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Web blight (*Thanatephorus cucumeris*): a new disease on leaves of okra plants

*Mancha areolada (***Thanatephorus cucumeris***): nova doença das folhas do quiabeiro*

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ABSTRACT: In an experiment on organic production of okra (*Abelmoschus esculentus* (L.) Moench) that was carried out from September 2013 to January 2014, in Manaus, Amazonas state, Brazil, we observed large chlorotic, necrotic, helical, discontinuous, dark or light-brown lesions with partial detachment of the injured area on the adaxial surface of leaves located in the median and basal portions of the plants. A whitish mycelium mantle covers the lesions on the leaves at the abaxial surface at high moisture conditions. Using morphological characteristics, Koch's postulates, and phylogenetic analyses of the ITS-5.8S rDNA region, we identified that the fungus causing the lesions on the okra leaves was *Thanatephorus cucumeris* (Frank) Donk (asexual stage of *Rhizoctonia solani* Kuhn of the anastomosis group AG-1 ID). This is the first report of *T. cucumeris* causing web blight on okra in Brazil, and probably in the world. So far, *T. cucumeris* was described on okra only on post-harvest pods rotting and seedlings' damping off.

KEYWORDS: *Thanatephorus cucumeris*; *Abelmoschus esculentus*; etiology; Amazon.

RESUMO: Em um experimento sobre a produção orgânica do quiabeiro (*Abelmoschus esculentus* (L.) Moench), que foi instalado em Manaus, Amazonas, Brasil, no período de setembro de 2013 a janeiro de 2014, observou-se, na face adaxial do limbo foliar das folhas medianas e baixeiras, a ocorrência de lesões cloróticas e necróticas grandes, helicoidais, de coloração marrom escuro ou marrom claro e descontínuas, com desprendimento parcial da área lesionada. Na face abaxial, sobre as manchas, em condições de alta umidade, constatou-se a presença de um manto micelial esbranquiçado do patógeno, facilmente visível, recobrindo a área colonizada. Por meio da análise de características morfológicas, postulados de Koch e análise filogenética da região ITS-5.8S do rDNA do fungo isolado, identificou-se *Thanatephorus cucumeris* (Frank) Donk (fase assexuada *Rhizoctonia solani* Kuhn grupo de anastomose AG-1 ID) como o agente causal da doença. Este é o primeiro relato de *T. cucumeris* causando mancha foliar em quiabeiro no Brasil e, provavelmente, no mundo. Até então, sua ocorrência em quiabeiro estava restrita à podridão pós-colheita em frutos e tombamento de mudas.

PALAVRAS-CHAVE: *Thanatephorus cucumeris*; *Abelmoschus esculentus*; etiologia; Amazônia.

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Okra (*Abelmoschus esculentus* (L.) Moench), which has its origin in the African continent, possibly in Ethiopia, is part of the Malvaceae family. It is a semi-woody, annual, shrubby and erect plant that may grow to 3 m (FILGUEIRA, 2003). Fruits of these leafy vegetables are used in the preparation of typical regional dishes and are rich in vitamins (A and B) and minerals (TRANI et al., 2007).

In an experiment on organic production of okra carried out in an experimental area at Embrapa Amazônia Ocidental, Manaus, Amazon state, Brazil, from September 2013 to January 2014, we observed large chlorotic, necrotic, helical, discontinuous, dark or light-brown lesions with partial detachment of the injured area on the adaxial surface of leaves located in the median and basal portions of the plants (Fig. 1). An easily seen whitish mycelium mantle covered the spots on leaves of the abaxial surface at high moisture conditions, which covered the whole colonized area (Fig. 2).

The fungus was isolated using a potato dextrose agar (PDA) medium. The pathogenicity test was made under greenhouse conditions. The mycelium found in the abaxial surface of young okra leaves inoculated the PDA blocks. After the inoculation process, the plants were kept in a humidity chamber for 24 hours. We identified the pathogen based on morphological characteristics, Koch's postulates, and on the ITS-5.8S rDNA sequence region of fungus.

We observed the morphological characteristics in the asexual phase culture grown on the PDA medium and on the basidiospores, by adapting the technique used by TRINDADE et al. (1983) to produce these spores. We used distilled water to wash parts of leaves showing fully developed lesions and containing the pathogen's mycelium on the abaxial surface collected at the field. Then, we took 2-cm^2 pieces of the lesioned parts of the leaves. Each piece was placed at the inner side of the lid of a Petri dish along with a cotton sliver, and secured using adhesive tape. The leaf's abaxial surface faced the bottom of the dish. After moistening the cotton sliver using sterile water, we placed the lid onto the Petri dish that contained the PDA medium. After preparation, the Petri dishes were incubated at 23ºC under environmental lighting at the laboratory. Under these conditions, we noticed the ejection of basidiospores after two hours.

PDA-grown hyphae, at their asexual phase, showed wide-angle branches (nearly 90º) featuring a small constriction at their point of origin, which is a characteristic of *Rhizoctonia* species. On the pieces of leaves containing the pathogen's mycelium that were placed in the humidity chamber, the hymenium's hyphae produced 6 to 18 µm diameter and 9 to 26 µm height basidia — from barrel-shaped to cylindrical basidia — arranged individually or in arrays similar to clusters. Each basidium produced an average of four sterigmata (varying from 3 to 7), which measured from 35 to 53 µm in length. The ejected basidiospores were hyaline, oblong, with a thin and flat wall, and measured 6–13 µm × 4–9 µm.

The pathogenicity test showed symptoms and signs of the pathogen in the third and fifth days after inoculation, respectively. The pathogen was then re-isolated in the PDA medium for confirmation of Koch's postulates.

To obtain the pathogen's phylogenetic position, we determined the rDNA ITS-5.8S sequence region of the fungus. The fungus' lyophilized mycelial DNA was extracted using the Genelute kit (Sigma-Aldrich Brasil), and following the manufacturer's instructions. The DNA extraction procedure was performed in triplicate. To amplify the polymerase chain reaction (PCR) and the rDNA's ITS region sequencing, we applied the ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGT CGTAACAAGG-3') pair of primers (WHITE et al., 1990).

The PCR products were sent to Macrogen® (Korea) and submitted to a sequencing reaction using the PE Applied Biosystems ABI-3700 automatic sequencer. We analyzed the sequences using the Geneious software (Biomatters Limited,

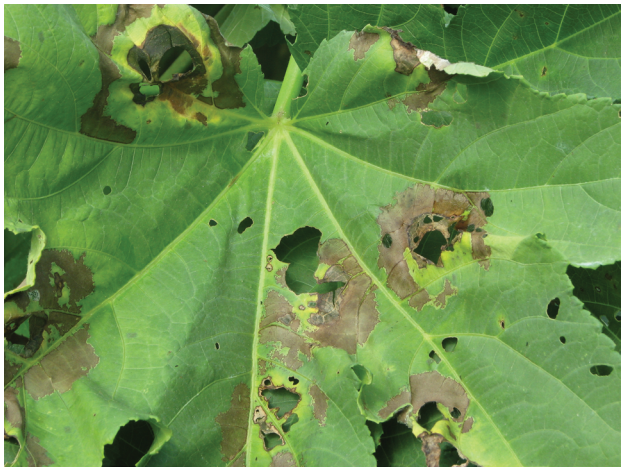

Figure 1. Symptoms of web blight (*Thanatephorus cucumeris*) on the adaxial surface of okra leaves.

Figure 2. Abaxial surface of an okra leaf showing web blight symptoms and signs of the *Thanatephorus cucumeris* pathogen that is characterized by the growth of the pathogen's mycelium.

Table 1. DNA sequences of rDNA's ITS1-5.8S-ITS2 region from standard isolates of AG-1 and AG-4 HGI complexes from *Rhizoctonia solani* that is used to determine the phylogenetic position of the pathogen's isolate associated with web blight on okra.

Anastomosis group	Sequence code in GenBank (NCBI)	Isolates	Host	Place of origin	Source[a]
AG-1 IA	AB000010	1A A-10	-	Japan	S. Kuninaga
AG-1 IA	DQ173048	AG1IArJ	Rice	Switzerland, Zurich	M. B. Ciampi
AG-1 IA	AJ000199	IMI 360366	-	United Kingdom	A. Johanson
AG-1 IA	AJ000200	IMI 360021	-	United Kingdom	A. Johanson
AG-1 IA	AJ000197	IMI 358761	-	United Kingdom	A. Johanson
AG-1 IA	AF308631	Cuba2	Beans	USA, Lincoln	G. Godoy-Lutz
AG-1 IA	DQ173047	AG1IAmJ	Maize	Japan, Tohoku	M. B. Ciampi
AG-1 IA	DQ173063	SJ047	Soybean	Brazil	M. B. Ciampi
AG-1 IA	AY270011	SJ067	Soybean	Brazil, Goiânia	R.C. Fenille
AG-1 IA	DQ173064	SJ048	Soybean	Brazil	M. B. Ciampi
AG-1 IA	DQ301757	SJ057	-	Holland	A.K. Nakatani
AG-1 IA	AB000016	1A Cs-Gi	-	Japan	S. Kuninaga
AG-1 IA	AB000017	1A Cs-Ka	-	Japan	S. Kuninaga
AG-1 IA	AF354097	2Rs	Rice	USA	D. Gonzalez
AG-1 IA	AF354060	1Rs	Soybean	USA	D. Gonzalez
AG-1 IB	AB000025	1B 001-7	-	Japan	S. Kuninaga
AG-1 IB	AB000038	1B SFBV-1	-	Japan	S. Kuninaga
AG-1 IB	AB000039	1B SHIBA-1	-	Japan	S. Kuninaga
AG-1 IC	AF354058	3Rs	*Pinus*	Canada	D. Gonzalez
AG-1 IC	EU591807	R107	Cabbage	USA	G. S. Abawi
AG-1 ID	MF497483	-	Okra (*Abelmoschus Esculentus*)	Manaus, Brazil	The authors of this paper
AG-1 ID	AB122125	RCP1	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122131	RCP15	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122127	RCP4	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122130	RCP13	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122132	RCP21	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122126	RCP3	Coffee	Japan	M. Hyakumachi
AG-1 ID	AB122129	RCP11	Coffee	Japan	M. Hyakumachi
AG-1 ID	Bel59, Bel60, Bel61 and Bel71	Bel59, Bel60, Bel61 e Bel71	Several hosts	Belém, Brazil	L.S.Poltronieri, A.P.S.C. Gaino
AG-1 ID	KX674530.1	SPM3	-	Philippines	O.Z.A.Rashed
AG-1 ID	EF197798	SR61	*Durio zibethinus* (Malvaceae)	Vietnam	T.T.M. Thuan
AG-1 ID	KF907725.1	HGPH01-3	*Brassica juncea*	Vietnam	G.K.H. Hua
AG-1 ID	AB122128	RCP7	Coffee	Japan	M. Hyakumachi
AG-1 IE	JF946719.1	L2	Beans	Los Limones, Honduras	G. Godoy-Lutz
AG-1 IE	JF946736.1	H25	Beans	Jamastran, Honduras	G. Godoy-Lutz
AG-1 IF	Bel56a, Bel68	Bel56, Bel68	Several hosts	Belém, Brazil	L. S. Poltronieri, A.P.S.C. Gaino
AG-1 IF	Bel56b	Bel56	Several hosts	Belém, Brazil	L. S.Poltronieri, A.P.S.C. Gaino
AG-1 IF	JF946727.1	PR0671	Beans	La Isabela, Porto Rico	G. Godoy-Lutz
AG-4 HGI (b)	AB000018	HG-I GM-3	-	Japan	S. Kuninaga
AG-4 HGI (b)	AB000007	HG-I 78-23R-3	-	Japan	S. Kuninaga
AG-4 HGI (b)	Bel54, Bel64b and Bel66	Bel54, Bel64 and Bel66	Several hosts	Belém, Brazil	L. S Poltronieri, A.P.S.C. Gaino
AG-4 HGI (b)	Bel62a, Bel62b and Bel64a	Bel62 and Bel64	Several hosts	Belém, Brazil	L. S. Poltronieri, A.P.S.C. Gaino

[a]Sequences of rDNA's ITS-5.8S from standard isolates obtained from: A. Johanson, Pest Management Department, Natural Resources Institute, Central Avenue, Chatham Maritime, Chatham, Kent, ME4 4TB, United Kingdom; A. K. Nakatani, Comparative Genomics and Bioinformatics, Centraalbureauvoor Schimmecultures-Fungal Biodiversity Center, Uppsalalaan 8, Utrecht 3584CT, Holland; A. P. S. C. Gaino, UNESP – Campus de Ilha Solteira, Depto. Fitos., Eng. Rural e Solos, Ilha Solteira, SP, Brazil; D. E. Gonzáles, Sistematica Vegetal, Instituto de Ecologia, A. C., Km 2.5 Antigua Carretera a Coatepec, Xalapa, Veracruz 91000, Mexico; G. Godoy-Lutz, Plant Pathology, University of Nebraska-Lincoln, 406 Plant Sciences Hall, Lincoln, NE 68583-0722, USA; G. S. Abawi, Department of Plant Pathology, Cornell University, New York State Agric. Exp. Station, Geneva, 14456, USA; G. K. H. Hua, Crop Protection, Ghent University, Coupure Links 653, Ghent 9000, Belgium; L. S. Poltronieri, EMBRAPA Amazonia Oriental, Belém, Pará, Brazil; M. B. Ciampi, UNESP – Campus de Jaboticabal, Depto. Tecnologia, Jaboticabal, SP, Brazil; M. Hyakumachi, Gifu University, Faculty of Agriculture; Yanagido 1-1, Gifu, Gifu, 501-1193, Japan; O. Z.A.Rashed, Plant Protection, Faculty of Agriculture, University Putra Malaysia, Serdang, Selangor, Malaysia; R. C. Fenille, Ministério da Agricultura, Goiás, Brazil; S. Kuninaga, Health Sciences University of Hokkaido; Tohbetsu, 1757, Ishikari-gun, Hokkaido 061-0293, Japan; T. T. M. Thuan, Department of Plant Protection, Nong Lam, University, Hochiminh 84, Vietnam; [b]Used as outgroup.

New Zealand), to determine quality and to edit. Then, we aligned them using the ClustalX software (THOMPSON et al., 1997), and we finally compared them to sequences of the rDNA's ITS region from all *R. solani*'s anastomosis groups deposited in the GenBank (NCBI) database. We searched for similar sequences using BLASTN (nucleotide-nucleotide), version 2.2.27 from September 10, 2012 (ALTSCHUL et al., 1997). The isolate's sequence was coded MF497483 in the GenBank. Due to its similarity to sequences of the rDNA's ITS-5.8S region of *R. solani*'s AG-1 anastomosis group, we conducted a phylogenetic analysis comparing the AG-1 IA, IB, IC, ID, IE and IF groups — already described in Brazil —, using *R solani*'s AG-4 HGI as an outgroup, for the tree rooting. The sequences used are described in Table 1. The phylogenetic analysis was performed using the *Geneious R9.1.8* software and its tool *Geneious Tree Builder*, as well as the HKY evolution model and the UPGMA method to build the tree. To obtain the consensus phylogenetic tree, data were resampled through bootstrapping with 1,000 replicates.

Considering the morphologic characteristics and the phylogenetic position of the okra isolate's rDNA ITS-5.8S region (Fig. 3), which features sequences identical to AG1 ID,

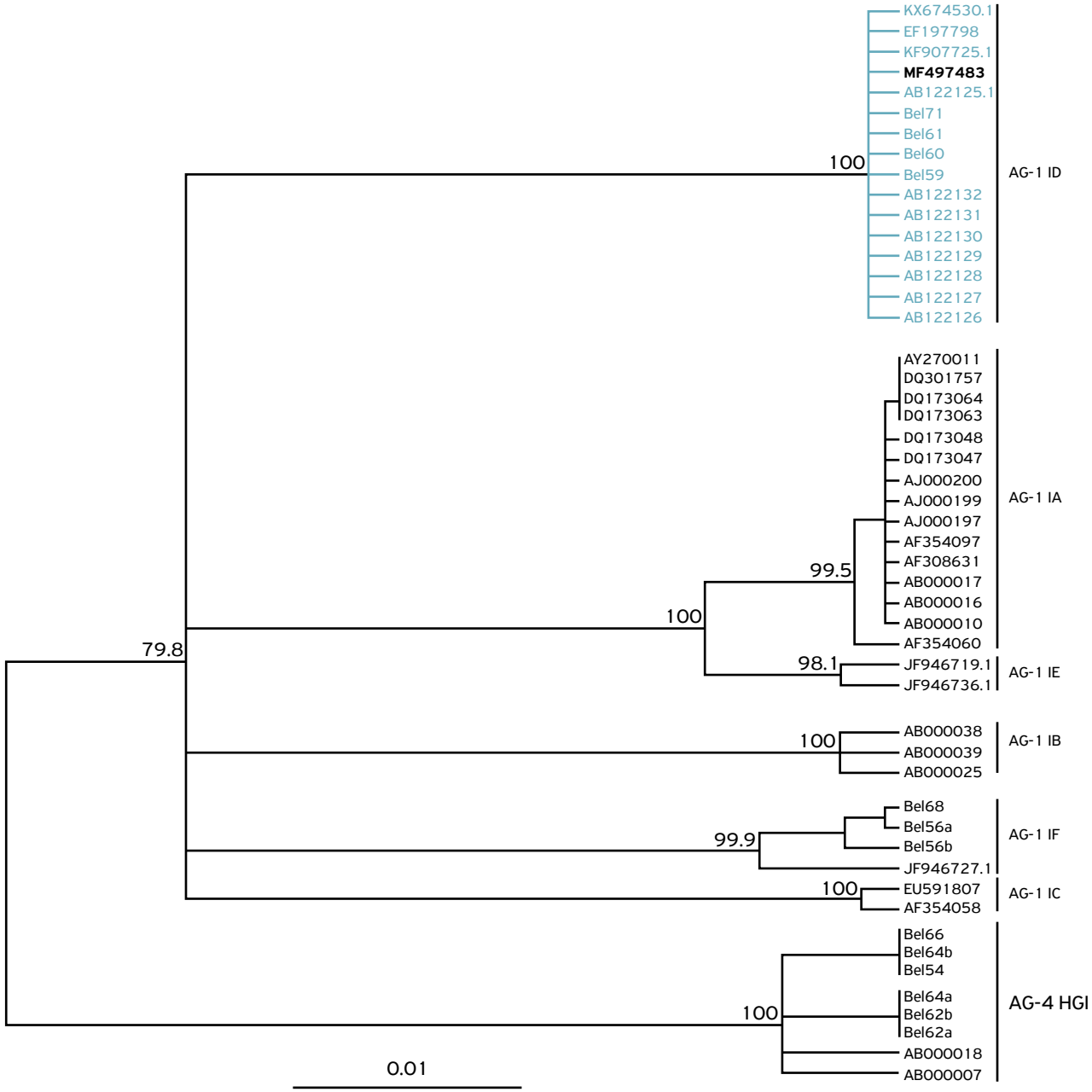

Figure 3. Phylogenetic analysis (UPGMA distance) of the rDNA's ITS-5.8S region from *Rhizoctonia solani* isolates of the AG-1 anastomosis group (AG-1 IA, IB, IC, ID, IE and IF) to identify the isolate associated with web blight on okra (MF497483 sequence, in bold) at Manaus, Brazil. Sequences obtained from *R. solani* AG-4 HGI isolates were used as outgroup. Consensus tree was obtained by bootstrap for 1,000 replicates. The values in branches indicate the bootstrap support in percentage.

T. cucumeris (asexual phase of *R. solani* AG-1 ID) was the fungus identified as the agent causing the disease. This is the first report of an association between *R. solani* AG-1 ID and okra. The occurrence of *R. solani* AG-1 ID as a pathogen associated with web blight, however, was reported in Amazonia during an association with passion fruit (*Passiflora edulis* f. *flavicarpa* Deg., Bel59 isolate), beans (*Phaseolus vulgaris* L., Bel60), shellflower [*Alpinianutans* (L.) Roscoe, Bel61], and black pepper (*Piper nigrum* L., Bel71) in 2010 (GAINO et al., 2010).

In the Amazon, *T. cucumeris* causes web blight in several hosts, such as Para rubber tree (*Hevea* spp.) (DESLANDES, 1944) and orange (*Citrus* spp.) (LOURD et al., 1984). According to CAMPOS (2006) and GAINO et al. (2010), the Para rubber tree hosts several other anastomosis groups (AGs), among them AG2-2 Hb, the most frequent one, and AG-1 1D and AG-1 IF (recently reclassified, now encompasses isolates previously classified as AG-1 IB). These isolates infect Para rubber tree and occur in other hosts (native or in crops). This wide array of hosts reflects the pathogen's potential to infect different crops.

In okra, the pathogen had been reported to cause pre- and post-emergence rots, damping off (MASSOLA; BEDENDO, 2005), and post-harvest fruit rots (HENZ et al., 1996). A strong attack of *T. cucumeris* happened throughout the okra plant's developmental stages, causing defoliation. It was more frequent during the rainy period (from January to May), when the conditions for infection are more favorable.

This is the first report of *T. cucumeris* causing web blight and strong defoliation in okra in Brazil, and probably in the world.

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