding of the importance of reducing the rate of increase and its effect on the development of a disease.

Thiabendazole had control after methyl thiophanate, so they could be considered as an alternative to disease control. In the same sense, thiabendazole could be considered for a second application, as it has an effect on reducing the rate of increase, in this study presented an rL of 0.049764, which is similar to that presented by methyl thiophanate. The witness exhibited an increase rate of 0.223954%, which resulted in an ABCPE of 336936.28%-day, which described an obvious development of the epidemic.

The contact fungicides chlorothalonil and mancozeb had a higher rate of increase, 0.164006 and 0.114338%-day respectively, which resulted in a higher ABCPE, therefore, these fungicides should not be considered for the curative management of *Lasiodiplodia* spp., Valle-de la Paz *et al*. (2019b) report that isolated *L. theobromae* and *L. citricola* in 'Persian' lemon from Morelos, Mexico and under *in vitro* conditions, are sensitive to methyl thiophanate and thiabendazole, which is consistent with this research in field, while *in vitro* conditions they report good control with the use of *Trichoderma* but in field conditions there was no good control.

Similarly, Masood *et al*. (2014), reported high effectiveness for the control of descending death in mango crops in Pakistan, with the use of methyl thiophanate, by injection into xylem and foliar applications. On the other hand, Khanzada *et al*. (2005) found that carbendazim had a greater effect than methyl thiophanate, when evaluated in the same system. In the case of mango, injecting methyl thiophanate directly into xylem three months after starting treatment reduces defoliation in trees. However, injuries with the presence of gummosis cannot be eliminated.

Khanzada *et al*. (2005) when applying carbendazim, methyl thiophanate and fosetyl aluminum for the control of descending death of mango in Pakistan, they found acceptable effectiveness of methyl thiophanate in the control of this disease (only surpassed by carbendazim) and in addition to that, with this study, they managed to reduce fungal infection, suppressed the exudation of rubber, death and wilting of mango branches, in addition there was a significant increase in vegetative growth of the plants. In late orange was reported high effectiveness of thiabendazole for the control of *Guinardia citricarpa* (Yan *et al*., 2016).

On the other hand, in this sense, Tovar-Pedraza *et al*. (2013) managed to reduce 62% the incidence of descending death in the grafting phase in mamey sapote nurseries by treating immersion sticks with thiabendazole and mancozeb, they reported that thiabendazole showed greater effectiveness in disease control.

Likewise, Tovar-Pedraza *et al*. (2013) mention that the combination of fungicides and washing prevented *L. theobromae* infection during the mamey sapote grafting process. Shahbaz *et al*. (2009); Navqui *et al*. (2015) reported that under *in vitro* conditions methyl thiophanate has high effectiveness in the control of *Lasiodiplodia*. Wang *et al*. (2007) indicated that prochloraz, iprodione and tebuconazole were the most effective fungicides to inhibit the mycelial growth of *L. theobromae* in papaya, similarly Bester (2007) reported the use of prochloraz and tebuconazole for the control of *Lasiodiplodia* in grapes.

In this work *Trichoderma* did not have a good control, this may be because it was applied foliar form, compared to other researchers where they apply it in soil to control pathogens that affect root, in addition optimal conditions for the development of this control agent were not guaranteed (Harman *et al*., 2004; Infante *et al*., 2009; Martinez *et al*., 2013).

Some studies in 'Persian' lemon and tangerine indicated that *Trichoderma* spp., may have a similar effect as methyl thiophanate to control root rot caused by *Fusarium* (El-Mohamedy *et al.*, 2012; El-Mohamedy *et al*., 2013). In *in vitro* studies, Bhadra *et al*. (2015) reported high effectiveness of *Trichoderma viridae* in controlling *Lasiodiplodia theobromae.*

Considering the results obtained in this experiment, the use of *Trichoderma* for the control of *Lasiodiplodia* would not be recommended, because the infection is given in the branches and when the biological control agent is applied here, does not have all the necessary conditions to develop and be present for *Lasiodiplodia* control.

This research found that chlorothalonil had a better effect up to 28 days, because reduced the development of the disease more efficiently. After 28 days, this product loses its effectiveness, while methyl thiophanate has control throughout the evaluation time. Mancozeb was the least

effective fungicide in this experiment. In trees treated with methyl thiophanate, increased scarring of these lesions was observed followed by thiabendazole, *Trichoderma* spp., chlorothalonil and mancozeb, compared to untreated trees. However, after 45 days of the first application (ddpa), some of the lesions that were already healing, began to have little rubber runoff. In untreated trees, the disease increased over time, until some of the stumps collapsed.

In the study region it is observed that some practices such as conserving lemon on the tree until it increases its value cause the increase in incidence of *Lasiodiplodia* and peduncle rot. In addition, the mummified fruits and pruning remains left on the ground are source of inoculum, impacting the initial intensity of the epidemic, in addition to the rate of increase in tree reinfection cycles, to avoid this, it is advisable to carry out the necessary fungicide applications, in addition to removing pruning residues and avoid leaving the fruit in the trees for an unnecessary time, since it also has an impact on the increase in the susceptibility of the trees to *Lasiodiplodia* infection.

It is usually associated with *Phythopthora* spp., as the causal agent of citrus gummosis, and for its control metalaxyl and fosetyl-Al treatments are carried out (Farih *et al*.,1981; Jadeja *et al*., 2000 and Raina *et al*., 2012). In the study area of Martínez de la Torre, Tlapacoyan, Papantla, fosetyl-Al and metalaxyl are regularly applied for the control of gummosis, but there are no favorable results, since the incidence of gummosis in the orchards exceeded 92.5% and economic losses increase considerably. In this research, methyl thiophanate was the best product to control *Lasiodiplodia*, followed by thiabendazole.

Conclusions

The fungicide that had the best effectiveness in controlling the descending death of 'Persian' lemon in field conditions, was methyl thiophanate, followed by thiabendazole. Performing two fungicide applications significantly reduces descending death; however, it is suggested that in an integrated management of the 'Persian' lemon, to consider the climatic conditions in which pruning is carried out and the application to decrease the source of inoculum and the rate of increase, since in rainy season would increase incidence and severity of *Lasiodiplodia* spp.

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