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Evaluation of chemical resistance inducers in maradol papaya against *Phytophthora nicotianae* var. *parasitica*

Rodríguez-Falconi, Rebeca[1*]; **Ortiz-García, Carlos F.**[1*]; **Moscoso-Ramírez, Pedro A.**[1]; **Lagunez-Espinoza, Luz del C.**[1]; **Osorio-Acosta, Francisco**[2*]

[1] Área de Ciencia Vegetal. Colegio de Postgraduados Campus Tabasco. Periférico Carlos A. Molina, Km 3.5. Carret. Cárdenas-Huimanguillo. H. Cárdenas, Tab. C.P. 86500.
[2] Área de Ciencia Vegetal. Colegio de Postgraduados Campus Veracruz. Carr. Xalapa Veracruz km 88.5 Pazo de ovejas, Ver. C.P. 91700.
* Correspondence: cfortiz@colpos.mx; rebecarodriguezfalconi1986@hotmail.com

ABSTRACT

Objective: To test the efficiency of four chemical resistance inducers on Maradol papaya to reduce *Phytopthora nicotianae* var. *parasitica* infections in rainfed crops at Chontalpa, Tabasco, Mexico.

Design/methodology/approach: Three doses of four resistance inducers were tested on 60-day-old papaya plants in a greenhouse with a randomized design, with four replications and 10 plants as experimental plots. Three days after the inducers' application inoculations with mycelium discs were made, there were negative and positive control treatments to evaluating their efficiency by applying Abbott's formula.

Results: The four chemical inducers for resistance (sodium silicate (SS), potassium silicate (PS), potassium phosphite (PF) and acibenzolar-s-methyl (ASM)) were statistically different from the control ($P<0.0001$**). The inducers SS 1%, PS 1%, FP 0.35% and ASM 0.1 mM showed higher effectiveness (81.2, 75.9, 74.7 and 74.0 %).

Study limitations/implications: The retained effective concentrations were tested in a single application, and their durability is unknown, so this point should be broadened. however, it may be an alternative for repeated use after transplanting.

Findings/conclusions: Optimal concentrations of SS, PS, FP, and AMS, that respond against *P. nicotianae* var. *parasitica* infections can reduce damages in rainfed crops.

Keywords: *Carica papaya*, root rot, *Phytopththora* n. var. *parasitica*.

INTRODUCTION

Papaya root rot (*Carica papaya* L.) is caused by different *Phytophthora* species and is worldwide recognized as one of the most important diseases of this crop, as it can occur at any stage of development, from seedling to harvest (Vázquez *et al.*, 2010). This disease was first reported in 1916 in the Philippines, later in 1924 in Ceylon, and then after in other countries such as Malaysia, Hawaii, Australia, Brazil, Spain and Taiwan caused by *P. palmivora* (Ho, 1990). Nevertheless, in India, *P. nicotianae*. var *parasitic* was reported as the main causal agent of papaya fruit and root rot (Sukhada *et al.*, 2011).

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In Mexico, in 1976, Garcia-Alvarez reported the presence of root and stem base rot caused by *P. parasitica* in Colima, Guerrero, and San Luis Potosi. Likewise, in Tabasco, Saldaña *et al.* (1986), informed the presence of root rot in papaya caused by *Phytophthora* sp. It was not until 2002 that *P. nicotianae* var. *parasitica* was confirmed as the causal agent of the papaya foot rot. Also, Fernández-Pavía *et al.* (2015) included reports of *Phytophthora parasitica* as the causal agent of root and stem base rot on a report of plant species diseases in Mexico (Fernández-Pavía *et al.*, 2015). Vázquez *et al.* (2010) reported the presence of *P. palmivora* at the Huasteca region as the responsible for stem and root rot caused in papaya; although this species is not found in Mexico and is included in the list of pathogens under phytosanitary surveillance (SENASICA, 2016).

In Tabasco, papaya is grown in rainfed conditions, on flatlands with no drainage which is considered as of low technology; using direct sowing or seedlings transplanting, manual weeding control, and a limited application of pest management and disease control (Guzmán *et al.*, 2009). Therefore, during rains, soils are easily saturated and waterlogged, which favors the root rot incidence by up to 11% (Saldaña, 2002).

Currently, there are control alternatives, such as the induction of systemic resistance, which involves activating plants' natural defense mechanisms through chemical inducers of resistance (Ozeretskovskaya and Vasyukova, 2002), by the pathogens themselves, other organisms, or environmental factors (Park and Paek, 2007). The papaya crop grown in rainfed conditions does not have sustainable management strategies to reduce root damage caused by *Phytophthora*, and its cultivation process demands using chemical products with low toxicity for mammals and is environmentally friendly. Under this premise, the present research was conducted to assess the effectiveness of chemical inducers for resistance to *Phytophthora nicotianae* var. *parasitica*, in transplanted (60 days) Maradol papaya.

MATERIALS AND METHODS

Isolation. From November to January, four papaya plantations were located in the municipalities of Cunduacán and Huimanguillo. From these municipalities comes the CPA1504 strain of *P. nicotianae* var. *parasitic*, isolated from Maradol papaya plants with root rot (Figure 1), proven as its causal agent at the Chontalpa subregion, Tabasco (Rodríguez, 2017).

Production of papaya seedlings in greenhouses. The production of papaya seedlings followed the techniques by Rodríguez and Cruz (2003). Plant emergence was carried out in germination trays with 72 wells, filled with a substrate (COSMOPEAT® COSMOCEL) sterilized (autoclave at 15 lb per half hour) two days before used and kept cold; one seed was sown in each well at a one-centimeter depth. The germination trays were protected from the sun, under partial shade, keeping a constant substrate humidity; seedling emergence began after three days.

***In vivo* test effectiveness of the resistance inducers**. The experiment was conducted in a greenhouse, on Maradol papaya seedlings, 60 d of growth. Four chemical resistance inducers (RI) and three doses of each, were tested: sodium silicate ([Silicatos y Derivados S.A de C.V. A subsidiary of PQ Corporation, density of 1.5 kg/L] were 0.1, 0.75 and 0.5 % [SS]), potassium silicate ([Silicatos y Derivados S. A de C.V. A subsidiary

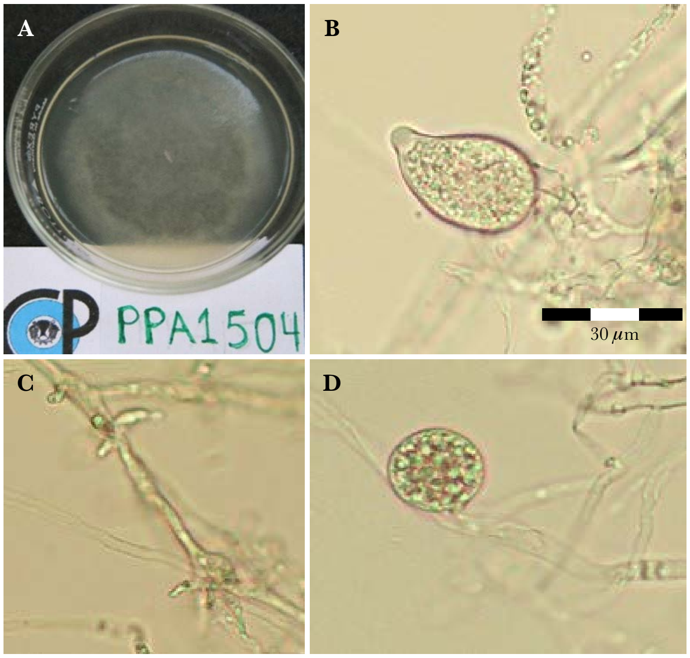

Figure 1. A) General aspect of the CPA1504 colony (*P. nicotianae* var. *parasitica*) 4 days after seeding. B) globose sporangium, limoniform with large papilla C) branched mycelium, turulose and swollen hyphae. D) terminal chlamydospore.

of PQ Corporation, Sil-MATRIX®, 29.1 % a.i.] were 1.25, 1 and 0.75 % [SP]), potassium phosphite ([ALIETTE® WDG, Bayer: FOSETIL-Al 80%. GD]were 0.35, 0.30 and 0.25 % [FP]) and acibenzolar-s-methyl ([Syngenta, Actigard® 50 GS] were 1, 0.1 and 0.01 mM [AM]). In addition, a negative control treatment (papaya seedlings sprayed with sterile distilled water, without inoculation [Te]) and a positive control treatment (papaya seedlings artificially inoculated with *Phytophthora* sp. [Te-]) were included.

The experiment was established with 14 treatments, and four replications, in a randomized complete experimental block design, using sets of 15 papaya plants as the experimental units. The treatments were applied by spraying the plants with a manual atomizer, until they reached dew point, three days before the artificial inoculation. The treated plants, before inoculation, were placed in a humid chamber equipped with a Vitalys^{plus} ultrasonic humidifier. Mycelial discs from the CPA1504 pathogen strain were used for artificial inoculation (2.5 mm in diameter) at the base of the papaya seedling stems, placed in a humid chamber, maintaining the humidity for four consecutive days.

Study variables. Daily, for a 10 d period, the number of dead plants was counted and their survivorship and dead percentages, average days to death per treatment, and the efficacy of the inductor were calculated, using the Abbott formulas (1925) corrected by Rosenhein and Hoy (1987).

$$Pcorr = \left(\frac{Psti - Pste}{1 - Pste} \right)$$

Where: *Pcorr* (corrected survival percentage), *Psti* (survival percentage of treatment with inducer), *Pste* (survival percentage of the positive control).

Statistical analysis. Survival percentage data were transformed to Arcosine $\sqrt{Y}$, prior to performing an ANOVA with the SAS V9 statistical software. Statistical significance was considered at a p=0.05 level. Tukey's test was used to separate means. The values shown are untransformed means. Covariance tests between survival and height, survival, and leaf area were also performed in the same statistical software.

RESULTS AND DISCUSSION

Table 1 shows that the four evaluated resistance inducers were effective and responded, as indicated by Walter *et al.* (2005), who states that, induced resistance being of broad-spectrum, should not always be expected to completely control the infection. Pointing out that the efficacy of chemical inducers on the causal agent of wheat blight disease varied from 20 to 85%. They emphasize that, when the pathogen is highly aggressive, as is the wheat blight case, resistance inducers such as SS and FP did not reduce the disease severity, as they had done in a previous cycle with a less aggressive version of the disease (Pagani *et al.*, 2014).

Overall, in this research, all treatments with chemical inducers for resistance were significantly effective (37-81% effectiveness) compared to the negative control, where

Table 1. Chemical resistance inducers effect on root rot in 'Maradol' papaya plants.

Treatment*		**Survival %**	**Efficacy %**	**Days of dead plants**
Inducers	**Doses**			
SS$_1$	1.00%^{x}	82.17 ab	81.26	8
SS$_2$	0.75%	45.72 b	42.95	5
SS$_3$	0.50%	71.79 ab	70.35	7
SP$_1$	1.25%^{x}	76.22 ab	75.01	8
SPi$_2$	1%	77.12 ab	75.96	7
SPi$_3$	0.75%	71.90 ab	70.47	6
FP$_1$	0.35%^{x}	75.92 ab	74.70	6
FP$_2$	0.30%	53.38 b	51.00	7
FP$_3$	0.25%	64.68 ab	62.88	7
AM$_1$	1mM^x	60.65 ab	58.64	7
AM$_2$	0.1mM	75.28 ab	74.02	7
AM$_3$	0.01mM	40.82 b	37.81	8
Te	0	95.15 a	94.90	0
Te-	0	0.00 c	0.00	5

*Inductors: SS (sodium silicate), PS (potassium silicate), PF (potassium phosphite), AM (acibenzolar-S-methyl), Te (uninoculated control and Te- (negative control). X percentage weight/volume ratio. Groups (a, b, ab and c), equal letters are not significantly different between treatments (Tukey $\alpha=0.05$). The arcsine transformation was applied to the survival percentage values. Untransformed means are shown.

100% of dead plants were quantified in a five-day period. The 1% sodium silicate treatment was the most effective (81%) and delayed the first dead plants eight days (Table 1). Likewise, the 1% dose of the three tested SS treatments was also the most effective against the root rot, suggesting that SS probably acts as a plant growth regulator which is effective at optimal doses, contrary to conventional fungicides, which increase their effectiveness as the dose increase. This result concurs with those by Li *et al.* (2012), who used 100 mM SS, on the postharvest to induce resistance in melon (*Cucumis melon*) to *Trichothecium roseum*, which reduced the diameter of lesions during storage. Additionally, Moscoso-Ramírez and Palou (2013) reported a SS 90% efficacy when used at 1000 mM to green and blue rots in 'Valencia' oranges; however, they did not recommend using it, due to the presence of phytotoxicity in the fruit rind during the postharvest at that dose. It is worth pointing out that, in the above studies, higher SS concentrations than those used in this study were used (1%).

Potassium silicate at a 1% concentration was the second-best chemical inducer of resistance in this research, with a 76% effectiveness. In the three tested concentrations, it had a stable effect on the plants, with a survival range of 71 to 77% (Figure 2) and an efficiency of 70 to 76%; although there were no significant differences among them. Likewise, this chemical inducer showed a mean number of dead plants up until the 7th day. Reports mention that PS affects *Sphaerotheca fuliginea* applied on cucumber plants at Culiacán, Sinaloa, with 9.45 g L^{-1} and 18.90 g L^{-1} doses, the latter with a 96.4% efficacy, although this dose showed phytotoxicity in the crop (Pérez-Angel *et al.*, 2010). Nevertheless, Ramírez *et al.* (2013) reported that neither SP nor FP had a detrimental effect on *P. cinamomi* applied on avocado, regarding other organic practices.

Papaya plants treated with FP had a 53 to 76% survival rate (Figure 2), the 0.35% dose being the best, with a 75% effectiveness. The 0.30% dose showed lower effectiveness, 51%. Whereas the minimum dose treatment showed 0.25% and 64.6% effectiveness, with an average death of the first plants within seven days.

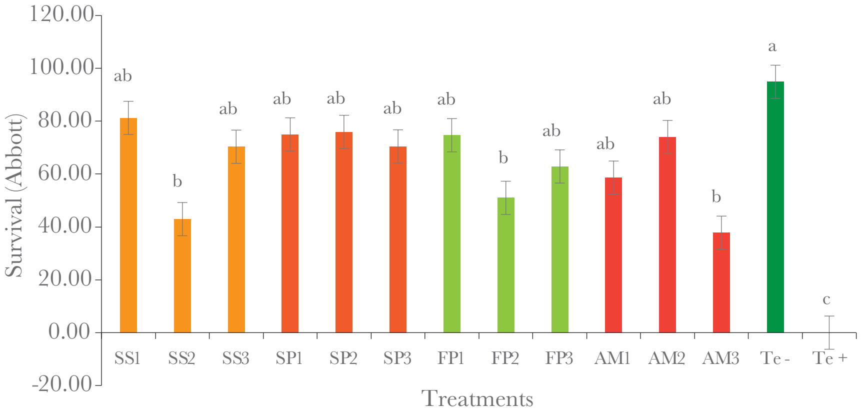

Figure 2. Average survival percentages of 60-day-old Maradol papaya plants, by IR resistance, inoculated with *P. nicotianae* var. *parasitica* Groups (a, b, ab and c), equal letters are not significantly different between treatments (Tukey α=0.5).

These results suggest that PF probably worked as a growth regulator, effective at optimum doses (in this case, PF at 0.35%). Multiple researchers show the FP efficacy as a control product for important diseases caused by oomycetes, not only on the *Phytopthora* genus, but also on *Peronospora*, *Plasmopara*, and *Pythium* genera (Groves *et al.*, 2015). Similar results were obtained for wheat blight with PF when the disease is mild; however, its effect is constrained when the pathogen shows greater aggressiveness (Pagani *et al.*, 2014). In plants of *Pinus radiata* other wild species, inoculated with *P. cinnamomi* (soil), at high PF concentrations, showed no significant effect on the pathogen but did cause phytotoxicity on some plants. However, stem injections and PF spraying inhibited *P. cinnamomi* (Shearer and Crane, 2014).

The third most effective treatment (74%) was acibenzolar-s-methyl at 0.1 mM. The three applied concentrations had effectiveness from 37 to 74%, with the first plants dying within seven days, being ASM at 0.1 mM the effective dose in the control of papaya root rot. These results also suggest that this resistance inducer acted as a growth regulator. Our results are similar to those reported by Vawdrey and Westerhuis (2007), who showed that ASM (0.025g L^{-1}) significantly reduced root rot incidence caused by *P. palmivora* on papaya in Australia. Gilardi *et al.* (2014), on other hand, found that AM at 0.025 and 0.0125 g i.a. L^{-1} applied three times before inoculation, produced 100% efficacy in disease mitigation in the first test. The difference with this research was that it was done with only one application. In the present investigation, the effectiveness (75%) with ASM (0.1 mM) against the infection of *P. nicotianae* var. *parasitica* was higher than that reported by Macedo *et al.* (2009), who observed a survival of 68.3 to 65.3% in the plants, after three days of inoculation, with a previous application of 0.30 g L^{-1} of ASM in the Golden papaya variety against the pseudofungus *P. palmivora*. Concerning the present research, the highest concentration obtained 16% more survival, however, in both cases, the survival was higher than 40% for this resistance inducer. Moscoso-Ramírez and Palou (2013) conducted preventive studies through primary *in vivo* experiments with resistance inducers to *Penicillium* spp. in 'Valencia' oranges. They found that ASM at 0.9 mM (0.2 g L^{-1}) reduced the green rot incidence by 15%. Furthermore, this resistance inducer also reduced the severity of green rot, but not of the blue rot causal agent. Despite the above, ASM does not always act as an inhibitor of all pathogens. For example, Méndez *et al.* (2010) evaluated conventional fungicides and resistance inducers among them, where ASM at a concentration of 0.02 kg a.i. ha^{-1}, against *Pseudoperonospora cubensis* infection responsible for downy mildew in melon, and the results showed inefficiency and behavior as the positive control.

CONCLUSIONS

Mortality of 60 d of age Maradol papaya plants inoculated with *P. nicotianae* var. *parasitica* occurs on average five days post-infection, demonstrating the high susceptibility of the papaya to this pathogen. The four tested chemical resistance inducers: sodium silicate, potassium silicate, potassium phosphite, and acibenzolar-s-methyl, showed significant efficacy in inducing resistance on 60 d old papaya seedlings inoculated with *P. nicotianae* var. *parasitica*.

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