Short communication

Severe outbreaks of strawberry crown and root charcoal rot caused by *Macrophomina phaseolina* in Tucumán, Argentina

Ataques severos de podredumbre carbonosa de corona y raíz en frutilla causada por *Macrophomina phaseolina* en Tucumán, Argentina

J. Viejobueno[1#]; A.C. Ramallo[2#]; D.S. Kirschbaum[1]; O.M. Baino[2]; S.M. Salazar[1,2*]

[1] INTA EEA Famaillá. Ruta Prov. 301 km 32, (4132) Famaillá, Tucumán. Argentina. *E-mail: salazar.sergio@inta.gob.ar
[2] Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán. Avda. Kirchner 1900, (4000) San Miguel de Tucumán. Tucumán, Argentina.
[#] Viejobueno, J. and Ramallo, A.C. contributed equally to this work.

Abstract

Tucumán province is one of the most important strawberries growers in Argentina. The strawberry crown and root charcoal rot is a soilborne disease caused by the fungus *Macrophomina phaseolina*. This disease is spread worldwide and *M. phaseolina* one of the most economically important fungal pathogens. The objective of this study was to determine the susceptibility of different strawberry cultivars to *M. phaseolina* under field conditions. The evaluation was carried out at INTA-Famaillá in Tucumán, Argentina. Fresh-dug strawberry plants form cultivars 'Macarena', 'Treasure', 'Pájaro', 'Ruby Gem', 'Carmela', 'Camino Real', 'Albion', 'Ventana', 'Camarosa' and 'Palomar' were evaluated. All evaluated plants showed decline and death but the pathogens isolated differed among cultivars. *Fusarium oxysporum* was co-isolated with *M. phaseolina* in 'Camarosa', 'Camino Real', 'Albion', 'Carmela', 'Treasure' and 'Pájaro'. Pure isolates of *M. phaseolina* were obtained from 'Ruby Gem' and 'Macarena'. Pure isolates of *Fusarium* spp. were obtained from 'Palomar'. Plant losses and disease ratings due to *M. phaseolina* were greater for 'Macarena', 'Treasure' and 'Pajaro', followed by, 'Ruby Gem' and 'Carmela' than for 'Palomar' and 'Camarosa', which appeared to be more tolerant to plant decline. This study demonstrates the differential tolerance to *M. phaseolina* of currently available strawberry cultivars in Tucumán, Argentina.

Keywords: *Fragaria ananassa*; Host specificity; *Macrophomina phaseolina*.

Resumen

La provincia de Tucumán es una de las productoras más importantes de frutilla de Argentina. La podredumbre carbonosa de corona y raíz es una enfermedad transmitida por el suelo causada por el hongo *Macrophomina phaseolina*. Este enfermedad se encuentra distribuida en todo el mundo y *M. phaseolina* uno de los hongos patógenos más importantes desde el punto de vista económico. El objetivo de este estudio fue determinar la susceptibilidad de diferentes cultivares de frutilla a *M. phaseolina* bajo condiciones de campo. La evaluación se realizó en el INTA-Famaillá en Tucumán, Argentina. Se utilizaron plantines de frutilla frescos de los cultivares 'Macarena', 'Treasure', 'Pájaro', 'Ruby Gem', 'Carmela', 'Camino Real', 'Albion', 'Ventana', 'Camarosa' y 'Palomar'. Todas las plantas evaluadas mostraron decaimiento y muerte pero los patógenos aislados difirieron entre variedades. *Fusarium oxysporum* fue co-aislado con *M. phaseolina* en 'Camarosa', 'Camino Real', 'Albion', 'Carmela', 'Treasure' y 'Pájaro'. Los aislados puros de *M. phaseolina* se obtuvieron de 'Ruby Gem' y 'Macarena', y el aislado puro de *Fusarium* spp. se obtuvo de 'Palomar'. Las pérdidas de plantas y las tasas de enfermedad debidas a *M. phaseolina* fueron mayores para 'Macarena', 'Treasure' y 'Pajaro', seguidas por 'Ruby Gem' y 'Carmela' que para 'Palomar' y 'Camarosa', que parecían ser más tolerantes al declive de la planta. Este estudio demuestra la tolerancia diferencial de los cultivares de frutilla actualmente disponibles a *M. phaseolina* en Tucumán, Argentina.

Palabras clave: *Fragaria ananassa*; Especificidad hospedante; *Macrophmomina phaseolina*.

Strawberries (*Fragaria ananassa* Duch.) are cultivated worldwide, including tropical, subtropical, and temperate regions, with an annual production estimated in three million tons. Argentina produces strawberry during the whole year due to its wide range of climates, presenting three dif-

Received 09/06/17; Accepted 11/22/17.
The authors declare to have no conflict of interest.

ferent production regions: northern, central, and southern, based on their particular climates and harvest seasons. In the northern region, the province of Tucumán is one of the most important producers of this crop (Kirschbaum and Hancock, 2000; Rodríguez *et al.*, 2009, Salazar *et al.*, 2012).

The charcoal rot is a soilborne disease caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich. The disease is spread worldwide and *M. phaseolina* is one of the most economically important fungal pathogens, infecting about 500 plant species in more than 100 plant families (Wyllie, 1993; Mihail and Taylor, 1995). Charcoal rot becomes important during periods with dry weather or when other unfavorable environmental conditions cause stress in the plants. Genetic resistance to charcoal rot has not been identified and it is difficult to control with crop rotation due to the wide host range of the pathogen. Morphological differences between *M. phaseolina* isolates were related with differences in pathogenicity and genetic diversity (Almeida *et al.*, 2008). Besides, isolates from a given host were genetically similar to each other but distinct from those obtained from other hosts (Su *et al.*, 2001). Despite that, no host specificity was observed in strawberry plants inoculated with *M. phaseolina* isolates obtained from other plant species, suggesting that strawberry crops should not be considered in crop rotation strategies with other host crops of the pathogen (Zveibil *et al.*, 2012). The massive outbreaks of *M. phaseolina* in different strawberry growing regions of the world have been associated with the replacement of methyl bromide by other soil fumigants. (Avilés *et al.*, 2008; Koike, 2008; Mertely *et al.*, 2005; Zveibil and Freeman, 2005).

In Tucumán, this fungus has previously been recorded as *Macrophomina* sp. on strawberry (Baino *et al.*, 2011). Disease outbreaks were observed in isolated groups of plants in the field. Symptoms consisted of foliage wilting, plant stunting, and drying and death of older leaves, though the central youngest leaves often remained green and alive. Plants eventually collapsed and died. When plant crowns were transversally dissected, internal vascular and cortex tissues were dark brown to orange brown. In locations where the disease has occurred for more than one season, patches can be quite large and appear to have spread from the initial infection focus. This pattern was consistent with the spread of a soilborne pathogen.

The objective of this study was to determine the susceptibility of different strawberry cultivars to *Macrophomina phaseolina* under field conditions.

The evaluation was carried out at INTA-Famaillá Experiment Station (27° 03 S, 65° 25 W, 363 m elevation) in Tucumán, Argentina. Fresh-dug strawberry plants from nurseries located in El Maitén (Chubut province) of the cultivars 'Macarena', 'Treasure', 'Pájaro', 'Ruby Gem', 'Carmela', 'Camino Real', 'Albion', 'Ventana', 'Camarosa' and 'Palomar' were planted on April 24th (2010) on a clay loamy soil (pH 6.5). Plants were established in offset 2-row raised beds (covered with 24 µ black polyethylene mulch) 0.50 m wide x 0.30 m high, using a 0.35-m between-row plant spacing and a 0.23-m in-row plant spacing. Water was provided through drip irrigation. Pre-plant fertilization consisted of 48 kg/ha N and 123 kg/ha P_2O_5. Additional N (144 kg/ha) and K (303 kg/ha K_2O), as well as P (57 kg/ha P_2O_5), Ca (125 kg/ha CaO), and Mg (25.5 kg/ha MgO), were applied twice a week during the season through drip irrigation system. Plants were irrigated three to four times a week. For each cultivar three replicates of 20 plants each were planted in a complete randomized block design.

Macrophomina phaseolina isolates were obtained from roots and crown of the strawberry plants. Root and crown of individual plants were excised into small pieces, sterilized in alcohol 70° for 1-2 s, in sodium hypochlorite at 2% for 30 s, washed three times with sterile distilled water for 1 min each and transferred to PDA (Potato Dextrose Agar) plates containing streptomycin (0.1 mg/ml). Plates were incubated at 26 ºC in dark for 7 days. Small pieces of medium containing microsclerotia were examined under stereoscopic microscope and a single microsclerotium was collected and transferred to PDA slants. Three single-microsclerotium isolates were obtained from each plant. From each colony, single discs were transferred to potato-dextrose broth for 14 days at 26 ºC when mycelium and microsclerotia were collected, washed in distilled water and dried at room temperature.

All cultivars presented a pathogen but the pathogens isolated differed between cultivars. *Fusarium oxysporum* was co-isolated with *M. phaseolina* in 'Camarosa', 'Camino Real', 'Albion', 'Carmela', 'Treasure' and 'Pájaro'. Pure isolates of *M. phaseolina* were obtained from 'Ruby Gem' and 'Macarena', and *Fusarium* isolate was obtained from 'Palomar' (Table 1).

Table 1. Pathogens isolated from different strawberry cultivars.

Pathogen	Strawberry cultivar
Macrophomina phaseolina + *Fusarium oxysporum*	'Treasure' 'Pájaro' 'Carmela' 'C. Real' 'Albion' 'Camarosa'
Macrophomina	'Ruby Gem' 'Macarena' 'Ventana'
Fusarium	'Palomar'

Plant losses and disease ratings due to *M. phaseolina* were greater for 'Macarena', 'Treasure' and 'Pajaro', followed by, 'Ruby Gem' and 'Carmela' than for 'Palomar' and 'Camarosa', which appeared to be more tolerant to plant decline (Table 2). Plant decline was accelerated at the end of the season (Nov-Dic) as a result of the typical increase of fruit production and matching with the isolation of *M. phaseolina* from dying plants. It is noteworthy that in these cases we have never isolated other important, well-known pathogens such as *Colletotrichum* spp., *Phytophthora* spp., or *Verticillium* spp., but it was associated with *Fusarium oxysporum*.

Table 2. Strawberry plant death rates for *Macrophomina phaseolina* during 2010 growing season.

Cultivar	Death rate*
'Macarena'	73.3 ± 6.2 b
'Treasure'	71.7 ± 9.1 a
'Pájaro'	68.3 ± 6.6 b
'Ruby Gem'	56.7 ± 8.2 b
'Carmela'	53.3 ± 10.8 a
'C. Real'	40.8 ± 5.1 b
'Albion'	36.7 ± 4.9 a
'Ventana'	24.2 ± 3.3 a
'Camarosa'	16.7 ± 3.1 b
'Palomar'	5.0 ± 2.3 b

*Death rate was estimated as the average of collapsed or died plants/total plants per plot. Values that do not share the same letter are statistically different (LSD test, $P < 0.05$).

Current results suggest a differential tolerance of strawberry cultivars to *M. phaseolina.* These results differ from those reported by Fang *et al.* (2011), where 'Albion' was the most resistant and 'Camarosa' the most susceptible to *M. phaseolina* isolate. This discrepancy could be related to differences in pathogenicity and the genetic diversity of the pathogen, confirmed by various molecular methods (Jana *et al.*, 2005; Su *et al.*, 2001), which could make a given cultivar more tolerant to an isolate and more susceptible to another. The great genetic diversity among *M. phaseolina* isolates makes difficult to find genetic resistance to the fungus. There are also other factors that make *M. phaseolina* difficult to control, such as the phase-out of methyl bromide, the heat tolerance of the pathogen and the fact that sclerotia are resilient resting structures (McCain *et al.*, 1982; Zveibil *et al.*, 2012; Chamorro *et al.*, 2015).

Chemical and biosolarization treatments are being used in Spain to control *M. phaseolina* (Chamorro *et al.*, 2015) and recently, the use of biological control tools has shown promising results against other pathogens (Salazar *et al.*, 2007; 2013; Gonzalez *et al.*, 2012). However, there is still no effective biological control tool against *M. phaseolina*.

This study demonstrates differential tolerance of currently available strawberry cultivars to *M. phaseolina* in Tucumán, Argentina. Our results might help strawberry growers to take accurate decisions about the election of cultivars in order to reduce losses by crown and root rot.

Acknowledgements

This study is part of joint project between INTA Famaillá and Facultad de Agronomía y Zootecnia (UNT), and it was partially supported by the Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT, 26/A422) and Instituto Nacional de Tecnología Agropecuaria (INTA-PNHFA 061281/1106073). JV is CONICET fellow.

References

Almeida Á.M., Sosa-Gómez D.R., Binneck E., Marin S.R., Zucchi M.I., Abdelnoor R.V., Souto E.R. (2008). Effect of crop rotation on specialization and genetic diversity of *Macrophomina phaseolina*. Tropical Plant Pathology 33 (4): 257-264.

Avilés M., Castillo S., Bascon J., Zea-Bonilla T., Martín-Sánchez P.M., Pérez-Jiménez R.M. (2008). First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathology 57 (2): 382.

Baino O.M., Salazar S.M., Ramallo A.C., Kirschbaum D.S. (2011). First Report of *Macrophomina phaseolina* causing strawberry crown and root rot in Northwestern Argentina. Plant Disease 95 (11): 1477-1477.

Chamorro M., Domínguez P., Medina J.J., Miranda L., Soria C., Romero F., De los Santos B. (2015). Assessment of chemical and biosolarization treatments for the control of *Macrophomina phaseolina* in strawberries. Scientia Horticulturae 192: 361-368.

Fang X.L., Phillips D., Sivasithamparam K., Barbetti M.J. (2011). Comparisons of virulence of pathogens associated with crown and root diseases of strawberry in Western Australia with special reference to the

effect of temperature. Scientia Horticulturae 131: 39-48.

Jana T.K., Singh N.K., Koundal K.R., Sharma T.R. (2005). Genetic differentiation of charcoal rot pathogen, *Macrophomina phaseolina*, into specific groups using URP-PCR. Canadian Journal of Microbiology 51 (2): 159-164.

Kirschbaum D., Hancock J. (2000). The strawberry industry in South America. Horticultural Science 35: 807-811.

Koike S.T. (2008). Crown rot of strawberry caused by *Macrophomina phaseolina* in California. Plant Disease 92 (8): 1253.

McCain A.H., Bega R.V., Jenkinson J.L. (1982). Solar heating fails to control *Macrophomina phaseolina*. Phytopathology 72: 985-988.

Mertely J., Seijo T., Peres N. (2005). First report of *Macrophomina phaseolina* causing a crown rot of strawberry in Florida. Plant Disease 89: 434.

Mihail J.D., Taylor S.J. (1995). Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production, and chlorate utilization. Canadian Journal of Botany 73 (10): 1596-1603.

Rodríguez G., Pérez D., Paredes V. (2009). El cultivo de frutilla en Tucumán: superficie implantada, rendimientos, destino de la producción, precios y gastos de producción. Reporte Agroindustrial EEAOC 32: 1-6.

Salazar S.M., Castagnaro A.P., Arias M.E., Chalfoun N., Tonello U., Díaz Ricci, J.C. (2007). Induction of a defense response in strawberry mediated by an avirulent strain of *Colletotrichum*. European Journal of Plant Pathology 117: 109-122.

Salazar S.M., Grellet C.F., Chalfoun N.R., Castagnaro A.P., Ricci J.C. (2013). Avirulent strain of *Colletotrichum* induces a systemic resistance in strawberry. European Journal of Plant Pathology 135 (4): 877-888.

Salazar S.M., Lovaisa N.C., Guerrero-Molina M.F., Ragout A.L., Kirschbaum D.S., Díaz-Ricci J.C., Pedraza R.O. (2012). Fruit yield of strawberry plants inoculated with *Azospirillum brasilense* RLC1 and REC3 under field conditions. Revista Agronómica del Noroeste Argentino 32: 63-66.

Su G., Suh S.O., Schneider R.W., Russin J.S. (2001). Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology 91: 120-126.

Wyllie T.D. (1993). Charcoal rot. In: Compendium of soybean diseases. Sinclair J.B., Backman P.A. (Eds.). APS Press, USA. Pp. 30-33.

Zveibil A., Freeman S. (2005). First report of crown and root rot in strawberry caused by *Macrophomina phaseolina* in Israel. Plant Disease 89: 1014.

Zveibil A., Mor N., Gnayem N., Freeman S. (2012). Survival, hostpathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. Plant Disease 96: 265-272.