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AN EFFECTIVE STRATEGY TO REDUCE THE INCIDENCE OF *Phytophthora* ROOT AND CROWN ROT IN BELL PEPPER

Esteban Sánchez-Chávez, Hilda Victoria Silva-Rojas, Gerardo Leyva-Mir, Federico Villarreal-Guerrero, Jorge A. Jiménez-Castro, Eduardo Molina-Gayosso, Alfonso A. Gardea-Béjar and Graciela Dolores Ávila-Quezada

SUMMARY

This work evaluated the effectiveness of combining grafted bell pepper and metam sodium fumigated soil to reduce the incidence of Phytophthora root and crown rot, a disease that significantly reduces crop yields. The experiment was carried out during 2011 in Chihuahua, Mexico. Six trials were established in a previously fumigated soil using 'Facinato' variety grafted onto 'Robusto' and 'Terrano' rootstocks, besides four self-rooted varieties. To identify the pathogen, ribosomal loci corresponding to the ITS region were amplified and sequenced. The general linear model described the increase in disease incidence in all trials. Grafted plants had a lower slope of disease incidence than the four self-rooted varieties. Results indicated that final disease incidence (Yf) of the varieties ranged 37-69%, while grafted plants exhibited a Yf of only 13-16%. Fumigation caused a better reduction of disease incidence when used for grafted plants. Disease onset and slope increase coincided with the possible fumigant degradation. These findings expand present knowledge regarding plant response to preventive fumigations and grafted plants use to decrease natural infection in bell pepper plants caused by this oomycete.

Introduction

Pepper (*Capsicum annuum* L.) is one of the main commodity crops worldwide. During 2013, Mexico was the second producer of fresh green peppers with 2.2×10^6t, behind China that produced 15.8×10^6t (FAOSTAT 2015). Bell or sweet peppers are different from hot peppers because they contain low quantities of the pungent substance capsaicin in the fruit parts (Chávez-Mendoza *et al*., 2013; Sora *et al*., 2015). In Mexico, most of the bell pepper crop is exported, mainly to Canada and the US (Ayala-Tafoya *et al*., 2015). During 2012, over 75% of fresh bell pepper imports into the US came from Mexico. Pepper crops are commonly subjected to the attack of soil-borne pathogens, which are very difficult to control, and are responsible for high disease incidences and heavy economic losses worldwide (Kamoun *et al*., 2015). One of the main diseases seriously impacting bell pepper yields is root and crown rot caused by *Phytophthora capsici*. Around the world, the oomycete *P. capsici* Leonian, is one of the most destructive soil-borne pathogens on peppers (Hwang and Kim, 1995; Ristaino and Johnston, 1999). On chili pepper, this pathogen can cause root rot and crown rot (Hausbeck and Lamour, 2004; Dunn and Smart, 2015), while other researchers have reported that this pathogen attacks the base of the stem (Ristaino and Johnston, 1999).

Management of *P. capsici* relies on integrated approaches such as cultural practices, chemical control, host resistance (Gevens *et al*., 2006) and grafting (Morra and Bilotto, 2010). Chemical treatments to reduce the incidence of root and crown rot relied in the past on methyl bromide

KEYWORDS / *Capsicum annuum* L. / ITS Region / Metam Sodium / *P. capsici* / 'Robusto' Rootstock / 'Terrano' Rootstock /

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UNA ESTRATEGIA EFECTIVA PARA REDUCIR LA INCIDENCIA DE LA PUDRICION DE RAÍZ Y CORONA POR *Phytophthora* EN PIMIENTOS

Esteban Sánchez-Chávez, Hilda Victoria Silva-Rojas, Gerardo Leyva-Mir, Federico Villarreal-Guerrero, Jorge A. Jiménez-Castro, Eduardo Molina-Gayosso, Alfonso A. Gardea-Béjar y Graciela Dolores Ávila-Quezada

RESUMEN

En este trabajo se evaluó la efectividad de la combinación de pimientos injertados y fumigación del suelo con metam sodio para reducir la incidencia de la pudrición de raíz y corona por Phytophthora, una enfermedad que reduce significativamente los rendimientos de los cultivos. El experimento se realizó durante el 2011 en Chihuahua, México. Seis ensayos se establecieron en el suelo previamente fumigado utilizando la variedad 'Facinato' injertada en los portainjertos 'Robusto' y 'Terrano', además de cuatro variedades sin injertar. Para identificar el patógeno, los genes ribosómicos correspondientes a la región ITS fueron amplificados y secuenciados. El modelo Lineal General describió el incremento de la incidencia de la enfermedad en todos los ensayos. Las plantas injertadas mostraron un menor parámetro de pendiente de incidencia de la enfermedad que las cuatro variedades sin injertar. Los resultados indicaron que la incidencia final de la enfermedad (Yf) de las variedades osciló entre el 37-69%, mientras que las plantas injertadas mostraron una Yf de 13-16%. La fumigación redujo la incidencia de la enfermedad cuando se usó en combinación con plantas injertadas. La aparición de la enfermedad y el aumento de la pendiente coincidieron con la posible degradación del fumigante. Estos hallazgos amplían el conocimiento actual sobre la respuesta de las plantas a tratamientos de fumigación preventivos y al uso de plantas injertadas para disminuir la infección natural por este oomiceto en plantas de pimiento.

UMA ESTRATÉGIA EFETIVA PARA REDUZIR A INCIDÊNCIA DA PODRIDÃO DE RAIZ E COROA POR *Phytophthora* EM PIMENTAS

Esteban Sánchez-Chávez, Hilda Victoria Silva-Rojas, Gerardo Leyva-Mir, Federico Villarreal-Guerrero, Jorge A. Jiménez-Castro, Eduardo Molina-Gayosso, Alfonso A. Gardea-Béjar e Graciela Dolores Ávila-Quezada

RESUMO

Neste trabalho foi avaliada a efetividade da combinação de enxertos de pimenta e fumigação do solo com metam sódio para reduzir a incidência da podridão de raiz e coroa por Phytophthora, uma enfermidade que reduz significativamente os rendimentos dos cultivos. O experimento foi realizado durante o ano 2011 em Chihuahua, México. Seis ensaios se estabeleceram no solo previamente fumigado utilizando a variedade 'Facinato' enxertada nos porta-enxertos 'Robusto' e 'Terrano', além de quatro variedades sem enxerto. Para identificar o patógeno, os genes ribossômicos correspondentes à região ITS foram amplificados e sequenciados. O modelo Linear Geral descreveu o incremento da incidência da enfermidade em todos os ensaios. As plantas enxertadas mostraram um menor parâmetro de pendente de incidência da enfermidade que as quatro variedades sem enxerto. Os resultados indicaram que a incidência final da enfermidade (Yf) das variedades oscilou entre 37 e 69%, enquanto que as plantas com enxerto mostraram uma Yf de 13 a 16%. A fumigação reduziu a incidência da enfermidade quando usada em combinação com plantas enxertadas. A aparição da enfermidade e o aumento da inclinação coincidiram com a possível degradação do fumigante. Estes achados ampliam o conhecimento atual sobre a resposta das plantas durante tratamentos de fumigação preventivos e durante a utilização de plantas enxertadas para diminuir a infecção natural por este oomiceto em plantas de pimenta.

fumigation (Wang *et al*., 2014). An alternative to substitute this fumigant is metam sodium (sodium N-methyl dithiocarbamate; metam-Na; Amvac Chemical Corp., Newport Beach, CA), which has a broad spectrum in biocidal activity in soil (Kreutzer, 1963). This registered fumigant (Duniway, 2002) is widely used in agricultural production for controlling soil-borne pathogens (Klose *et al*., 2008). Soil fumigation is one of the most effective methods to control pathogens and consequently maintain good yields (Hamm *et al*., 2003) and prevent environmental pollution (Arbeli and Fuentes, 2007). Even though studies have shown that metam sodium significantly reduces the inoculum of soil-borne pathogens (Hamm *et al*., 2003; Gerik, 2005; Gerik *et al*., 2006), little has been investigated on the effectiveness of this fumigant to reduce the incidence *P. capsici* in Mexico.

Another approach to manage this disease is the use of resistant pepper plant materials (Gilardi *et al*., 2013). In recent years, grafting of pepper commercial valued types on resistant pepper rootstocks has been used to reduce the root rot and to increase yields (Rouphael *et al*., 2010). This practice has been successfully applied for bell pepper in Korea (Jang *et al*., 2012) and in Italy (Morra and Bilotto, 2010; Gilardi *et al*., 2013).

In several studies about root rot in pepper plantations in Mexico, the causal agent of this disease has been attributed to diverse pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani* (Gonzalez *et al*., 2004; Mojica-Marín *et al*., 2009), *Pythium* spp., *Sclerotium rolfsii* (Gonzalez *et al*., 2004) and *Phytophthora capsici* (Romero-Cova, 1988; Zapata-Vázquez *et al*., 2012).

Most studies report *P. capsici* as the causal agent of root rot in the Mexican states of Durango (Mojica-Marín *et al*., 2009), Zacatecas, Guanajuato, San Luis Potosi (Anaya-López *et al*., 2011), Chihuahua (Guigón-López and Gonzalez-Gonzalez, 2004; Ávila-Quezada *et al*., 2005; Silva-Rojas *et al*., 2009) and Aguascalientes (Velásquez-Valle *et al*., 2003). In Chihuahua only *P. capsici* has been considered to cause root rot, and two compatibility types have been documented from naturally infested pepper fields (Silva-Rojas *et al*., 2009). Nevertheless, few studies have been conducted to identify the populations of this pathogen affecting different types of pepper in this state (Anaya-Lopez *et al*., 2011).

The aim of this work was to determine if soil fumigation

and use of resistant rootstocks have the capability to reduce root and crown rot incidence in bell pepper plots.

Materials and Methods

A field experiment was carried out in Delicias, Chihuahua, Mexico, in a *P. capsici* naturally infested site at an elevation of 1180masl.

Soil physical-chemical characteristics

Four samples from superficial soil (0-30cm) were taken for physical-chemical analysis in early April 2011. The soil of the experimental site had a sandy clay loam texture (29.84% clay, 12.08% silt and 58.08% sand), with pH of 7.72 and organic matter content of 1.68%. In addition, soil content (ppm) reached 50.17 inorganic N, 64.14 P, 912.51 Na, 1994.56 K, 4021.55 Ca, 408.42 Mg, 7.44 Fe, 5.47 Mn, 7.02 Zn and 2.17 Cu. Moreover, CIC values showed 32.5me/100g, and electrical conductivity was 0.84ds·m^{-1}.

Metam sodium application

The advantage of soil applied pesticides is that air pollution is avoided (Arbeli and Fuentes, 2007). Metam sodium (MS) was applied into pre-formed beds under a plastic mulch via the drip irrigation system at a dose of 600 lit·ha^{-1}. This application method enhances the chemical efficiency, as reported by Overman (1982) and Overman and Price (1983). The dose was based on label application instructions for this fumigant in the field (Gan *et al*., 1999). Given MS biocidal effects its application took place 30 days before transplanting to prevent seedlings damage.

Transplantation

Four commercial bell pepper varieties and one grafted onto two rootstocks were used to study the incidence of root and crown rot. All the seedlings were transplanted on April of 2011. The four non-grafted varieties were 'Fascinato', 'Janette', 'Lyzania' and 'Camila'. The 'Fascinato' variety was also grafted onto 'Robusto' and 'Terrano' rootstocks. All these plant materials or genotypes were bred by Syngenta Seeds, Houston, TX, USA. Seedlings were transplanted at a 0.45m spacing along into raised double-row 27m long beds. The trials had 120 or 240 plants (Table I).

Fertilization and irrigation

Fertilizers and water were applied via a drip irrigation system. Irrigations were done three days per week. Each day included two events of 1h duration; the first one at 8:00 and the second 2h later. Irrigation was suspended on those days when rainfall occurred. The growing season lasted 220 days. The following dosages (g·m^{-2}) of fertilizers were soil-applied: NH_4NO_3 (50.4), UAN32 (37.7), 5-30-00 (N-P-K) (56), KNO_3 (44.8), Ca $(NO_3)_2$ (162.3), K_2SO_4 (201.3), and $MgSO_4$ (107.5).

All the experimental area was covered with a fixed shade net, which was installed previous to the beginning of the experiment. It kept air temperature between 30ºC at day to 18ºC during the night.

Disease incidence and statistical analysis

To quantify changes in incidence over time, the disease was assessed visually. Assessments were made in intervals of 14 days during the harvest period, from July 12 to October 3, 2011. The variable recorded was disease incidence, represented by the proportion of plants expressing wilt or being dead in the area as a whole (Campbell and Madden, 1990).

Disease incidence was adjusted over time through the following general linear model by logistic regression analysis:

$$\text{Log}(Pi/1\text{-}Pi) = \beta_{0+}\beta_1$$

where Pi: probability of Yi=1, and 1-Pi: probability of Yi=0. The disease progression model was tested for each trial.

Pathogen isolation from soil

Seven soil samples were collected in July 2011 for pathogen isolation and characterization. Soil cores (500g each) were randomly sampled, at least 10m apart within the experimental area and taken from the superficial soil layer (3-20cm). Once collected, samples were transported to the laboratory.

A 10g subsample was then taken from each sample. The subsample was suspended in 90ml of sterile distilled water. One ml of the suspension (1:100) was placed into an empty Petri dish and PARPH selective media was poured and distributed throughout the dish (Erwin and Ribeiro, 1996), forcing the mycelium to grow from underneath the media towards the surface, free from bacteria (Martin *et al*., 2012).

Petri dishes were incubated at 28ºC and examined daily for colony growth during one week. Growth samples were placed on slides and examined under a Zeiss microscope (Carl Zeiss, New York) at ×40 and ×100 magnification to verify presence of pathogen mycelia and structures.

Once colonies of *Phytophthora* developed sufficiently, isolates were prepared and then plated out onto corn meal agar media (CMA; Fluka, Sigma-Aldrich) for morphological and molecular characterization. Three replications were made for each soil sample.

Pathogen isolation from plants

Symptomatic entire bell pepper plants were randomly collected in the experimental site to isolate the pathogen. The plants were transported to the laboratory, washed with tap water and cut in half. Then, small pieces of tissue were cut from the margins of lesions located on root, crown and stem.

These tissue pieces were surface disinfested as reported by Foster and Hausbeck (2010), and blotted dry with filter paper to avoid bacterial contamination (Martin *et al*., 2012). Tissues were then plated out on PARPH medium. Isolates were transferred to Petri dishes with CMA and incubated for

TABLE I
COMPARISON OF SLOPES FROM THE GENERAL LINEAR MODEL FOR DISEASE INCIDENCE OF *Phytophthora* ROOT AND CROWN ROT ON BELL PEPPER AMONG TRIALS

Trial	Self-rooted variety (V) or grafted plants (G)	N	Y_i % Jul 12th[a]	Y_f % Oct 3rd[b]	Intercept β_0	Slope β_1	Pr > χ^2
1	Robusto (G)	120	0.27	13.74	-3.7831	0.2312 a	P< 0.0001
2	Terrano (G)	120	0.83	16.13	-3.7463	0.2407 a	P< 0.0001
3	Fascinato (V)	240	0	61.66	-2.9479	0.4139 c	P< 0.0001
4	Janette (V)	240	0.55	52.77	-3.1105	0.3811 c	P< 0.0001
5	Lyzania(V)	240	0	37.07	-3.2755	0.3248 b	P< 0.0001
6	Camila (V)	120	0	69.16	-2.7774	0.4470 c	P< 0.0001

Six trials of bell pepper were compared as a strategy for controlling *P. capsici*. Four self-rooted varieties (V) and two grafted plants (G) were established in a metam sodium pretreated soil.
N=120 or 240 bell pepper plants per trial, Yi: initial disease incidence, Yf: final disease incidence.
The two parameters of Hick paradigm were estimated: intercept (β_0) and slope (β_1).
Pr > χ^2 significantly different at P>0.0001
Ha: β_1 of the trials 1 and 2≠β_1 of the trials 3-6; Ho: β_1 of the trials 1 and 2=β_1 of the trials 3-6.
a: 90 days after transplanting, b: 174 days after transplanting.

7-15 days at room temperature (21 ±2ºC). Pure cultures were obtained by hyphal tips.

Morphological and molecular characterization

A total of 86 isolates were obtained; 65 from root, 16 from crown, 2 from stem, and 3 from soil. Fourteen isolates were selected for molecular characterization: six from root, five from crown, two from stem and one from soil.

The 14 selected isolates were grown on CMA medium during one week at 24ºC. Afterwards, mycelia of each isolate were scraped and transferred to a sterile mortar. Liquid N_2 was added to ground the sample with a sterile pestle. Genomic DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen; Valencia, CA, USA) according to the manufacturer's instructions. DNA concentration and purity were measured using a NanoDrop ND 1000 spectrophotometer with the NanoDrop 2.4.7c software (NanoDrop Technologies Inc.; Wilmington, DE, USA).

Ribosomal loci of the Internal Transcribed Spacers (ITS) region were amplified and sequenced, as previously reported for some species of oomycetes (Díaz-Nájera *et al.*, 2015). The ITS region was amplified with the universal primers ITS5 and ITS4. We were able to amplify the extreme 3' of 18S, ITS1, 5.8S, ITS2 and 28S regions of ribosomal DNA. Reactions were performed as reported by Quesada-Ocampo *et al.* (2011). Polymerase chain reaction (PCR) products were separated by electrophoresis. The PCR products were located in an electrophoresis chamber with 1.5% (wt/vol) agarose gel, 0.5 Tris-borate-EDTA buffer, and a 1kb ladder (Invitrogen, USA) for 40min. The gel was stained with GelRed ($5\mu l \cdot ml^{-1}$) and photographed under UV light. Bands were considered for sequencing analysis. PCR products were sequenced in both directions using Big Dye Terminator v3 in a 3130 DNA Analyzer (Applied Biosystems, USA).

Electropherogram trimming and sequence alignments were performed using the Bioedit program (Hall, 1999). All alignments were exported as FASTA files and imported into Mega 6 software (Tamura *et al.*, 2011) to perform subsequent analysis. The obtained sequences were compared with the National Center for Biotechnology Information (NCBI) nucleotide database using the BLASTN program version 2.2.18 (Altschul *et al.*, 1990) with the default options. The known reference sequence GU259193 was included as well as two *P. capsici* sequences from Chihuahua obtained in a previous study. All the sequences were deposited in the GenBank of the NCBI.

Phylogenetic re-constructions were performed using the ITS rDNA datasets through the maximum parsimony method. To this end, we used the close neighbor interchange (CNI) search option (level= 1) with initial tree by random addition (10 reps), and gaps/missing data were considered a complete deletion. To determine the confidence values for clades within the resulting tree, bootstrap was calculated for 1000 replicates (Felsenstein, 1985). *Pythium aphanidermathum* KF667387 was used as an out-group genus.

Results

Disease temporal progress and statistical analysis

According to the graph exploration, the patterns of the disease epidemics differ between the group of varieties and the group of grafted plants with respect to epidemic onset and Yf (Table I).

Initially, disease incidence increased slowly in all trials. Disease occurrence on self-rooted bell pepper varieties was first detected 90 days after transplanting. In the two grafted plants trials the disease started 104 days after transplanting. The grafted plants group showed an exponential increase on the disease incidence only until 132 days after transplanting. By comparing the group of self-rooted and grafted plants, it is noticeable that in the latter the proportion of diseased plants remained low for a period of approximately 174 days.

Disease incidence rose significantly until day 132 for grafted plants and until 146 days for self-rooted plants. It reached an asymptote in rootstocks trials toward the end of the epidemics (Figure 1) whereas no asymptote was reached on the disease incidence in the case of the varieties.

The general linear model appropriately fits the disease progress data over time. The model effects were found to be statistically significant at $P<0.0001$. When 'Robusto' and 'Terrano' rootstocks were planted on the fumigated soil some control of *P. capsici* was achieved. In this case, only few plants showed symptoms, representing 16% incidence in 'Terrano', and 13.7% in 'Robusto'. Disease incidence followed a sigmoid function.

In general, the four varieties had a high disease incidence. However, differences among varieties can also be appreciated. For instance, self-rooted Lyzania plants (trial 5) were the most tolerant variety with the lowest slope $\beta_1= 0.3248$. In contrast, plants grafted onto 'Robusto' rootstock were the most tolerant in all evaluation dates with the lowest Yf (13.7%) and the lowest slope ($\beta_1= 0.2312$) in log odds ratio. The general lineal model fitted well the measured data for the two rootstocks and the four self-rooted varieties (adjusted R^2, $P<0.0001$).

Morphological and molecular characterization

Our results confirmed that the 86 obtained isolates from crown and root rotted tissues were P. capsici, according to the following morphological features: caduceus sporangia with papillae and pedicels longer than 20µm, formed in sporangiophores in simple sympodia. Plerotic oospores with amphygenous antheridia were observed in laboratory crosses (Erwin and Ribeiro, 1996).

Blast analysis of the ITS rDNA amplified regions for the 14 selected isolates showed 100% similarity to sequences of *P. capsici* deposited in GenBank (NCBI). In addition, all the sequences clearly clustered into one clade using the maximum parsimony method. These isolates were also grouped with the published type reference sequence GU259193 of *P. capsici* (Figure 2).

Discussion

The results indicate that the use of grafted bell pepper varieties on resistant pepper

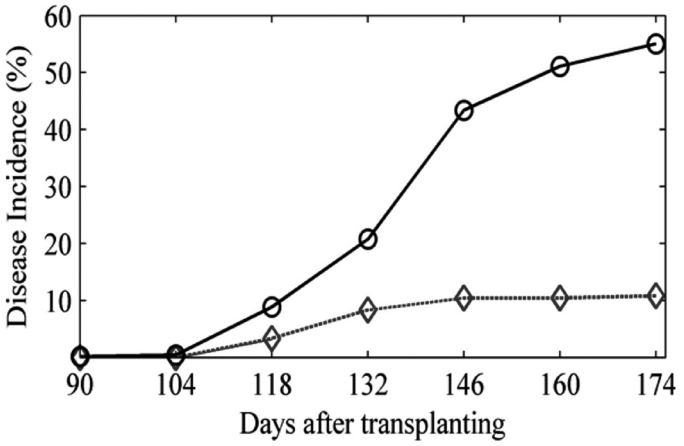

Figure 1. Disease incidence of *Phytophthora* root and crown rot over time. Four self-rooted varieties (line with circles) and plants grafted onto two resistant rootstocks (dotted line with diamonds) are grouped and compared.

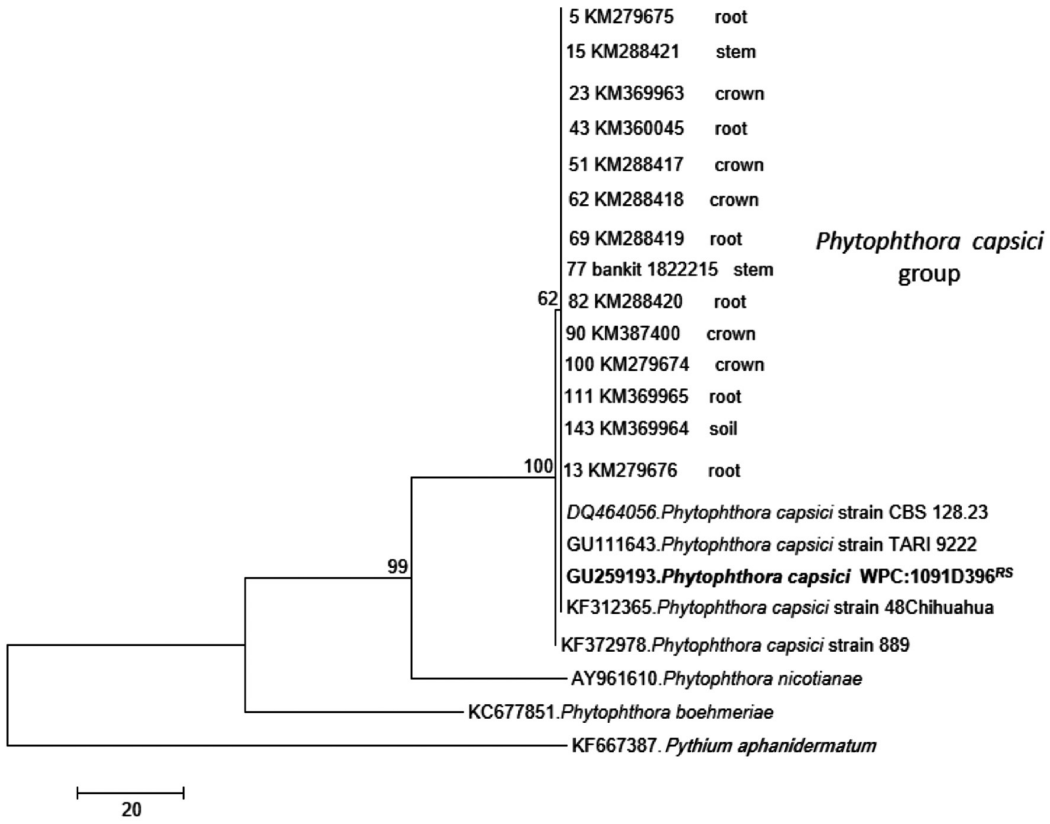

Figure 2. Phylogenetic tree inferred with using values created with maximum parsimony from ITS sequences, which were aligned automatically with CLUSTAL-W (Thompson *et al*., 1994). The numbers above the branches are fast bootstrap support values equal to or larger than 60% from 1000 replicates. Tissue where *P. capsici* was isolated is included. The tree was rooted with *Pythium aphanidermatum* as an outgroup genus.

rootstocks planted on a pre-fumigated soil could provide an effective management strategy to reduce *Phytophthora* root and crown rot incidence.

The linearization of disease progress curves was essential to determine the epidemic speed. We fit the epidemics with the general linear model, which is recognized as an appropriate model to describe soilborne diseases (Liu *et al*., 1995) such as *Phytophthora* root and crown rot (Ristaino, 1991).

It is likely that the effect of metam sodium resulted in slow disease progress at the beginning of the epidemic. Symptoms of the disease appeared 104 and 90 days after transplanting in grafted and non-grafted plants, respectively. It is likely that the application of metam sodium resulted in a slow epidemic onset. Later, the non-grafted varieties showed a sharp upward 'inflection' of disease incidence, probably caused by reduction of metam sodium effects. The loss of metam sodium effectiveness has been reported previously due to fumigant degradation in soil. In a study by Triky-Dotan *et al*. (2009) metam sodium significantly reduced the incidence of *Pythium* rot in peanut after one application. However, fumigant effectiveness was greatly reduced after the second application. The same situation occurred when *Verticillium* was controlled with single and double applications of metam sodium; this fumigant was even less effective in the third application. Caution should be taken on the extensive use of a fumigant for disease management as it can can render *P. capsici* field populations resistant (Triky-Dotan *et al*., 2009).

Even though bell pepper production was obtained in this study (data not shown), the disease incidence increased with time (Yf=13.7% for 'Robusto' rootstock). This percentage was reached 146 days after transplanting.

Since *P. capsici* is one of the most destructive pathogens to chili pepper and bell pepper (Hausbeck and Lamour, 2004) studies of soil fumigants (Linderman and Davis, 2008) and the use of resistant rootstocks (Louws *et al*., 2010) are essential to reduce disease incidence to assure crop yield. A considerable amount of research has been done on chemical control (Silvar *et al*., 2006; Keinath, 2007; Dunn *et al*., 2010; Foster and Hausbeck, 2010) and resistant pepper cultivars, although only a few have been conducted on combining resistant rootstocks planted on prefumigated soil (Morra and Bilotto, 2006). In the present study, results revealed that bell pepper grafted on resistant rootstocks could be an alternative to reduce the incidence of *Phytophthora* crown and root rot. Our findings confirm those of Gilardi *et al*. (2013), where 'Terrano' and 'Robusto' bell pepper rootstocks showed resistance to *P. capsici*.

The use of grafted pepper cultivars onto resistant pepper rootstocks in combination with metam sodium soil applications before transplanting could be an effective alternative to suppress disease progress caused by *P. capsici*. Rotation schemes are a cultural practice that somehow controls the disease. Nevertheless, management strategies that rely only on crop rotation may not provide an effective control of *P. capsici*, given the wide host range that this pathogen has, and the long time that propagules are cable to survive (Hwang and Kim, 1995). It should also be considered that the experimental site had a high propagule population, as given by the high incidence in the self-rooted control plants.

The phylogenetic analysis containing 14 sequenced *P. capsici* isolates of bell pepper, and other references showed that the dataset fitted into one clade. Previous research work on *P. capsici* found temperate isolates from the Solanaceae family to be grouped together in the same clade (Bowers *et al*., 2007).

Our results provide compelling evidence that the *P. capsici* population showed no genetic differences within this experimental area. This supports the statement of Lamour and Hausbeck (2003) and Hu *et al*. (2013) who mentioned that *P. capsici* isolates from a single field experiment outcrossed within the population and, as a result, unique genotypes are present. Previous studies in Mexico (Silva-Rojas *et al*., 2009) have found both *P. capsici* compatibility types, which indicates that sexual reproduction is involved.

Since *P. capsici* is a destructive pathogen on bell pepper, avoiding the introduction of soil from another infested area is a required action to prevent genetic recombination (Gevens *et al*., 2006). Some future research priorities are identified that would be valuable in a

better understanding of the epidemic. Large pathogen samples from different locations in northern Mexico would increase the robustness of these findings.

Studies on managing *Phytophthora* root and crown rot must continue to improve integrated management practices. This may include fumigants combination, fungicides, use of grafted plants, appropriate soil drainage and crop rotation.

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