Etiology of and Effect of Environmental Factors on Damping-Off and Stem Rot of Cowpea in Benin

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Damping-off and stem rot are two types of diseases affecting cowpea (*Vigna unguiculata* (L.) Walp.) in the Ouémé Valley, Benin. Of the fungal species isolated from diseased plants in the field during a 2-year experiment (2001 and 2002), *Sclerotium rolfsii* Sacc. was found to be solely responsible for these diseases. The disease incidence decreased with increasing distance of the field from the river. Measurement of *S. rolfsii* initial inoculum, soil moisture and disease incidence in cowpea field plots revealed a positive correlation among these parameters. The multiple regression analysis showed that the disease incidence increase was 0.4% for one unit increase in soil moisture percent, whereas the disease incidence increase was 19.8% for one unit increase of the density of initial inoculum of the pathogen. This is the first comprehensive study of the effects of environmental factors on the incidence of cowpea damping-off and stem rot caused by *S. rolfsii* in Benin, and shows that the density of the initial inoculum is the main contributing factor of the disease in the field in the Ouémé Valley.
KEY WORDS: Initial inoculum; soil moisture; susceptibility; temperature; *Vigna unguiculata.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume in West Africa (23), providing an inexpensive source of protein for the poor urban and rural population (1). Unfortunately, cowpea yield is low due to its susceptibility to many diseases (3,7,22), such as damping-off and stem rot. These diseases have been reported in many countries in tropical Asia, the USA, Nigeria (13,23), Benin (9,17) and South Africa (3). Damping-off and stem rot diseases are caused by many different species of fungi (3,6,23), including *Pythium aphanidermatum* (Edson) Fitzp., *Rhizoctonia solani* Kühn, *Phytophthora* sp., *Fusarium solani* (Mart.) Sacc. and *Sclerotium rolfsii* Sacc. (20).

Moreover, outbreaks of disease are often triggered by specific environmental events that can occur long after initial inoculum infection (8,15,16,25). Pieczarka and Abawi (16) and Wakeham *et al.* (25) reported that when environmental conditions prevailing in both air and soil are suitable, disease development can be greatly affected and colonization of host tissues and disease spread can be rapid. Similarly, Singh *et al.* (21) indicated that most

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Pythium species and *R. solani* cause major damage in rain-forest and guinea savanna with cool and wet weather conditions similar to those prevailing in Nigeria.

Damping-off and stem rot of cowpea are the most important diseases during cowpea cultivation, which starts after natural flooding in the Ouémé Valley, Benin. These diseases result in yield losses ranging from 19% to 40% (17). *Sclerotium rolfsii* was reported to be the causal agent of the disease in the Valley (9), but little is known about the disease in that area. The effect of environmental conditions on the severity of the disease has not yet been studied. The objective of the present study was to study aspects of the epidemiology of the disease in the Ouémé Valley, including the influence of initial inoculum, rainfall, and soil moisture on the incidence of damping-off and stem rot of cowpea.

MATERIALS AND METHODS

Collection of plant material Surveys were conducted in farmers' fields in six different villages (Agbonou, Agonguey, Agonlin, Dannou, Gangban and Wozounme) during the cowpea planting seasons in 2001 and 2002. Three bands about 150 m apart, each representing a block and running perpendicular to the river, were demarcated. Five fields at 200-m intervals were located along each band. The fields were planted with 'Tchawé kpayo', the local cowpea cultivar, and cultivated and weeded by farmers using traditional cultural practices. In each field, a plot of 10 × 20 m was marked, within which six 2-m^2 subplots were demarcated. The number of plants showing symptoms of the diseases was recorded and tape-marked at 7, 15 and 30 days after planting. Diseased plants were randomly collected from the 2-m^2 subplots and transported to the laboratory for fungal pathogen isolation and identification.

Isolations Diseased stem tissue was cut into small segments (~2 mm^2), surface disinfected with 0.5% sodium hypochlorite (NaOCl) for 15 s, rinsed twice in sterile distilled water (SDW), blotted dry and plated onto potato dextrose agar (PDA) amended with 0.025% chloramphenicol. After 2 days' incubation at 25±1°C under fluorescent light, pure cultures were obtained by transferring colonies to new PDA and water agar plates. The pure fungal cultures were maintained on PDA slants at 4°C.

Identification of fungal isolates Fungal colony morphological characteristics: The radial growth of the colonies was measured after 3 days' incubation, and the colony color and number of produced sclerotia were recorded. Identification was done using the Mycology Guidebook (24), the system of Nelson *et al.* (12) and keys of Domsch *et al.* (5). One pure culture was deposited in the National Collection of Fungi, Biosystematics Division, Plant Protection Research Institute, Pretoria (PREM 57252), and the remainder in the Collection of Fungi, Plant Health Management Division, International Institute of Tropical Agriculture (IITA), Cotonou, Benin (isolate codes: IITA 407, IITA 408, IITA 409, IITA 410, IITA 411, IITA 412).

Pathogenicity tests Pathogenicity of each isolate was tested in the greenhouse at IITA, Cotonou Station, Benin, using the *Sclerotium*-susceptible cowpea cv. Tchawé kpayo. Millet seed (*Panicum miliaceum* L.) soil inoculum techniques were used to inoculate the soil (26). Five 5-mm-diam discs cut from the actively growing edge of each colony on PDA were used to inoculate 50 g of the millet seeds. Prior to the inoculation, the millet seeds were steeped in water for 48 h and autoclaved for 45 min at 120°C. Inoculated-millet seeds were incubated for 21 days at 27°C and then used to inoculate the soil. Sandy loam soil was

pasteurized by aerated steam (60°C for 60 min) and stored for 21 days before inoculation with the millet seed inoculum (16). Inoculum was air-dried in a paper bag, lightly ground and passed through nested sieves with an opening of 3 mm. The soil inoculation was done 2 days before planting by mixing 10 g of the millet seed inoculum of each isolate with 1 kg of steam-disinfected sandy loam soil in a pot. Controls consisted of pasteurized soil (1 kg) unmixed or mixed with 10 g of uninoculated steeped millet seed, respectively.

Cowpea seeds of cv. Tchawé kpayo were obtained from the Ouémé Valley village. The seeds were surface-disinfected in 1% NaOCl for 2 min, rinsed twice in SDW and then planted in pots (14 cm diam × 18 cm height), using four seeds per pot filled with inoculated soil. Pots were kept at 21–30°C in the greenhouse, watered every 2 days and the number of seedlings showing disease symptoms in each pot was recorded at 7 and 30 days after planting. To fulfill Koch's postulates, at least two diseased plants were removed at each observation and plated onto PDA medium. Cowpea seedlings and plants were inoculated with the fungal culture. A fungal species was considered to be pathogenic when its inoculation to cowpea seedlings or plants in the greenhouse resulted in at least one diseased seedling or plant with the typical symptoms of the disease. The re-isolated fungus was cultured on PDA, and colony characteristics were recorded and compared to the original isolates.

Field evaluation of the effects of environmental conditions on disease development
On-farm trials were conducted during 2001 and 2002 in a village (Agonguey) in the Ouémé Valley to determine the effect of environmental conditions and natural field initial inoculum density on disease incidence in cowpea. The village is transected by a river that overflows and floods the fields of the village each year, toward the end of the rainy season (August–September). The weekly mean air temperatures and humidity recorded during the field experiment varied between 26.1° and 26.3°C, and 69.0% and 75.0%, respectively, in 2001, and between 25.8° and 26.9°C, and 71.1% and 73.6%, respectively, in 2002. Cowpea is grown after the water has drained from the fields. The fields are known to have a history of damping-off and stem rot of cowpea (17). During the 2001 cowpea planting season experiment, three bands, each representing a block and running perpendicular to the river, were demarcated approximately 150 m apart. Five fields at 200-m intervals were located along each band and planted with cv. Tchawé kpayo. The cultural practices were the same as described earlier. The experimental design was a complete block with each band representing a block, replicated three times along the river. A 1.0-kg soil sample from a depth of 20 cm was taken from each subplot at planting, for initial inoculum density determination. The wet-sieving method described by Punja *et al.* (19) was used to recover directly and enumerate sclerotia from the soil samples. Soil moisture was measured gravimetrically from a 20-cm-deep soil sample taken per subplot at planting and at 7-day intervals until harvest, using the technique of Nielsen *et al.* (14). Seven days after planting and subsequently at 7-day intervals until harvest, all cowpea plants within each subplot were examined and the number of seedlings or plants showing symptoms of damping-off or stem rot was recorded and tape-marked to establish disease incidence. Rainfall was recorded daily using a funnel-type gauge positioned 1.5 m above the soil surface in the field.

In the second experiment during the 2002 cowpea planting season, two cowpea cultivars, Tchawé kpayo and IT83D-326-2, were planted per block in the field. IT83D-326-2 is known to be moderately tolerant to damping-off and stem rot (17). The experimental

fields were located along the bands demarcated along the river as described earlier and each cultivar was grown separately in a 10 × 20-m plot (per field). The cultural practices, sampling and collection of environmental data were as described above.

Statistical analysis The percentage field data were arcsine (Y1/2) transformed. The analysis of variance was performed using the general linear model (GLM) procedure in the SAS System (20) and mean separations were done using the Student-Newman-Keuls (SNK) option. Correlation matrix and multiple regression analyses were performed, taking into account all measured variables of the system. The estimated multiple regression equation was used:

$$\hat{y} = \hat{y}_o + ax_1 + bx_2 + cx_3 + \ldots$$

where $\hat{y}$ represents the disease incidence; $\hat{y}_o$ the intercept; a, b and c are the increase in $\hat{y}$ resulting from one unit increase of the factors x_1, x_2 and x_3, respectively. To avoid overestimating the variable impact, the multiple coefficient of determination was adjusted and computed as follows (2):

$$R_a^2 = 1 - (1 - R^2)/(n - p - 1),$$

where $R_a{}^2$=adjusted multiple coefficient of determination; R^2=multiple coefficient of determination; n=number of observations; and p=number of independent variables.

RESULTS

Identification of fungal isolates Four fungal species, *Fusarium oxysporum* Schlecht., *Fusarium scirpi* Lamb. and Faut., *Trichoderma harzianum* Rifai and *Sclerotium rolfsii* Sacc. were isolated from plant samples collected from the field in 2001. Although all these species were present in all six villages, their prevalence was not consistent. In most cases, *S. rolfsii* was the only fungus isolated from the diseased plants. *S. rolfsii* and *F. oxysporum* were isolated from 2.91% of the same plant samples, *S. rolfsii* and *F. scirpi* from 1.5%, *S. rolfsii*, *T. harzianum* and *F. oxysporum* from 4.32%, and *S. rolfsii*, *F. scirpi* and *T. harzianum* from 0.5%. Incidences of the fungal species showed a similar trend during the second field experiment.

Pathogenicity tests None of the *F. oxysporum*, *F. scirpi* or *T. harzianum* isolates caused disease on cowpea in these experiments. Damping-off and stem rot occurred when cowpea seeds were planted in soil inoculated with *S. rolfsii* only. Some plants showed stem infection but remained upright. Symptoms corresponded with those observed in the field and also with those described by Singh and Rachie (23) on cowpea. *S. rolfsii* was re-isolated from the seedlings and produced cultures identical to those of the original isolate which was used as inoculum.

Field evaluation of the effects of environmental conditions on disease development Damping-off symptoms observed in the fields included necrotic, water-soaked and brown lesions on the hypocotyls. The diseased seedlings wilted and fell over onto the ground. When cowpea plants are affected at a later growth stage, the disease is called stem rot. The affected plants developed the above-mentioned symptoms, but remained upright. The lesions were covered with white mycelium in which brown sclerotia were embedded.

Soil moisture (%) recorded in the field diminished over time. The percent soil moisture data, either over time or at harvest (last measurement), were higher closer to the river and

TABLE 1. Soil moisture, initial inoculum, and incidence of *Sclerotium rolfsii* damping-off and stem rot of cowpea recorded in 2001 and 2002 in Agonguey, Ouémé Valley, Benin

Distance from river (m)	Soil moisture[z] (%)		Initial inoculum[y] (no. of sclerotia kg^{-1} soil)		Diseased plants (%)		
					Cultivar		
					Tchawé kpayo (susceptible)		IT83D-326-2 (moderately tolerant)
	2001	2002	2001	2002	2001	2002	2002
200	24.81[x]c	25.43c	24.6c	34.7c	8.51c	7.28c	4.37b
400	24.68bc	23.94b	20.5bc	25.1b	6.54b	5.86bc	3.42b
600	23.61ab	24.03bc	18.3b	19.9b	4.82b	4.39b	1.06a
800	23.88abc	22.21a	11.3a	12.4a	2.19a	1.99a	1.26a
1000	23.53a	21.11a	12.6a	12.2a	0.8a	1.04a	0.53a

[z]Means of measurements at 7-day intervals until harvest.
[y]Recorded prior to planting in each year. Six 1-kg soil samples were taken from each of five plots within bands running perpendicular to the river; number of sclerotia was determined using the wet-sieving method (19).
[x]Within columns, means followed by a common letter do not differ significantly (P=0.05) according to the General Linear Model test.

TABLE 2. Correlation matrix from parameters measured during the cowpea damping-off and stem rot survey in Benin during 2001 (01) and 2002 (02)

Parameters	Distance from river	[z]Soil moist. 01	Soil moist. 02	[y]Initial inoc. 01	Initial inoc. 02	[x]Incid. Tcha kpayo 01	Incid. Tcha kpayo 02	Incid. IT83D (02)
Distance from river	1							
Soil moist. 01	-0.665*	1						
Soil moist. 02	-0.876*	0.297	1					
Initial inoc. 01	-0.989*	0.689*	0.563*	1				
Initial inoc. 02	-0.968*	0.759*	0.519*	0.412	1			
Incid. Tcha kpayo 01	-0.954*	0.856*	0.803*	0.874*	0.955*	1		
Incid. Tcha kpayo 02	-0.965*	0.905*	0.843*	0.966*	0.984*	0.560*	1	
Incid. IT83D 02	-0.875*	0.524*	0.636*	0.779*	0.937*	0.380	0.445	1

[z]Soil moisture.
[y]Initial inoculum.
[x]Incidence recorded on cv. Tchawé kpayo (susceptible) and cv. IT83D-326-2 (moderately tolerant).
*Indicates the correlation coefficient is significant.

lowest in the plot farthest from the river (Table 1). A similar trend was observed for the initial inoculum density of *S. rolfsii* and the incidence of damping-off and stem rot during the 2-year experiment in the field (Table 1). During the field experiments, no rainfall was recorded in the Ouémé Valley.

There was a negative correlation between distance from the river and soil moisture, initial inoculum and disease incidence, as shown by the correlation matrix (Table 2). The regression analyses showed that inoculum density, soil moisture or disease incidence as dependent variables were each in a significant relationship, with the distance from the river used as independent variable. The estimated adjusted coefficient of determination R_a^2 was significant ($P<0.05$) and greater than 0.85 for all used dependent variables except that (0.595) of incidence recorded with cv. IT83D-326-2.

The multiple regression analysis, using incidence as dependent variable and soil moisture and initial inoculum density as independent variables, was significant ($P<0.05$)

with an estimated coefficient of determination R_a^2 higher than 0.901. However, the soil moisture 1 (first year) had *P*-values greater than 0.05. Initial inoculum was the only independent variable in a significant ($P<0.01$) relationship with the disease incidence in the first year. In the final step of the backward elimination procedure, soil moisture 2 (second year) had a *P*-value that was less than 0.05 for incidence on cv. Tchawé kpayo, but was not significant for cv. IT83D-326-2. In the specific case of the initial inoculum density, the *P*-values were less than 0.01, indicating a highly significant relationship between the inoculum density and the disease incidence. The estimated multiple regression equation, $\hat{y}=0.103 + 0.004x_1 + 0.198x_2$ (where $\hat{y}$ represents the disease incidence, in the second year, on cv. Tchawé kpayo; x_1 is the soil moisture; and x_2 is the initial inoculum density) was highly significant ($P<0.01$) with an adjusted coefficient of determination, $R_a^2=0.995$, indicating a good fit of the data.

DISCUSSION

Several soilborne fungi have been reported to be associated with damping-off and stem rot of cowpea (23). In the current study, four major fungal species, *F. oxysporum*, *F. scirpi*, *T. harzianum* and *S. rolfsii*, were isolated from plant samples collected from the field. The isolation concentrated on fungal pathogens only. Results showed that, of all the four fungal species, only *S. rolfsii* caused damping-off and stem rot of cowpea during the pathogenicity tests in the greenhouse, indicating that a mere association of fungi with diseased plants does not necessarily indicate that they are pathogens. *S. rolfsii* has been recorded in many countries in Africa, in tropical Asia, in Brazil and in the USA (23). *S. rolfsii* has been reported to be a destructive soilborne pathogen of numerous crops, especially in the tropics and subtropics (11,18). In the present study *S. rolfsii* was identified as the causal agent of damping-off and stem rot, as recorded earlier in the Ouémé Valley (9). *Fusarium oxysporum*, *F. scirpi* and *T. harzianum* isolated in the present study did not individually cause damping-off and stem rot symptoms, indicating that they might not be considered pathogenic on cowpea.

It is common knowledge that environmental conditions can influence the development of disease (16). The etiology of a particular plant disease has important implications in the cultivation of the crop and an understanding thereof is crucial in order to be able to control and eliminate the disease before it becomes established in the crop. Diseases caused by *S. rolfsii* are especially rampant in the tropics and subtropics, where temperatures are sufficiently high to permit the growth and survival of the fungus (11,18). During the present field experiment, air temperature and relative humidity did not vary significantly and might not be the dominant determinators influencing progress of cowpea damping-off and stem rot diseases in the Ouémé Valley. In the naturally infested field experiment, a gradient of *S. rolfsii* initial inoculum was recorded and the highest density was found closest to the river. Soil moisture and disease incidence followed a similar trend. The regression analysis showed that soil moisture was related to the disease incidence, and might be one of the factors influencing disease development in the valley. The fact that there was no rainfall during the 2-year field experiment is typical for the production period of cowpea in this study area. Moisture available in the soil after natural flooding was the primary source of moisture that might have influenced disease development. The positive correlation between soil moisture and disease incidence in the present study corresponds with findings on other soilborne diseases (16). However, in the multiple regression equation

analysis, one unit increase of the percent soil moisture resulted in a 0.4% increase in the disease incidence. This increase is very low compared to one unit increase of the initial inoculum density resulting in a 19.8% increase of the disease incidence. Thus, although soil moisture was found in the present study to be related to disease development, the analyses showed that the driving factor is the density of initial inoculum of the pathogen. In an earlier study, Punja *et al.* (19) recorded the same spatial pattern of inoculum of *S. rolfsii* and indicated that the inoculum was clustered or clumped and that a similar trend was observed for diseased plants. In the Ouémé Valley, the soil preparation by farmers before planting always comprises cutting harvested maize stems and weeds and making holes in which cowpea seeds are sown directly. The stems and weeds are placed on the field during the cowpea development period and are remoistened by dew, thus increasing the amount of organic substrate for mycelial growth. This may lead to increased disease incidence and severity, as reported earlier (4,18). However, disease incidence recorded with cv. IT86D-326-2 was lower than that with cv. Tchawé kpayo, confirming the moderately tolerant status of IT86D-326-2. Using this cultivar should increase yield, which would be improved further by integrated disease management combining other control methods.

Results from this study provide the first documentation on the field factors favoring cowpea damping-off and stem rot development in the Ouémé Valley. *Sclerotium rolfsii* initial inoculum, and to some extent soil moisture, were among the main factors influencing disease development during the study. The present results are important in the context of disease control methods and prediction, and development of an infection efficiency model (10) based on environmental data.

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