Article

Sensitivity to metalaxyl and pathogenic potential of *Phytophthora hydropathica* isolated from irrigation canals of the Valley of Culiacán

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Abstract

Phytopthora hydropathica is a species with few reports related to its ability to infect agricultural crops in a natural way, so it can be inferred that it has not been in contact with chemical agents; therefore, the objective of this study was to determine the pathogenicity and sensitivity to metalaxyl of 18 strains of *P. hydropathica* from irrigation canals in Culiacán, Sinaloa. The pathogenicity evaluation of the isolates was carried out on zucchini and cucumber leaves and on zucchini, cucumber and tomato fruits. Resistance to metalaxyl was evaluated *in vitro* in PDA medium added with metalaxyl. The isolates of *P. hydropathica* caused symptoms of necrosis and softening in fruits and symptoms of leaf necrosis of the evaluated plant species; no resistant strains were found, seven strains showed intermediate sensitivity and the rest were susceptible to metalaxyl. The CE_{50} of the isolates ranged from 0.000013 $\mu g\ L^{-1}$ to 1 $\mu g\ L^{-1}$. It can be concluded that the use of metalaxyl would be effective in controlling some disease outbreak caused by *P. hydropathica*.

Keywords: *Phytophthora hydropathica*, CE_{50}, Oomycetes, Ridomil Gold.

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Introduction

In the genus *Phytophthora*, there are about 124 species described (Martin *et al*., 2014), which have the capacity to infect hundreds of plant species around the world (Gallegly and Hong, 2008). At present, the presence of *Phytophthora* species in aquatic environments has increased (Zappia *et al*., 2014), for example, in recent years the presence of *Phytophthora hydropathica* stands out mainly in irrigation water (Hulvey *et al*., 2010; Hüberli *et al*., 2013; Bienalfp and Balci, 2014; this is to be expected, since etymologically, the name of this microorganism derives from the Greek words '*hydro*' which refers to its aquatic nature and '*pathica*' to its pathogenic nature (Hong *et al*., 2010). Most of the reports of *P. hydropathica* are related to infections in ornamental plants, such as foliar necrosis in azalea (*Rhododendron catawbiense*), neck rot in mountain laurel (*Kalmia latifolia*) (Hong *et al*., 2010), as well as wilting and regressive death of wild laurel (*Viburnum tinus*) (Vitale *et al*., 2014).

Before being formally described as *P. hydropathica*, Hong *et al*. (2008) showed that this species is capable of causing 'damping off' in cucumber seedlings, while in tomato and pepper plants it caused root infections; In addition, they mention that *P. hydropathica* penetrates in fruits of tomato and chili by means of wounds. In Mexico, the presence of *P. hydropathica* was reported in irrigation water in the Valley of Culiacan and was shown to cause necrosis in tomato and chili leaves (Álvarez *et al*., 2017).

To control diseases caused by *Phytophthora*, the chemical ingredient metalaxyl, which is a phenylamide-type fungicide that protects plants systemically is mainly used for the control of oomycetes (Urech *et al*., 1977). Metalaxyl acts on specific sites of the pathogen by preventing protein biosynthesis through interference in the synthesis of ribosomal RNA (Nunninger *et al*., 1995); however, excessive use of this product can cause pathogens to generate resistance (Damicone, 2004). An example is the rapid development of resistance in populations of *P. infestans*, which was detected in the 80s in Europe (Davidse *et al*., 1981) and in the 90s in the USA, Canada and Mexico (Matuszak *et al*., 1994; Power *et al*., 1995).

Resistant isolates are equal to or more aggressive than susceptible isolates, thus converting metalaxyl resistance into an important agronomic characteristic in the integrated management of diseases caused by *Phytophthora*, especially in late potato blight caused by *P. infestans* (Forbes *et al*., 1998); in addition, *in vitro* bioassays have been used to characterize and classify *Phytophthora* isolates and other oomycetes according to the level of susceptibility to metalaxyl (Peters *et al*., 2001; Fontem *et al*., 2005; Tian *et al*., 2016).

The objectives of the present study were: 1) to determine the pathogenic potential of isolates of *P. hydropathica* in leaves and fruits of tomato, cucumber and zucchini; and 2) determine the sensitivity or resistance of said isolates to metalaxyl.

Materials and methods

Pathogenic potential. To determine the pathogenic potential, three representative strains of *P. hydropathica* were used [13F2 (KX298864), 16-1F2 (KX298868) and 18-2F1 (KX298873). Strains were reactivated in potato dextrose agar medium (PDA). In the pathogenicity experiments three fruits of tomato (Saladette), cucumber (SFPP) and pumpkin (Nurizeli) were used, as well as three leaves of pumpkin (Nurizeli) and cucumber (SFPP) of 1 month of age. The plant materials were washed with running water and disinfested with 70% ethanol. Both leaves and fruits were wounded with a sterile dissection needle and inoculated by placing 5 mm diameter discs of PDA medium with active growth of the three strains. The inoculated leaves and fruits were placed in a humid chamber for 120 h (Orlikowski *et al*., 2012). As control, fruits and leaves were used in which wounds were made and discs of culture medium were placed.

Metalaxyl sensitivity. To evaluate the susceptibility of the strains of *P. hydropathica* to metalaxyl, an in vitro study was carried out, which consisted of adding 10 mg L^{-1} of metalaxyl to the PDA culture medium (Shattock, 1988; Rekanovic *et al*., 2011), the commercial formula Ridomil Gold 480 EC (480 g of active ingredient of metalaxyl) was used, which was added to the culture medium after sterilization and just before emptying it in Petri dishes. From the border of the colonies of each strain, 5 mm diameter discs of culture medium with mycelial growth were taken and placed in Petri dishes with PDA medium added with metalaxyl and without metalaxyl (control). The in vitro growth of each isolate was evaluated after six days at 25 °C (Paez *et al*., 2001).

The growth was determined by measuring the diameter of the colony perpendicularly. To determine the relative growth, the formula PC= (DMC-5mm/DMCA)100 was used; where PC= percentage of growth, DMC= diameter of the colony with metalaxyl and DMCA= diameter of the colony without metalaxyl. A percentage of growth in diameter of the colony equal to or greater than 60% is considered to be resistant isolate, a growth of the colony between 10-60% is classified as intermediate and when the colony is less than 10% it is considered as susceptible (Shattock, 1988; Deahl *et al*., 1995; Riveros *et al*., 2003).

To determine the CE_{50} values in each strain, four concentrations of metalaxyl 0.1, 1, 5 and 10 mg L^{-1} were used; the CE_{50} was calculated by a linear regression between the log_{10} of the relative radial growth and the log10 of the concentration of the fungicide (Paez *et al*., 2001). Three repetitions were performed for each isolate and concentration of metalaxyl.

Design of experiments. In the assay with PDA culture medium with 10 mg L^{-1} of metalaxyl, a totally random factor design was used, where the factor was the 18 isolates. An analysis of variance and comparisons of means were made using the Tuckey test. For the in vitro assay in which the resistance or sensitivity of the isolates to metalaxyl was determined, a completely randomized two-factor design was used; one factor consisted of the isolates with 18 levels and the other factor in the concentration of metalaxyl. The response variable was the growth of the colony of the strains in millimeters.

Results and discussion

Pathogenic potential. In cucumber and zucchini leaves, symptoms of disease were visible at 48 h after inoculation (hdi). In cucumber leaves, the three inoculated isolates induced symptoms of necrosis with wrinkling and darkening of the affected tissues (Figure 1). There were some differences between the three inoculated isolates, the main one occurred in the leaf inoculated with strain 18-2F1, in which signs of the pathogen (whitish mycelium) were observed (Figure 1B).

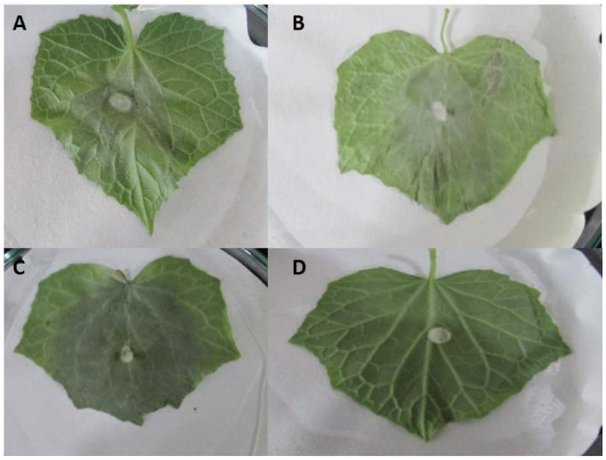

Figure 1. Cucumber leaf lesion caused by *P. hydropathica* 48 hdi. A) isolated 16-2F1; B) isolated 18-2F1; C) isolated 13-F2; and D) control leaf.

In zucchini leaves, the isolates also caused symptoms of necrosis (Figure 2). In zucchini there are no reports of studies that consider it as a host of *P. hydropathica*. In contrast, cucumber *P. hydropathica* has been reported to cause damping-off in seedlings (Hong *et al*., 2008).

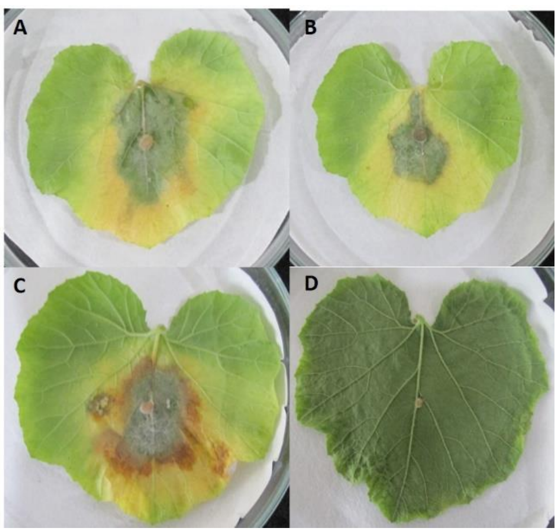

Figure 2. Symptoms on zucchini leaves caused by *P. hydropathica* 48 hdi. A) isolated 13-F2; B) isolated 18-2F1; C) isolated 16-2F1; and D) control leaf.

In zucchini fruits showed symptoms of necrosis; while, in tomato and cucumber there were symptoms of softening (Figures 3, 4 and 5). Strain 13F2 was the most aggressive in cucumber and tomato fruits, even at 72 hdi, signs of the pathogen were observed (Figures 3B and 3C). In contrast, strain 16-2F1 caused symptoms of barely visible necrosis in zucchini fruit and symptoms of softening in tomato (Figures 4A and 4B). Strain 18-2F1 was the most aggressive in zucchini fruits (Figure 5A), where as in tomato fruits, at 72 hdi signs of the pathogen were observed (Figure 5B), these differences observed between the different isolates were can attribute to the different degree of virulence of each strain.

Figure 3. Symptoms in fruits caused by *P. hydropathica* isolated 13-F2 72 hdi. A) zucchini; B) tomato; and C) cucumber.

Figure 4. Symptoms in fruits caused by *P. hydropathica* isolated 16-2F1 72 hdi. A) zucchini; B) tomato; C) cucumber.

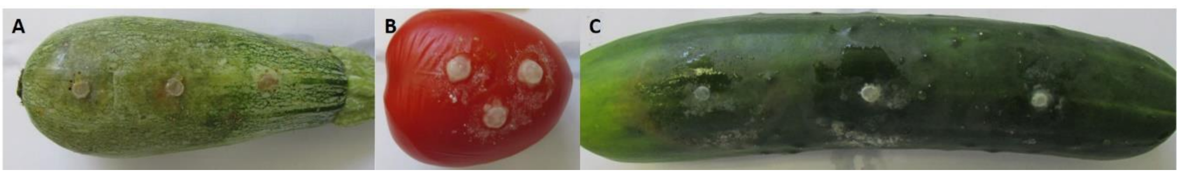

Figure 5. Symptoms in fruits caused by *P. hydropathica* isolated 18-2F1 72 hdi. A) zucchini; B) tomato; C) cucumber.

Metalaxyl sensitivity. The 18 strains used in the trial showed a reduction in their growth when exposed to 10 mg L^{-1} of metalaxyl compared to growth in medium without metalaxyl (Figure 6); no resistant strains were found; this means that no isolate grew more than 60% in the medium added with 10 mg L^{-1} of metalaxyl. Seven strains showed intermediate resistance and 11 were susceptible according to the values proposed by Shattock, (1988) (Table 1). Of the 11 sensitive strains, six of them were totally inhibited at the concentration of 10 mg L^{-1} of metalaxyl, these data are similar to those obtained in tests conducted by Riveros *et al*. (2003), where the isolate used as control (sensitive to metalaxyl) was completely inhibited at the concentration of 10 mg L^{-1}. In some cases, *in vitro* sensitivity results have been presented in oomycetes, which do not accurately predict the sensitivity they will have to chemicals *in vivo* conditions (Moorman and Kim, 2004).

For example, in *P. infestans* there was no good correlation between the results obtained in tuber discs with the results in culture medium (Straub *et al*., 1979). Despite this, the high degree of sensitivity presented by *P. hydropathica* isolates may be due to the fact that these isolates have not been exposed to this chemical, as there are no reports of this species causing damage to agricultural crops in the region.

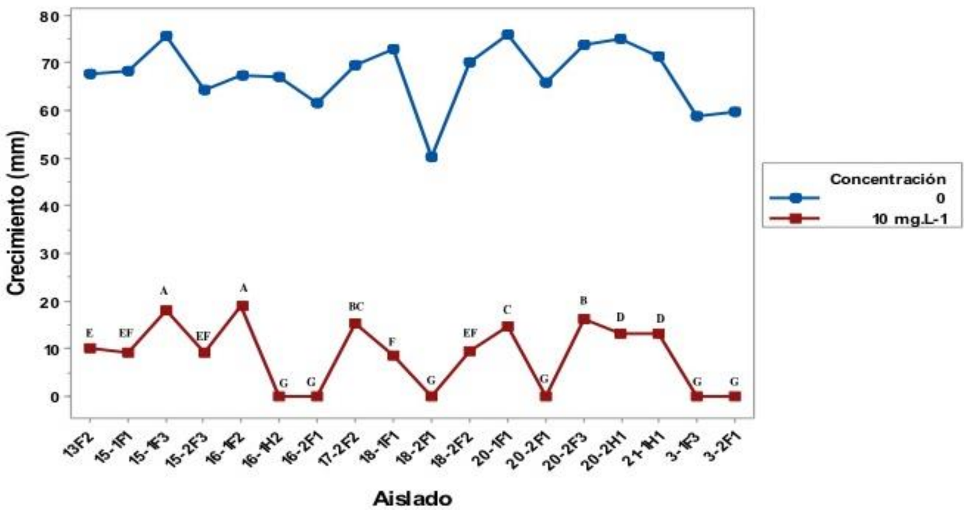

Figure 6. Growth of *P. hydropathica* isolates in culture medium with 10 mg L^{-1} of metalaxyl and in a metalaxyl-free culture medium after 6 days of incubation. Different letters indicate significant differences (Tuckey $p < 0.05$).

Table 1. Sensitivity and effective concentration of metalaxyl in *P. hydropathica* isolates.

Isolated	Type of resistance			CE_{50} (µg L^{-1})
	Resistant	Intermediate	Sensitive	
3-1F3			●	<0.000013
3-2F1			●	<0.000013
13-F2			●	0.0040
15-1F1			●	0.0054
15-2F3			●	0.0055
15-1F3		●		1
16-1F2		●		0.0034
16-1H2			●	<0.000013
16-2F1			●	<0.000013
17-2F2		●		0.26
18-1F1			●	0.000086
18-2F1			●	0.017
18-2F2			●	0.079
20-1F1		●		0.14
20-2F1			●	0.000013
20-2F3		●		0.52
20-2H1		●		0.074
21-1H1		●		0.1

CE_{50}= effective concentration at which the microorganism grows 50% compared to the control; R^2= 94.5%.

The CE_{50} of the 18 strains were very low, fluctuating in concentrations less than 0.000013 μg L^{-1} and up to 1 μg L^{-1} (Table 1), similar CE_{50} were determined in strains of *Phytophthora infestans* considered sensitive to metalaxyl where the lowest concentration was 0.02 μg L^{-1} (Power *et al*., 1995), whereas, in a study with the species *P. nicotianae*, maximum CE_{50} concentrations of 0.04 μg mL^{-1}were obtained for isolates classified as sensitive. (Hu *et al*., 2008), higher concentrations of CE_{50} have also been found in isolates classified as sensitive, such is the case of those obtained by Paez *et al*. (2001) where they determined minimum concentrations of 1 000 μg mL^{-1} for isolates of *P. infestans*.

Conclusion

P. hydropathica has the potential to infect leaves and fruits of tomato, cucumber and zucchini, plants that are important in the horticultural sector in Sinaloa. The 18 strains of *Phytophthora hydropathica* are sensitive to metalaxyl.

Acknowledgments

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