

Rootstock selection of avocado (*Persea americana*) tolerant-resistant to *Phytophthora cinnamomi*

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Abstract

Worldwide, the effort to control root rot caused by *P. cinnamomi* has had a limited success. The objective of this work was to select avocado genotypes that are tolerant-resistant to *P. cinnamomi*. The selection was done by inoculating trees of the genotypes Tepetl, Atlixco, Tepeyanco, Thomas, and Duke clone 7, ranging from 5 to 8 months and 2 year-old, with a mycelia solution (10^4 /mL) of *P. cinnamomi*, grown in a V8 juice medium. The selection was done at controlled temperatures, 17 and 28°C, and open air temperature (18.6°C). In a second experiment, seedlings of Colin, Tepetl, Toliman, Atlixco, Tepeyanco and Thomas ranging in height from 5 to 20 cm were inoculated. Regarding the effect of inoculation at all three temperatures, Thomas, Duke 7 and Atlixco showed a low death ratio. Based on the obtained results, the appropriate temperature for tolerant-resistant rootstock selection is 28 and 17°C, in Atlixco and Tepeyanco, which had a high percentage of asymptomatic plants and having 0 to 10% of the symptom. In the selection test for tolerance-resistance to *P. cinnamomi* in 5 cm high seedlings, the death ratio of Thomas, Tepetl, and Toliman was 100%. Atlixco, Tepeyanco, and Colin had low death percentages. Stem canker symptoms were only present in Atlixco and Tepeyanco. However, Colin, Tepeyanco, and Atlixco formed new seedlings shoots and strong roots, suggesting tolerance to *P. cinnamomi*, this selection test induces susceptibility. In 15 to 20 cm tall seedlings selection, Tepetl and Toliman showed 90% death ratio; while Atlixco, Tepeyanco, and Colin had low percentages. Thomas was resistant to plant inoculation, showing 0% mortality. Regarding seedlings with stem canker symptoms, Atlixco shed 28.78%. However, Tepeyanco, Atlixco, and Thomas had a high percentage of asymptomatic plants.

Key words: *P. cinnamomi*, tolerance-resistance, avocado, seedlings, temperature, canker.

Selección de portainjertos de aguacate (*Persea americana*) tolerantes-resistentes a *Phytophthora cinnamomi*

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Resumen

A nivel mundial, el intento por controlar la pudrición de la raíz causada por *P. cinnamomi* ha tenido un éxito limitado. El presente trabajo tuvo como objetivo seleccionar genotipos de aguacate tolerantes-resistentes a *P. cinnamomi*. La selección se realizó inoculando árboles de 5 a 8 meses y 2 años, con solución micelial (10^4 /mL) *P. cinnamomi*, crecido en medio líquido jugo V8, a los genotipos Tepetl, Atlixco, Tepeyanco, Thomas y clon Duke 7. La selección se realizó con temperaturas controladas, 17 y 28°C, además de temperatura ambiente (18.6°C). En un segundo experimento, se seleccionaron plántulas inoculando a Colin, Tepetl, Toliman, Atlixco, Tepeyanco y Thomas con una altura entre 5 a 20 cm. El efecto de la inoculación en las tres temperaturas, Thomas, Duke 7 y Tepetl presentaron porcentajes altos de mortalidad con relación a Tepeyanco y Atlixco que presentaron baja mortalidad. En base a los resultados obtenidos, la temperatura apropiada para la selección de portainjertos tolerantes-resistentes es de 28 y 17°C, en Atlixco y Tepeyanco por presentar porcentajes altos de plantas asintomáticas y por presentar de 0 a 10% de síntomas. En la prueba de selección para tolerancia-resistencia a *P. cinnamomi* en plántulas con 5 cm de altura, la mortalidad en Thomas, Tepetl y Toliman fue 100%. Atlixco, Tepeyanco y Colin presentaron porcentajes bajos de mortalidad. Los síntomas de cancro de tallo únicamente los presentaron Atlixco y Tepeyanco. Sin embargo, Colin, Tepeyanco y Atlixco formaron nuevos brotes de plántulas y raíces vigorosas, sugiriendo tolerancia a *P. cinnamomi*, esta prueba de selección induce a susceptibilidad. La selección en plántulas inoculadas de 15 a 20 cm de altura, Tepetl y Toliman presentaron 90% de mortalidad, Atlixco, Tepeyanco y Colin obtuvieron bajos porcentajes. Thomas fue resistente a la inoculación en plántula mostrando 0% de mortalidad. En cuanto al porcentaje de plántulas con síntomas de cancro de tallo, Atlixco mostró 28.78%. Sin embargo, Tepeyanco, Atlixco y Thomas presentaron alto porcentaje de plantas asintomáticas.

Palabras clave: *P. cinnamomi*, tolerancia-resistencia, aguacate, plántulas, temperatura, cancro.

Introduction

Root rot caused by *Phytophthora cinnamomi* Rands is one of the main problems that affects avocado (*Persea americana* Mill) production. Another important disease is the trunk canker, also caused by *P. cinnamomi*. Trunk Canker may attack the bottom of the trunk causing dark rotting (Téliz et al, 2007, p. 192). Root rot causes declination and deaths plant. Resistance is one of the best promising method with a long run success to control Root rot disease (Menge et al, 1992, p. 53-59). For many years the development of a resistant rootstock has been a priority as a solution to the problems caused by *Phytophthora*. Among the varieties that have showed a moderate resistance to this disease are Duke 6 and Duke 7. Thomas and Duke 7 rootstock are considered resistant to this disease at California, U.S.A. fields (Zentmyer 1978, p. 87-89; Menge et al, 1992, p. 53-59). Duke 7 rootstock has turned into the most common rootstock in the industry because it produces healthy, non varied and productive trees. Thus, the potential production of avocado depends on the selection of the rootstock due to its resistance to the disease.

The selection of a resistant rootstock to root rot by *P. cinnamomi* has been done in a partial way; this selection must be the priority to deal with this disease. Recently there is a concern about the lack of solutions to the problems caused by *P. cinnamomi*, (Téliz et al, 2007, p. 52). Therefore the selection of rootstock coming from seeds must be done in order to use all the genetic variation and identifying and using the resistant rootstock against *P. cinnamomi*.

Materials and Methods

Samples of roots with symptoms of root rot on avocado from cultivar Hass were collected in Uruapan, Michoacán, México. From the roots were taken 2 cm long sections and were disinfected with sodium hypochlorite at 10% for one minute. Then the ten roots sections were rinsed with distilled water and collocated in V8 culture. PARPH (pimaricin, ampicillin, rifampicinllin, PCNB and hymexazol). (Tsao et al, 1977, p. 796-801). The culture with the ten root sections was incubated at 28°C during four days. After the incubation, the isolation medium was transferred to a Petri dish with V8 juice agar culture where it grew at 28°C during eight days. Then the inoculum was increased in a liquid V8 culture medium (Figure 1). The isolation was characterized morphologically (Erwin et al, 1996, p. 270-271, Gallegly 2008, p 38). An extraction and sequence of *P. cinnamomi*'s DNA was made at the Biotechnology Laboratory of seeds in Colegio de Postgraduados, Campus Montecillo Méx. The evaluation of tolerance-resistance was made by two methods. In the first method of controlled temperatures 28°C, 17°C and room temperature (Bunker green house at 18.6°C), were inoculated 250 ml of a mycelium suspension (10^4 /mL) of *P. cinnamomi*. The oomycete was developed in a liquid V8 culture medium. The seeds of Mexican race, Atlixco and Tepeyanco cultivars were collected in Atlixco Puebla and Tepeyanco Tlaxcala. The rootstocks Duke 7 (2 years) and Thomas (8 months); Tepetl, Toliman and Colin (5 months) from Mexican race seeds were given by Fundación Salvador Sánchez Colín CICTAMEX, Coatepec Harinas, Méx.

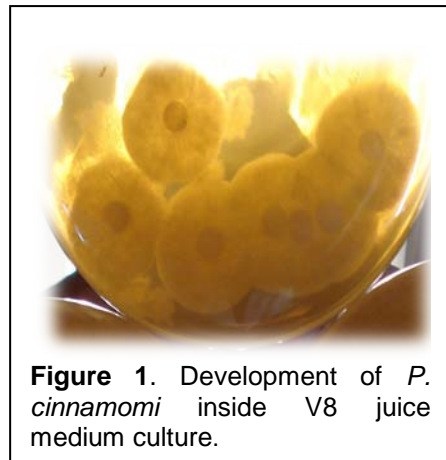


Figure 1. Development of *P. cinnamomi* inside V8 juice medium culture.

The second method selection among seedlings of 5 cm and 15 to 20 cm tall, this method was developed in a bunker greenhouse. Seedlings of 5 cm and 15 to 20 cm tall were inoculated with a mycelium solution (10^4 /mL), 200 ml of the mycelium solution were required to inoculate. Before the inoculation of Thomas, Colin, Tepetl, Toliman, Atlixco y Tepeyanco, four holes of 3cm depth were made in the soil near the root. After the inoculation, the soil was irrigated until saturation point. Between the days 8 and 15 of the inoculation, the percentage of seedling with symptoms, without symptoms, mortality, steam canker and seedlings with secondary sprouts was evaluated. The experimental design was a random factorial assignment and the results were analyzed by using the software SAS® (Statistical Analysis System) V. 9.0.

Results and discussion

The morphological characteristics observed in the oomycete match with the ones reported by Gallegly (2008, p. 38); Erwin and Ribeiro (1996, p. 270-271). Hyphal swellings, chlamyospore germination, chlamyospore presence and sporangium of *P. cinnamomi* were observed (Figure 2). The phylogenetic tree built with the sequences CO I y CO II, showed that the isolations of avocado roots taken from trees with symptoms of root rot on avocado correspond to *P. cinnamomi*. The access number for Genbank is in process.

The effect of temperatures 28°C, 17°C and room temperature in the bunker green house (18.6°C) without *P. cinnamomi*, showed that the percentage of infection in the witness was 0% and did not cause mortality in plants. In the inoculated plants, the infection was present eight months later with temperatures of 17 and 18.6°C. The variation rootstock and inoculation was significant (Table 1). The percentage of wilting symptoms in Tepetl, Duke 7 and Thomas were 20, 50 y 51% at 17°C. In the Atlixco and Tepeyanco cultivars at 17°C there were no infections symptoms however at 18.6°C the symptomatology for Atlixco and Tepeyanco was of 20 and 10 % respectively.

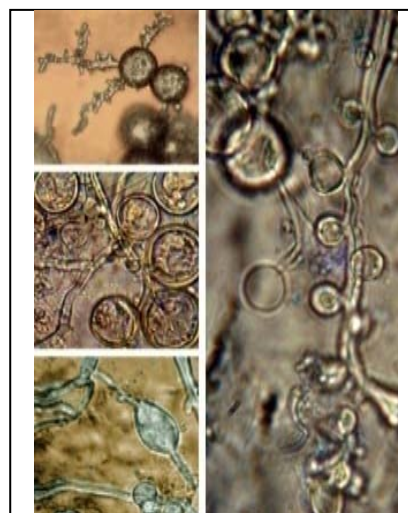


Figure 2. *P. cinnamomi*. chlamyospore germination, chlamyospores, sporangium, hyphal swellings.

Tepetl and Thomas at 18°C showed symptoms of 30 and 42.8 %. The symptomatology at 28°C does not show any statistically difference in Thomas and Tepetl with 42.8 and 60% of wilting symptoms. The lowest percentage of symptoms was 10 % in Atlixco and Tepeyanco. There was no wilting symptoms at 28°C in Duke 7 (Table 1.) due to the 75% of plant mortality at this temperature. There was no statistically difference in mortality range at the cultivars Tepetl and Thomas ($P \geq 0.05$) with 20 y 28.57%, Menge (1999, p. 69-72) mentioned that Thomas is the most resistant rootstock against root rot.

The percentage of mortality did not was significantly different between the rootstock Thomas (14.29%) and the Mexican Atlixco race cultivar (10%) at the room temperature of the bunker greenhouse (18.6 %), while at the Mexican Tepeyanco race there was a lack of mortality (0%) at the three temperatures. Table 1 show that Atlixco and Tepeyanco had a range from 70 to 100% of non-symptomatic plants at the three temperatures. However at the three temperatures, the rootstock Thomas showed a range of 28.6 to 42.9% of plants without symptoms, the Duke 7 and Tepetl cultivars at the three temperatures showed 10 to 50% of plants without symptoms. However the Mexican Atlixco race and Tepeyanco cultivars showed 70 to 100% of plants without symptoms in the tree temperatures, this point to lowest percentages of mortality and wilting symptoms at the three temperatures. The effect of inoculation at the three temperatures (necessary conditions for *P. cinnamomi* development) caused significant percentages of mortality ($P \geq 0.05$), Thomas, Duke 7 and Tepetl showed higher mortality percentages than Tepeyanco and Atlixco which had a low mortality and mostly had an expression of 0%. However, the resistance of the plant and the defense mechanisms allowed them to heal the infections in a quick and effective way. The plants may also produce toxic substances. These general defense mechanisms may appear due to the selection originated by potential pathogens such as *P. cinnamomi* (Niks 2004, p. 15).

Table 1. Temperature and mortality and infection percentage caused by *P. cinnamomi* at the resistance–tolerance test in avocado rootstock.

		% of mortality and infection								
		Mortality			Wilting symptoms			Without symptoms		
		28°C	17°C	†18.6°C	28°C	17°C	†18.6°C	28°C	17°C	†18.6°C
Thomas	Inoculated	28.57 ^{bcd}	14.29 ^{cde}	14.29 ^{cde}	42.8 ^{abc}	57.1 ^{ab}	42.8 ^{abc}	28.6 ^f	28.6 ^f	42.9 ^d
	Uninoculated	0 ^e	0 ^e	0 ^e	0 ^c	0 ^c	0 ^c	100 ^a	100 ^a	100 ^a
Duke-7	Inoculated	75 ^a	50 ^{abc}	50 ^{abc}	0 ^c	50 ^{ab}	0 ^c	25 ^h	0 ^k	50 ^d
	Uninoculated	0 ^e	0 ^e	0 ^e	0 ^c	0 ^c	0 ^c	100 ^a	100 ^a	100 ^a
Tepetl	Inoculated	20 ^{cde}	40 ^{abcd}	60 ^{ab}	60 ^a	20 ^{abc}	30 ^{abc}	20 ⁱ	40 ^e	10 ^j
	Uninoculated	0 ^e	0 ^e	0 ^e	0 ^c	0 ^c	0 ^c	100 ^a	100 ^a	100 ^a
Atlixco	Inoculated	0 ^e	0 ^e	10 ^{de}	10 ^c	0 ^c	20 ^{abc}	90 ^b	100 ^a	70 ^c
	Uninoculated	0 ^e	0 ^e	0 ^e	0 ^c	0 ^c	0 ^c	100 ^a	100 ^a	100 ^a
Tepeyanco	Inoculated	0 ^e	0 ^e	0 ^e	10 ^c	0 ^c	10 ^c	90 ^b	100 ^a	90 ^b
	Umimoculatedr	0 ^e	0 ^e	0 ^e	0 ^c	0 ^c	0 ^c	100 ^a	100 ^a	100 ^a
LSD (0.05)		39.84			47.37			0		

LSD: Least Significant Difference.

†= Greenhouse room temperature; all of them were measured with the same letter, therefore there is no significant difference, Tukey (Pr≥0.05).

According with the results, the deduction of the selection of rootstocks resistant and tolerant in conditions of controlled temperature was that the Mexican race, Atlixco and Tepeyanco cultivars are tolerant to *Phytophthora cinnamomi* infection. 25 to 28°C is the right range of temperature for the development of *P. cinnamomi*, The results in this test were similar to obtained by Zentmeyer (1978, p. 87-89), where Duke 7 was selected at 25°C showing 45% of resistance to root rot. The results suggest that for the selection of rootstock tolerant- resistance to *P. cinnamomi* with controlled temperatures, the suitable temperature for the selection with inoculums is between 28°C y 17°C, for the Mexican, Atlixco and Tepeyanco races because they have symptom percentages between 0% and 10% and they show high percentage of plants without symptoms, which indicate different responses to the effect of temperature and defense reaction of the plants (Zentmyer et al, 1960, p. 107-109). The utilization of rootstocks tolerant to disease caused by *P. cinnamomi* is an important preventive practice to control this fungus, thus this practice offers possibilities of success in the management of this disease (Leach et al, 2001, p. 187-224).

Rootstock selection inoculating seedlings of 5 cm height, in the test of seedling selection of 5 cm height, the percentage of mortality in Thomas and in the genotypes of the Mexican races Tepetl and Toliman was of 100% (Table 2) after 30 days of the inoculation. This selection test induces the susceptibility, due the incapacity of the seedling to activate its defense mechanisms (new root formation and new seedling shoots), before the presence of the pathogen.

The lowest percentages of mortality were found in Atlixco, Tepeyanco and Colin (Table 2). Regarding to the presence of wilting symptoms, characteristic of *P. cinnamomi*, Colin, Tepeyanco and Atlixco showed 47.82, 33.33 and 25.55% mortality, respectively. The stem canker symptoms were only presented in Atlixco and Tepeyanco in low percentages of 8.7 and 10%, respectively. The results are consistent in Atlixco and Tepeyanco, presenting 28.9 and 20% of seedlings without symptoms pointing out that there was more tolerance to the infection (Table 2).

Table 2. Percentage of mortality and infection in the selection of seedlings of 5 cm height inoculated with *P. cinnamomi*.

		% of mortality and infection			
		Mortality	Wilting symptoms	Stem canker	Without symptoms
Thomas	Inoculated	100 ^a	0 ^b	0 ^a	0 ^e
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
Tepetl	Inoculated	100 ^a	0 ^b	0 ^a	0 ^e
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
Toliman	Inoculated	100 ^a	0 ^b	0 ^a	0 ^e
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
Colin	Inoculated	43.47 ^b	47.82 ^a	0 ^a	8.7 ^d
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
Atlixco	Inoculated	36.66 ^b	25.55 ^{ab}	8.89 ^a	28.9 ^b
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
Tepeyanco	Inoculated	36.66 ^b	33.33 ^a	10 ^a	20 ^c
	Uninoculated	0 ^c	0 ^b	0 ^a	100 ^a
LSD (0.05)		23.11	30.8	20.7	0

LSD = Least Significant Difference. Means with the same letter in each column does not differ significantly, Tukey (Pr \geq 0.05).

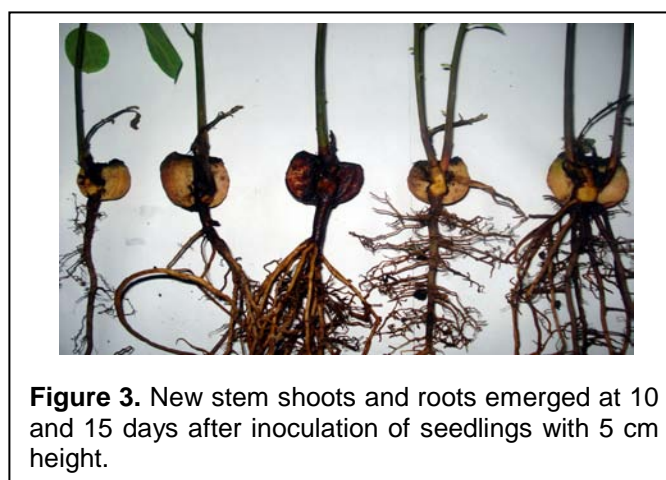
Formation of new seedling shoots before the pressure of the inoculation of *P. cinnamomi* in the test of seedling selection of 5 cm height. In the test of the selection of inoculated seedlings of 5 cm height, it was found that after 10 to 15 days of the inoculation and death of the first seedling in the cultivars of the Mexican races Tepetl, Colin, Tepeyanco and Atlixco (Table 3), there was emergence of one or two seedling shoots. In these, there was a second inoculation to prevent the seedlings to escape infection. In this second inoculation, the highest mortality was 20, 17.39, 7.77 and 6.66% in Tepetl, Colin, Tepeyanco and Atlixco, respectively. 40 days after reinoculation, the new reinoculated seedling shoots showed necrotic spots. However, where there were two shoots, one of them dies, and sometimes the other one had symptoms. These observations the cultivars of the Mexican races Colin, Atlixco and Tepeyanco showed a lower incidence of damage.

Table 3. Percentage of new seedling shoots and infection in the selection of seedlings of 5 cm height inoculated with *P. cinnamomi*.

		% of new seedling shoots		
		Mortality	With Symptoms	Without symptoms
Tepetl	Inoculated	20 ^a	0 ^a	0 ^e
	Uninoculated	0 ^b	0 ^a	100 ^a
Colin	Inoculated	17.39 ^a	4.34 ^a	8.7 ^c
	Uninoculated	0 ^b	0 ^a	100 ^a
Tepeyanco	Inoculated	7.77 ^{ab}	3.33 ^a	5.6 ^d
	Uninoculated	0 ^b	0 ^a	100 ^a
Atlixco	Inoculated	6.66 ^{ab}	4.44 ^a	8.9 ^b
	Uninoculated	0 ^b	0 ^a	100 ^a
LSD (0.05)		17.21	11.82	0

LSD = Least Significant Difference. Means with the same letter in each column does not differ significantly, Tukey (Pr ≥ 0.05).

Still are unknown the mechanisms of the resistance of the avocado seedling to the infection of *P. cinnamomi*. In Figure 3 are shown the death of the first seedling and the new seedling shoots with new vigorous roots. According to the obtained results Tepeyanco, Atlixco and Colin can be tolerant and resistant rootstocks to *P. cinnamomi*.



Selection in inoculated seedlings of 15 to 20 cm height. The mortality of the inoculated seedlings of 15 to 20 cm height show significant differences (Pr ≥ 0.05). The cultivars Tepetl and Toliman showed a high mortality (90%) and the lowest mortality were obtained in the Atlixco, Tepeyanco and Colin cultivars with 6.18, 8.00, and 17.9%, respectively. Thomas was resistant to the inoculation in seedlings showing 0% of mortality. However, it showed a 64.28% of wilting symptoms in the seedlings of 15 to 20 cm height. Colin showed a 64.28% of the symptoms. As for the percentage of seedlings with symptoms of stem canker, Atlixco showed the highest value (28.78%). However, Tepeyanco, Atlixco and Thomas obtained the highest percentage of seedlings without symptoms (Table 4). These results suggest that the selection of the seedlings of 15 to 20 cm height is another option to obtain tolerance–resistance to the wilt of the root and the stem canker caused by *P. cinnamomi*.

Table 4. Percentage of mortality and infection in the seedling selection of 15 to 20 cm height inoculated at room temperature of 18.5 °C with *P. cinnamomi*.

		% of mortality and infection			
		Mortality	Wilting symptoms	Stem canker	Without symptoms
Thomas	Inoculated	0 ^c	64.286 ^a	10 ^a	30 ^{cd}
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
Tepetl	Inoculated	90 ^a	10 ^b	0 ^b	0 ^e
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
Toliman	Inoculated	90 ^a	10 ^b	0 ^b	0 ^e
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
Colin	Inoculated	17.9 ^b	64.28 ^a	7.14 ^{ab}	10.71 ^{de}
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
Tepeyanco	Inoculated	8 ^{bc}	16.21 ^b	8.78 ^{ab}	66.89 ^b
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
Atlixco	Inoculated	6.18 ^{bc}	16.66 ^b	28.78 ^a	47.72 ^{bc}
	Uninoculated	0 ^c	0 ^b	0 ^b	100 ^a
LSD (0.05)		17.46	26.78	26.03	20.39

LSD = Least Significant Difference. Means with the same letter in each column does not differ significantly, Tukey (Pr ≥ 0.05).

The symptoms of the stem canker in seedlings of 15 to 20 cm height was observed at 25 days after inoculation showing necrosis of 1 cm long confined to the stem epidermis. At 60 days, the necrosis reached the 20 cm in length in the stem (Figure 4), showing wilt symptoms at 30 days and finally plants death (Gallegos 1983, p. 317).

CONCLUSIONS

The adequate temperatures for the selection of tolerant-resistant rootstocks to *P. cinnamomi* are 28 °C and 17 °C indicate different rates of infection and mortality, in response to the defense mechanisms of the plant to the oomycete.

In the tests of inoculated seedling selection 5 cm and 15 to 20 cm height, the genotypes of the Mexican races Atlixco and Tepeyanco can be promising tolerant-resistant rootstocks, by showing consistence to the wilt of the root and stem canker caused by *P. cinnamomi*.

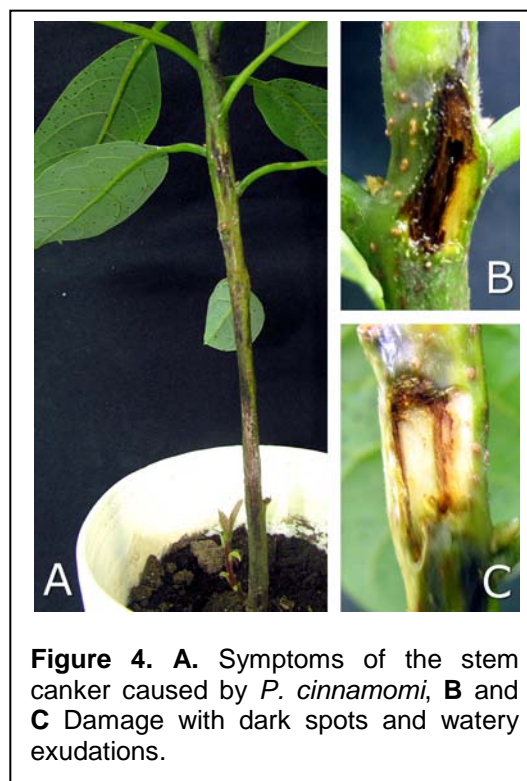


Figure 4. A. Symptoms of the stem canker caused by *P. cinnamomi*, B and C Damage with dark spots and watery exudations.

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