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Geographical distribution and prevalence of the main tomato fungal wilt diseases in Benin

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ABSTRACT

Tomato is one of the most economically important vegetable crops in Benin and its production represents more than 51% of the total production of vegetable crops. The ecological peculiarity of its farming exposes it to diseases and pests. Tomato wilt constitutes the major phytosanitary constraint for its production. To identify the causal agents, a survey was carried out across the 12 departments of Benin. Three districts were selected per department and three villages in each district were surveyed. Two farmer's fields were visited per village where five diseased plants were collected per field. Three pathogens identified from the samples collected in the diseased fields: *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *lycopersici* and *F. solani*. Pathogenicity test conducted for each of the isolated pathogens was positive. The symptoms observed in greenhouse were exactly the same as those recorded in the field. The prévalence of tomato wilting recorded in farmer's fields varies from 0.1% to 27% for *S. rolfsii*, from 3% to 20% for *F. oxysporum* f. sp. *lycopersici*, and from 1% to 3% for *F. solani*. The most disseminated and most devastating pathogen was *S. rolfsii*. *F. oxysporum* f. sp. *lycopersici* and *F. solani* were less disseminated and less devastating pathogens.

Keywords: Tomato, fungal wilts, incidence, distribution, Bénin.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the main vegetable crop in Benin and is an important source of income for farmers (Agossou et al., 2001; James et al., 2010). Tomato is grown in rain and dry seasons in all the 12 departments of the country. However, phytosanitary problems limit its production and reduce farmers' income. Among these problems include diseases caused by soil-borne pathogens which are the most limiting factors to tomato production and are the main cause of economic losses recorded by farmers (Sikirou et al., 2001).

Wilting of tomato reduces or prevents its production in some areas in Benin. For

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instance, in the valley of Ouémé, the pathogens can jointly cause yield losses up to 100% (Sikirou et al., 2001). Tomato wilt is frequently caused by five different pathogens including bacteria such as *Ralstonia solanacearum* (Wydra and Dannon, 2006; Sikirou et al., 2009) and *Clavibacter michiganensis* (Blancard, 2015) and fungi such as *Fusarium oxysporum* f. sp. *lycopersici* (Osuinde and Ikediugwu, 2002), *Sclerotium rolfsii* (Sikirou et al., 2001; Adandonon, 2006), and *F. solani* (Cucuzza and Watterson, 1991). *S. rolfsii* and *F. oxysporum* f. sp. *lycopersici* are among the most aggressive soil-borne fungi causing wilt and rot of tomato plants (Khaled et al., 2005). The symptom of *S. rolfsii* is often manifested by yellowing accompanied by wilting of plants. At the later stage, the fungus develops sclerotia at the collar of the plant. *F. oxysporum* causes yellowing of the lower leaves on one side of the plant followed by wilting (Smith et al., 1988). *F. solani* attacks more often plant roots and cause wilting. These fungi survive in the soil and also in plant debris (Smith et al., 1988). As mentioned above, wilting of Solanaceae in general and of tomato in particular is a major concern for farmers in Benin. Apart from the work of Sikirou et al. (2001, 2009) who reported the presence of *S. rolfsii*, *F. oxysporum* and *R. solanacearum* as responsible for wilt of tomato in some localities of Southern parts of Benin, no known data is available on their geographical distribution and prevalence. The objectives of the present study were to (i) locate fungal diseases of wilting of tomato in Benin, and (ii) to evaluate the pathogenicity of the causal agents of these diseases.

MATERIALS AND METHODS

Origin and collection of samples

A survey was carried out across the 12 departments of Benin (Atlantique, Littoral, Mono, Couffo, Plateau, Ouémé, Zou, Collines, Alibori, Borgou, Atacora and Donga) (Figure 1) in 2006 and 2007. In each department, three districts were selected and three villages were inspected in each of them. In each village, two tomato fields were visited and in each field, five wilted tomato plants were sampled and placed in labeled envelopes. In each field, three density squares of 50 m^2 (5 m x 10 m) each were delimited to evaluate the prevalence of wilted tomato plants. According to the cropping system of regions, the number of plants per density square in the fields varies between 50 and 200. The prevalence was assessed as the number of wilted plants over the total number of plants in the delimited density square. Villages and fields surveyed were randomly selected. The collected samples were carried to the laboratory of diagnosis of plant diseases at IITA - Benin.

Isolation of fungi

Fungi were isolated according to the techniques of Fox (1993). The techniques consist of washing infested plant parts with running tap water to remove soil surface; and to superficially clean them with cotton soaked in 70% diluted ethanol for 3 min. The roots were cut into sections of 0.5 cm long which were then soaked in a solution of 1% sodium hypochlorite for 5 min and then rinsed with sterilized distilled water. These sections were then dried on sterilized filter papers under the laminar. After drying, the root sections were placed on the water agar in Petri dishes (Fox, 1993). The Petri dishes were then incubated at a temperature of 25 °C for 3 to 5 days. The mycelium was collected and then cultured on PDA medium (prepared as follows: 39 g of PDA were put in 1 liter of distilled water and autoclaved for 30 min at 120 °C).

Identification of isolated fungi

Fungi were identified on the basis of macroscopic characteristics (color, colony

pigmentation, elevation, mycelium and its aspect) and microscopic characteristics (color, shape and size of spores and conidiophores). The identification of fungi was carried out with the aid of the identification key (Barnett and Hunter, 1987; Samson et al., 1995; Kirk et al., 2001).

Fusarium species were identified by a serial dilution technique of isolates' suspensions (Toussoun and Nelson, 1976). One milliliter of an initial spore suspension was taken and then diluted four times in a ratio of 1:10. Each level of dilution was used to inoculate the water agar. After 24 hours, the mycelium from a single spore was transferred on nutrient medium, Potato Dextrose Agar (PDA) (9 g in 1 L of distilled water and 20 g of agar) then on Carnation Leaf Agar (CLA) (15 g of agar in 1 L of distilled water and then autoclaved for 30 min at 120 °C and after cooling at 55 °C add 2 to 3 pieces of carnation leaf). The latter medium was specific to identify *F. oxysporum* f. sp. *lycopersici*.

Pathogenicity test

Fusaria

The inoculum was prepared by harvesting with sterilized distilled water, spores, mycelium and chlamydospores from different isolates of *Fusarium* grown on PDA. The resulting suspensions were diluted and filtered using a sieve. For each isolate, the spore concentration was evaluated using the hemacytometer.

Five 3-weeks-old tomato plants cv. Tounvi, were transplanted into individual plastic pots (14 cm x 16 cm) containing sterilized field soil. Plants were inoculated the same day by spraying at the collar with 30 ml of an inoculum of $1x10^6$ spores/ml (Santos, 1997). Plants inoculated with only sterilized distilled water served as controls. Out of the 23 *F. oxysporum* f. sp. *lycopersici* recorded isolates, eight were tested with two per department. Three of the 5 collected *F. solani* isolates were also tested. Three classes of pathogenicity were determined. Isolate was (1) highly pathogenic when the first symptoms appear before 15 DAI, (2) moderately pathogenic when the first symptoms appear after 15 DAI and (3) non-virulent when no symptoms appear until 28 DAI.

S. rolfsii

With the isolates of *S. rolfsii*, each plant was inoculated at the collar with 50 sclerotia. The sclerotia were counted and taken together with the PDA medium on which the fungus was grown. Five plants were inoculated per isolate. Control plants were inoculated with uncultured PDA medium. The same classes of pathogenicity as mentioned above were considered for *S. rolfsii* species tested. All the inoculated plants were kept in the greenhouse and monitored for the appearance of wilting. The assessment of wilting disease was carried out for 28 days. Out of the 149 recorded isolates, 23 were selected for the pathogenicity test. Fungi were recovered from a symptomatic inoculated plants using the previous isolation methods.

Statistical analysis

The analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) Version 8.1 on the prevalence of wilted tomato in the field. The Student-Newman-Keuls test was used to compare mean values of prevalence at 5%. Values given in the table are means with corresponding standard errors.

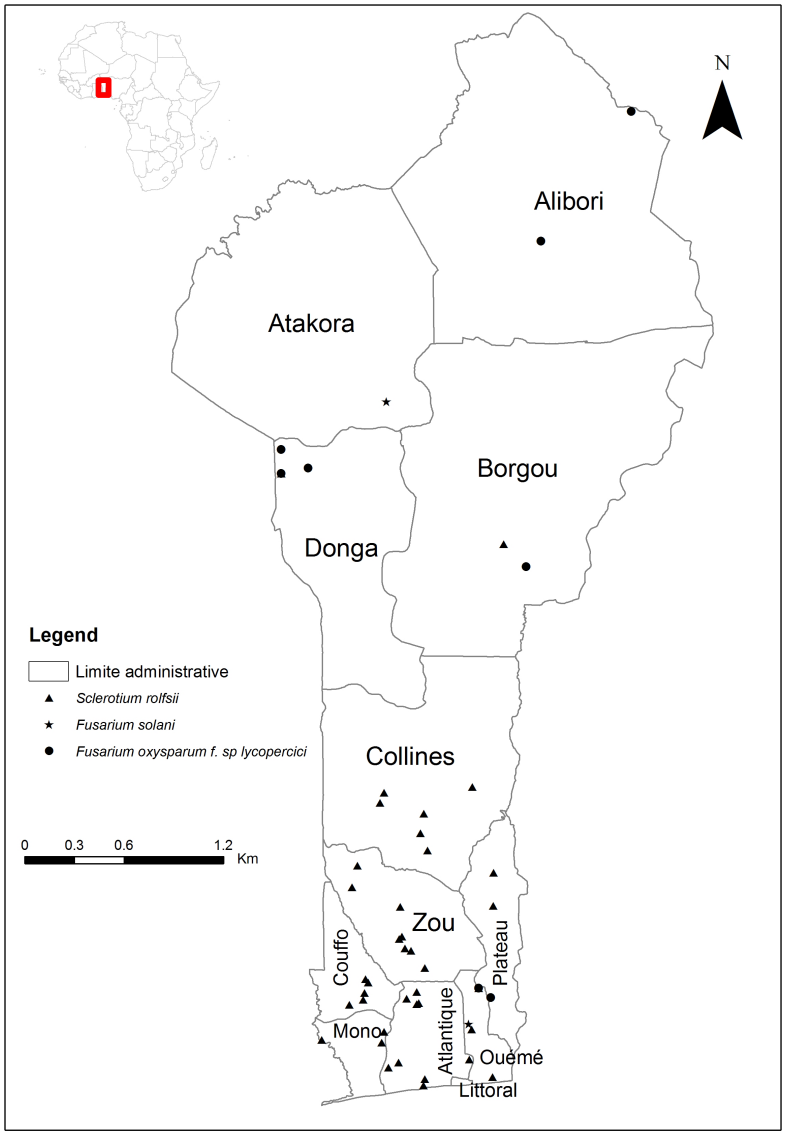

Figure 1: Map of Benin showing the distribution of *S. rolfsii*, *F. oxysporum* f. sp. *lycopersici* and *F. solani* in tomato fields.

RESULTS

Identification of fungi

Identification of S. rolfsii

The mycelium that grew from diseased roots was placed on PDA medium and after 2 to 4 days the whole Petri dishes were rapidly covered with whitish mycelium. Sclerotia of 0.5 to 2 mm in diameter grew after 7 days of incubation. Initially, they were of white color but later became brown.

Identification of F. oxysporum f. sp. *lycopersici*

On PDA culture medium, the colonies were either white or whitish-pink and with purple ting and the reverse were colorless or pink. The mycelium was sparse and flaky. On the culture medium of CLA, microconidia were abundant oval shape, 0-1 septate, measuring 2-5 x 1.5-2 µm. Macroconidia were 3-4 septate, fusiform, less curved, fine tips.

They measure 6-10 x 2-3 µm. Chlamydospores were spherical measuring 3-4 µm in diameter. The monophialides were very short.

Identification of F. solani

On PDA culture medium, the colonies were white or whitish-purple; the reverse was yellowish or whitish. The microconidia were abundant, nonseptate or with a septate, measuring 3-6 x 1.5-2 µm on CLA. Macroconidia were 3-7 septate, with rounded ends, measuring 6-10 x 2-3 µm. Chlamydospores in couples measured 3-4 µm in diameter; they were formed after 2 weeks. The monophialides were very long.

Distribution of fungal pathogenic agents responsible for tomato wilts in Benin

The fungus, *S. rolfsii*, was identified in the following departments: Atlantique, Collines, Zou, Donga, Plateau, Ouémé, Couffo, Mono and Borgou (Figure 1). In the department of Atlantique, it was identified in the villages of Ouègbo-aliho, Ouègbo center and Akpé (Toffo district) in Adékou, Agamalomey villages (Kpomassè district) and in the village of Pahou-Avlékété (Ouidah district). In the department of Collines, the fungus was identified in Ouessè-Tchogoudo and Dohissa-Hounoukon villages (Savalou district), and in Dani village (Savè district).

In the department of Zou, the disease was identified in all the villages surveyed. There were Cannan, and Houessouho villages (Bohicon district); Amouta, Agbohountogon, and Dridji villages (Djidja district); and Hlagbadénou and Avlamey villages (Zogbodomey district). In the department of Donga, it was identified in the village of Tchacléro (Copargo district). In the department of Plateau, it was identified in the villages such as Awaya and Dogo (Kétou district) and in the village of Tatonnonkon (Adja - Ouèrè district). In the department of Ouémé, this fungus was identified at Agongo village (Sèmè - Kpodji district), Gangban (Adjohoun district) and Agonguè village (Dangbo district). In the department of Couffo, it was identified in the villages of Madjrè, Dékandji and Agbédranfo (Dogbo district) in Lokogba and Koutimey villages (Lalo district) and in the village of Tchikpé (Klouékanmey district). In the department of Mono, it was identified in the villages of Agbadji and Anadji (Bokpa district) and in the village of Dédékpoé (Athiémé district). In the department of Borgou, Banikani was the only village where the fungus was identified.

Fusarium wilt caused by *F. oxysporum* was identified in the departments of Alibori, Donga, Borgou and Plateau (Figure 1). In the department of Alibori, Noureni (Malanville district) and Tissarou (Kandi district) were the villages where the disease was identified. In the department of Donga, the disease was identified in Tchacléro and Sérotargo villages (Copargo district). In the department of Borgou, Kika (Okpara district) was the only village where the disease was found. In the department of Plateau, it was recorded in Igboabikou and Tatonnonkon villages (Sakété district).

Fusarium wilt caused by *F. solani* was identified in Atacora and Ouémé (Figure 1). In the department of Atacora, it was identified in the village of Bokossi (Péhunco district) and in Ouémé, it was found only in the village of Dannou (Adjohoun district).

Prevalence of the diseases

Of the 12 surveyed departments (Table 1), *S. rolfsii* was isolated from 9 departments, *F. oxysporum* from 4 departments and *F. solani* from 2 departments. The prevalence of wilt caused by *S. rolfsii* varied between 0.1% and 27%. As for *F. oxysporum*, its prevalence varied between 3% and 20% while that of *F. solani* ranged from 1% to 3%.

Pathogenicity of *S. rolfsii*, *F. oxysporum* f. sp. *lycopersici* and *F. solani*

All the eight tested isolates of *F. oxysporum* and the 23 tested isolates of *S. rolfsii* induced wilting of tomato plants before 15 days after inoculation. While the three tested isolates of *F. solani* on the same tomato variety showed wilting after 15 days of inoculation (Table 2).

Table 1: Percentage of wilted tomato plants in field by *S. rolfsii*, *F. oxysporum* f. sp. *lycopersici* or *F. solani.*

Department	Village	[1]Mean	P value
Alibori	Toumboutou	16.7±10.5a	0.108
	Tantadji	1.2±0.3a	
	Fandoboh-carnon	0.8±0.8a	
	Garou	0.0±0.0a	
Atacora	Péhunco	3.0±0.3b	0.003
	Toroubou	1.0±0.3a	
	Moussansamou	0.0±0.0b	
Atlantique	Ouidah-centre	10.0±5.4a	0.155
	Agamalomè	6.7±3.1a	
	Pahou-Hounjava	5.4±2.7a	
	Adékou	1.0±0.4a	
	Ouègbo Aliho	0.7±0.3a	
	Etoko	0.0±0.0a	
Borgou	Banikani	0.8±0.2a	0.0009
	Okpara-Kika	0.8±0.2a	
	Tissarou	0.3±0.2ab	
	Bahounkpo	0.0±0.0b	
	Tamarou	0.0±0.0b	
Collines	Dani	11.2±5.7bc	0.163
	Dohissa	7.0±4.7c	
	Tchogoudo	1.3±0.6c	
	Katakou	0.3±0.1c	
Couffo	Koutimè	16.3±1.9b	<0.0001
	Madjrè	13.3±1.7b	

	Lokogba	4.7±2.2b	
	Tchikpé	4.0±3.2b	
	Dékandji	3.8±1.3b	
	Agbodohoui	0.3±0.2b	
	Agbédranfo	0.0±0.0b	
	Lalo-centre	0.0±0.0b	
Donga	Anandana	20.0±5.8a	0.355
	Tchacléro	18.1±4.6a	
	Sérotargo	10.0±4.7a	
Mono	Anadji	26.7±13.6ab	0.004
	Kpinou	3.2±1.6b	
	Dédékpoé	3.0±1.9b	
	Agbodji	0.1±0.1b	
	Ayiginnou	0.0±0.0b	
	Ewékondji	0.0±0.0b	
	Nicouécondji	0.0±0.0b	
	Gbakpodji	0.0±0.0b	
Ouémé	Djrègbé	1.3±0.7d	0.082
	Agongon	0.5±0.3d	
	Gbéko	0.5±0.2d	
	Site maraîcher VIMAS	0.0±0.0d	
Plateau	Ayétoro	17.3±2.2bc	<0.0001
	Ikpilè	2.5±1.7c	
	Fouditi	0.8±0.8c	
	Awaya	0.2±0.2c	
	Dogo	0.2±0.2c	
	Kpankoun	0.0±0.0c	

Zou	Cannan	17.8±6.0a	0.001
	Avlamè	2.7±1.8b	
	Hlagbadénou	1.7±0.6b	
	Dridji	0.3±0.2b	
	Agbohountogon	0.0±0.0b	
	Dahnonkpota	0.0±0.0b	
	Hlagba-Adogbé	0.0±0.0b	

Means of 6 replications, SE = Standard Error, Means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at 5% level.

Table 2: Pathogenicity of isolates of *S. rolfsii*, *F. oxysporum* f. sp. *lycopersici* and *F. solani*.

Isolate designation	Pathogen name	Pathogenicity group	Pathogenicity level
37N, 11N, 5N, 7N, 72S, 73S, 48N and 52S	*F. oxysporum* f. sp. *lycopersici*	1	Highly pathogenic
97c, 88c, 52c, 137S, 57c, 4S, 53N, 54N, 39S, 40S, 82S, 89S, 110S, 105S, 125S, 127S, N3, N4, 44S, 101Se, 68V and 140S	*S. rolfsii*	1	Highly pathogenic
67N, 68N and 69N	*F. solani*	2	Moderately pathogenic

1 = highly pathogenic, 2 = moderately pathogenic, 3 = non-virulent.

DISCUSSION

The present study demonstrates that tomato fungal wilt is caused in Benin by the fungi *S. rolfsii*, *F. oxysporum*, and *F. solani*. Among these pathogens, *S. rolfsii* is the most prevalent and widely disseminated in Benin with decreasing concentration gradient from the south to the north. In this country, *F. oxysporum* and *F. solani* were found in the north and south. These results confirm the conclusions of Deepthi and Eswara Reddy (2013) who reported that *Sclerotium rolfsii* is an important pathogen which attacks different crops and causes serious yield losses. Of these three pathogens, *S. rolfsii* was the most distributed. A high prevalence was noticed in the fields infected by *S. rolfsii* compared to those infected by *F. oxysporum*, and *F. solani*. This could be explained by the cropping practices in the south of Benin. Tomato monoculture is largely practised by farmers in the south during the two consecutive rainy seasons while cultivated once a year and during the dry season in the north. In the south, most of farmers cultivate tomato local varieties (Tounvi, Akikon, Kèkèfo, Ahougbo) and produce themselves the seeds. When tomato slumped in price during the rainy seasons, farmers abandon tomato fruits in the fields. This practice favours a contact of tomato fruits with the soil, promoting the multiplication of *S. rolfsii* and its dissemination through the fields. By contact with the soil, tomato fruits are quickly attacked by the fungus *S. rolfsii* when it is present. In the north, farmers cultivate hybrids varieties of tomato (Tropimech, Petomech, Roma, Rossol, Elgon, Caraïbe, Mongal, Heat master, Padma, etc.) and are compelled to buy seeds every year. In this dry season, the trading value of tomato fruits increases and are seriously managed for marketing. This explains the reason why the pathogens mainly *S. rolfsii* is scarcely distributed in the northern Benin.

During the survey, it was observed that *S. rolfsii* attacked tomato fruits which were in contact with the soil by causing their rot. Also, in several events, white mycelia with sclerotes covered in full the stems of the tomato plants at vegetative and reproductive stages above soil surface.

Our results are similar to those of Momol and Pernezny (2006) who found that the mycelia of *S. rolfsii* are often produced over the diseased tissue and surrounding soil forming a white mat of mycelial threads with the typical tan-to-brown, mustard-seed-sized sclerotia.

Isolates of *S. rolfsii* and *F. oxysporum* showed the first wilt symptom between 14 and 15 days after inoculation. For *F. solani*, wilting appeared at 20 days of inoculation. This study confirms the findings of Jones and Overman (1986) who reported that *S. rolfsii* (southern blight) and *F. oxysporum* are fungi that cause wilt of tomatoes. Djordjević et al. (2011) showed that race 3 of Fusarium wilt is a limiting factor for successful tomato production.

The results of the present study showed that *F. oxysporum* induced wilting before 15 days after inoculation. Our results are consistent with those of Ignjatov et al. (2012) who reported that pathogenicity of isolates TFW1-TFW12 appeared 14 days after inoculation.

In some fields, two pathogens lived together. For example at Igboabikou, Tatonnonkon, Dannou, Agonguè and Ouègbo villages, *R. solanacearum* cohabited either with *F. oxysporum* or *S. rolfsii* on the same field. Two pathogens were not isolated from the same plant in any case.

The work carried out previously by Sikirou et al. (2009) showed that wilting of tomato is also caused by the bacteria *R. solanacearum*. When compared the results of Sikirou et al. (2009) on *R. solanacearum* to the present study, *R. solanacearum* caused more damage to tomato production than the three fungal pathogens identified. From the best of our knowledge, wilting of tomato caused by *F. oxysporum* and *F. solani* is the first study conducted in Benin Republic.

Conclusion

Among the three different pathogens identified as responsible for tomato wilt in Benin, *S. rolfsii* is the most prevalent and widely disseminated across the country. *F. oxysporum* and *F. solani* are found in the north and south of Benin.

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