Article

Antiserum *vs* phytopathogenic fungi in the tomato crop in Sonora, Mexico

Francisco Eleazar Martínez-Ruiz[1]
Gabriela Andrade-Bustamante[1]
Emmanuel Aispuro Hernández[1]
Luis Guillermo Hernández-Montiel[2]
Ramón Jaime Holguín Peña[2]
Edgar Omar Rueda-Puente[3§]

[1]Institute of Agricultural Sciences-Autonomous University of Baja California. Ejido Nuevo León, Mexicali, Baja California, Mexico. CP. 21705. [2]Center for Biological Research of the Northwest. Mar Bermejo No. 195, Col. Beach Palo Santa Rita, La Paz, Baja California Sur. CP. 23090. [3]Department of Agriculture and Livestock-University of Sonora. Highway to Kino Bay km 21, Hermosillo, Sonora, Mexico. CP. 23000.

[§]Corresponding author: erueda04@santana.uson.mx.

Abstract

Phytosanitary problems are to a large extent the cause of global economic losses in agricultural crops, which are mainly caused by fungi. In the Mexican Republic, specifically in the state of Sonora, Mexico, the area directed to the tomato crop has increased considerably in recent years. In the last production cycles, a problematic of phytosanitary control has been generated, where the symptoms of known pathogens, are confused with those of 'new' phytopathogens that are arriving to the agricultural areas, allowing the technician to have uncertainty of which pathogen treats and therefore does not succeed in applying a control in the crop. Going to professional services in phytosanitary laboratories, the demand too much, is usually an unaffordable way to the producer. The objective of the present investigation was to identify the causative agents of diseases: *Fusarium oxysporum* f. sp. *lycopersici* (Race 1), *Alternaria solani* and *Botritys cinerea* on the sampled organs to know the current phytosanitary status of the tomato crop in the state of Sonora, to seed, seedling, foliage and physiological maturity, isolating and identifying them by means of the combination of diagnosis (production of antisera in New Zealand race rabbits, techniques for the use of culture media, Elisa and pathogenicity tests). The sampling for the identification of phytopathogenic fungi was carried out representatively in tomato producing regions. The results obtained show the identification of three representative fungi of economic importance in the tomato crop distributed in the state of Sonora. It is concluded that separate screening tests should not be used as a single detection method.

Keywords: antiserum, detection, fungi, phytopathogen.

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Introduction

Among the disease-causing fungi found in tomato crops mentioned *Fusarium oxysporum* causing vascular wilt disease, *Alternaria* blight disease causing early and *Botrytis cinerea* causes gray mold (Grijalva-Contreras *et al*., 2010; Grijalva-Contreras *et al*., 2011; Grijalva-Contreras *et al*., 2014; SAGARPA, 2015).

Fusarium oxysporum is one of the causative agents of more important diseases in the tomato crop since it can reach 60% decrease in production, its initial symptom is yellowing of the oldest leaves, this wilting progresses until the plant dies; *Alternaria*, is capable of diminishing yields between 20-30%, mainly damaging the leaves, in which it generates necrotic spots, which reduces the photosynthetic capacity of the plant, the injuries in the fruit normally occur at the tip of the calyx, and they are dark, leathery and sunken (Martínez-Ruiz *et al*., 2016). On the other hand, *Botrytis cinerea* is the second most important phytopathogenic fungus in tomato, during the development of the crop it can cause canker in the stem and rot of freshly set leaves, flowers and fruits (Dal Bello *et al*., 2012).

For the detection of microorganisms one of the diagnostic methods is the conventional one that is based on biochemical tests. Another of the methods used in the diagnosis is the DAS-Elisa, a technique with sensitivity and replicability, but that does not meet one of the requirements of a diagnostic method such as economic when it requires to be used in a large number of samples; the constant use of the kits (commercial components) DAS-Elisa, turns out to be unaffordable for the producer (Alvarado-Martínez *et al*., 2013).

Based on the principle of immunization and antigen-antibody interaction and based on the above described (economic infeasibility of the DAS-Elisa, for the detection of phytopathogens), in the present investigation is suggested the production of an antiserum against *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria* sp. and *Botritys cinerea* and its detection in the tomato crop (*Solanum lycopersicum*), during the vegetative development in tomato crops in the state of Sonora.

Materials and methods

The investigation was carried out in two phases. The first consisted in the production of antiserum for *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* and the second in obtaining commercial seed, sampling of seedlings, developed leaf and tomato fruit in three regions of the state of Sonora, and its phytopathological analysis for the detection of the above-mentioned fungi in the Department of Agriculture and Livestock of the University of Sonora in Hermosillo, Sonora, Mexico. The fungal specimens of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* were provided by the company Nutrimentos Sustentables para la Agricultura, located in Cuautla, Morelos.

Increase in *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea*

The first stage started with the inoculum increase. According to the protocol established by Cañedo and Ames (2004), the fungus was obtained from the infected material, either by sowing pieces of potato-dextrose-agar (PDA).

The identification for each fungus was made according to the Jarvis monograph (1975), in addition to the keys of 'CMI descriptions of pathogenic fungi and bacteria' belonging to the Commonwealth Micological Institute. In the same way, the support of the keys for identification of fungi was used by Barnett and Hunter (1998); Gilman (1963); Romero (1993). To have the fungi with the minimum genetic variation, monosporic cultures of all the strains were made, from which the sample was taken.

The suspensions of conidia in tubes were stored under refrigeration at 4 °C until use. Subsequently, this material were performed in the pathogenicity tests seed for *Fusarium oxysporum* f. sp. *lycopersici* (R1)= (Folr1), was developed in tomato vegetative material Bonny Best and Manapal (without resistance genes and resistant to Folr1, respectively), for *A. solani* was in leaves and *B. cinerea* in fruit being made in the variety 'Río Grande' whose characteristics is that it is of a certain type and fruit type saladette and susceptible to both phytopathogenic agents.

The pathogenicity tests consisted in an inoculation of Folr1 in differential materials; for the case of seed, 20 seeds were imbibed in 200 ml in a conidial suspension (Folr1) of 10^8 conidia ml^{-1} and the seeds were seeded in unicel cups (½ liter) containing sterile peat-moss substrate (Sunshine, Sun Gro Horticulture Canada, Ltd.); likewise, it was carried out in seedlings with a development of 20 days after sowing, through a conidial suspension of 10^8 ml^{-1} conidia through the immersion of roots to which small wounds were previously made with a hypodermic needle.

They were immediately transplanted into ½ liter beaker vessels containing the same peat-moss type sterile substrate (Rueda *et al*., 2006). The plants were maintained at an ambient temperature of approximately 30 ±2 °C. Four repetitions of a plant in each material were used for the data collection, the response of the differential materials was observed and recorded to ensure also and autoinfection by Folr1. The irrigation was carried out with sterile distilled water, according to the technical recommendations (INIFAP, 2005). Wilt symptoms in inoculated seedlings were presented 30 days after inoculation. The evaluation was made again based on the presence or absence of the disease.

Regarding the tests on leaves and fruits, the first ones were inoculated with *A. solani* with the help of a hand sprayer-sprinkler, of 250 ml, while the fruit was inoculated with *B. cinerea* by first puncturing them and adding with a 1 ml cotton swab of conidial suspension at a concentration of 10^8 ml^{-1}. After the inoculations, the organs were placed in humid chambers at a temperature of 30 ±2 °C in a period of 30 days (Jarvis and Hargreaves, 1973; Jarvis, 1975; Davised, 1988; Eckert, 1988; Tello and Lacasa, 1988; SAGARPA, 2005), which are appropriate conditions to induce the signs of the disease. In each of the tests, a control was included (negative control - use of sterile water). Confirmation of the pathogens was carried out using the Elisa serological technique following the general protocol of identification of fungi AGDIA.

Production of antiserum

The immunization was carried out in rabbits of the New Zealand breed with a weight of 3 kg, an age of 9-24 months and avoiding that the females were pregnant (Kirali, 1974; Bokx, 1980; Valdes, 1995; Villarreal, 1980). The immunization plan was carried out in three schemes, with five rabbits per scheme (Table 1), according to the methodology recommended by Flores (1994); Rueda *et al*. (2006).

Table 1. Three immunization schemes used in the production of antiserum against *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in New Zealand type rabbits.

Day	Volume of the vaccine (ml)	Route of injection
Scheme 1		
1	1	Intramuscular
7	1	Intramuscular
14	2	Intramuscular
Scheme 2		
1	0.1	Intramuscular
3	0.3	Intramuscular
7	0.3	Intramuscular
10	1	Intramuscular
15	2	Intramuscular
Scheme 3		
1	0.1	Intravenous
3	0.3	Intravenous
6	0.5	Intravenous
10	1	Intramuscular
15	2	Intramuscular
20	2	Intravenous

Obtaining imported tomato seed that is planted in the state of Sonora

To obtain the seed, we had the collaboration of the National Confederation of Horticultural Producers (CNPH) Sonora region, where each producer provided an amount of 100-150 seeds for the analysis.

Sampling in plants (seedling, developed leaf and fruit)

For the sampling of lots, the national potato sampling carried out by the SARH (1994) was reproduced. Sampling was carried out on 10% of the total arable land of three of the tomato producing municipalities (Table 2). In 10% of the surface of each region divisions of 5 ha were made that would be considered as a hypothetical plot to be sampled. At each point an imaginary diagonal line was drawn from corner to corner, and 10 samples were collected on that line, the samples collected, previously identified, were wrapped with moist paper and placed in a cooler to be transferred to the laboratory for analysis.

Detection of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in seed of import in culture media

According to the technique of Randhawa (1996) and Rueda *et al*. (2006), each sample of seed was washed in tap water for 30 min in order to eliminate chemicals (ie. fungicides) and placed in plastic trays with a capacity of 2 L. Each tray with the seed was added 2 ml of phosphatase buffer solution

with a pH= 7. The mixture of water with phosphatase containing each seed sample was called 'mother suspension', the trays were incubated for 12 h in refrigeration at 4 °C in order to be released conidia of Folr1, *A. solani* and *B. cinerea* to the mother suspension.

After incubation, 10 ml of mother suspension was taken from each of the trays, four dilutions were made to such suspension (10:1, 10:2, 10:3, 10:4) and the last dilution was took 0.1 ml that was seeded in PDA culture medium in Petri dishes by the rod dispersion method (Roger *et al*., 1981). The media were incubated for seven days at 34 °C. Once the conidia were germinated and there was mycelial growth with reproductive structures, these were identified considering the keys indicated in the phase of increase of the strains.

Detection of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in seed, seedling, developed leaf and fruit with the antiserum produced

The seedlings, developed leaves and sampled fruits were subjected by a process of cuts of 0.5 to 1 cm in diameter and introduced directly into a saline solution at 0.85% NaCl, called the mother solution. For the detection of Folr1, *A. solani* and *B. cinerea*, the slide agglutination technique was developed as indicated by Kiraly (1974).

Detection of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in seed, seedling, developed leaf and fruit by Elisa technique

For the detection of Folr1, *A. solani* and *B. cinerea*, with respect to the Elisa serological technique, the protocol described in seed detection was considered.

Pathogenicity tests of those samples positive for *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea*, with different detection methods

For the reaffirmation of the fungi Folr1, *A. solani* and *B. cinerea* that turned out to be positive in the previous detection methods, the pathogenicity tests were carried out using the technique of Randhawa (1996) and Rueda *et al*. (2006), as described in the purification and increase of inoculum to produce antigen.

Results and discussion

Identification tests

When carrying out the previous tests of inoculum increase, and identification (specific culture media and ELISA) and of pathogenicity, the result was the same as those indicated by Jarvis (1973); Cañedo and Ames (2004); Ascencio-Álvarez *et al*. (2008); López (2012), State Committee for Plant Health of Guanajuato, AC (CESAVEG, 2016); Robles-Carrion *et al*. (2014); Valencia *et al*. (2016a), indicating that for Folr1, the characteristics are to produce colonies of fast growth, they present an aerial mycelium, cottony and of white coloration; microscopically they show macroconidia, fusiform, curves at the ends and septate; the dimensions of the macro and microconidia (29.1-45 X 2.9-4.7 μm), the development of conidia was observed at nine days, item that agrees with Valencia *et al*. (2016b).

In relation to the fungus *Alternaria solani*, the development of conidia was achieved after 8 days; result that agrees with Cazar *et al*. (2014), this presented a cottony aerial mycelium, which soon turned black when sporulated. The conidiophores are dark measuring 12-20 X 120-296 µm, dark, with the appearance of a mallet and have longitudinal and transverse septa (Martínez-Ruiz *et al*., 2016).

On the other hand, *Botrytis cinerea*, presented gray colonies. On the fifth day, well-differentiated, simple, straight conidiophores of 205-210 x 18 -20 µm, branches of 18-20 x 6-8 µm were observed. Ellipsoidal, smooth, almost hyaline conidia, 7-14 x 5-8 µm, often with a 3 µm long appendage according to Farrera *et al*. (2007); Robles-Carrion *et al*. (2014).

Regarding pathogenicity tests, the symptoms of seeds embedded in the conidial suspension of 10^8 ml^{-1} conidia, with Folr1, 100% germinated between 36 and 48 h under favorable conditions of the disease in both materials used (Bonny Best and Manapal), the seedlings after 18 days showed a slight wilting on the stem. The cotyledons showed irregular spots with a darker and more greasy appearance in relation to the healthy area. This same symptomatology was identified in seedlings that once dead were observed as the fungus fructified on the surface of the stem under humid conditions. The opposite occurred with the Manapal material, where the manifestation of symptoms was not detected or when developing trans and horizontal cuts along the conductive tissues in root and seedling stem. The results obtained agree when carrying out inoculations in the same material Bonny Best and Manapal, with Ascencio-Álvarez *et al*. (2008).

For *Alternaria solani* are the symptoms on leaves, at nine days those were characteristic circular, close to 1.5 cm in diameter, brown containing concentric rings. The results agree with Martínez-Ruiz *et al*. (2016), indicating that the first symptoms appear on the oldest leaves and progress towards the newer leaves; from there you can also see brown spots on the pedicels and on the chalices when they are attached to the flower or fruit. In this way, the fruits become infected; through the calyx or the pedicel both when they are green or mature.

For *Botritys cinerea* the symptomatology began with the formation of small concentric rings; the first odors of putrefaction were detected and on the 13th day there were already conidiospores. The results obtained agree with Martínez-Ruiz *et al*. (2016).

Through confirmation by the Elisa serological technique, a positive result was obtained for the fungal specimens provided by the donor company, as well as suspensions of cellular juice and infected tissue rich in conidia obtained from the aforementioned pathogenicity tests.

Antigen production against *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea*

Because not all warm-blooded animals react as an antigen, only Scheme 3 (Table 1) was found to be efficient to produce a high antibody content. The agglutination was reaffirmed by making readings under the compound microscope to observe a microagglutination with the help of a compound microscope (Table 2) (Bokx, 1980).

Table 2. Presence of *Fusarium oxysporum* f. sp. *lycopersici* (*R1*), *Alternaria solani* and *Botritys cinerea* in tomato seed *Solanum lycopersicum* (L.), provided by producers from the regions sampled in the state of Sonora, Mexico.

Region	Variety	Production system	Surface total in the region (ha)	Sampled surface (ha)	Sampled organ	PDA culture medium			ELISA			Antiserum produced		
						Folr1	As	Bc	Folr1	As	Bc	Folr1	As	Bc
Coast of Hermosillo	DMX1	Shadow mesh	27	18	Seed	+	-	-	+	-	-	+	+	-
	DMX2	Shadow mesh		6	Seed	+	-	-	+	-	-	+	+	-
Valley of Guaymas	DMX1	Shadow mesh	478.8	22	Seed	+	-	-	+	-	-	+	-	-
	RUE	Shadow mesh		2	Seed	-	-	-	-	-	-	-	-	-
	ZAP	Shadow mesh		1.5	Seed	-	-	-	-	-	-	-	-	-
	LEO	Open field		34	Seed	-	-	-	-	-	-	-	-	-
	DAR	Shadow mesh		16	Seed	-	-	-	-	-	-	-	-	-
Valley of the Yaqui	BER	Open field	954	54	Seed	-	+	-	+	+	-	+	+	-
	GLO	Open field		65	Seed	-	+	-	+	+	-	+	+	-
	DMX1	Shadow mesh		12	Seed	+	-	-	+	-	-	+	+	-
	DMX2	Shadow mesh		17	Seed	+	-	-	+	-	-	+	+	-
	Positive control				Seed	+	+	+	+	+	+	+	+	+
	Negative control				Seed	-	-	-	-	-	-	-	-	-

+= positive test; -= negative test; medium agar potato-dextrose culture (PDA); *Fusarium oxysporum* f. sp. *lycopersici* (R1) = (Folr1); *Alternaria solani= As*; *Botritys cinerea= Bc*.

Detection of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in the state of Sonora

In the case of seed, the results are shown in Table 2. Under the technique of Diagnosis using solid means papa-dextrosaagar (PDA), the presence of Folr1 in the seed donated by producers belonging to the Coast of Hermosillo region was demonstrated. Valley of Guaymas and Yaqui, with the materials DMX1 and DMX2, results similar to those indicated by Valencia *et al*. (2016a), Robles-Carrion et al. (2014). The same result occurred in the seed obtained from Valley of Yaqui in the BER and GLO material, but with the difference that they turned out to be positive for *Alternaria solani*, since the observed conidia corresponded to that according to Robles-Carrion *et al*. (2014) and Martínez-Ruiz *et al*. (2016).

With respect to the Elisa technique, the results indicate that, by this technique, the seed obtained from the three regions with the DMX1 and DMX2 materials turned out to be positive to the presence of Folr1, being confirmed with the samples processed with respect to the control.

With respect to the agglutination and microagglutination technique, the results were positive for Folr1, in seed samples obtained from producers in the sampled regions (DMX1 and DMX2, BER and GLO), with the exception of the Guaymas region with RUE, ZAP materials. , LEO and DAR that also turned out to be negative. In Table 2, the results vary only for the pathogen *A. solani*, where the diagnostic technique with the antiserum produced is detected in the DMX1 and DMX2 materials, the opposite occurred in the Elisa, hence the importance of the difference of results and to define if those samples presented or not conidial cells belonging to the fungi studied (Rueda *et al*., 2006).

Sampling of seedling, developed leaf and fruit in commercial lots

Regarding the study of seedling, leaf and fruit made by means of the PDA culture medium technique, the Elisa technique and antiserum produced for the detection of Folr1, *A. solani* and *B. cinerea*, the results are shown in Table 3. In can appreciate that the vegetative materials of the three sampled regions that were found to be positive to the presence of Folr1, in seed, when detected in seedling or in the subsequent phenological stages, the result was negative; this may be due to the fact that the varieties that are sown are resistant to Folr1 or because the cultural and management conditions, as well as those within the production areas, such as high temperature and low atmospheric humidity, could cause the pathogen does not have the ability to survive and therefore not produce an infection in the tomato plant. *Alternaria solani* and *Botritys cinerea* under the techniques carried out, vary among the techniques; it can be seen that the technique of Elisa and Antiserum produced, were sensitive to the presence of these two phytopathogens, otherwise the PDA medium occurred (Table 3).

Table 3. Presence of *Fusarium oxysporum* f. sp. *lycopersici* (*R1*), *Alternaria solani* and *Botritys cinerea* in seedling, leaves and tomato fruits *Solanum lycopersicum* (L.), sampled in the regions sampled in the state of Sonora, Mexico.

Region	Variety	Sampled organ	PDA culture medium			ELISA			Antiserum produced		
			Folr1	As	Bc	Folr1	As	Bc	Folr1	As	Bc
Coast of Hermosillo	DMX	Seedling	-	-	-	-	+	-	-	+	-
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	DMX	Seedling	-	-	-	-	+	-	-	+	+
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
Valley of Guaymas	DMX	Seedling	-	+	-	-	+	-	-	+	-
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	RUE	Seedling	-	-	-	-	+	-	-	+	-
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	-	-	+	+	-	+	+
	ZAP	Seedling	-	+	-	-	+	-	-	+	-
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+

Region	Variety	Sampled organ	PDA culture medium			ELISA			Antiserum produced		
			Folr1	As	Bc	Folr1	As	Bc	Folr1	As	Bc
Valley of the Yaqui	LEO	Seedling	-	-	-	-	+	-	-	+	+
		Leaf	-	-	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	DAR	Seedling	-	+	-	-	+	-	-	+	-
		Leaf	-	+	-	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	BER	Seedling	-	-	-	-	+	-	-	+	-
		Leaf	-	+	+	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	GLO	Seedling	-	+	-	-	+	-	-	+	-
		Leaf	-	+	+	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	DMX	Seedling	-	-	-	-	+	-	-	+	-
		Leaf	-	+	+	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	DMX	Seedling	-	+	-	-	+	-	-	+	-
		Leaf	-	+	+	-	+	+	-	+	+
		Fruit	-	+	+	-	+	+	-	+	+
	Positive control	Seedling	+	+	-	+	+	+	+	+	+
		Leaf	+	+	+	+	+	+	+	+	+
		Fruit	+	+	+	+	+	+	+	+	+
	Negative control	Seedling	-	-	-	-	-	-	-	-	-
		Leaf	-	-	-	-	-	-	-	-	-
		Fruit	-	-	-	-	-	-	-	-	-

+= positive test; -= negative test; medium agar potato-dextrose culture (PDA); *Fusarium oxysporum* f. sp. *lycopersici* (R1) = (Folr1); *Alternaria solani= As*; *Botritys cinerea= Bc*.

Tests of pathogenicity to positive fungi in vegetative samples by the three methods of detection

These tests were only carried out on those samples obtained from the sampling sites and which turned out to be positive in the diagnostic techniques used. Pathogenicity tests showed that, when inoculated into seed, 20-day-old seedlings, leaves and fruit, seed and seedling for *Fusarium oxysporum* f. sp. *lycopersici* (*R1*), leaf for *Alternaria solani* and fruit for *Botritys cinerea* with a respective positive and negative control, under favorable conditions of the disease, turned out to be positive according to the characteristics described by Ascencio-Álvarez *et al*. (2008), in vegetative material Bonny Best and Manapal. On the other hand, *A. solani* was in leaves and *B. cinerea* in fruit being made in the variety 'Rio Grande'.

Conclusions

Only one of the immunization schemes used was suitable for the production of antigen against *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea*. The presence of the causative agent of *Fusarium oxysporum* f. sp. *lycopersici* (R1) and *Alternaria solani* was detected in seed that is directed to planting in the state of Sonora. This presence was verified by the three detection techniques that were used: nutritive media, Elisa, antiserum produced and pathogenicity tests, in the different materials used in the diagnosis.

Likewise, *Botritys cinerea* was identified, present in the production areas of the three regions sampled; however, it is important to indicate that the presence of the pathogens studied were not symptomatically reflected, this result is attributed to the cultural practices that are applied in these production systems, do not present the necessary conditions for the development of the disease.

Being positive the presence of *Fusarium oxysporum* f. sp. *lycopersici* (R1), *Alternaria solani* and *Botritys cinerea* in the seed of planting and vegetative samples during the development of the crop, represent a risk of an eventual manifestation of disease, for which it is necessary that all the producing regions continue carrying out prevention activities; separate detection tests should not be used as a single method of detection, it is necessary to combine them when performing diagnostic tests to apply appropriate and preventive controls and thus decrease large amounts of chemicals and decreasing genetic resistance and pollution to the environment.

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Cited literature

Ascencio-Álvarez, A.; López-Benítez, A.; Borrego-Escalante, F.; Rodríguez-Herrera, S. A.; Flores-Olivas, A.; Jiménez-Díaz, F. y Gámez-Vázquez, A. J. 2008. Marchitez vascular del tomate: I. Presencia de razas de *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder y Hansen en Culiacán, Sinaloa, México. Rev. Mex. Fitopatol. 26:114-120.

Barnett, H. L. and Hunter, B. B. 1998. Ilustrated genera of imperfect fungi. 4 (Ed.). APS. The Am. Phytopathol. Soc.). USA. 258 p.

Bokx, J. 1980. Virosis de la papa y de la semilla de papa. Hemisferio Sur, SA. Buenos Aires, Argentina. 303 p.

Cañedo, V. y Ames, T. 2004. Manual de laboratorio para el manejo de hongos entomopatógenos. Lima, Perú; Centro Internacional de la Papa (CIP) ©Centro Internacional de la Papa (CIP). Lima, Perú. 62 p.

Cázar, M. E.; Villena, P.; Parra, J.; Espinoza, V.; Larriva G. y Caldas, A. 2014. Eficacia de extracto etanólico de eucalipto (*Eucaliptus globulus*) en el control de *Alternaria* sp. en cultivos de col y patata. Maskana. 5(1):33-41.

CESAVEG. 2016. Comité Estatal de Sanidad Vegetal de Guanajuato, AC. Manual de plagas y enfermedades en jitomate. *In*: SAGARPA- SENASICA. 29 p.

Dal Bello, G.; Nico, A. y Mónaco, C. I. 2012. Hongos saprófitos como herramientas de control biológico de '*Botrytis cinerea'* en tomate. Horticultura global. 303:64-68.

Davised, C. L. 1988. Benzimidazole fungicides: mechanism of action and resistance. *In*: fungicides resistance in North America. Delp. J Ch. Ed. Am. Phytopathol. Soc. St. Paul Minnesota, USA. 25-27 pp.

Eckert, J. W. 1988. Historical development of fungicide resistance in plant pathogens. *In*: fungicides resistance in North America. Delp, J. Ch. Ed. American Phytopathological Society. St. Paul, Minnesota, USA. 1-3 pp.

Farrera, R. E.; Zambrano, A. E. y Ortiz, F. A. 2007. Identificación de hongos asociados a enfermedades del fruto de la fresa en el municipio Jáuregui del estado Táchira. Rev. Fac. Agron. 24(2):269-281.

Flores, O. A. 1994. Producción de antisuero contra *Agrobacterium tumefaciens*. Universidad Autónoma Agraria Antonio Narro (UAAAN). Buenavista, Saltillo, Coahuila, México. 9 p.

Gilman, J. C. 1963. A manual of soil fungi. Compañía Editorial Continental. México. 873 p.

Grijalva-Contreras, R. L.; Macías-Duarte, R. y Robles-Contreras, F. 2011. Comportamiento de híbridos de tomate bola en invernadero bajo condiciones desérticas del noroeste de Sonora. Trop. Subtrop. Agroecosyst. 14(2):675-682.

Grijalva-Contreras, R. L.; Macías-Duarte, R.; Grijalva-Durón, S. A. y Robles-Contreras, F. 2010. Evaluación de densidades y arreglos de plantación en tomate bola en condiciones de invernadero en el Noroeste de Sonora. Biotecnia. 12(2):20-28.

Grijalva-Contreras, R. L.; Macías-Duarte, R.; Grijalva-Durón, S. A.; Núñez-Ramírez, F. y Robles-Contreras, F. 2014. Productividad de cultivares de tomate Cherry bajo condiciones de invernadero en el noroeste de Sonora. Biotecnia 16(2):27-30.

INIFAP. 2005. Instituto Nacional de Investigaciones Forestales y Agropecuarias. Manejo integrado del cultivo del jitomate en el estado de San Luis Potosí. Fundación Produce. Folleto técnico núm. 22. 18 p.

Jarvis, W. R. 1975. Tolerance of *Botrytis cinerea* and rose powdery mildew to benomyl. Plant. Dis. 55:44-49.

Jarvis, W. R. and Hargreaves, A. J. 1973. Tolerance to benomyl in *Botrytis cinerea* and *Penicillium corymbifenum.* Plant Pathol. 22:139-141.

Kiraly, G. Z. 1974. Methods in plant pathology. Elsevier Scientific Publishing Company. New York, USA. 509 p.

Martínez-Ruiz, D. E.; Cervantes-Díaz, L.; Aíl-Catzím, C. E.; Hernández-Montiel, L. G.; Sánchez, C. L. D. T. y Rueda-Puente, E. O. 2016. Hongos fitopatógenos asociados al tomate (*Solanum Lycopersicum* L.) En la zona árida del noroeste de México: la importancia de su diagnóstico. Eur. Sci. J. 12(18):232-256.

Randhawa, P. 1996. Fruit blotch testing protocol. California Seed and Plant Lab. Inc. Roseville, California, USA. 5 p.

Robles-Carrión, A.; Salinas-Serrano, D.; Armijos-Armijos, W.; Sánchez-Rodríguez, A. y Torres-Gutiérrez, R. 2014. Estudio de la variabilidad morfológica de aislados fúngicos asociados con la enfermedad de la marchitez vascular del babaco (*Vasconcellea heilbornii* var. *pentagona*) en Loja, Ecuador. Centro de Biotecnología. 2(2):33-44.

Roger, Y.; Stainer, Doudoroff, M. y Adelberg, E. 1981. Microbiología. Segunda (Ed). Prentice_Hall Inc. Madrd, España. 932 p.

Romero, C. 1993. Hongos fitopatógenos. Universidad Autónoma Chapingo (UACH). México, DF. 347 p.

Rueda-Puente, E. O.; Tarazón-Herrera, M. A.; García-Hernández, J. L.; Murillo-Amador, B.; Holguín-Peña, R. J.; Flores-Hernández, A.; Sánchez-Arizpe, A.; Flores-Olivas, A. y Preciado-Rangel, P. 2006. Producción de antisuero contra la mancha bacteriana del fruto (*Acidovorax avenae* pv. *citrulli* (Schaad, Sowell, Goth, Colwell y Webb) Willems, Goor, Thielemans, Gillis, Kersters y De Ley) y su detección en el cultivo de sandía (*Citrullus vulgaris* Schrad.) en la Comarca Lagunera, México. Rev. Mex. Fitopatol. 24(2):129-136.

SAGARPA. 2005. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Análisis Agropecuario del Tomate. Boletín informativo. Culiacán, Sinaloa, México. 9 p.

SAGARPA. 2015. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Agenda Técnica Agrícola de Sonora. Segunda edición. México. 244 p.

SARH. 1994. Secretaría de Agricultura y Recursos Hidráulicos. Manual de muestreo y procesamiento para la identificación de los principales patógenos de la papa. Dirección General de Sanidad Vegetal. México, México, DF. 16 p.

Tello, J. C. y Lacasa, A. 1988. Evolución racial de poblaciones *Fusarium oxysporum* f. sp. *lycopersici.* Boletín de Sanidad Vegetal-Plagas. 14:335-341.

Valdés, D. R. 1995. Cría y explotación del conejo. Secretaría de Agricultura y Recursos Hidráulicos. Secretaría de Fomento Agropecuario de Coahuila. Saltillo, Coahuila, México. 115 p.

Valencia, S.; Hurtado, G.; Pérez, K. y Vilaplana, R. 2016b. Evaluación de las cepas fúngicas más agresivas de banano (*Musa acuminata* L.) en la poscosecha. Agron. Colomb. 34(1):702-705.

Valencia, S.; Pérez, K.; Hurtado, G. y Vilaplana, R. 2016a. Evaluación de la severidad de las podredumbres causadas por hongos aislados de piña (*Ananas comosus* L.) en poscosecha. Agron. Colomb. 34(1):763-766.

Villarreal, G. L. A. 1980. Supervivencia, Dispersión y Patogenicidad de *Erwinia carotovora* y su Relación con *Sclerotium rolfsii* y *Sclerotinia sclerotiorum* Tesis de Maestría en Ciencias. Colegio de Postgraduados. Montecillo, Estado de México. México. 92 p.