Article

Identification and alternative management of downy mildew in rose

Pablo Israel Álvarez Romero[1]
Rómulo García Velasco[1§]
Martha Elena Mora Herrera[1]
Martha Lidya Salgado Siclan[2]
Daniel Domínguez Serrano[1]

Tenancingo University Center-Autonomous University of the State of Mexico. Road Tenancingo-Villa Guerrero km 1.5, Tenancingo, State of Mexico. ZC. 52400. Tel 01 (714) 1407724. (pablo-alvarez-r@yahoo.com, marthaelenam@gmail.com, danusso10@hotmail.com). Faculty of Agricultural Sciences-Autonomous University of the State of Mexico. The Cerrillo Piedras Blancas, Toluca, State of Mexico, ZC. 50200. Tel. 01 (722) 2965529. (mlsalgados@uaemex.mx).

[§]Corresponding author: rgarciave@uaemex.mx.

Abstract

The downy mildew caused by *Peronospora sparsa* Berkeley is one of the most important diseases of the rose bush in Mexico, causing 100% losses. Its management is based on fungicides in continuous applications that tend to generate resistant populations. The search for alternatives is indispensable. In the present work, the morphometric and molecular identity of the agent associated with the downy mildew of the rose was confirmed, the effectiveness of the potassium phosphite (K_3PO_3), chitosan, silicon and mefenoxam was evaluated for the management of the disease and its effect on the length and diameter of floral stem. The study was conducted in summer and fall of 2013 in Tenancingo, State of Mexico. The morphometric characterization was carried out under a compound and scanning electron microscope. For molecular characterization, the ribosomal DNA of the ITS region was amplified with primers PS3 and PS1. The treatments were: potassium phosphite (K_3PO_3), chitosan, silicon, mefenoxam and control and were applied at weekly intervals. The experimental design was randomized blocks and the mean comparison was by Tukey (α= 0.05). Morphometric and molecular data corresponded to *Peronospora sparsa*. K_3PO_3 and silicon reduced the incidence and severity with respect to the control. The treatment with K_3PO_3 showed increases of 24.8 and 97.5% in the length of stems with diameters of 7.5 and 6.2 mm in summer and autumn respectively, comparing with the control. Thus, K_3PO_3 and silicon can be alternatives in the management of downy mildew of the rose bush under greenhouse conditions.

Keywords: *Peronospora sparsa*, potassium phosphite, incidence, severity.

Reception date: October 2018
Acceptance date: December 2018

Introduction

The *Rosa* spp. It is one of the most important ornamental crops and greenhouse production is estimated at around 8 500 ha, with an annual production of approximately 15 to 18 billion cut stems in the world (Blom and Tjusita, 2003). In 2017, in Mexico, 1 127.1 ha were cultivated in the greenhouse and its yield was 6 985.98 t ha^{-1} (SIAP, 2018). However, the crop is susceptible to various diseases such as downy mildew caused by the oomycete *Peronospora sparsa* Berkeley (Debener and Byrne, 2014), one of the main phytosanitary problems of this crop worldwide, which causes losses in production by decreasing the quality of the floral stems and increase the cost of production, especially by the increase in the number of applications of fungicides (Ayala *et al*., 2008; Castillo *et al*., 2010).

The management of *Peronospora sparsa* is based fundamentally on the application of fungicides (Aegerter *et al*., 2002; Quiroga and Arbelaez, 2004) with up to three applications per week, with a drastic increase in production costs and impacts on human health and the environment, besides originating resistant variants of the pathogen (Kuck *et al*., 2011; Rebollar-Alviter *et al*., 2012). Due to market pressure on the safe handling of less polluting and dangerous crops, it is important to find alternatives to fungicides for the management of downy mildew, such as salts of phosphorous acid (phosphites) that have shown potential to control diseases caused by oomycetes, in particular the genera *Peronospora*, *Plasmopara*, *Phytophthora* and *Pythium* (Lobato *et al*., 2010; Silva *et al*., 2011; Burra *et al*., 2014; Brunings *et al*., 2015).

Its mode of action is complex; present direct effects on pathogens (King *et al*., 2010) and indirect effects by stimulating host defense responses (Machinandiarena *et al*., 2012; Burra *et al*., 2014). In this regard, Lobato *et al*. (2010) showed that applications of phosphites reduced the symptoms caused by *Phytophthora infestans* (Mont.) of Bary, *Fusarium solani* (Mart.) Sacc. and *Rhizoctonia solani* Kühn in potato tubers (*Solanum tuberosum* L.).

In addition, that the phosphites act as a potential inducer of beneficial metabolic responses in plants, their efficacy on the quality and yield of some crops has been confirmed (Lovatt and Mikkelsen, 2006; Gómez-Merino and Trejo-Téllez, 2015). Also, silicon applications have been shown to be effective in suppressing fungal diseases; when deposited in intra and inter cellular spaces, acting as a physical barrier against the infection of pathogens (Ma and Takashi, 2002).

Also the resistance mediated by silicon to phytopathogens has been demonstrated in several pathosystems such as cucumber-*Podosphaera fuliginea* (Schltdl.) U. Braun & S. Takam. (*Liang et al*., 2005), zucchini-*P. xanthii* (Castagne) U. Braun & Shishkoff (Sawas *et al*., 2009), wheat-*Blumeria graminis* (DC.) Speer (Belanger *et al*., 2003) and pepper-*Phytophthora capsici* (Leonian) (Lee *et al*., 2004) by the induction and accumulation of low molecular weight antifungal metabolites in response to infection during the development of the disease (Fawe *et al*., 1998; Fauteux *et al*., 2005). It has also been shown that the application of silicon promotes the growth and quality of roses (Hwang *et al*., 2005; Reezi *et al*., 2009). The control of oomycetes has also been achieved by treatment with chitosan, acting directly on the pathogen or by stimulating responses of plant defenses (Iriti *et al*., 2011). In this regard, it was reported

that the treatment with chitosan inhibited the mycelial growth of *P. capsici* in peppers (Xu *et al*., 2007), whereas in seedlings of *Solanum tuberosum* the enzymatic activity of chitinase was increased in response to infection by *P. infestans* (O'Herlihy *et al*., 2003).

On the other hand, Wojdyla (2004) reported that the application of chitosan at a concentration of 0.025% in rose, showed a biological effectiveness higher than 72% in the control of *Peronospora sparsa* and was similar to the fungicide. Regardless of the effects of chitosan on diseases, significant improvements in growth and quality have been reported in various ornamental crops (Wanichpongpan *et al*., 2000; Ohta *et al*., 2001; Nge *et al*., 2006; Ramos-García *et al*., 2009).

Based on the above, the objectives of this study were to morphologically and molecularly characterize the agent associated with downy mildew in *Rosa* spp., evaluate the efficacy of four commercial products (Metalaxyl, potassium phosphite, silicon and chitosan) in the management of downy mildew in field conditions and determine its effect on the length and diameter of the floral stem.

Materials and methods

Morphometric characterization

It is collected in glasshouse rose leaves var. Lupita® (Meilland International) with symptoms and signs of downy mildew in the municipality of Tenancingo, Mexico. Structures such as sporangiophores and sporangia were detached from the leaflets and with them semipermanent preparations were made with Scotch tape in 50% glycerol acidified with 12N HCl, these were observed and measured in the compound microscope (Carl Zeiss® Axiostar plus). morphometric of 30 sporangiophores and 50 sporangia. The identification of the genus and species was carried out according to Achard (1997); Horts and Cloyd (2007).

Scanning electron microscopy

Fragments of young leaves (0.5 cm^2) of rose with signs of downy mildew were fixed in 3% glutaraldehyde for 24 h, then washed with Sorensen's phosphate buffer (0.1 M). The samples were dehydrated by immersion in ethanol at gradual concentrations (30, 40, 50, 60, 70, 80, 90) for 40 min each and 100% three times for 20 min. Subsequently, they were dried in CO_2 in a critical point desiccator (Sandri-780A®, USA) for 40 min, mounted in copper sample holders and coated with gold in an ionizer (Ion Sputter JFC-1100, JEOL®, Japan) for 1 min. Finally, the preparations were observed and photographed in a scanning electron microscope (JEOL JSM-6390®, Japan).

Extraction of DNA

The extraction of DNA was carried out from leaves with signs of downy mildew, using the Plant DNAzol Reagent® reagent (Invitrogen™) according to the protocol described by the manufacturer, with modifications to avoid the effect of phenols, so that five washed with 300 µL of 75% ethanol. DNA integrity was observed on a 1% agarose gel (Ultrapure™), DNA bands were visualized in a transilluminator (Syngene® GVM20), the quality and concentration were determined in a biophotometer (Eppendorf® D-5000-3000). The obtained DNA was resuspended in 50 µL of molecular biology water and stored at -20 °C for later use.

PCR and DNA sequencing

For the PCR test, the specific primers PS3 (5'ATTTTGTGCTGGCTGGC3') and PS1 (5'TGCCACACGACCGAAGC3') (Aegerter *et al*., 2002) were used to differentially amplify the ITS1, 5.8S and ITS2 region of the rDNA of the pathogen under study. The PCR reactions were performed in a final volume of 20 µL of the mixture: 2.6 µL of sterile deionized water (Gibco®), 10 µL of 2X Phire Plant PCR Buffer (includes 200 µM of each dNTPs and 1.5 mM of MgCl2), 2 µL of each first PS3 and PS1 (10 ρmol), 2 µL of sterile non-fat milk (10 mg mL^{-1}), 1 µL of DNA and 0.4 µL of DNA polymerase (Phire® Hot Start II). The amplification was performed in a thermocycler (MJ Research Thermal® PTC-100) according to the procedure described by Ayala *et al*. (2008). The product of the amplification was verified by electrophoresis at 90 V for 30 min in 1% agarose gel and staining with 1 µL of ethidium bromide, the visualization was carried out in a transilluminator (Syngene® GVM20). The DNA was purified with the commercial kit DNA Clean & ConcentratorTM-5 (Zymo Research®).

Subsequently, fragments amplified by PCR were sequenced in both directions in a genetic analyzer (Applied Biosystem® ABI Prism 3130XL). The sequence obtained was aligned in the NCBI database. The sequence was deposited at the GenBank base.

Greenhouse experiments

In rose plants var. Lupita® grown in greenhouse conditions were conducted two trials: the first in the summer season and the second in autumn 2013. Both were performed under a randomized block design with five treatments and four repetitions. Twenty experimental units were used, each experimental unit consisted of a plot of 2.7 m long and 1 m wide with 27 rose plants distributed in a row. By means of a pruning, the production of shoots was stimulated in a homogeneous way, on which the treatments were evaluated.

Treatments

The treatments were: potassium phosphite, silicon, chitosan, the fungicide mefenoxam and a control based on distilled water (Table 1). The treatments were randomly assigned to each experimental unit, its application started eight days after pruning and later at weekly intervals until the end of the trials. The application was made with a motorized spray pump (Maruyama® MS072H) with a fan nozzle.

Table 1. Description of the treatments applied on rose plants var. Lupita® for the management of *Peronospora sparsa*.

Treatment	Tradename	Concentration	*Doses (mL L^{-1})
Control	Distilled water	------	---
Potassium phosphate	Nutriphite magnum®	2% N, 40% P_2O_5, 16% K_2O	2.5
Silicon	Armurox®	2% peptide complex with soluble silicon	2.5
Chitosan	Biorend®	2.5% poly-d-glucosamine	3
Mefenoxam	Ridomil Gold® 480 SL	480 g ia L^{-1}	2

*Doses recommended by the manufacturer.

Variables evaluated

Ten stems were selected and labeled randomly per experimental unit for the evaluation of the incidence and severity of the disease, length and diameter of floral stems.

Evaluation of the incidence and severity

To favor the natural development of downy mildew during the trials, the relative humidity (90-100%) was increased by means of a nebulization system. The incidence and severity were evaluated immediately after the onset of the first symptoms, subsequently at weekly intervals. The percentage of incidence was calculated by counting the number of stems with symptoms in relation to the 10 stems evaluated by experimental unit. The severity of the disease was determined by a percentage scale according to Rebollar *et al*. (2012) with the following classes: 0= (no symptoms), 1= up to 5%, 2= 5-10%, 3= 10-25%, 4= 25-50%, 5= 50-75% and 6=> 75% leaf area covered with lesions. The values were transformed to percentage of severity by the equation of Townsend and Heuberger (1943). The incidence and severity data were transformed to area under the disease progress curve (ABCPE), applying the trapezoidal integration method (Campbell and Madden, 1990).

Evaluation of length and diameter of floral stem

At the cutting point, the stem length (cm) was measured with a flexometer, from the base to the apex thereof. The diameter was determined in the middle part of the stem with a digital vernier (Truper® CALDI-6MP).

Analysis of data

The data of the variables were subjected to an analysis of variance and comparison of means Tukey (α= 0.05%) through the statistical program InfoStat, student version 2015.

Results and discussion

Morphometric characterization

The pathogen associated with the disease was identified as *Peronospora sparsa* Berk. based on the symptomatology shown in the leaves, which was characterized by the development of irregular purple to dark brown spots on the leaves (Figure 1A and 1B), while on the underside the signs of the pathogen (sporangiophores) and esporangios) were observed, later abscission of the leaves was evidenced. These symptoms agree with those reported by Horst and Cloyd (2007). Hyaline sporangiophores were observed emerging from the stomata on the underside of the leaves, 150-240 x 7.5-12 µm, with dichotomous branching 3 to 4 times with bifurcated tips (Figure 1C and 1D). The sporangia were hyaline, subglobose to ellipsoidal, of 17.5-25 x 12.5-17.5 µm, with rough texture wall and in the base presented a germination papilla (Figure 1E and F). Morphometric characteristics that coincide with the reports of Achar (1997), Horst and Cloyd (2007) and López-Guisa *et al*. (2013).

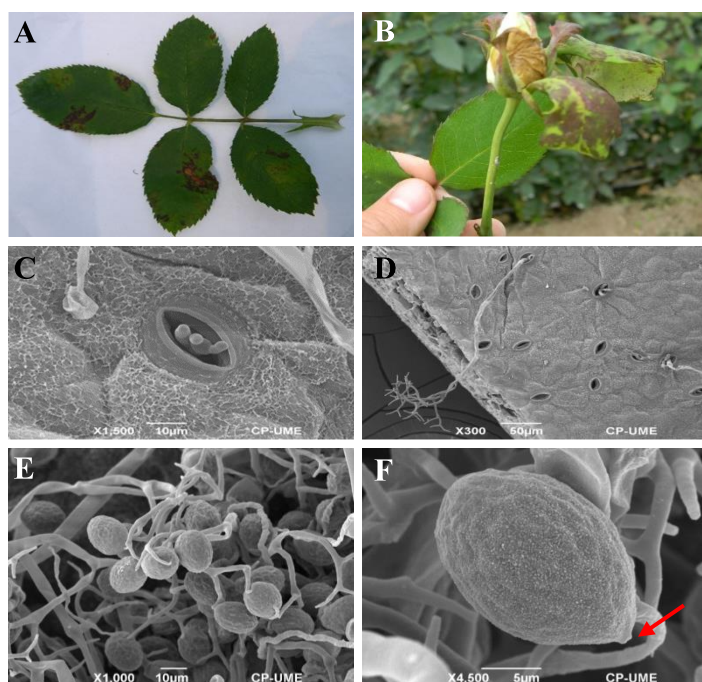

Figure 1. A and B) symptoms caused by *Peronospora sparsa* in rose bush. var. Lupita®; C and D) hyaline sporangiophores emerging from stomata, with dichotomous branches and bifurcated tips; E and F) hyaline sporangia with rough wall and germination papilla.

Molecular characterization

Sequencing of the product of the ITS region of the rDNA amplified by PCR using primers PS3 and PS1 (Aegerter *et al*., 2002) allowed to amplify a nucleotide sequence of 689 bp. When comparing the nucleotide sequence of this study (deposit number KJ817198), with those deposited in GenBank, the BLAST analysis showed a 99% identity with accessions of *Peronospora sparsa* on *Potentilla reptans* (DQ874342) in Korea (Choi *et al*. , 2007) and in *Rubus* sp. (EU369694) from Mexico (Revollar-Alviter *et al*., 2008) and 98% with isolated sequences from *Rosa multiflora* (AY608610) in Korea, *Rubus fruticosus* (EU391654) from Denmark and in *Rosa* sp. (AF266783) from England (Cooke *et al*., 2000; Choi *et al*., 2005; Sundelin *et al*., 2009).

Incidence and severity assessment

The ABCPE of the incidence and severity showed significant differences ($p > 0.05$) between treatments (Table 2). The treatment with potassium phosphite at a dose of 2.5 mL L^{-1} significantly reduced the incidence (7.6% in summer and 58% in autumn) and the severity (50.3% in summer and 84.2% in autumn) of *Peronospora sparsa*, this with respect to the witness Similar results have been reported by Chavarro-Carrero *et al*. (2012), who demonstrated that periodic applications of potassium phosphite (2.5 mL L^{-1}) on rose var. Bingo White® reduced the incidence up to 35% and the severity in 6.3% of *P. sparsa*.

Recently, Boyzo-Marin *et al*. (2015) reported that treatment with potassium phosphate in blackberry reduced the incidence of *P. sparsa* from 0.66 to 13% compared to the control. The effect of the phosphites is due to the fact that they are compounds that move systemically through the xylem and the phloem (Cooke and Little, 2001), acting directly in the development of the pathogen, inhibiting the mycelial growth, causing the deformation of the hyphae and lysis of the cell wall (King *et al.,* 2010), or indirectly through the activation of plant defense mechanisms through the salicylic acid pathway (Massoud *et al*., 2012).

The results of this study also indicate that silicon can be an alternative for the management of this disease, since in the summer there was a significant reduction in incidence (4.5%) and severity (20.3%), with respect to the control. In the fall season the incidence decreased 13.1%, but without statistical difference, while in the severity a significant reduction of 66.8% was observed, in contrast to the control (Table 2). In this regard, Ratnayake *et al*. (2016), reported that the application of potassium silicate (200 ppm) in plants of *Momordica charantia* L., significantly reduced the severity of *Pseudoperonospora cubensis* (Berk. & MA Curtis) Rostovzev, from 37-53%, compared with the control.

Table 2. Area under the curve of the progress of the disease (ABCPE) for the incidence and severity of *P. sparsa* in the cultivation of rose var. Lupita®.

Treatment	Incidence		Severity	
	Summer	Autumn	Summer	Autumn
Potassium phosphate	2 135 A	1 286.2 A	1 014.4 A	258.1 A
Silicon	2 205 A	2 660 BC	1 624.8 B	542.5 A
Chitosan	2 187.5 A	2 817.5 BC	1 676.5 BC	1 255.6 B
Mefenoxam	2 187.5 A	2 852.5 BC	1 739.9 BC	1 255.6 B
Control	2 310 B	3 062.5 C	2 039.7 C	1 631.8 B

Means with a letter in common are not significantly different, Tukey ($p > 0.05$).

Similar results were obtained by Garibaldi *et al*. (2012), who reported that the application of potassium silicate, reduced the incidence and severity of downy mildew caused by *Bremia lactucae* Regel, in lettuce plants (*Lactuca sativa* L.). The effect of silicon is attributed to the creation of physical barriers (thickness of the cuticle) by the accumulation of the same in the leaves, which prevents the penetration of pathogens and by the direct stimulation of responses in the host (Rodrígues *et al*., 2005; Ma and Yamaji, 2006; Ratnayake *et al*., 2016).

On the other hand, the treatment with chitosan only significantly reduced the incidence of *P. sparsa* in the summer season, contrasting with the control, while the severity was not reduced in any of the two seasons (Table 2). The results are possibly related to the concentration applied, the molecular weight, the degree of deacetylation and the type of chitosan, as reported by Bautista-Baños *et al*. (2006); Kong *et al*. (2010).

Similarly, the fungicide mefenoxam did not absolutely inhibit the development of *P. sparsa*, since the ABCPE values were statistically equal to the control, except for the incidence observed in the summer season (Table 2). A similar trend was observed by Quiroga and Arbelaez (2004), who reported that applications of mefenoxam to soil and foliage did not effectively control the

development of *P. sparsa* in the rose crop in Colombia. Resistance to mefenoxam has also been observed in an isolation of *Peronospora belbahrii* Thines on basil (*Ocimum basilicum* L.) in Israel (Cohen *et al*., 2013).

The loss of sensitivity of the pathogen to mefenoxam found in the present work, may be due to the development of *P. sparsa* resistance, which suggests analyzing the use of mefenoxam in the production of rose, mainly because this fungicide is widely used in Mexico for the management of this pathogen. Another factor that could influence was the pressure of the disease, as reported by Walter *et al*. (2004) who demonstrated that metalaxyl did not have a significant effect for the control of *P. sparsa* in bramble (*Rubus hybrid*) when high disease pressure was observed.

Evaluation of length and diameter of floral stem

The treatment with potassium phosphite in rose var. Lupita® plants significantly increased the length of stems of 24.8% and 97.5% in the summer and fall season respectively, contrasting with the control, which induced the shortest length in both seasons (Table 3). Similarly, plants treated with potassium phosphite, showed the largest stem diameters with 7.5 and 6.2 mm in the summer and autumn season respectively, and were statistically different ($p > 0.05$) with respect to the control (Table 3). Despite the fact that there is no evidence to show the positive effects of potassium phosphite on flowers and ornamental species (Gómez-Merino and Trejo-Téllez, 2015), the results of the present investigation allow us to infer that applications of potassium phosphite in plants of rose, increases the length and diameter of stems; so it becomes an alternative for the integrated management of said crop. Similar results have been reported in other crops, for example; Glinicki *et al*. (2010) reported beneficial effects of potassium phosphite on the growth parameters of three strawberry cultivars, on the other hand, Tambascio *et al*. (2014) documented that the application of potassium phosphite in tubers (seed) of *Solanum tuberosum* L. accelerated the emergence and increased leaf area and dry matter.

Table 3. Effect of treatments on the length and diameter of floral stems of the Lupita® variety.

Treatment	Length (cm)		Diameter (mm)	
	Summer	Autumn	Summer	Autumn
Potassium phosphate	76.1 A	39.3 A	7.5 A	6.2 A
Silicon	61.8 B	22.9 B	6.8 BC	4.6 BC
Chitosan	61.9 B	21.7 B	7.1 B	4.8 B
Mefenoxam	66.1 B	23.7 B	7.4 A	4.7 BC
Control	61 B	19.9 B	6.6 C	4.4 C

Values with a letter in common are not significantly different, Tukey ($p > 0.05$).

In addition, in citrus and avocado, it has been shown that a single foliar application of phosphites increases floral intensity, yield, fruit size, total soluble solids and anthocyanin concentration (Lovatt and Mikkelsen, 2006).

Likewise, it has been demonstrated that silicon applications have beneficial effects on the growth and quality of roses (Hwang *et al*., 2005; Reezi *et al*., 2009). However, in our tests the application of silicon did not show significant differences on the length and diameter of floral stems, this with respect to the control (Table 3). The results could be related to the dose of application, since there is evidence that high doses of silicon cause a reduction in the length and diameter of rose stems (Reezi *et al*., 2009), in *Gerbera jamesonii* L. Bolus, it decreases stem length and causes flower deformation (Kamenidou *et al*., 2010).

With regard to chitosan, an increase in length of stem 1.5% was observed in summer and 9% in autumn, but without significant difference with respect to the control. While increments of 7.6 and 9.1% were observed in the stem diameter for summer and autumn respectively, and were statistically different ($p > 0.05$) when compared with the control (Table 3). Some reports indicate that the application of chitosan presents significant improvements on growth and development in ornamental crops, such as gerbera (Wanichpongpan *et al*., 2000), orchids (Chandrkrachang, 2002), lisianthus (Ohta *et al*., 2001), *Lilium* spp. (Kim *et al*., 2005) and Gladiola (Ramos-García *et al*., 2009). However, it is important to note that the success of chitosan may be associated with the concentration used, the molecular weight and the degree of deacetylation (Aranaz *et al*., 2009; Salachna and Zawadzinska, 2014).

Conclusions

The morphometric and molecular characterization confirmed that *Peronospora sparsa* Berkeley is the agent associated with the downy mildew of roses in the municipality of Tenancingo, State of Mexico, Mexico. The applications with potassium and silicon phosphite reduced the incidence and severity of *P. sparsa*, so they should be considered as viable alternatives for the management of the disease. The weekly application of potassium phosphite (Nutriphite magnum®) has positive effects on stem length and diameter. The fungicide mefenoxam did not absolutely inhibit the development of *P. sparsa*.

Cited literature

Achar, P. N. 1997. First report of downy mildew disease of rose caused by *Peronospora sparsa* in KwaZulu Natal, Southern Africa. Plant Dis. 81(6):695.

Aegerter, B. J.; Nuñez, J. J. and Davis, R. M. 2002. Detection and management of downy mildew in rose rootstock. Plant Dis. 86(12):1363-1368.

Aranaz, I.; Mengibar, M.; Harris, R.; Panos, I.; Miralles, B.; Acosta, N.; Galed, G. and Heras, A. 2009. Functional characterization of chitin and chitosan. Current Chem. Biol. 3(2):203-230.

Ayala, V. M.; Argel, R. L. E; Jaramillo, V. S. y Marín, M. M. 2008. Diversidad genética de *Peronospora sparsa* (Peronosporaceae) en cultivos de rosa de Colombia. Acta Biol. Colomb. 13(1):79-94.

Bautista, B. S.; Hernández, L. A. N.; Velázquez, del V. M. G.; Hernández, L, M.; Ait, B. E.; Bosquez, M. E. and Wilson, C. L. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection. 25(2):108-118.

Bélanger, R. R.; Benhamou, N. and Menzies, J. G. 2003. Cytological evidence of an active role of silicon in wheat resistance of powdery mildew (*Blumeria graminis* f. sp. *tritici*). Phytopathology. 93(4):402-412.

Blom, T. J. and Tsujita, M. J. 2003. Cut rose production. *In*: Encyclopedia of rose science. Roberts, A. V.; Debener, T. and Gudin, S. (Eds.). Elsevier Academic Press. Amsterdam, Netherland. 594-600 pp.

Boyzo, M. J.; Silva, R. H. V. and Rebollar, A. A. 2015. Biorational treatments to manage dryberry of blackberry caused by *Peronospora sparsa*. Crop Protection. 76:121-126.

Brunings, A. M.; Liu, G.; Simonne, E. H.; Zhang, S.; Li, Y. and Datnoff, L. E. 2015. Are phosphorous and phosphoric acids equal phosphorous sources for plant growth? UF IFAS Extension, University of Florida. 1-8 pp.

Burra, D. D.; Berkowitz, O.; Hedley, P. E.; Morris, J.; Resjö, S.; Levander, F.; Liljeroth, E.; Andreasson, E. and Alexandersson, E. 2014. Phosphite-induced changes of the transcriptome and secretome in *Solanum tuberosum* leading to resistance against *Phytophthora infestans*. BMC Plant Biology. 14:254.

Campbell, C. L. and Madden, V. L. 1990. Introduction to plant disease epidemiology. John Whiley and Sons Inc. New York, USA. 532 p.

Castillo, C. F.; Álvarez, E.; Gómez, E.; Llano, G. A. y Castaño, Z. J. 2010. Mejoramiento nutricional de la rosa para el manejo de *Peronospora sparsa* Berkeley, causante del mildeo velloso. Rev. Acad. Colomb. de Ciencias Exactas, Físicas y Naturales. 34(131):137-142.

Chandrkrachang, S. 2002. The applications of chitin and chitosan in agriculture in Thailand. Adv. Chitin Sci. 5:458-462.

Chavarro, C. E. A.; García, V. R.; González, D. J. G.; González, C. L. E. y Jiménez, Á. L. J. 2012. Uso del fosfito de potasio para el manejo de *Peronospora sparsa* en *Rosa* spp. Fitopatol. Colomb. 36(2):53-56.

Choi, Y. J.; Constantinescu, O. and Shin, H. D. 2007. A new downy-mildew of the Rosaceae: *Peronospora oblatispora* sp. nov. (Chromista, Peronosporales). Nova Hedwigia. 85(1-2):93-101.

Choi, Y. J.; Hong, S. B. and Shin, H. D. 2005. A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. Mycological Res. 109(7):841-848.

Cohen, Y.; Vaknin, M.; Ben, N. Y.; Rubin, A. E. and Galperin, M. 2013. First report of the occurrence and resistance to mefenoxam of *Peronospora belbahrri*, causal agent of downy mildew of basil (*Ocimum basilicum*) in Israel. Plan Dis. 97(5):692.

Cooke, D. E. L.; Drenth, A.; Duncan, J. M.; Wagels, G. and Brasier, C. M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Gen. Biol. 30(1):17-32.

Cooke, L. R. and Little, G. 2001. The effect of foliar application of phosphonate formulations on the susceptibility of potato tubers to late blight. Pest Manag. Sci. 58(1):17-25.

Debener, T. and Byrne, D. H. 2014. Disease resistance breeding in rose: Current status and potential of biotechnological tools. Plant Sci. 228:107-117.

Fautex, F.; Rémus, B. W.; Menzies, J. G. and Bélanger, R. R. 2005. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol. Letters. 249(1):1-6.

Fawe, A.; Abou, Z. M.; Menzies, J. G. and Bélanger, R. R. 1998. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathol. 88(5):396-401.

Garibaldi, A.; Gilardi, G.; Cogliati, E. E. and Gullino, M. L. 2012. Silicon and increased electrical conductivity reduce downy mildew of soilless grown lettuce. Eur. J. Plant Pathol. 132(1):123-132.

Glinicki, R.; Sas-Paszt, L. and Jadczuk-Tobjasz, E. 2010. The effect of plant stimulant-fertilizer 'Resistim' on growth and development of strawberry plants. J. Fruit Ornamental Plant Res. 18(1):111-124.

Gómez, M. F. C. and Trejo, T. L. I. 2015. Biostimulant activity of phosphite in horticulture. Sci. Hortic. 196:82-90.

Horst, K. and Cloyd, R. 2007. Compendium of rose diseases. The American Phytopathological Society. Second Edition. St. Paul, Minnesota, USA. 96 p.

Hwang, S. J.; Han-Min, P. and Jeong, B. R. 2005. Effects of potassium silicate on the growth of miniature rose 'Pinocchio' grown on rockwool and its cut flower quality. J. Japan. Soc. for Hort. Sci. 74(3):242-247.

Iriti, M.; Vitalini, S.; Di Tommaso, G.; D'Amico, S.; Borgo, M. and Faoro, F. 2011. New chitosan formulation prevents grapevine powdery mildew infection and improves polyphenol content and free radical scavenging activity of grape and wine. Austr. J. Grape Wine Res. 17(2):263-269.

Kamenidou, S.; Cavins, T. J. and Marek, S. 2010. Silicon supplements affect floricultural quality traits and elemental nutrient concentrations of greenhouse produced gerbera. Sci. Hortic. 123(3):390-394.

Kim, J. H.; Lee, A. K. and Suh, J. K. 2005. Effect of certain pretreatment substances on vase life and physiological character in *Lilium* spp. Acta Hortic. 673(39):307-314.

King, M.; Reeve, W.; Van der Hoek, M. B.; Williams, N.; McComb, J.; O'Brien, P. A. and Hardy, G.E. 2010. Defining the phosphite-regulated transcriptome of the plant pathogen *Phytophthora cinnamomi*. Mol. Genet. Genomics. 284(6):425-435.

Kong, M.; Chen, X. G.; Xing, K. and Park, H. J. 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int. J. Food Microbiol. 144(1):51-63.

Kuck, K. H.; Leadbeater, A. and Gisi, U. 2011. FRAC mode of action classification and resistance risk of fungicides. *In*: modern crop protection compounds. Krämer, W.; Schirmer, U.; Jeschke, P. and Witschel, M. (Eds.). Second Edition. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany. 539-558 pp.

Lee, J. S.; Seo, S. T.; Wang, T. C.; Jang, H. I.; Pae, D. H. and Engle, L. M. 2004. Effect of potassium silicate amendments in hydroponic nutrient solution on the suppressing of *Phytophthora* blight (*Phytophthora capsici*) in pepper. Plant Pathol. J. 20(4):277-282.

Liang, Y. C.; Sun, W. C.; Si, J. and Römheld, V. 2005. Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. Plant Pathol. 54(5):678-685.

Lobato, M. C.; Olivieri, F. P.; Daleo, G. R. and Andreu, A. B., 2010. Antimicrobial activity of phosphites against different potato pathogens. J. Plant Dis. Prot. 117(3):102-109.

López, G. D.; Yáñez, M. M. J. and Alanis, M. I. 2013. First report of *Peronospora sparsa* on *Rosa* spp. in Mexico. J. Plant Pathol. 95(4):73.

Lovatt, C. J. and Mikkelsen, R. L. 2006. Phosphite fertilizers: What are they? can you use them? what can they do?. Better crops with Plant Food. 90(4):11-13.

Ma, J. F. and Takahashi, E. 2002. Soil, fertilizer and plant silicon research in Japan. Elsevier Science. Amsterdam, Holland. 294 p.

Machinandiarena, M. F.; Lobato, M. C.; Feldman, M. L.; Daleo, G. R. and Andreu, A. B. 2012. Potassium phosphite primes defense responses in potato against *Phytophthora infestans*. J. Plant Physiol. 169(14):1417-1424.

Massoud, K.; Barchietto, T.; Le Rudulier, T.; Pallandre, L.; Didierlaurent, L.; Garmier, M.; Ambard, B. F.; Jean, M. S. and Saindrenan, P. 2012. Dissecting phosphite-induced priming in arabidopsis infected with *Hyaloperonospora arabidopsidis*. Plant Physiol. 159(1):286-298.

Nge, K. L.; New, N.; Chandrkrachang, S. and Stevens, W. F. 2006. Chitosan as a growth stimulator in orchird tissue culture. Plant Sci. 170(6):1185-1190.

O'Herlihy, E. A.; Duffy, E. M. and Cassells, A. C. 2003. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. Folia Geobotanica. 38(2):201-207.

Ohta, K.; Asao, T. and Hosoki, T. 2001. Effects of chitosan treatments on seedling growth, chitinase activity and flower quality in *Eustoma grandiflorum* (Raf.) Shinn. 'Kairyou Wakamurasaki'. J. Hortic. Sci. Biotechnol. 76(5):612-614.

Quiroga, B. N. J. y Arbeláez, T. G. 2004. Evaluación de la eficacia de fungicidas aplicados al suelo y al follaje para el control de mildeo velloso, ocasionado por *Peronospora sparsa* en un cultivo comercial de rosa. Agron. Colomb. 22(2):110-118.

Ramos, G. M.; Ortega, C. S.; Hernández, L. A. N.; Alia, T. I.; Bosquez, M. E. and Bautista, B. S. 2009. Response of gladiolus (*Gladiolus* spp.) plants after exposure corms to chitosan and hot water treatments. Sci. Hortic. 121(4):480-484.

Ratnayake, R. M. R. N. K.; Daundasekera, W. A. M.; Ariyarathne, H. M. and Ganehenege, M. Y. U. 2016. Soil application of potassium silicate reduces the intensity of downy mildew in bitter gourd (*Momordica charantia* L.) leaves. Ceylon J. Sci. 45(1):23-31.

Rebollar, A. A.; Silva, R. H. V. and Zelaya, M. L. X. 2008. Detection and management of downy mildew of blackberry caused by *Peronospora sparsa* in Michoacan, México. Phytopathology. 98:S131-S131.

Rebollar, A. A.; Silva. R. H. V.; López, C. I.; Boyzo, M. J. and Ellis, M. A. 2012. Fungicide sprays programs to manage downy mildew (dryberry) of blackberry caused by *Peronospora sparsa*. Crop Protection. 42:49-55.

Reezi, S.; Babalar, M. and Kalantari, S. 2009. Silicon alleviates salt stress, decreases malondialdehyde content and affects petal color of salts-tressed cut rose (*Rosa* x *hybrida* L.) 'Hot Lady'. Afr. J. Biotechnol. 8(8):1502-1508.

Rodrigues, F. A.; Jurick, W. M.; Datnoff, L. E.; Jones, J. B. and Rollins, J. A. 2005. Silicon-influences cytological and molecular events in compatible and incompatible rice-*Magnoporthe grisea* interactions. Physiol. Mol. Plant Pathol. 66(4):144-159.

Salachna, P. and Zawadzińska, A. 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. J. Ecol. Eng. 15(3):97-102.

Sawas, D.; Giotis, D.; Chatzieustratiou, E.; Bakea, M. and Patakioutas, G. 2009. Silicon supply in soilless cultivations of zucchini alleviates stress induced by salinity and powdery mildew infections. Environ. Exp. Bot. 65(1):11-17.

SIAP. 2018. Servicio de Información Agroalimentaria y Pesquera. http://www.siap.gob.mx.

Silva, O. C.; Santos, H. A. A.; Dalla Pria, M. and May-De Mio, L. L. 2011. Potassium phosphite for control of downy mildew of soybean. Crop Protection. 30(6):598-604.

Sundelin, T.; Collinge, D. B. and Lübeck, M. 2009. A cultivation independent, PCR-based protocol for the direct identification of plant pathogens in infected plant material. Eur. J. Plant Pathol. 123(4):473-476.

Tambascio, C.; Covacevich, F.; Lobato, M. C.; de Lasa, C.; Caldiz, D.; Dosio, G. and Andreu, A. 2014. The application of K phosphites to seed tubers enhanced emergence, early growth and mycorrhizal colonization in potato (*Solanum tuberosum*). Am. J. Plant Sci. 5(1):132-137.

Townsend, G. R. and Heuberger, J. W. 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis. 27(17):340-343.

Walter, M.; Harris, V. P.; Thomas, W.; Tate, G.; Waipara, N. W. and Langford, G. 2004. Agrochemicals suitable for downy mildew control in New Zealand boysenberry production. Crop Protection. 23(4) 327-333.

Wanichpongpan, P.; Suriyachan, K. and Chandrkrachang, S. 2000. Effects of chitosan on the growth of gerbera flower plant (*Gerbera jamesonii*). *In*: Chitin and chitosan in life science. Uragami, T.; Kurita, K. and Fukamizo, T. (Eds.). Kodansha Scientific. Yamaguchi, Japan. 198-201 pp.

Wojdyla, A. T. 2004. Chitosan (biochikol 020 PC) in the control of some ornamental foliage diseases. Commun. Agric. Appl. Biol. Sci. 69(4):705-715.

Xu, J.; Zhao, X.; Han, X. and Du, Y. 2007. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. Pesticide Biochem. Physiol. 87(3):220-228.