GUAVA DECLINE: EVIDENCE OF NATIONWIDE INCIDENCE IN BRAZIL

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ABSTRACT

Gomes, V.M., R.M. Souza, G. Midorikawa, R. Miller and A.M. Almeida. 2012. Guava decline: evidence of nationwide incidence in Brazil. Nematropica 42:153-162.

In Rio de Janeiro State (Brazil), guava decline is a complex disease in which parasitism by *Meloidogyne enterolobii* predisposes *Fusarium solani*-immune trees to extensive root decay caused by this fungus. On the shoot, the symptoms include chlorosis, wilting, scorching of leaf margins and leaf drop, yield reduction, and plant death within months. Since in many States *M. enterolobii*-infested orchards have been decimated with the same symptoms, this study aimed to assess the incidence of guava decline nationwide. Root samples from declining, nematode-infested orchards were received from Southern, Northeastern and Midwestern Brazil. Of the 682 root fragments processed for fungal isolation, about half were positive for *Fusarium* sp., and only 5% were positive for other fungi. Sixty-nine *Fusarium* sp. isolates were established as representatives of those Brazilian regions. To expedite the evaluation of virulence to guava of all these isolates, a growth chamber assay was developed using as reference 11 *Fusarium* sp. isolates whose virulence to guava plants had been assessed in previous microplot experiments. Of the 69 *Fusarium* sp. isolates assessed, 87% - from all Brazilian regions - were virulent to stem cuttings used in the growth chamber assay, with 42% of these being classified as highly virulent. All virulent isolates were preliminarily identified as *F. solani* through morphological characterization. To confirm this, 12 representatives from different Brazilian regions were selected for molecular identification through ITS4 and ITS5 gene sequencing, which confirmed their identity as *F. solani*.

Key words: complex disease*, Fusarium solani,* guava root-knot nematode*, Meloidogyne enterolobii, Psidium guajava.*

RESUMEN

Gomes, V.M., R.M. Souza, G. Midorikawa, R. Miller and A.M. Almeida. 2012. Deterioro del guayabo: evidencia de la incidencia en Brasil. Nematropica 42:153-162.

En el Estado de Rio de Janeiro (Brasil), el deterioro del guayabo es una enfermedad compleja en la cual el parasitismo por *Meloidogyne enterolobii* predispone a los árboles al ataque por *Fusarium solani,* causando pudriciones radicales. En la parte aérea, los árboles muestran síntomas de clorosis, marchitez, quemazón de las márgenes foliares y defoliación, además de reducción de la productividad y muerte. Debido a que estos síntomas se han observado en plantaciones de guayabo infestadas con *M. enterolobii* en varios estados, se llevó a cabo este estudio para determinar la incidencia del deterioro del guayabo en Brasil. Se recibieron muestras de raíz provenientes de plantaciones sintomáticas, y con presencia del nematodo, del sur, noreste y occidente de Brasil. De los 682 fragmentos de raíz que se examinaron para aislamiento de hongos, aproximadamente la mitad fueron positivos para *Fusarium* sp., y sólo 5% fueron positivos para otros hongos. Se establecieron 69 aislamientos de *Fusarium* sp. como representativos de estas regiones en Brasil. Se desarrolló un bioensayo en cámara de crecimiento para acelerar la evaluación de la virulencia de estos aislamientos, usando como referencia 11 aislamientos de *Fusarium* sp. cuya virulencia había sido previamente establecida en experimentos de microparcelas. De los 69 aislamientos evaluados, 87% - provenientes de todas las regiones de Brasil – mostraron virulencia a los esquejes en el bioensayo, con 42% de ellos mostrando ser altamente virulentos. Con base en caracterización morfológica, se identificaron preliminarmente todos los aislamientos virulentos como *F. solani.* La identificación por morfología se confirmó con pruebas moleculares usando secuencias de los genes ITS4 e ITS5, para las cuales se seleccionaron 12 aislamientos de las diferentes regiones y que coincidieron con la identificación de *F. solani*.

Palabras clave: enfermedad compleja, *Fusarium solani,* nematodo agallador del guayabo*, Meloidogyne enterolobii, Psidium guajava.*

INTRODUCTION

In Brazil, one of the main phytosanitary problems in guava (*Psidium guajava* L.) is the death of trees associated with parasitism by *Meloidogyne enterolobii* Yang and Eisenback, 1983 (syn *Meloidogyne mayaguensis* Rammah and Hirschmann, 1988). The decimation of orchards extends over about 5000 hectares in several States, causing direct damage estimated to cost more than $ 112 million reais (about US$ 69 million) and job losses of more than 3,700 full-time rural workers (Pereira *et al.*, 2009). Several attempts to control *M. enterolobii* have failed, and most research efforts in Brazil are now focused on screening *Psidium* sp. genotypes for resistance to this nematode (e.g., Almeida *et al.*, 2009; Burla *et al.*, 2010; Miranda *et al.*, 2010).

In the State of Rio de Janeiro, Gomes *et al.* (2011) showed that progressive root rot in guava trees parasitized by *M. enterolobii* was caused by *Fusarium solani* (Mart.) Sacc. In this disease complex, known as guava decline, parasitism by the nematode predisposes the otherwise *F. solani*-immune trees to extensive fungal root decay. *Meloidogyne enterolobii*-parasitized trees may have galled roots and some root rot for several months without secondary symptoms on the shoots. The onset or worsening of shoot symptoms, which results in plant death within months, often occurs following a high production season or drastic pruning, which is used by some growers to synchronize production. Declining trees have leaf chlorosis, scorching of margins and wilting, which result in total defoliation of the tree. According to Gomes *et al.* (2008), these symptoms are associated with foliar deficiency in nitrogen, phosphorus and potassium, and accumulation of manganese, chlorine and sodium to near-phytotoxic levels.

The etiology of the extensive decimation of guava orchards in the State of Rio de Janeiro seems, therefore, well established. Nonetheless, the role of *F. solani* has not been confirmed in the widespread decimation of orchards across several Brazilian States, which is always associated with parasitism by *M. enterolobii* and with the same symptoms as those described in Rio de Janeiro (e.g., Carneiro *et al.*, 2006; Silva *et al.*, 2008; Silva and Oliveira, 2010). To confirm the nationwide incidence of guava decline based on many *Fusarium* sp. isolates from different regions in Brazil, a two-week-long growth chamber assay was developed to assess virulence of all isolates simultaneously, as a less time-consuming proxy for the 6-month-microplot experiment conducted twice by Gomes *et al.* (2011) to assess just 11 isolates.

Hence, this work aimed: i) to assess the incidence of *Fusarium* sp. in root samples collected in declining, *M. enterolobii*-parasitized guava orchards in different Brazilian regions, ii) to evaluate the *Fusarium* sp. isolates obtained from these regions for their virulence to guava, using the growth chamber assay, and iii) to identify to species level the most virulent *Fusarium* sp. isolates through morphological and molecular criteria.

MATERIALS AND METHODS

Field sampling

Requests were sent to nematologists across Brazil to provide root samples from *M. enterolobii*-infested guava orchards. The samples, collected from declining trees, were received from the municipalities of Petrolina (Northeastern Brazil, three samples from the localities N8, N10 and N11), Ivinhema (Midwest, two samples from the localities of Vitória and Piraverde), Carlópolis (South, one sample) and Santa Maria (South, one sample). The samples weighed about 4 kg each, and they contained roots and rootlets with nematode-induced galls and typical rot. The samples were collected under the tree canopy, at 0-30 cm depth.

Fungal isolation, identification and selection

Many 5 mm-fragments were randomly taken from roots and rootlets of each sample, close to nematode-induced galls. The fragments were externally cleansed, disinfested in 70% alcohol for 1 min and with Qboa® commercial bleach (about 2% sodium hypochlorite) for 3 min, and rinsed with sterile distilled water (Dhingra and Sinclair, 1995). The fragments were incubated for 7 days at 27ºC and 12 h-photoperiod on potato-dextrose-agar (PDA) medium amended with 500 ppm of streptomycin sulfate.

After seven days of incubation, fungal colonies were subcultured and, upon sporulation, fungi were transferred into a drop of lactophenol mounted on a glass slide and identified at the genus or species level under a light microscope, with or without staining with cotton blue or acid fuchsin, according to the descriptions by Booth (1971), Ellis (1971; 1976), Barnett and Hunter (1972), Sutton (1980) and Ventura (2000).

Based on the morphology of the colonies in PDA medium, macro and micro conidia and chlamydospores, and sampling location, 69 *Fusarium* sp. isolates (referred to hereafter as UENF/CF 234 to 301) were selected as representatives of the diversity found in the different Brazilian regions. These isolates were stored on colonized wheat grains, which were stored in glass vials maintained at 4ºC, as well as mixed with silica gel and maintained at room temperature, in the dark (Dhingra and Sinclair, 1995).

Growth chamber assay for virulence

Guava stem cuttings (10 cm long) with two leaves were collected in a commercial orchard of the cultivar 'Paluma'. The cuttings were rinsed in tap water and separately immersed for 5 s in a conidial suspension (10^7/ml) of one of the 11 *Fusarium* sp. isolates (UENF/CF 160-170) whose virulence to guava plants had

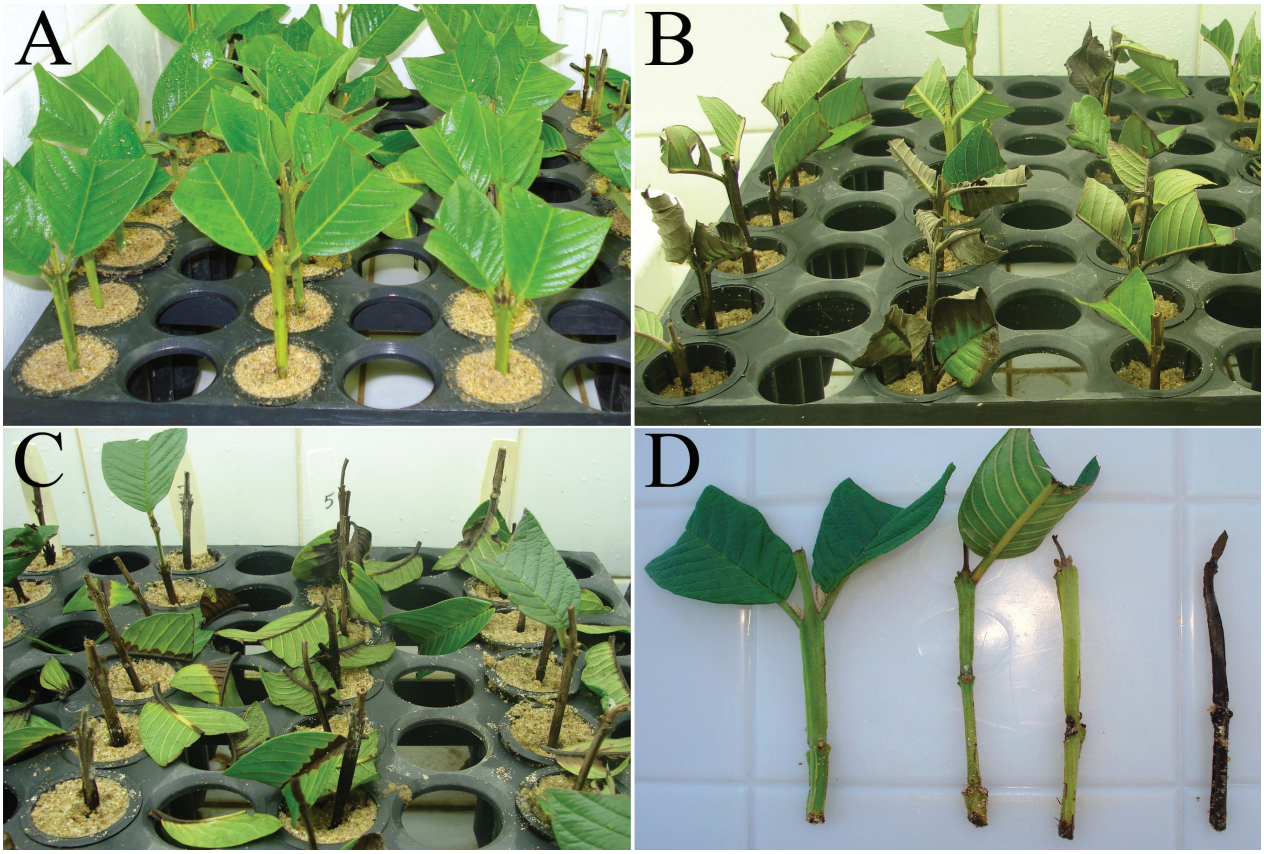

Fig. 1. Symptoms and virulence grades of *Fusarium* sp. on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95% relative humidity, 27 ± 2ºC temperature and 12-h photoperiod. A: grade 0 (no symptoms). B: grade 1, necrotic lesions covering small areas of leaves, leaf petioles and stem. Slight wilting of leaves, with occasional leaf drop. C: grade 2, necrotic lesions covering large areas of (or entire) leaves, leaf petioles and stem. Stem often turns black and becomes distorted. Severe wilting and distortion of leaves, often both of which falling. D: Grading of symptoms from 0 through 2.

been assessed in microplots (Gomes *et al.*, 2011). The cuttings were then placed individually in plastic plant cells with sterile sand as substrate, and transferred to a plant growth chamber at 27 ± 2ºC, 95% relative humidity, and photoperiod of 12 hr. Eight cuttings were inoculated per isolate, and eight were immersed in sterile, distilled water as a control. For inoculum production, the *Fusarium* sp. isolates were cultivated for 7 days at 27ºC and 12 h-photoperiod with PDA medium.

Fifteen days after inoculation, each cutting was evaluated for incidence and extension of leaf lesions, distortion and falling; lesions in leaf petioles; and stem lesions, darkening and distortion. These symptoms were expressed in a 0-2 scale (Table 1; Fig. 1). This assay was repeated once.

For each *Fusarium* sp. isolate, a virulence grade (VG) was calculated as follows: VG = (n x 0) + (n x 1) + (n x 2)/16, in which "n" was the number of cuttings graded 0, 1 or 2. The degree of virulence was expressed as null, low, medium or high according to the

Table 1. Virulence scale of *Fusarium* sp. on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95 % relative humidity, 27 ± 2ºC temperature and 12-h photoperiod

Grades	Symptoms
0	No symptoms (healthy)
1	Necrotic lesions covering small areas of leaves, leaf petioles and stem. Slight wilting of leaves, with occasional falling of one of them.
2	Necrotic lesions covering large areas of (or entire) leaves, leaf petioles and stem. Stem often turns black and becomes distorted. Severe wilting and distortion of leaves; often both of which fall.

following VG values: 0, 0.1-0.66, 0.67-1.33 and 1.34-2, respectively. To validate this growth chamber assay, the degree of virulence obtained for each isolate was compared to that observed in the microplot experiments conducted by Gomes *et al.* (2011).

Virulence of Fusarium *sp. isolates*

Upon validation of the growth chamber assay this approach was employed to assess the virulence of 69 *Fusarium* sp. isolates from different Brazilian regions. The assay was conducted and evaluated as described before, and repeated once. The highly virulent isolate UENF/CF 163 was included in the assays as a pathogenic standard.

Identification of selected Fusarium *sp. isolates*

Of the 69 *Fusarium* sp. isolates from different Brazilian regions, those virulent to guava were identified to species based on morphological and colony characters (Ventura, 2000). To confirm the morphological identification, the 12 isolates of *Fusarium* sp. that were most aggressive to the guava cuttings in the growth chamber test, and originated from Southern, Southeastern, Midwestern and Northeastern Brazil, were selected for specific identification through PCR and gene sequencing of ITS4 and ITS5. These isolates were UENF/CF 160, 163, 164, 170, 241, 244, 251, 263, 268, 275, 279 and 295.

The genomic material of the isolates was extracted using a phenol-chloroform method (Raeder and Broda, 1985). The isolates were separately cultivated in Erlenmeyer flasks of 250 ml with 100 ml of CYA liquid culture, and incubated at 25ºC at 120 rpm, for 5 days. The mycelium of each isolate was recovered by manual filtering and washed with distilled water to remove all culture medium residues. The isolates were conditioned in Eppendorf® tubes of 1.5 ml, lyophilized and macerated in a porcelain crucible. To extract the genomic DNA, an extraction buffer containing Tris HCl 200 mM, NaCl 250 mM, EDTA 25 mM and SDS 0.5% at pH 7 was used. The DNA was precipitated with isopropanol at -20ºC in the proportion of 54% of the solution volume and re-suspended in TE (10 mM Tris-HCl at pH 8 and 1 mM EDTA). The extracted genomic DNA was visualized by electrophoresis in agarose 1% gel (5V/cm) in the presence of ethidium bromide (1 µg/ml). The low DNA mass ladder® (LDML) (Invitrogen) was used as the standard marker.

To amplify the rDNA ITS region of the isolates of *Fusarium* sp., the PCR reaction was carried out using the following primer set: [5'-GGA AGT AAA AGT CGT AAC AAGG-3'] and [5'-GGT CCG TGT TTC AAG ACGG-3']. The PCR reaction was carried out with a final volume of 25 µl, containing 14.37 µl of Milli-Q water, 2.5 µl of 1X buffer, 1 µl of primer ITS4 (10mM) and 1 µl of primer ITS5 (10mM), 2 µl of dNTPs (2.5 mM), 0.125 µl of Taq Pht (5 U/µl) and 4 µl of DNA (concentration of 5 ng/µl). The PCR was performed on the thermocycler ABI 377 (Applied Biosystems, Foster City, CA), using the following reaction conditions: 94ºC (4 min); 39 cycles of 94ºC (1 min), 50ºC (1 min), 72ºC (1 min); followed by 72ºC (5 min); 10ºC (hold). Amplified DNA fragments were size-fractionated by electrophoresis in 1% agarose gels (5V/cm) containing ethidium bromide (1 µg/ml). LDML was used as the standard marker.

PCR products were forward-sequenced, without purification, following the method of Sanger *et al.* (1977). Each sequencing reaction was performed with a final volume of 10 µl, containing 2 µl of DynamicTM ET Terminator kit (Pharmacia Biotech), 1 µl of primer (2 µM), 60 ng of PCR product and Milli-Q water (Millipore) to complete the final volume. Thermal cycling parameters were 30 cycles of 95ºC for 20 s, 50ºC for 15 s and 60ºC for 1 min. Following the purification, the sequenced products were re-suspended in 3-4 µl of DynamicTM ET loading solution (Amersham Pharmacia Biotech, Piscataway, NJ). DNA sequencing was carried out in an Applied Biosystems ABI 377 sequencer (Applied Biosystems, Carlsbad, CA). To analyze the quality of the sequences and form a consensus sequence the Sequencer V4.8 program (Gene Codes Corporation, Ann Arbor, Michigan) was used. Analysis of sequences homologous to the isolates of *Fusarium* sp. was carried out using BLASTn software based on the Genbank® (http://www.ncbi.nlm.nih.gov/genbank/).

RESULTS

Of the 682 root fragments randomly selected from different Brazilian regions, 47% were positive to *Fusarium* sp. isolates. Per region, 58%, 37.5% and 44% were positive in Southern, Midwestern and Northeastern Brazil, respectively. Collectively, *Chaetomium* sp., *Penicillium* sp., *Trichoderma* sp., *Aspergillus niger, Nigrospora* sp., *Monilia* sp. and *Rhizoctonia* sp. were isolated in less than 5% of the root fragments.

The virulence grade and virulence degree of *Fusarium* sp. isolates on guava varied (Tables 2 and 3). The amplification products (bands) of the 12 *F. solani* isolates from different Brazilian regions were similar (Fig. 2). The ITS sequences of these isolates are available at GenBank (acession numbers JN006807 through JN006818).

DISCUSSION

The samples employed in this study were collected from all Brazilian regions where declining orchards have been reported. The methodology employed in this work to isolate fungi from root fragments was similar to that used by Gomes *et al.* (2011); both studies revealed similar frequency of positive isolations for *Fusarium* sp. in Rio de Janeiro State and in other Brazilian

Table 2. Virulence grade and virulence degree of *Fusarium* sp. isolates on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95% relative humidity, 27 ± 2ºC temperature and 12-h photoperiod, as compared with virulence to guava plants in previous microplot experiments.

Isolates	Sampling site (municipality/region)	Virulence grade[w]	Degree of virulence[x]	Virulence in microplot[y]
UENF/CF 160	São João da Barra/Southeastern Brazil	1.56	High	+
UENF/CF 161	"	1.13	Medium	+
UENF/CF 162	"	0	Null	-
UENF/CF 163	"	1.88	High	+
UENF/CF 164	"	1.81	High	+
UENF/CF 165	"	0	Null	-
UENF/CF 166	"	1.56	High	+
UENF/CF 167	"	0.25	Low	-
UENF/CF 168	"	0	Null	-
UENF/CF 169	"	1.5	High	+
UENF/CF 170	"	1.63	High	+
Control[z]	--	0	Null	-

[w]Weighted mean of virulence grades in a 0-2 scale calculated upon two assays with eight cuttings for each *Fusarium* sp. isolate.
[x]Based on the range of virulence grades 0 (null), 0.1-0.66 (low), 0.67-1.33 (medium) and 1.34-2 (high).
[y]Microplot experiments conducted by Gomes et al. (2011). "+" denotes virulence to guava plants, "-" denotes avirulence.
[z]Immersion of cuttings in sterile, distilled water.

Table 3. Virulence grade and virulence degree of *Fusarium* sp. isolates on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95% relative humidity, 27 ± 2ºC temperature and 12-h photoperiod.

Isolates	Sampling site (municipality/region)	Virulence grade[x]	Degree of virulence[y]
UENF/CF 245	St Maria/Southern Brazil	0.31	Low
UENF/CF 246	"	1.38	High
UENF/CF 247	"	1	Medium
UENF/CF 248	"	1.3	Medium
UENF/CF 249	"	0	Null
UENF/CF 250	"	0.8	Medium
UENF/CF 251	"	1.69	High
UENF/CF 252	"	0	Null
UENF/CF 253	"	1.13	Medium
UENF/CF 254	"	1.38	High
UENF/CF 255	"	0.44	Low
UENF/CF 256	Carlópolis/Southern Brazil	0.94	Medium
UENF/CF 257	"	1.13	Medium
UENF/CF 258	"	0.56	Low
UENF/CF 259	"	1.19	Medium
UENF/CF 260	"	1.56	High
UENF/CF 261	"	0.69	Medium

Table 3. Virulence grade and virulence degree of *Fusarium* sp. isolates on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95% relative humidity, 27 ± 2ºC temperature and 12-h photoperiod.

Isolates	Sampling site (municipality/region)	Virulence grade[x]	Degree of virulence[y]
UENF/CF 262	"	1.63	High
UENF/CF 263	"	1.5	High
UENF/CF 264	"	0.56	Low
UENF/CF 234	Ivinhema/Midwestern Brazil	1.13	Medium
UENF/CF 235	"	0.25	Low
UENF/CF 236	"	0	Null
UENF/CF 237	"	0	Null
UENF/CF 238	"	1.31	Medium
UENF/CF 239	"	1.44	High
UENF/CF 240	"	1.56	High
UENF/CF 241	"	1.69	High
UENF/CF 242	"	0.19	Low
UENF/CF 243	"	1.19	Medium
UENF/CF 244	"	1.69	High
UENF/CF 265	"	0.31	Low
UENF/CF 266	"	1.56	High
UENF/CF 267	"	1.63	High
UENF/CF 268	"	1.94	High
UENF/CF 269	Petrolina/Northeastern Brazil	1.63	High
UENF/CF 270	"	1.69	High
UENF/CF 271	"	0.44	Low
UENF/CF 272	"	0.5	Low
UENF/CF 273	"	0	Null
UENF/CF 274	"	1.88	High
UENF/CF 275	"	1.63	High
UENF/CF 276	"	0	Null
UENF/CF 277	"	0.94	Medium
UENF/CF 278	"	1.88	High
UENF/CF 279	"	2	High
UENF/CF 280	"	0.69	Medium
UENF/CF 281	"	0	Null
UENF/CF 282	"	1.25	Medium
UENF/CF 283	"	0.75	Medium
UENF/CF 284	"	0	Null
UENF/CF 285	"	0.06	Low
UENF/CF 286	"	1.31	Medium
UENF/CF 287	"	0.75	Medium
UENF/CF 288	"	0.75	Medium
UENF/CF 289	"	0	Null

Table 3. Virulence grade and virulence degree of *Fusarium* sp. isolates on guava stem cuttings immersed in conidial suspension (10^7/ml) for 5 s and maintained for 15 days in plastic plant cells with sterile sand in a growth chamber at 95% relative humidity, 27 ± 2ºC temperature and 12-h photoperiod.

Isolates	Sampling site (municipality/region)	Virulence grade[x]	Degree of virulence[y]
UENF/CF 290	"	0.69	Medium
UENF/CF 291	"	1.19	Medium
UENF/CF 292	"	1.44	High
UENF/CF 293	"	0.25	Low
UENF/CF 294	"	1.63	High
UENF/CF 295	"	1.5	High
UENF/CF 296	"	1.38	High
UENF/CF 297	"	0.5	Low
UENF/CF 298	"	1.56	High
UENF/CF 299	"	1.06	Medium
UENF/CF 300	"	0.88	Medium
UENF/CF 301	"	1.38	High
UENF/CF 163	São João da Barra/Southeastern Brazil	1.87	High
Control[z]	--	0	Null

[x]Weighted mean of virulence grades in a 0-2 scale calculated upon two assays with eight cuttings for each *Fusarium* sp. isolate.
[y]Based on the range of virulence grades 0 (null), 0.1-0.66 (low), 0.67-1.33 (medium) and 1.34-2 (high).
[z]Immersion of cuttings in sterile, distilled water.

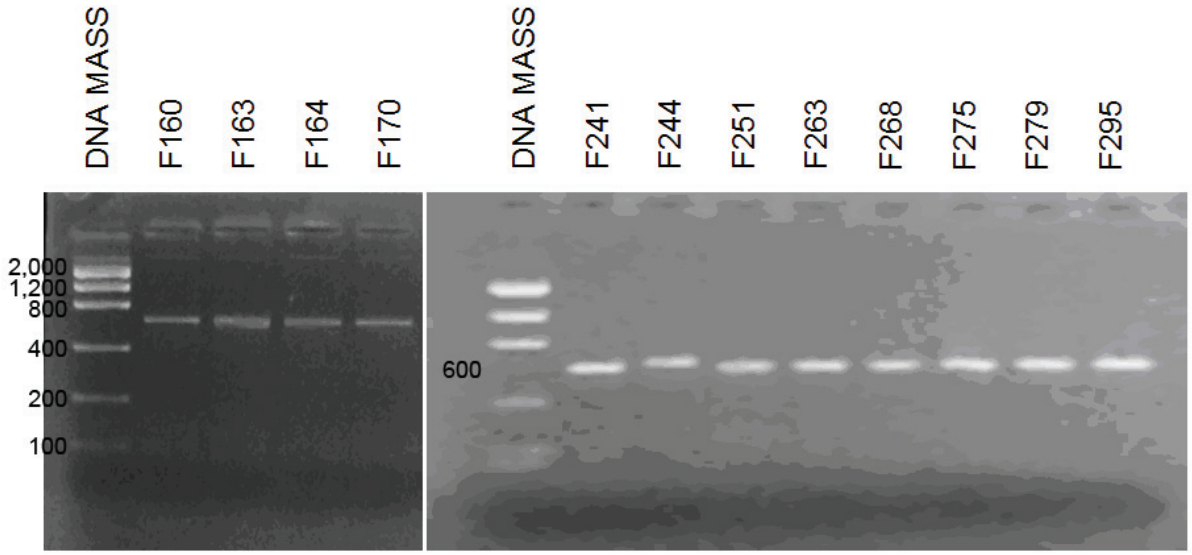

Fig. 2. Agarose gel with PCR amplification of fragments of rDNA ITS region of 12 isolates of *Fusarium solani* virulent to guava plants. First and sixth lanes from left are low DNA mass ladder®, with molecular weights (in base pairs) indicated. All *F. solani* fragments are ~600 bp.

regions. This demonstrates that in declining orchards, *M. enterolobii*-parasitized roots are commonly colonized by *Fusarium* sp., as opposed to no isolation of this fungus from hundreds of root fragments from nematode-free guava trees examined by Gomes *et al.* (2011).

Of the 11 *Fusarium* sp. isolates assessed twice in microplot experiments by Gomes *et al.* (2011), seven were pathogenic to guava. In this work, the same isolates were virulent to stem cuttings in the growth chamber assay, while the others induced no or only mild symptoms. Hence, there seems to exist factor(s) that enable some *Fusarium* sp. isolates to decay guava roots in the presence of *M. enterolobii* as well as stem cuttings. This correlation validated the growth chamber assay as a tool to expedite the assessment of incidence of guava decline in different Brazilian regions based on many *Fusarium* sp. isolates. Indeed, microplot experiments are much more time-, space- and resource-consuming because guava is a perennial with relatively slow growth. Because the growth chamber assay was not meant to examine the guava-*M. enterolobii*-*F. solani* interaction, nematodes were not co-inoculated with the fungus. Greenhouse and in vitro experiments and bioassays are in progress to elucidate several aspects of this interaction, which is mediated by chemical alterations in root exudates of *M. enterolobii*-parasitized plants (Gomes, 2011).

This growth chamber assay showed that 87% of *Fusarium* sp. isolates were virulent to guava stem cuttings, with 42% of these being classified as highly virulent. This indirectly confirms the nationwide incidence of *Fusarium* sp. root rot in declining, *M. enterolobii*-infested guava orchards.

The analysis of sequence homology of the isolates UENF/CF 160, 163, 164, 170, 241, 244, 251, 263, 268, 275, 279 and 295, using the BLASTn program, confirmed they were indeed *F. solani*. The relatively high cost of this procedure precluded the molecular identification of all *Fusarium* sp. isolates. In conclusion, this work presents strong evidence of the nationwide incidence of guava decline, which is responsible for the decimation of many orchards across several Brazilian States.

Guava decline adds to several reports of synergistic interaction between *F. solani* and plant-parasitic nematodes, such as with *M. incognita* on papaya (Khan and Hussain, 1991a;b), on tomato (Yeboah and Opoku-Asiamah, 2004; Ganaie and Khan, 2011), on ginger (Doshi and Mathur, 1987, cited by Evans and Haydock, 1993), on pepper (Hamada *et al.*, 1985, cited by Evans and Haydock, 1993), and on chickpea (Mani and Sethi, 1987); with *Meloidogyne* spp. on mungbean (Ehteshamul-Haque and Ghaffar, 1994, cited by Siddiqui *et al.*, 2001) and on eggplant (Ghaffar, 1995, cited by Siddiqui and Shaukat, 2002); with *Radopholus similis* on banana (Stover, 1966, cited by Evans and Haydock, 1993); with *Heterodera glycines* on soybean (Roy *et al.*, 1989); with *Rotylenchulus reniformis* on cotton seedlings (Palmateer *et al.*, 2004), and with *Tylenchulus semipenetrans* on citrus seedlings (Van Gundy and Tsao, 1963).

In addition to being of prime importance to the guava crop in Brazil, the synergistic interaction between *F. solani* and *M. enterolobii* may also be responsible for orchard decimation in Venezuela and Mexico. In Venezuela, Suárez *et al.* (1999) claimed that *Macrophomina* sp., *Fusarium* sp. and *Meloidogyne* spp. act synergistically to debilitate guava orchards. However, their experimental results do not support their claim of a disease complex involving these pathogens because no significant difference ($P > 0.05$) was observed between the different combinations of those pathogens. Also, the authors did not identify to species level the fungi and nematodes involved. In Mexico, Mejía *et al.* (2001) reported declining guava orchards with symptoms somewhat different from those observed in Brazil, such as rolled leaves, rosettes of leaves on the apical region of shoots, and a grayish trunk. The authors were unable to determine the causal agent, but reported the association of *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, *M. javanica* (Treub, 1885) Chitwood, 1949 and *M. arenaria* (Neal, 1889) Chitwood, 1949 with the fungi *F. solani, Verticillium dahliae* Kleb., *Pythium aphanidermatum* (Edson) Fitzp., *Trichoderma roseum* (Pers.) Link and *Trichoderma* spp. The authors considered the fungi to be secondary players in the disease, although no data were presented to substantiate this and other claims.

It is plausible to hypothesize that properly designed experiments could reveal the role of *F. solani* in guava decline in Mexico and Venezuela. Also, as pointed out by Carneiro *et al.* (2001), *M. mayaguensis* (= *M. enterolobii*) is a polyphagous and morphologically variable species, and may have been misidentified as *M. javanica, M. arenaria* or *M. incognita* in many survey studies in which isozyme or molecular taxonomic methods have not been used. In Brazil, several surveys have showed *M. enterolobii* to be the only parasitic species of guava. Therefore, although several reports list different *Meloidogyne* species parasitizing this crop in Latin America, a specialized survey might reach a similar conclusion as in Brazil.

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