Prevalence and Severity of *Natrassia mangiferae* Root and Stem Rot Pathogen of Cassava in Bénin

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ABSTRACT

Msikita, W., Bissang, B., James, B. D., Baimey, H., Wilkinson, H. T., Ahounou, M., and Fagbemissi, R. 2005. Prevalence and severity of *Natrassia mangiferae* root and stem rot pathogen of cassava in Bénin. Plant Dis. 89:12-16.

Root rot pathogens were found through diagnostic surveys in all departments (regions) of Bénin, West Africa, to affect 86 to 100% and 96 to 100% of cassava fields during the dry and rainy seasons, respectively. Disease incidence in individual fields ranged between 0 and 53%, and averaged 16 to 27% per department. *Natrassia mangiferae* was consistently the most frequently isolated root rot pathogen (56% in the dry season and 22 to 52% in the rainy season). Pathogenicity of *N. mangiferae* was confirmed on four cultivars of cassava using stem cuttings and storage roots. For all four cultivars, *N. mangiferae* significantly reduced the number of roots. Lesions (3 to 15 cm long) formed on the lower stem portion of all inoculated plants, whereas control plants remained symptom free. On storage roots, the disease profile was similar to that formed on stem cuttings. Other root rot pathogens detected during the dry season were *Macrophomina phaseolina* (14.2%), *Fusarium* spp. (11.8%), *Botryodiplodia theobromae* (7.7%), and *Pythium* spp. (2.9%). During the rainy season, *Fusarium* spp. were the second most commonly isolated root rot pathogens in three departments (Atlantique, Borgou, and Mono). In Oueme and Zou, *B. theobromae* was the second most isolated root rot pathogen (ranging between 24 and 28%) during the rainy season. During the same season, *Pythium* spp. were pronounced in Borgou (18%), followed by Mono (11%), Atlantique (9%), Atacora (8%), Oueme (5%), and Zou (6%). Results of the study are discussed with a view to creating awareness of the destructive power of *N. mangiferae*, a hitherto poorly recognized root rot pathogen of cassava in Bénin and West Africa in general.

Additional keywords: disease resistance, susceptibility

Cassava (*Manihot esculenta* Crantz) is a major food crop for an estimated 260 million people in Africa (4). Production in Africa is hampered by diseases caused by viruses (e.g., the Africa Cassava Mosaic disease, cassava brown streak), bacteria (e.g., cassava bacterial blight), and a wide range of fungi. Among the serious fungal diseases, root rots affect young and old cassava plants as single or as several genera of fungi in both the rainy and dry seasons. Symptoms are variable and include lodging, partial or complete wilting of the plant, and rotting (dry or soft) of roots, which may be accompanied by visible fungal structures (e.g., mycelia, sclerotia, pycnidia) attached to the roots. Cassava

root rot pathogens are classified into dry root rots (e.g., *Armillariella mellea* (Vahl:Fr.) P. Kumm., *Sclerotium rolfsii* Sacc., *Rosellinia necatrix* Prill.) and soft root rots (e.g., *Fusarium moniliforme* J. Sheld., *F. oxysporum* Schlechtend.:Fr., *F. solani* (Mart.) Sacc., *Phytophthora erythroseptica* Pethybr., *P. cryptogea* Pethybr. & Lafferty, *P. drechsleri* Tucker, *P. nicotianae* pv. *nicotianae* G.M. Waterhouse, and *Pythium* spp.) based on prevalence in the dry or wet environments and symptoms (10,19).

Because root-rotting pathogens affect mostly the underground portion of the plant (and are hence out of sight), and because the cassava plant has an extensive root system (and thus can remain standing despite a significant portion of its roots being rotted), the nature and extent of cassava root rot problems is poorly understood and quantified in Africa. Further, for a large part of Africa, little diagnostic survey work has been done to identify and quantify cassava root rot pathogens, resulting in paucity of information regarding

their extent and nature. In extensive diagnostic surveys carried out in Bénin and Nigeria in 1996 to 1997, *Natrassia mangiferae* (Syd. & P. Syd.) Sutton & Dyko, hitherto unreported in Africa, was discovered to seriously affect cassava (15,16,18). *N. mangiferae*, formally *Hendersonula toruloidea* (anamorph *Scytalidium dimidiatum* (Penz.) Sutton & Dyko) (28), is a cosmopolitan fungus with a wide host range that includes fruit trees (2,23), banana (13,29), eucalyptus (11), ornamental trees (3), and man (7,8,12, 20,22,27,30). On humans, *N. mangiferae* causes various skin and nail infections and corneal ulcerations (6). *N. mangiferae* is a recognized pathogen of cassava, where it causes black root and stem rot disease (10). In northeastern Brazil, for instance, the synomorphic state (which the authors identified as *Scytalidium* sp.) was reported to cause up to 85% root yield loss (1). In Africa, however, there is paucity of information regarding *N. mangiferae* prevalence or severity on cassava (17), largely because no extensive diagnostic surveys on cassava root rot pathogens have been conducted. Therefore, following the discovery of the disease in Bénin, this study was undertaken to determine the extent and severity of *N. mangiferae* and to elucidate the identity of other pathogens causing cassava root rot during the dry and rainy seasons in Bénin.

MATERIALS AND METHODS

Field surveys. During part of the dry season in 1996 (November to December), diagnostic surveys were carried out on cassava plants (≥ 3 months old) to determine the incidence of root and stem rot (typified by brown to black discolorations on the lower stem and root portions of lodged, wilted, and/or dead plants) in 79 fields of cassava randomly selected between latitudes 6°36' N and 11°04' N in Bénin. Sites were selected to ensure uniform coverage in each of the six regions or departments (Atacora, Atlantique, Borgou, Mono, Oueme, and Zou) of the country. During part of the rainy season in 1997 (July to August), the surveys were repeated in the same fields, and expanded to include 11 more fields for a total of 90 fields of

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cassava between latitudes 6°16' N and 11°08' N. In each field, 60 plants were examined for root rot symptoms in two diagonals (30 plants per diagonal were randomly selected) cutting across the field. Wilted and/or dead plants showing termite damage were excluded in the estimation of root rot disease incidence. Samples from the dry season surveys were all grouped into one collection, whereas the ones from the rainy season were separated by departments of Bénin. In the laboratory, infected root and stem portions (0.5 to 1 cm) were cut out, surface-disinfested (10 min) in 10% bleach (0.6% sodium hypochlorite), rinsed in sterilized distilled water, and cultured on potato dextrose agar (PDA) acidified to pH 4.5 with 0.4% (vol/vol) lactic acid. Cultures were incubated at 25°C, under 12-h light provided by cool-white fluorescent lamps. After 1 week, mycelia, conidiophores, and conidia were observed at $\times 30$ to $\times 40$ magnification under a compound microscope.

Pathogenicity studies. Stem cuttings and storage roots were inoculated with *N. mangiferae* in separate experiments.

(i) *Stem inoculation.* Cassava cultivars 'Agric', 'Ben 86052', 'Tchukunochi', and 'TMS 30572', selected based on their large-scale production in Bénin, were used for the study. Two weeks prior to the experiment, inocula of *N. mangiferae* were prepared by incubating 5-mm-diameter mycelial plugs ($\sim 3 \times 10^3$ conidia) of *N. mangiferae* (isolate Nm 14) locally collected (8°01' N, 2°27' E) from infected cassava plants with 500 ml of autoclaved rice seed for 10 days at 25°C, followed by air-drying in a laminar flow hood for 2 days. Five 30-cm-long stem portions were cut from healthy plants of each cassava cultivar, surface-disinfested in hot water (52°C, 5 min), and transplanted into sterilized (autoclaved, 1 h) sand in 1-liter pots to which 10 ml of the *N. mangiferae*-colonized rice inoculum had been added. There were five control stems for each cultivar, similarly treated, but not inoculated. Plants were maintained in a greenhouse under natural light at 28 to 30°C. Thirty days after planting, plant height, lesion length, and number of shoots and roots were recorded. The experiment, repeated two times, was arranged in a randomized complete block design, and data were analyzed by the General Linear Model procedure of SAS 1996 (26).

(ii) *Root inoculation.* Six similarly sized (100 g, 5 to 10 cm long), freshly harvested cassava roots, with peels intact, for each of the four cultivars were used in the stem inoculation study. Soil was washed off under running tap water; then they were washed in 5% bleach (0.3% sodium hypochlorite) and rinsed three times with sterilized distilled water. Using a number 2 cork borer, a hole was drilled in the center of the roots. Three roots for each of the

cultivars were inoculated with *N. mangiferae* (isolate Nm 14). Inoculum was prepared by placing a 5-mm-diameter PDA mycelial plug cut out on the edge of the petri plate with a number 2 cork borer, added to 5 ml sterilized distilled water, and the conidia adjusted to 4×10^3 ml⁻¹. One milliliter of *N. mangiferae* conidial suspension was applied to the wounds. The other three roots were treated with sterilized distilled water (control treatments). Roots were then kept for 5 days in separate sterile plastic bags, in the dark, in an incubator set at 25°C. To allow for the differences in root lengths, lesions formed were expressed as infection index (length of lesion formed on the root per total root length), and data were analyzed as described under (i) above.

RESULTS

Field surveys. In the field, *N. mangiferae*-infected plants appeared wilted, and on close examination, the stems tended to be blackened with dark discolorations and were often cracked (Fig. 1). Bark blisters were also commonly noticed on the stems, and in the cracks were masses of black fungal structures (mycelia and conidia). Infected storage roots were dry and simi-

larly showed symptoms of cracking with visible fungal structures (Fig. 1).

Root rots were found in all six departments of Bénin. In the dry season, root rots affected 100% of fields surveyed in Atacora and Atlantique, 86% of fields in Oueme, 88% in Zou, and 86% in Borgou and Mono. In the rainy season, root rots affected all fields surveyed in Atacora, Atlantique, Oueme, and Zou, 96% of fields surveyed in Borgou, and 97% of Mono (Table 1). In individual fields, incidence of root rots ranged from 0 (one field each in Borgou and Mono) to as high as 53% (in a field in Zou). The mean of disease incidence per field was significantly higher (27.3%) in Zou than in all the other five departments (which had a mean range of 7 to 22%) (Table 1).

Fungal identifications. Out of the 169 symptomatic samples collected from the dry season surveys, nine fungal genera were isolated: *Aspergillus* spp. (1% of fungi observed), *Botryodiplodia theobromae* (7.7%), *Fusarium* spp. (11.8%), *Macrophomina phaseolina* (14.2%), *N. mangiferae* (56.2%), *Penicillium* spp. (0.6%), *Pythium* spp. (2.9%), *Rhizopus* spp. (1.7%), and *Trichoderma* spp. (2.4%). One percent of the fungi isolated did not

Fig. 1. *Nattrassia mangiferae*-infected cassava storage roots (left and center) and stem cutting (right). The fungus causes drying, hardening, and cracking of the root and stem. Fungal structures (mycelia and conidia) are visible in the cracks.

Table 1. Prevalence of cassava root rots during the dry and rainy seasons in departments (regions) of Bénin

Department	No. of fields surveyed		Infected fields (%) ^y		Disease incidence ^z
	Dry season	Rainy season	Dry season	Rainy season	
Atacora	13	15	100.0 a	100.0 a	17.33 b (9-19)
Atlantique	6	14	100.0 a	100.0 a	18.81 b (7-21)
Borgou	11	13	86.7 c	96.8 b	20.71 b (18-22)
Mono	11	12	86.7 c	96.5 b	17.64 b (14-19)
Oueme	23	12	93.9 b	100.0 a	18.89 b (11-20)
Zou	15	23	88.5 c	100.0 a	27.39 b (23-31)
Total	79	90			

^y Percentage of total fields surveyed with root rot. Means followed by the same letter within a column are not significantly different ($P = 0.05$) using Fishers least significant difference (LSD) test. LSD value for comparing percentage of infected fields between departments in different seasons = 2.21. Percentages were transformed (logarithmic) before statistical analysis.

^z Data collected only for the rain season. Disease incidence is expressed as the total number of plants infected by root rots and without termite infestation expressed as a percentage of the total number of plants surveyed. Means followed by the same letter are not significantly different ($P = 0.05$) using Fishers LSD. Numbers in parentheses are ranges of disease incidence for the fields in each department.

sporulate in culture and were not identified (Fig. 2).

Out of the 1,135 symptomatic samples collected during the rainy season, 12 fungal genera were isolated: *Aspergillus* spp., *B. theobromae*, *Fusarium* spp., *M. phaseolina*, *N. mangiferae*, *Penicillium* spp., *Pestalotia* spp., *Pythium* spp., *Rhizopus* spp., *Sclerotium* spp., *Sphaeropsis* spp., and *Trichoderma* spp. Among the 12 genera, *N. mangiferae* was significantly more prevalent (averaging between 22 and 52%)

than all other fungi isolated in all six departments of the country. In three departments (Atlantique, Borgou, and Mono), *Fusarium* spp. were the second most frequently isolated fungi, ranging between 23 and 32%), while *B. theobromae* was the second (ranging between 24 and 28%) in two departments (Oueme and Zou). *Pythium* spp. were most prevalent in Borgou (18%), followed by Mono (11%), Atlantique (9%), Atacora (8%), Oueme (5%), and Zou (6%) (Table 2).

Fig. 2. Frequency of isolation of fungi associated with cassava root rot during the dry season in Bénin. Fungi were isolated from 169 symptomatic plants collected in 79 different fields. Each sample was cultured on three different plates containing potato dextrose agar, and fungi were identified under a microscope. Histograms are logarithmic transformed isolation frequencies (percentages) of the total fungi collected.

Pathogenicity tests. For all four cultivars of cassava tested, *N. mangiferae* significantly reduced number of roots compared with control plants (Fig. 3). Lesions (3 to 15 cm long) formed on the lower stem portions of all inoculated plants, resulting in variable degrees of wilting of the infected plants. Inoculated plants of two of the cultivars ('Agric' and 'Ben 86052') died within 3 weeks after planting. Control plants remained asymptomatic. *N. mangiferae* was consistently reisolated from infected plants, and the identification was independently confirmed by the International Mycological Institute, Surrey, UK.

On storage roots, a profile similar to that obtained with stem cuttings was observed. Lesions formed and covered the entire length of the root (mean infection index = 1) for 'Ben 86052', which was significantly greater than lesions formed on the other three cultivars. At 0.78 infection index, 'Agric' was the second most susceptible cultivar, followed by 'Tchukunochi' (0.56) and 'TMS 30572' (0.38), respectively (Fig. 4).

DISCUSSION

Root rots were found through the diagnostic surveys of this study to affect cassava in all the departments of Bénin in both the dry and rainy seasons. Factors such as moisture and cultural practices (e.g., planting on ridges as opposed to planting on flat beds) affect root rot pathogens (9). In Bénin, cassava is planted either on ridges or on flat beds. This practice likely accounts for the wide variability in the incidence of root rots among individual fields within and among departments. Other cultural practices (such as hot water treatment of planting stems, use of resistant cultivars, time of planting) are known to control cassava root rot pathogens (9,17,19). To date, the influence of such cultural practices in the control of *N. mangiferae* remain unknown. Variability in disease incidence among departments also may be attributed to variability in the intensity of cassava production among the

Table 2. Species composition (percentages) of fungi isolated from cassava tissues from different departments (regions) of Bénin

Fungal species/genera	Atacora	Atlantique	Borgou	Mono	Oueme	Zou
<i>Aspergillus</i> spp.	2.4 (0.2) ^y	0.8 (0.3)	8.0 (0.7)	0.0 (0.0)	2.1 (0.3)	1.9 (1.9)
<i>Botryodiplodia theobromae</i>	13.7 (0.7)	1.9 (0.3)	19.7 (1.3)	5.9 (1.2)	24.3 (1.3)	27.7 (0.7)
<i>Fusarium</i> spp.	14.1 (0.7)	27.4 (1.4)	22.8 (1.3)	31.8 (2.2)	6.5 (0.6)	8.4 (0.6)
<i>Macrophomina phaseolina</i>	0.8 (0.1)	0.9 (0.3)	1.2 (0.2)	0.0 (0.0)	0.5 (0.3)	0.0 (0.0)
<i>Nattrassia mangiferae</i>	52.4 (2.0)	48.7 (1.8)	22.2 (1.1)	45.9 (2.8)	49.6 (1.9)	50.4 (0.9)
<i>Penicillium</i> spp.	1.2 (0.2)	0.0 (0.0)	2.5 (0.4)	0.0 (0.0)	2.3 (0.4)	2.1 (0.2)
<i>Pestalotia</i> spp.	0.4 (0.3)	0.0 (0.0)	1.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<i>Pythium</i> spp.	8.2 (0.6)	8.8 (1.3)	18.5 (1.2)	10.6 (1.5)	5.2 (0.5)	6.3 (0.4)
<i>Rhizopus</i> spp.	5.2 (0.6)	3.5 (0.4)	1.3 (0.2)	0.0 (0.0)	6.5 (0.8)	1.9 (0.3)
<i>Sclerotium</i> spp.	0.4 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<i>Sphaeropsis</i> spp.	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (0.3)	0.0 (0.0)	0.0 (0.0)
<i>Trichoderma</i> spp.	1.2 (0.2)	8.0 (0.7)	2.5 (0.4)	4.6 (0.9)	3.0 (0.3)	1.3 (0.1)
N ^z	192	196	185	178	175	209

^y Numbers in parentheses are standard deviations of treatment means.

^z Total number of symptomatic cassava samples collected. Fungi were isolated from 1,135 symptomatic plants collected in 90 different fields. Each sample was cultured on three different plates containing potato dextrose agar, and fungi were identified under a microscope. Percentages are logarithmic transformed isolation frequencies of the fungi.

departments. For example, Zou, which is the largest and most intense cassava growing department in Bénin, had the highest incidence of root rots. Departmental differences or similarities in the incidence of specific genera of root rot pathogens may also be attributed to exchange of germ plasm among farmers, leading to planting of genetic stock similarly susceptible to the pathogens. It is not uncommon for cassava farmers to share their planting stock with their neighbors. *N. mangiferae* affects both the root and stem, and therefore, moving and planting infected planting stems will introduce the pathogen to new fields.

N. mangiferae was prevalent in both dry and rainy seasons. However, a higher incidence of root rots was observed in the rainy than in the dry season. This observation may be attributed to increased prevalence of moisture-loving types of root rots such as *Fusarium* and *Pythium* spp. (19).

N. mangiferae emerged as the most serious cassava root rot pathogen, accounting for more than half the percentage of root rots isolated both in the dry and rainy seasons. The high prevalence may be related to the fact that *N. mangiferae* has a wide host range (2) and is a facultative parasite that often grows on dead branches (21,24) and on dried, stored cassava roots (17). This pathogen is very virulent on both stems and storage roots based on results from pathogenicity studies. This study appears to concur with reports from north-eastern Brazil, where *Scytalidium* sp., which is a synomorphic state of *N. mangiferae* (28), was reported to cause up to 85% yield loss of cassava (1). Among cultivars grown in Bénin, 'TMS 30572' appeared to

be more resistant to *N. mangiferae* than the other three cultivars used in the pathogenicity tests. This observation suggests a possibility for existence of cassava genotypes resistant to *N. mangiferae*.

M. phaseolina was found commonly during the dry season in Bénin, which confirms earlier reports of *M. phaseolina* as a pathogen of cassava (16) that prefers dry environments (5). Other pathogens of

Fig. 4. Development of root rot on cassava genotypes inoculated with *Natrassia mangiferae*. Infection index was calculated as the length of lesion formed on the root per total root length. Data are means of six replications per cultivar. Vertical bars represent standard deviations of treatment means. Means not followed by the same letter are significantly different ($P \leq 0.05$).

Fig. 3. Effect of *Natrassia mangiferae* on root formation and disease development of cassava genotypes. Data are means of five replicates per cultivar. Vertical bars represent standard deviations of the treatment means. Number of roots in uninoculated (control) plants was not significantly different ($P \leq 0.5$). Number of roots and lesion length for inoculated plants were significantly different ($P \leq 0.05$).

cassava rots detected during this study were *Fusarium* spp., *B. theobromae*, *Pythium* spp., and *Sclerotium* spp., all of which are known pathogens of cassava (9,10,17). The study also revealed many other fungi (such as *Aspergillus* spp., *Penicillium* sp., *Rhizopus* spp., and *Trichoderma* spp.), which were present as saprophytes. It would be interesting to study these isolates as possible biocontrol agents for *N. mangiferae* and other cassava root rots.

Whereas *N. mangiferae* has been reported on cassava (1,15,17,18), yam in storage (25), and mango (23), all important crops in Africa, and medical literature has consistently demonstrated serious skin, nail, and toe infections (14), and even corneal ulceration (6) with *N. mangiferae* infection, little information is available on cross-infection by the fungus of both human and crops. Studies to elucidate this important relationship would generate useful information on safe handling of infected crops.

In conclusion, *B. theobromae*, *Fusarium* spp., *M. phaseolina*, and *Sclerotium* spp. were found to be important cassava root pathogens in both the dry and rainy seasons in Bénin, while a hitherto unrecognized pathogen in Bénin, *N. mangiferae*, was shown to be the most common and destructive root rot pathogen. Additional research is warranted to examine the dynamics of *N. mangiferae* as a pathogen of cassava, its role as a pathogen of many hosts, including humans, and to examine the role of cultural practices in managing the fungus.

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