Journal of Agricultural Science (2004), **142**, 561–566. © 2004 *Cambridge University Press*
doi:10.1017/S0021859604004629 *Printed in the United Kingdom*

Occurrence and distribution of cowpea damping-off and stem rot and associated fungi in Benin

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(*Revised MS received 21 October 2004*)

SUMMARY

Damping-off and stem rot of cowpea is an important soil-borne disease worldwide. Cowpea fields were randomly chosen in each agro-ecological zone in Benin and surveyed in 2001 and 2002 to determine the occurrence of the diseases throughout the country. Diseased plants, prevailing environmental conditions and cowpea grower cultural practices were recorded and causal agents associated with the disease identified. Results indicated that damping-off and stem rot were distributed throughout Benin. The disease incidence was higher in the South (0·074) and Centre zones (0·063) than in the other zones (<0·02) in the country. Among factors influencing the disease incidence, cultural practices such as sole crop and no-till systems appeared to be most important. Isolated fungi included *Sclerotium rolfsii*, *Fusarium* spp., *Pythium ultimum*, *Rhizoctonia solani*, *Phoma* sp., *Rhizopus* sp. and *Trichoderma harzianum*. None of the *Fusarium*, *T. harzianum* or *Rhizopus* sp. isolates were pathogenic in the greenhouse. *Pythium ultimum*, *R. solani* and *Phoma* sp. were infrequently isolated and few isolates caused the disease symptoms in the greenhouse. However, this is the first report of *Phoma* sp. causing damping-off and stem rot of cowpea in Benin. *Sclerotium rolfsii* was by far the most common species isolated from all the agro-ecological zones and all isolates were pathogenic on cowpea in the greenhouse. *Sclerotium rolfsii* was considered to be the main causal agent of cowpea damping-off and stem rot in the Republic of Benin due to its wide distribution, high incidence and predominance on plants with damping-off and stem rot symptoms.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) yield is often low due to insect pests and many diseases including damping-off and stem rot, which have been reported in many countries (Singh *et al.* 1997; Aveling & Adandonon 2000) and associated with several causal agents (Singh *et al.* 1997). In Southern Africa the causal agents were identified as *Pythium ultimum* Trow (Aveling & Adandonon 2000) and *Rhizoctonia solani* Kuehn (Adandonon 2000). The diseases have also been reported on cowpea in Nigeria, Asia and Brazil, and are caused by fungi such as *Pythium aphanidermatum* (Edson) Fitzp., *Rhizoctonia solani* Kuehn., *Phytophthora* sp., *Fusarium solani* (Mart.) App. and Wol. and *Sclerotium rolfsii* Sacc. (Singh *et al.* 1997).

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Outbreaks of disease are often triggered by specific environmental events that can occur before or during initial inoculum infection (Pieczarka & Abawi 1978; Wakeham *et al.* 1997). These authors reported that when environmental conditions prevailing in both air and soil are suitable, disease development might be greatly affected and colonization of host tissues and disease spread can be rapid. Moreover, inappropriate cultural practices may lead to severe disease expression (Singh & Rachie 1985). In Benin, the cultural practices vary from one cowpea production area to another and farmers prepare soil differently before sowing (PEDUNE-Benin 1995).

In the Ouémé valley, Benin, damping-off and stem rot is a major constraint to the production of cowpea (PEDUNE-Benin 1995) and the diseases were reported to be caused by *S. rolfsii* (Adandonon 2000; Kossou *et al.* 2001). However, no information exists on the countrywide distribution of the diseases, and the associated causal agents in each zone have yet to be identified. The main objectives of the present study were to survey cowpea production areas throughout the different agro-ecological zones to determine the distribution of the diseases in Benin and identify the associated soilborne fungal pathogens and their relative importance in cowpea fields in the country.

MATERIALS AND METHODS

Distribution and incidence of damping-off and stem rot in Benin

Surveys were conducted throughout the Republic of Benin, a West African country bordered in the north by Niger and Burkina Faso, in the south by the Atlantic Ocean, in the east by the Federal Republic of Nigeria and in the west by Togo Republic. The country consists of five agro-ecological zones, namely South, Centre, South Borgou/South Atacora, North Borgou and Atacora (Fig. 1) (INRAB 1995). The surveys were done in the cowpea farmers' fields over 2001 and 2002. In each agro-ecological zone, 30 cowpea fields chosen at random were surveyed at seedling, flowering and pod stages, for the occurrence of damping-off and stem rot. In each field, six 2 m^2 plots were marked and all cowpea plants showing symptoms of the diseases were recorded. Two to four diseased plants were randomly selected, shoots were excised and hypocotyls or stems were collected for fungal identification in the laboratory at the International Institute of Tropical Agriculture (IITA), Cotonou, Benin. Isolation frequency (%) of a fungal species for a given zone was calculated as number of diseased plants with a specific fungal species divided by the total number of diseased plants collected from the specific agro-ecological zone multiplied by 100.

Field evaluations of the effects of environmental conditions and cowpea cropping systems on disease incidence

Rainfall (mm) was recorded daily using a funnel-type gauge positioned 1·5 m above the soil surface in the field. The air temperature (°C) and relative humidity (%) were measured in the field for the duration of the experiment using a hygrothermograph placed at a height of 30 cm above the cowpea plant canopy. To ensure measurement accuracy, the instrument was housed in a Stevenson screen. The environmental data were measured daily, and data sheets were replaced weekly. The housed instrument was protected from direct sunlight and rain. Cropping systems, such as sole cropping and no-till practices, were recorded.

Fungal identification and pathogenicity

The diseased samples were surface-sterilized using Rizvi & Yang's (1996) method. The diseased plants were rinsed in tap water, surface disinfected for 1 min in 0·53 % sodium hypochlorite (NaOCl), rinsed with sterile water and plated onto potato dextrose agar (PDA) and incubated in the dark at 27 ± 1 °C. Plates were examined 3–15 days later for identification of fungi associated with the symptoms observed (Domsch *et al.* 1980). Pathogenicity of each isolate was tested in the greenhouse at the International Institute of Tropical Agriculture (IITA), Cotonou Station, Benin. Soil inoculation with each isolated species was done by means of the millet seed inoculum technique (Weideman & Wehner 1993). Inoculated millet seed was added to pasteurized sandy loam soil at the rate of 17 g inoculum/kg soil. The control treatments consisted of pasteurized soil to which 17 g sterile millet seed was added and pasteurized soil without millet seed. Seeds of a local cowpea cultivar (Tchawe kpayo) susceptible to damping-off and stem rot (Adandonon 2000) were surface-sterilized and then planted in pots (14 cm diameter × 18 cm height) at four seeds per pot. Pots were kept in the greenhouse at temperatures varying from 21–28 °C. The experimental design was a randomized block design with four replicates of each isolate. Seedlings showing damping-off and plants

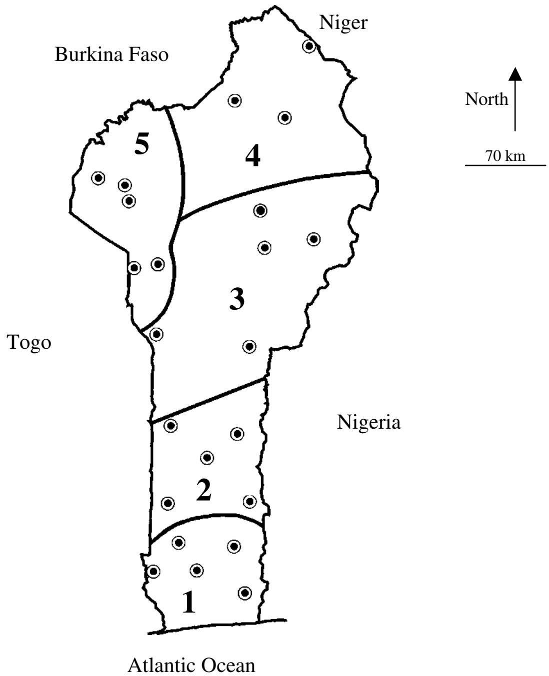

Fig. 1. Map of Benin with its five agro-ecological zones and the different regions surveyed for the occurrence and distribution of damping-off and stem rot of cowpea. 1 = South; 2 = Centre; 3 = South Borgou/South Atacora; 4 = North Borgou and 5 = Atacora (INRAB 1995). Dots represent surveyed centres.

Table 1. *Distribution, incidence of damping-off and stem rot of cowpea, cropping system and rainfall recorded per agro-ecological zone throughout Benin in 2001–2002*

	Disease incidence (%) (± S.E.M.)[1]				
Ecological zones (Arranged North to South)	Damping-off seedlings	Stem rot plants	Rainfall (mm) (± S.E.M.)	Sole crop[2] (%) (± S.E.M.)	No-till[3] (%) (± S.E.M.)
Atacora	1·9±0·21	0·3±0·01	1174±10·4	6±1·9	9±1·9
North Borgou	2·2±0·44	0·1±0·02	914±10·3	20±2·1	16±1·9
S Borgou /S Atacora	5·1±0·9	0·6±0·2	1105±9·9	3±2·2	53±1·8
Centre	6·3±0·54	0·8±0·10	1092±9·6	78±1·9	72±1·6
South	7·4±0·65	0·7±0·04	1378±10·6	93±2·4	85±1·9

[1] Stem rot plants were only observed at the flowering stage and no more disease plants were recorded afterwards in the field. Degree of freedom for the error term: 19.
[2] Sole crop (%) = Percentage of fields planted with cowpea as a sole crop.
[3] No-till (%) = percentage of cowpea fields without tillage.

with stem rot were recorded 3 days after planting and at 3-day intervals until 30 days after planting.

To fulfil Koch's postulates, dying seedlings and plants were removed at each observation and plated onto PDA media. 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 diseases. The re-isolated fungus was cultured on PDA and colony characteristics were recorded and compared with the original isolates.

Statistical analysis

The percentage field data were arcsine ($Y^{1/2}$) transformed. The analysis of variance was performed using the general linear model (GLM) procedure in the SAS System (SAS 1997) and mean separations were done using the Student Newman Keuls option. Correlation matrix and multiple regression analyses were performed, taking into account all variables of the system. To avoid overestimating the variable impact, the multiple coefficient of determination was adjusted and computed as follows (Anderson *et al.* 2003):

$$R^2_{\mathrm{a}} = 1-(1-R^2)(\mathrm{n}-1)/(\mathrm{n}-\mathrm{p}-1)$$

where R^2_{a} = adjusted multiple coefficient of determination, R^2 = multiple coefficient of determination, n = number of observation and p = number of independent variables.

RESULTS

Distribution and incidence of damping-off and stem rot in Benin

Seedling damping-off symptoms observed in the field included necrotic, water-soaked and brown discoloured lesions on the hypocotyls. The diseased seedlings wilted and fell over onto the soil surface. Stem rot was observed on mature cowpea plants, which showed whitish or brown lesions at the base of the stem and wilting, but remained upright. The lesions on these plants were covered with white mycelium in which brown sclerotia were often embedded. The disease symptoms were found in all five agro-ecological zones (Table 1) in Benin. The incidence of the damping-off and stem rot diseases varied from zone to zone and the highest incidence was recorded in the South and Centre zones of Benin (Table 1). Few diseased plants were recorded at flowering stage compared with the seedling stage, whereas no diseased plants were observed at the podding stage.

Field evaluations of the effects of environmental conditions and cowpea cropping system on disease incidence

Cowpea is cultivated mainly as sole crop (field with one crop species) in the South but the percentage of cowpea fields under sole cropping decreases to the North of the country (Table 1). The percentage of fields with no-till was the highest in the South and reduced progressively northwards. The rainfall was highest in the South and lowest in North Borgou (Table 1). There was a positive correlation between disease incidence and recorded minimum temperature, air humidity, rainfall, sole crop and no-till, as shown by the correlation matrix (Table 2). The estimated multiple regression equation, including all measured variables of the system, was significant ($P<0·05$) with an estimated coefficient of determination $R^2=0·987$. However, all the independent variables, except no-till and sole crop, had P values greater than 0·05. In the final step of the backward

Table 2. *Correlation matrix from variables measured during the cowpea damping-off and stem rot survey in Benin in 2001–2002*

Variables[1]	TMin. (°C)	TMax. (°C)	HMin. (%)	HMax. (%)	Rainy days (0/1)	Rainfall (mm)	Sole crop (0/1)	No-till (%)	Diseased plants (%)
TMin. (°C)	1								
TMax. (°C)	−0·035	1							
HMin. (%)	−0·920	0·077	1						
HMax. (%)	−0·924	0·120	0·963	1					
Rainfall (mm)	−0·269	0·057	0·519	0·412	1				
Rainy days (0/1)	−0·484	0·445	0·623	0·524	0·625	1			
Sole crop (0/1)	−0·865	0·049	0·943	0·966	0·504	0·500	1		
No-till (%)	−0·945	0·081	0·945	0·984	0·344	0·502	0·962	1	
Diseased plants (%)	−0·939*	0·124	0·936*	0·979*	0·357	0·520*	0·945*	0·990*	1

[1] TMin., Minimum temperature; TMax., Maximum temperature; HMin., Minimum humidity; HMax., Maximum humidity. Sole crop refers to fields planted with cowpea as a sole crop. * indicates the correlation coefficient is significant at $P<0{\cdot}05$.

elimination procedure, only no-till and sole crop had P values that were less than 0·05. The estimated multiple regression equation, $y=1{\cdot}295+0{\cdot}0707x_1+0{\cdot}0269x_2$ (where y represents the disease incidence, x_1 cowpea grown as a sole crop and x_2 the use of no-till) was significant ($P<0{\cdot}05$) with an adjusted coefficient of determination, $R^2=0{\cdot}995$.

Fungal identification and pathogenicity

Soilborne fungi isolated from diseased seedlings or plants included *S. rolfsii*, *Fusarium* spp., *Phoma* sp., *Pythium* sp., *R. solani*, *Rhizopus* sp. and *T. harzianum* (Table 3). *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*) was the predominant fungus isolated (isolation frequency of 98–100%) from samples from all the agro-ecological zones. A culture was deposited in the National Collection of Fungi, Biosystematics Division, Plant Protection Research Institute, Pretoria (PREM 57252). In all pathogenicity tests, soil inoculation with *S. rolfsii* alone always resulted in disease symptoms. None of the isolates of *Fusarium* spp., *T. harzianum* or *Rhizopus* sp. caused disease (Table 3). *Pythium* sp. and *R. solani* were isolated only from South Borgou/South Atacora and *Phoma* sp. only from Northern Borgou with isolation frequencies of 5·03, 3·14 and 8·16%, respectively (Table 3). Only two of the eight *P. ultimum*, one of the five *R. solani* and one of the four *Phoma* sp. isolates caused the disease symptoms in the greenhouse.

DISCUSSION

The occurrence and distribution of a specific disease varies from one place to another, but some diseases occur in and cause significant damage across many regions of the world (Emechebe & Florini 1997; Singh *et al.* 1997). Damping-off and stem rot is reported on cowpea in most continents (Onuorah 1973; Singh & Rachie 1985; Singh & Allen 1979). In the low-altitude rain forest of Africa, the greatest losses occur because of seed decay, damping-off and stem rot (Singh & Rachie 1985; Singh *et al.* 1997). For example, in Tanzania, cowpea stem rot was reported to be a major constraint to the production of the crop. Varieties resistant to the causal agent in Australia, were all destroyed by the disease in Tanzania (Singh *et al.* 1982). In Nigeria (neighbouring country to Benin) damping-off resulted in a seedling stand as low as 25% in cowpea sown during cool, wet weather in Ibadan (Singh & Rachie 1985), where a high percentage of stem rot was also recorded (Onuorah 1973; Singh *et al.* 1997). The present study is the first report of the countrywide distribution and incidence of damping-off and stem rot of cowpea in Benin. Although damping-off and stem rot symptoms were found in the present study in all five agro-ecological zones of Benin, the disease incidence was uneven, indicating that the environmental conditions favourable to the diseases varied over the five agro-ecological zones. The incidence of damping-off was greater than that of stem rot, indicating that cowpea is more susceptible to *Sclerotium* infection at the seedling stage (Adandonon 2000).

The disease incidence was the highest in the South and decreased to the North. The environmental data recorded showed that the South received the most rain and had the highest humidity (INRAB 1995). These conditions are said to be influenced by the presence of many lakes and lagoons and the proximity of the Atlantic Ocean (INRAB 1995). In general, the cultural practices varied from one cowpea production area to another and farmers prepared soil differently before sowing (PEDUNE-Benin 1995). Farmers in the South and Centre quite often practised no-tillage coupled with sole cropping. In the no-till system, plants are directly exposed to the existing initial inoculum of the pathogen and therefore disease

Table 3. *Isolation frequencies and* in vivo *pathogenicity of the fungi isolated from cowpea damping-off and stem rot seedlings or plants per agro-ecological zone throughout Benin in 2001–2002*

Ecological zones (Arranged North to South)	*Fusarium* spp. Isolation frequency (%) (±S.E.M.)	*Fusarium* spp. Pathogenicity*	*Phoma* sp. Isolation frequency (%) (±S.E.M.)	*Phoma* sp. Pathogenicity	*Pythium* sp. Isolation frequency (%) (±S.E.M.)	*Pythium* sp. Pathogenicity	*R. solani* Isolation frequency (%) (±S.E.M.)	*R. solani* Pathogenicity	*Rhizopus* sp. Isolation frequency (%) (±S.E.M.)	*Rhizopus* sp. Pathogenicity	*S. rolfsii* Isolation frequency (%) (±S.E.M.)	*S. rolfsii* Pathogenicity	*T. harzianum* Isolation frequency (%) (±S.E.M.)	*T. harzianum* Pathogenicity
Atacora	18·9±1·57	(4) 0	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	100±0·00	(22) 22	4·5±0·55	1 (0)
North Borgou	20·4±1·92	(10) 0	8·2±0·68	(4) 1	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	100±0·00	(49) 49	0±0	0 (0)
South Borgou/South Atacora	11·3±1·84	(18) 0	0±0	(0) 0	5·0±0·21	(8) 2	3·1±0·12	(5) 1	3·8±0·07	(6) 0	98·7±0·87	(80) 80	4·4±0·34	(7) 0
Centre	23·1±1·93	(46) 0	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	100±0·00	(80) 80	5·0±0·54	(10) 0
South	27·1±2·14	(45) 0	0±0	(0) 0	0±0	(0) 0	0±0	(0) 0	1·8±0·04	(3) 0	100±0·00	(80) 80	12·1±0·94	(20) 0

Degree of freedom for the error term: 19. * Pathogenicity is the number of isolates causing damping-off or stem rot symptoms on cowpea (not in parentheses) out of the total number of isolates tested (in parentheses).

is initiated quite rapidly (Singh & Rachie 1985). In contrast, tillage buries weeds and any infected plants remaining after harvest. Burial of infected plants favours the spread of soilborne diseases (Dekker 2003) and might help explain the level of disease incidence observed in the Centre zone. Furthermore, sole cropping allows the pathogen to spread easily to nearby plants. Thus, sole cropping coupled with the no-till system leads to a high disease incidence and severe epidemic conditions (Coakley 1988). In contrast, in the South Borgou/South Atacora, North Borgou and Atacora zones, the cropping system consisted of burning the infected plant residues before tillage (ploughing). Moreover, cowpea is intercropped with many crops such as maize, sorghum and yam in these zones.

Sclerotium rolfsii has an extensive host range of at least 500 species in 100 families (Punja 1985; Ferreira & Boley 1992; Okabe *et al*. 1998, 2001; Okabe & Matsumoto 2003). However, the most susceptible common hosts of *S. rolfsii* are the legumes, crucifers and cucurbits, and the diseases are said to be less severe on yam, some grasses and grains (Ferreira & Boley 1992). Burning infected plant residues before ploughing, coupled with the mixed crop system might lead to the elimination or reduction of the initial inoculum of soilborne pathogens (Summer *et al*. 1981) and could lead to low disease incidence (Singh *et al*. 1997) as observed in the current study.

Several early reports have shown that in an intercropping or mixed crop system, the populations of several pests and disease incidence are reduced and yields increased accordingly (Singh *et al*. 1997; Coakley 1988). Coakley (1988) and Huber & Gillespie (1992) indicated that when large acreages are planted to the same varieties of crop, a greater likelihood exists that the pathogen infection will result in a high disease incidence and even in an epidemic. In the present study, no-till and sole crop factors were positively correlated with the disease incidence, although the amount of rainfall recorded in the Atacora zone was relatively important.

Among the fungi isolated in the present study, *S. rolfsii* was predominant in all five agro-ecological zones and all isolates were pathogenic in the greenhouse. *Sclerotium rolfsii* has been reported to prevail in warm climates and is a destructive soilborne pathogen which causes severe damage in the Tropics and Subtropics (Punja 1985; Mukherjee & Raghu 1997). The five agro-ecological zones of Benin are characterized by annual temperatures ranging from 26–35 °C (INRAB 1995). The cowpea cropping soils in Benin are mostly ferruginous with some clay in the valley lowlands where cowpea is also cultivated after natural flooding (INRAB 1995). The low air humidity in North Borgou and high maximum temperatures (often 40 °C) may restrict the growth of the fungus. These results correspond to early reports on the ecology of *S. rolfsii* (Punja 1985; Mukherjee & Raghu 1997; Singh *et al.* 1997). Other fungi might be regarded as minor causal agents of these cowpea diseases in Benin and this is the first report of *Phoma* sp. causing damping-off and stem rot of cowpea in Benin.

Control measures could include the gathering and burning of the infected plants at one place, and where applicable, planting seeds on raised beds (till) with appropriate plant spacing, as suggested by Lucas *et al.* (1993).

Research was financed by the International Institute of Tropical Agriculture (IITA), Nigeria. Thanks are due to Dr Y. D. Arodokoun., INRAB, Cotonou, Dr J. van der Waals, University of Pretoria, and Mrs Hotegni J., IITA-Cotonou.

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