Integrated control of cassava bacterial blight in West Africa in relation to ecozones, host plant resistance and cultural practices

Vom Fachbereich Gartenbau
der Universität Hannover
zur Erlangung
des akademischen Grades eines

Doktors der Gartenbauwissenschaften

- Dr. rer. hort. -

genehmigte

Dissertation

von

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geboren in Adjengré, Togo

2003

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Tag der Promotion:	18. Juli 2003

Contents

Abbreviations

ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
AUSiPC	Area under severity index progress curve
BlLS	*Cercospora* blight leaf spot
BLS	*Cercospora* brown leaf spot
CAD	Cassava anthracnose disease
CANCORR	Canonical correlation
CBB	Cassava bacterial blight
Cfu	Colony forming units
CIAT	Centro Internacional de Agricultura Tropical
CMD	Cassava mosaic disease
DESA	Direction des Enquêtes et Statistiques Agricoles
DMN	Direction de la Météorologie Nationale
Dpi	Days post inoculation
DS	Dry savanna
DSID	Direction des Statistiques Agricoles, de l'Information et de la Documentation
Fig.	Figure
FST	Forest savanna transition
G	Gram
GLM	General linear model
GYCA	Glucose yeast calcium carbonate agar
Ha	Hectare
IITA	International Institute of Tropical Agriculture
IPCA	Interactions principal component analysis
ITRA	Institut Togolais de Recherche Agronomique
KCl	Potassium chloride
Kg	Kilogram
M	Meter
M^2	Square meter
$MgSO_4$	Magnesium sulphate
µl	Microlitre
Mm	Millimeter
MR	Medium resistant
N	Nitrogen
No.	Number
NPK	Nitrogen phosphate potassium
P	Probability
R	Resistant
S	Susceptible
SAS	Statistical Analysis System
Si	Severity index
WLS	*Cercospora* white leaf spot
WS	Wet savanna

Zusammenfassung

Die Maniokproduktion wird durch mehrere Krankheiten stark beeinträchtigt, unter ihnen der Maniokbakterienbrand (CBB) als zweitwichtigste Maniokkrankheit in Afrika. Eine Voraussetzung, integrierte Bekämpfungsmaßnahmen für den Bakterienbrand zu entwickeln, ist die Kenntnis der Verbreitung und der Befallsstärke der Krankheit in den verschiedenen Ökozonen Togos. Ein Hauptelement in der integrierten Bekämpfung ist die Entwicklung resistenter Sorten. Deshalb wurden die Reaktion von Sorten gegenüber CBB und ihre Interaktion mit der Umwelt in verschiedenen Ökozonen Togos getestet. Die Virulenz von *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) Stämmen aus allen Ökozonen Togos wurde bestimmt, und Sorten wurden nach Inokulation mit einem Set von Stämmen aus verschiedener geographischer Herkunft in Afrika charakterisiert, um Pathotyp x Sorte Interaktionen zu identifizieren. Als weitere Elemente der integrierten Bekämpfung wurden Hygienemassnahmen und an Ökozonen angepasste Kulturmassnahmen getestet, wie Mischkulturanbau, Mulchen und Düngergaben.

In einem Survey zur Festellung der Maniokkrankheiten in vier agro-ökologischen Zonen von Togo wurde ein starkes Auftreten von Maniokbakterienbrand, Maniokmosaikkrankheit (CMD) und *Cercospora*-Krankheiten beobachtet. In der Trockensavanne waren 90,5% der Felder mit CBB befallen, in der Waldsavannenübergangszone 70%, in der Feuchtsavanne 64% und in der Regenwaldzone 52,6 %, mit einem durchschnittlichen Befall der Einzelpflanzen pro Feld von 27,4% in der Regenwaldzone bis zu 72,7% in der Trockensavanne. CMD wurde in nahezu 100% aller Felder in den vier Ökozonen gefunden, mit einem hohen Einzelpflanzenbefall pro Feld von bis zu 86,9%. *Cercospora*-Blattkrankheiten – Braune Blattflecken (BLS), Blattbrand (BlLS) und Weisse Blattflecken (WLS) – traten in allen Ökozonen mit Häufigkeiten von 68% bis 100% der Felder auf. Negative Korrelationen zwischen CBB und CMD beziehungsweise CMD und WLS wurden beobachtet, während BLS und BlLS, BLS und WLS und auch BlLS und WLS positive korreliert waren. Die Befallshäufigkeit der Felder mit CBB war positiv korreliert mit dem Pflanzenalter, mit der Regenfallmenge – einem höheren Befall in den trockeneren Ökozonen ($p < 0,01$) – und der Stärke der Verunkrautung der Felder ($p < 0,05$). Weitere signifikante, aber negative Korrelationen traten zwischen CBB und dem Vegetationstyp (Anzahl Bäume) in der Umgebung der Felder auf. Zwischen dem Vorkommen von Braunen *Cercospora*-Blattflecken und der Anzahl Bäume in der Umgebung des Feldes und dem Faktor Anzahl Früchte pro Feld (intercopping) trat eine signifikant negative Beziehung auf, während Weisse

Cercospora-Blattflecken negativ mit dem Anteil Sand im Boden korreliert war. Ein weiterer Survey zur Bestätigung der Ergebnisse wird empfohlen. Maßnahmen zur Bekämpfung der Maniokkrankheiten, insbesondere des Bakterienbrandes, werden empfohlen um schwere Epidemien zu vermeiden.

Die Auswahl resistenter Sorten ist ein wichtiges Element in der Entwicklung einer integrierten Bekämpfungsstrategie von Maniokbakterienbrand. Deshalb ist die Kenntnis der Virulenz und Diversität der Stämme von *Xam* notwendig. Siebenundvierzig Stämme wurden aus Blattsymptomen von Blättern, die während des Survey in den Ökozonen Trockensavanne, Feuchtsavanne, Wald-Savanne-Übergang und Regenwald gesammelt wurden, isoliert und mittels Stängelinokulation auf der anfälligen Sorte Ben86052 auf ihre Virulenz getestet. Die meisten Stämme (94%) waren hoch virulent, und es wurden generell nur geringe Unterschiede zwischen den Stämmen festgestellt. Die Unterschiede waren unabhängig von der Ökozone, aus der die Stämme stammten.

Um die Resistenzeigenschaften von Manioksorten gegen Bakterienbrandbefall unter Feldbedingungen zu untersuchen, wurden 23 lokale Sorten und Zuchtsorten unter natürlicher Infektion und nach Sprüh-Inokulation in der Regenwaldzone und der Wald-Savannenübergangszone in den Jahren 1998 und 1999, und in der Feuchtsavanne im Jahr 1999 gescreent. Starke Sorten x Umwelt Interaktionen wurden beobachtet, und es wurde keine Sorte mit einer Krankheitsresistenz in den drei Standorten in den 3 Ökozonen gefunden. Die Sorten CVTM4, Main27, TMS30572 und TMS92/0429 zeigten jedoch eine resistente Reaktion in wenigstens einer Umgebung (Ort und/oder Jahr) und eine mittlere Resistenz in den anderen Standorten und Jahren, während die Sorten Lagos, Toma289 und Toma 378 unter allen Bedingungen anfällig waren. CBB war signifikant negative mit dem Erntegewicht der Maniokwurzeln in den sprüh-inokulierten Feldern in den Standorten in der Regenwaldzone in den Jahren 1998 und 1999, und in den nicht sprüh-inokulierten Feldern in der Wald-Savannen-Übergangszone und der Feuchtsavanne in den Jahren 1998 beziehungsweise 1999 korreliert. Die Analyse der Entwicklung der verschiedenen Symptomtypen – Flecken, Brand und Blattwelke – für jede Sorte ergab, dass generell die Stärke von Blattflecken und Blattbrand positiv korreliert waren, während keine oder eine negative Beziehung zwischen Blattsymptomen und Blattwelke auftrat. Dieselbe Beobachtung wurde bei der Gesamt-Analyse der Symptomdaten von allen Sorten unter allen Bedingungen gemacht. Eine signifikant negative Beziehung bestand zwischen der Anzahl Blätter mit Brand- und Welkesymptomen und dem Wurzelgesicht in jeder der 3 Ökozonen, und zwischen

der Anzahl Blätter mit Blattflecken und dem Erntegewicht der Wurzeln in der Regenwaldzone.

Zusätzlich zum Feldsrceenen der Sorten wurden 24 lokale Sorten und Zuchtsorten aus Togo auf ihre Reaktion nach Stängelinokulation mit 4 hoch virulenten *X. axonopodis* pv. *manihotis* Stämmen aus verschiedenen Afrikanischen Herkünften unter kontrollierten Bedingungen untersucht. Die lokalen Sorten Nakoko und Toma159 waren am anfälligsten gegen die 4 Stämme, während die meisten anderen Sorten einschließlich der Referenzsorte Ben86502 anfällig gegen wenigstens zwei und resistent gegenüber mindestens einem Stamm reagierten. Sechs Sorten waren resistent gegen alle 4 Stämme. Unter ihnen waren die lokale Sorte Gbazékouté und die Zuchtsorte CVTM4 die resistentesten. Sechs Gruppen von Sorten mit einer differentiellen Reaktion gegenüber den vier Stämmen wurden gebildet, und die Stämme konnten somit als Pathotypen definiert werden.

Eine Voraussetzung für den Aufbau einer gesunden Maniokplantage ist die Verwendung von nicht-infiziertem Pflanzgut. Deshalb wurde die Verteilung von *X. axonopodis* pv. *manihotis* in Maniokstängeln untersucht, mit dem Ziel, Empfehlungen für die Auswahl von gesundem Pflanzmaterial zu geben. *X. axonopodis* pv. *manihotis* wurde in den Stängeln der anfälligen Sorten Ben86052 und Fétonégbodji in einer diskontinuierlichen Verteilung und nicht auf einen Stängelabschnitt beschränkt gefunden. Die Anzahl von *X. axonopodis* pv. *manihotis* Zellen war im oberen Stängelabschnitt mit circa 10^7 cfu/g in Sorte Ben86052 und 10^6 cfu/g in Sorte Fétonégbodji, einschlisslich Pflanzen ohne systemische Symptome, höher als als in den mitteleren und unteren Stängelabschnitten, in denen die geringsten Anzahlen gefunden wurden. Obwohl in 90-100% und 50-90% der Stängelabschnittte der Sorten Ben86052 beziehugnsweise Fétonégbodji das Pathogen gefunden wurde, entwickelten sich nur aus 40-50% beziehungsweise 20-40% der Stängelabschnitte infizierte Sprösslinge. Aus den meisten Stängelabschnitten, in denen *X. axonopodis* pv. *manihotis* nicht nachgewiesen wurde, entwickelten sich gesunde Sprösslinge. An der Sorte TMS30572 traten im Feld keine Bakterienbrandsymptome auf, das Pathogen wurde in keinem Teil des Stängels gefunden, und keiner der neuen Sprösslinge zeigte Bakterienbrandsymptome. Daher können Stecklinge der symptomlosen, resistenten Sorte TMS30572 für pathogenfrei gehalten werden. Die Selektion von bakterienbrandfreiem Pflanzmaterial von resistenten Sorten kann den Bauern zur Eindämmung der Krankheit empfohlen werden.

Als weiteres Element der integrierten Bekämpfung des Bakterienbrandes wurde der Einfluss von Mischkulturanbau mit wichtigen Grundnahrungsfrüchten in Togo, der Effekt einer Kaliumdüngung mit Aufwandmengen von 60 und 120 kg/ha und das Mulchen mit *Cassia siamea* auf den Kranheitsbefall unter Feldbedingungen in 4 agroökologischen Zonen getestet. Die Befallsstärke wurde signifikant reduziert im Mischanbau Maniok-Taro und Maniok-Mais gegenüber Maniok Monokultur bei mittlerem und hohem Inokulumdruck in der Regenwald-Hochlandzone, im Maniok-Mais Mischanbau in der Wald-Savannenübergangszone bei mittlerem, aber nicht bei hohem ($p < 0,01$) Inokulumdruck, und im Maniok-Mais Mischanbau in der Feuchtsavanne bei hohem Inokulumdruck ($p \leq 0,05$). Obwohl die Befallsverminderungen signifikant waren, waren sie generell eher gering (6-23%), führten aber generell nicht zu einer Ertragsreduktion.

Die Kaliumgabe und das Mulchen zeigten nur unklare krankheitsreduzierende oder -fördernde Effekte und können deshalb nicht als Teil einer Bekämpfungsstrategie empfohlen werden.
Da keine Sorten mit einer hohen Resistenz unter den getesteten lokalen Sorten und den Zuchtsorten gefunden wurden, wird die Kombination von mittel-resistenten Sorten wie TMS92/0429, TMS30572 und TMS91/02316 mit niedrigem Befall und hohem Ertrag und der Mischanbau, beides angepasst an die jeweilige Ökozone, den Bauern zur Bekämpfung des Bakterienbrandes empfohlen. Die Sorten TMS92/0326, TMS92/0057, Cameroon und Ben86052, die sich als tolerant gegenüber CBB zeigten, sollten wegen der Gefahr der Verschleppung von Inokulum nicht angebaut werden. Die Sorten Main27 und CVTM4, resistent, aber mit geringem Ertrag, werden Züchtern zum Einbringen ihrer Resistenzcharakteristika in das Zuchtmaterial empfohlen. Auch die Sorten TMS30572 und TMS92/0429 sollten von Züchtern wegen ihrer hohen Resistenz gegen Welkensymptome zum Einkreuzen in Sorten mit hoher Anfälligkeit für systemische Infektion genutzt werden. Zur Identifizierung von Stamm-Sorte Interaktionen sollten Sorten mit verschiedenen Pathotypen inokuliert werden. Um Sorten zu identifizieren, die für die Produktion von gesundem Pflanzmaterial geeignet sind, sollten Züchter die Sorten auf ihre Eigenschaften zur Unterdrückung systemischer und latenter Infektion und der Ausbildung gesunder Schößlinge als zusätzliche Resistenzmerkmale untersuchen. Mischkulturanbau Maniok-Mais und/oder Maniok-Taro, je nach Ökozone, reduzierte den Krankheitsbefall gegenüber Monokultur in der Hochlandwaldzone, in der Feuchtsavanne und in der Wald-Savannen-Übergangszone, generell ohne negativen Einfluss auf den Ertrag, und kann deshalb den Bauern in diesen Ökozonen als Element einer integrierten Bekämpfung von Bakterienbrand empfohlen werden.

Schlagworte: Maniokbakterienbrand**,** integrierten Bekämpfung, Westafrika.

Abstract

Cassava production is reduced by several diseases among which cassava bacterial blight (CBB) is the second important in Africa. Prerequisite to develop integrated control measures of CBB is the knowledge on the distribution and the severity of the disease in different ecozones of Togo. A major element in the integrated control of CBB is host plant resistance. Therefore, genotypes were screened for durable resistance to CBB by characterizing their interactions with the environment in field trials in different ecozones. Virulence of *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) strains across ecozones was established. Selected genotypes were challenged by stem-inoculation with a set of representative, virulent *Xam* strains in order to identify possible pathotype x genotype interactions. As further elements of integrated control, crop sanitation and cultural measures adapted to ecozones e.g. intercropping, as well as soil amendments with mulch and potassium fertilizer were studied.

A cassava disease survey was conducted in four agroecological zones of Togo. High incidences of cassava bacterial blight (CBB), cassava mosaic disease (CMD) and cercosporioses were observed across ecozones. CBB field incidences of 90.5% in the dry savanna zone, 70% in the forest savanna transition zone, 64% in the wet savanna zone and 52.6% in the forest zone, were recorded, with plant incidences ranging from 27.4% in the forest zone to 72.7% in the dry savanna zone. CMD field incidences were nearly 100% in all the ecozones and high plant incidences up to 86.9% were found. *Cercospora* leaf diseases – brown leaf spot (BLS), blight leaf spot (BlLS) and white leaf spot (WLS) - occurred in all the ecozones with incidences ranging from 68% to 100%. Negative correlations between CBB and CMD, and between CMD and WLS were found, while BLS and BlLS, BLS and WLS, and BlLS and WLS were positively correlated. Field incidence of CBB was positively correlated with plant age, ecozones - higher severity in dryer ecozones - ($p < 0.01$), and weed density ($p < 0.05$). Further significant, but negative correlations occurred between CBB and vegetation type in surroundings of the field (number of trees) ($p < 0.05$). *Cercospora* brown leaf spot (BLS) was significantly negatively associated with the number of trees in the surroundings of a field and the number of crops in a field (intercropping) ($p < 0.05$), and *Cercospora* white leaf spot with more sandy soils ($p < 0.01$). A further survey is recommended to confirm the present data. Measures to control cassava diseases, especially cassava bacterial bight, should be taken to avoid severe epidemics.

The selection of resistant genotypes is a major element in the development of an integrated control system of CBB. Therefore, knowledge on the virulence and diversity of pathogen strains is important. Fourty-seven strains of *Xanthomonas axonopodis* pv. *manihotis* were isolated from leaf samples collected during the disease survey from the forest savanna transition, forest, wet savanna and dry savanna zones of Togo and tested for virulence by stem-inoculation of the susceptible cassava genotype Ben86052. Most (94%) strains were highly virulent, and generally only slight differences in virulence among strains were observed. Differences in virulence were independent of their origin in agroecological zones.

To monitor the resistance characteristics of cassava genotypes to CBB infection under field conditions, 23 improved and local genotypes from Togo were screened under natural infection and after spray-inoculation with *X. axonopodis* pv. *manihotis* in the forest and forest savanna transition zones in years 1998 and 1999, and in the wet savanna zone in year 1999. High genotype x environment interactions were observed, and no genotype with disease resistance in the three sites in the forest savanna transition and forest zones over a two year-experiments and wet savanna zone in a one-season trial was found. However, genotypes CVTM4, Main27, TMS30572 and TMS92/0429 were resistant in at least one environment and medium resistant in other environments, and Toma159 and TMS91/02316 were medium resistant across environments, while Lagos, Toma289 and Toma378 were over all susceptible. Cassava bacterial blight severity was significantly negatively correlated to cassava root yield in inoculated plots in the site in the forest zone in 1998 and 1999, and in non-inoculated plots in the forest savanna transition zone and the wet savanna zone in 1998 and 1999, respectively. Analysing the development of the different symptom types by genotypes, generally spot and blight symptom development was positively correlated, while there was no relation, or a negative correlation between leaf symptoms and the wilt symptom development. The same oberservation was made, when data were analysed across genotypes and environments. Significant negative correlations were observed between blight and wilt symptoms, and root yield in each of the three ecozones, and between spots and root yield in the forest zone.

Additonally to the field screening of cassava genotypes, 24 improved and local genotypes from Togo were screened for resistance to cassava bacterial blight by stem-inoculation with four highly virulent *Xanthomonas axonopodis* pv. *manihotis* strains from different geographic origins in Africa under controlled conditions. The local genotypes Nakoko and Toma159 were most susceptible against the four strains, while most other genotypes including the reference genotype Ben86052, with susceptible reaction against at least two strains were resistant to at

least one strain. Six genotypes showed a resistant reaction against the four strains. Among them, the local genotype Gbazékouté and the improved CVTM4 were the most resistant ones. Six groups of genotypes, with differential reactions to the strains were formed, and the strains were defined as pathotypes.

A prerequisite for a healthy cassava plantation is the use of non-infected planting material. Therefore, the distribution of *X. axonopodis* pv. *manihotis* in cassava stems was studied with the aim to develop recommendations for the selection of healthy stem material. *X. axonopodis* pv. *manihotis* was detected in stems of the susceptible varieties Ben86052 and Fétonégbodji, in a discontinuous colonization pattern and not restricted to any part of the stem. *X. axonopodis* pv. *manihotis* numbers were higher in the upper parts, with about 10^7 cfu/g in Ben86052 and 10^6 cfu/g in Fétonégbodji, including plants without systemic symptoms, than in the middle and basal parts, where the lowest numbers were found. Although 90-100% and 50-90% of cuttings of varieties Ben86052 and Fétonégbodji, respectively, harboured the pathogen, only 40-50% and 20-40%, respectively, of emerging sprouts were infected. From most of the cuttings in which *X. axonopodis* pv. *manihotis* was not detected, healthy sprouts emerged. No bacterial blight symptoms occurred on genotype TMS30572 in the field, and the pathogen was not found in any part of the plants, nor did any of the new shoots from the planted cuttings show bacterial blight symptoms. Thus, symptomless plants of the latter genotype could be considered free of *X. axonopodis* pv. *manihotis*. The selection of bacterial-blight-free cassava planting material from symptomless, resistant varieties is recommended to farmers to reduce disease incidence.

As further element in the integrated control of CBB, the influence of intercropping cassava with common staple crops in Togo on cassava bacterial blight, and the effects of potassium (KCl) fertilizer doses of 60 and 120 kg/ha and the application of *Cassia siamea* mulch on disease development were studied under field conditions in four agro-ecological zones of Togo. Bacterial blight severity was significantly reduced compared to sole cassava in the forest highland in cassava-taro and cassava-maize intercropping at medium and high inoculum levels; in cassava-maize intercropping in the forest savanna transition zone at medium, but not at high inoculum levels (p 0.01), and in cassava -maize intercropping in the wet savanna zone at high inoculum level (p 0.05), with generally no significant negative effect on yield. Though significant, disease reductions by intercropping generally were low (6-23%). The application of potassium and mulch revealed only unclear disease reducing and increasing effects and can, thus, not be recommended as part of a disease control strategy.

Since no varieties with complete resistance had been identified among local and local improved varieties across ecozones in Togo, the combination of medium resistant varieties and an intercropping system, both adapted to the respective ecozone, is recommended to farmers.

Since no genotypes with stable resistance were identified among local and local improved genotypes across ecozones in Togo, the combination of medium resistant genotypes such as TMS92/0429, TMS30572 and TMS91/02316 with low disease severity and high root yield and an intercropping system, both adapted to the respective ecozone, is recommended to farmers. Genotypes TMS92/0326, TMS92/0057, Cameroon and Ben86052, tolerant to the disease, should be avoided by farmers due to the risk of dissemination of inoculum. Genotypes Main27 and CVTM4, resistant, but with low root yield could be recommended to breeders to introduce their resistance characteristics into the breeding materials. Additionally, genotypes TMS30572 and TMS92/0429 should be used to introgress their higher resistance to the wilt symptom into genotypes with susceptibility to systemic symptoms. To identify strain x genotype interactions, genotypes should be screened for their reaction to inoculation with different pathotypes. To select genotypes which are suitable for production of healthy planting material, breeders should consider differences between genotypes in restriction of systemic infection, latent infection of stems and restriction of sprout symptoms as additional characteristics in selection for resistance. Intercropping cassava-maize and/or cassava-taro, according to the ecozone, significantly reduced disease severity compared to cassava monocropping in the forest highland, the wet savanna and the forest savanna transition zones, with generally no significant negative yield effect, and thus, can be recommended in these ecozones as part of an integrated control strategy for CBB.

Key words: Cassava bacterial blight, integrated control, West Africa.

General introduction

The cultivated forms of cassava belong to the species *Manihot esculenta* Crantz, which derive from the wild populations of *Manihot esculenta* subsp. *flabellifolia* (Olsen and Schaal, 1999). The crop is of Central and South America origin, from where it spread to other parts of the world. It was introduced to Africa in the 16th centry by Portugese traders (Jones, 1959). Cassava is a perennial shrub of 1 to 5 m height of the family Euphorbiaceae, cultivated mainly for its starchy roots. The roots are adventitious and develop to a fibrous root system. Some roots bulk and become storage roots, while the remaining ones are involved in water and nutrient absorption. Cassava has a sympodial branching. The stems are woody, cylindrical, and with alternating nodes and internodes. Leaves are simple, deep-lobed with palmated veins, and spirally arranged. Cassava is a monoecious plant, cross-pollinated by insects. The fruit is a globular, trilocular, dehicent capsule.

Cassava is a plant of the humid tropics, with an optimal rainfall of 1000-2000 mm per year, however, it is drought tolerant. Cassava needs an open position with no shade and with as much sunlight as possible. The crop is propagated vegetatively through stem cuttings or stakes. Cassava can be planted on a flat ground, as well as on ridges or mounds. In Africa, it is usually grown in mixed stands with other crops (IITA, 1997).

It is the sixth most important source of calories in the human diet, and one of the most important food staples in the tropics (FAO, 1999). Total world cassava production was estimated at 158,620,000 tons of root fresh weight (FAO, 1998), with Africa as the leading producing region (Hillocks, 2002). Cassava serves as primary staple food of millions of people in the tropics and subtropics, and is used as a carbohydrate source in animal feed. It is used as a raw material in the manufacture of processed food, animal feed and industrial products (Plucknett et al., 1998).

Pests and diseases are the most important production constraints of cassava all over the world.
The major pests of cassava include cassava green mites (*Mononychellus* spp.), elegant grasshopper (*Zonocerus elegans* Thunb. and *Z. variegatus* L.), cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) (Hillocks, 2002). Cassava is susceptible to various diseases of fungal, bacterial and viral origin and to nematodes (Hillocks and Wydra, 2002; Wydra and Verdier, 2002). Among all these diseases, cassava mosaic virus disease, cassava

bacterial blight, cassava root rots and cassava anthracnose disease are of major economic importance (CIAT, 1996; Fokunang et al., 2000).

Cassava mosaic disease (CMD) is the most widespread cassava disease and commonly found in Africa and Southern India causing important yield losses (Geddes, 1990; Thresh et al., 1994). The disease is caused by Begomoviruses [Geminiviridae: Geminivirus Sub-group III] (Otim-Nape et al., 1997) transmitted by the whitefly *Bemisia tabaci* Genn (Legg et al., 2001). CMD is characterized by a mosaic pattern of chlorotic areas of the leaves which vary in size depending on the severity of the disease, and stunting of the plant when severely infected.

Cassava root rots are caused by various pathogens often in a mixed infection. Roots infected by *Rosellinia necatrix* are initially surface-covered with white mycelial strands, which subsequently turn black. The disease is common in areas where cassava is planted after forest clearance and in soils with a high organic matter content (Hillocks and Wydra, 2002). The tissue of roots infected by *Sclerotium rolfsii* shows soft rotting with pale brown discoloration. White mycelial growth can be observed (Nwufo and Fajola, 1986). The disease is favored by warm, wet periods and the presence of non-decomposed organic matter. Further fungal pathogens causing root rots include *Phytophthora drechsleri*, *Pythium*, and *Fusarium* species (Hillocks and Wydra, 2002). *Botryodiplodia theobromae* is extremely common throughout the tropics. Infected roots may appear healthy externally, although the skin may be somewhat wrinkled. The internal tissue is dark-blue discolored (Akinyele and Ikotun, 1989) and, under humid conditions, the development of white and subsequently dark grey mycelia occurs.

Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* f. sp. *manihotis* Henn. (Penz) Sacc. is characterized by development of cankers on stems, branches and fruits, leaf spots and tip dieback (Théberge, 1985). The disease is favoured by humid, wet conditions (Fokunang et al., 1999). CAD incidence of up to 90% has been reported in Africa (Wydra and Msikita, 1998).

Cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *X. campestris* pv. *manihotis* (Bondar, 1915), is the most important bacterial disease of cassava with a worldwide distribution (Lozano, 1986; Maraite, 1993). CBB was observed in different countries of West Africa in all ecozones, with higher site incidence of more than 60% (Wydra and Msikita, 1998). Recently, CBB field and plant incidences of more

than 90% and 70%, respectively, were reported in Togo (Banito et al., 2001). Typical symptoms of CBB include water-soaked angular leaf spots, blighting, wilting, defoliation, vascular necrosis of the stem, production of exudates on leaves, petioles or stems, and stem dieback (Lozano and Sequeira, 1974; Maraite and Meyer, 1975). Root yield losses of more than 50% due to CBB were reported (Wydra et al., 2001a; Wydra, 2002). Since chemical control of the disease does not exist, integrated control measures were suggested (Wydra and Rudolph, 1999) including the use of resistant genotypes, crop rotation, weeding and mixed cropping associating cassava with maize (Fanou, 1999; Fanou et al., 2001). The importance of CBB across ecozones and the relationship between the disease and ecological and agronomic characteristics have never been established in Togo. Though trials on host-plant resistance were initiated (Boher and Agbobli, 1992), the selection of cassava cultivars for resistance to CBB in various ecozones, investigations on genotypes x environment interactions (Zinsou, 2003), on the association of cassava with other crops and the use of fertilization to control CBB were not conducted in Togo.

Objectives of the studies

While surveys on the status of cassava diseases were recently carried out in all ecozones of several African countries (Wydra and Msikita, 1998; Wydra and Verdier, 2002), the distribution of cassava diseases has never been established in all agroecological zones of Togo. Since the surveys on CBB by Boher and Agbobli (1992) covering some ecozones, no suitable control measures have been used in Togo. The present studies in **chapter 1** aimed at determining the incidence, severity and geographic distribution of cassava diseases in the major ecological zones of Togo, including a systems approach to elucidate conditions that could influence and determine disease outbreaks. Knowledge on the virulence of strains deriving from different ecozones in Togo is important for screening for resistance and the most virulent strains occurring in an area concerned should be used for inoculation (CIAT, 1978). Therefore, the pathological characterization of strains from Togo described in **chapter 2** is the prerequisite to select resistant cassava genotypes and recommend suitable genotypes to farmers.

Improved and local genotypes from Togo have never been characterized for their reaction to bacterial blight in various agroecological zones of Togo. Therefore, in the present studies, selected cassava varieties from Togo and from an international collection were evaluated for reaction to cassava bacterial blight under field conditions in different ecozones to select

resistant, high yielding genotypes suitable for farmers (**chapter 3**). For further characterization, the genotypes were evaluated for their reaction to cassava bacterial blight by inoculation of four highly virulent *X. axonopodis* pv. *manihotis* strains from different geographic origins in a glasshouse experiment (**chapter 4**).

The distribution of *Xam* in infected stems of field plants was reported for some varieties (Fanou, 1999), but never established in detail for varieties frequently grown in Togo. Also the incidence of infected sprouts deriving from infected cuttings has not been studied in detail. To develop sanitation measures in areas with a high pressure of cassava bacterial, the role of infected cuttings in disease dissemination has to be known. Therefore, (i) the distribution of *Xam* in different parts of stems of cassava varieties from Togo, and (ii) the incidence of infected sprouts, were determined in order to develop recommendations for the selection of healthy stem cuttings (**chapter 5**).

Since stable resistance to cassava bacterial blight has never been reported, measures contributing to an integrated control of cassava bacterial blight were investigated. Generally, intercropping has been reported as one of the measures to reduce CBB (Nyango, 1979; Terry, 1974). Ene (1977) reported that CBB was significantly reduced by providing shade or intercropping cassava with maize or melon. The use of intercropping was proposed as means to reduce CBB in the dry savanna (Tabot, 1995) and in the humid forest (Arene, 1976). Significant reduction of CBB severity in cassava intercropped with cowpea and maize compared to cassava monoculture were observed in the forest savanna transition zone of Nigeria, with the highest disease reduction of 53% in cassava-maize intercrop, without significant yield effect due to cropping system (Fanou, 1999). The latter author suggested that intercropping could have a barrier effect to inhibit the transport of the inoculum of *X. axonopodis* pv. *manihotis* since bacterial diseases are generally disseminated in the field by rainsplash and aerosols combined with wind. The effect of intercropping on CBB severity may vary with intercrops used and across ecozones. Therefore, as part of an integrated control system for cassava bacterial blight suggested by Wydra et al. (2001b; 2003), an intercropping system adapted to agroecological conditions should be developed in each cassava growing area. In Togo, studies on the use of intercropping to reduce CBB, have never been conducted. Thus, the effectiveness of intercropping cassava with common staple crops in controlling CBB under field conditions in various agroecological zones in Togo was investigated (**chapter 6**).

Rainsplashing is the most important mean of dissemination in the field or between fields over short distances (Lozano and Sequeira, 1974; Otim-Nape, 1976). Ene (1977) found that CBB could be controlled by the use of means such as mulching which reduce the impact of rain splash. Additionally, a green manure is known to release nutrients for the plant, suppress weeds, support root development and increase soil moisture (Maliki et al., 1997). The application of mulch produced significantly greater corm yield, but also showed a higher incidence of corm rots of taro compared to non-mulched plots (Miyasaka et al., 2001). The use of lower potassium rates in reducing CBB compared to those proposed by Arene and Odurukwe (1979), could be ideal to minimize the cost of fertilizer application. Therefore, KCl fertilizer doses of 60 and 120 kg/ha and the *Cassia siamea* mulch were tested for their effect on cassava bacterial blight development under field conditions in different ecozones of Togo (**chapter 6**).

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1 Assessment of cassava diseases in Togo in relation to agronomic and environmental characteristics in a systems approach

Abstract

A cassava disease survey was conducted in four agroecological zones of Togo. High incidences of cassava bacterial blight (CBB), cassava mosaic disease (CMD) and cercosporioses were observed across ecozones. CBB field incidences of 90.5% in the dry savanna zone, 70% in the forest savanna transition zone, 64% in the wet savanna zone and 52.6% in the forest zone, were recorded, with plant incidences ranging from 27.4% in the forest zone to 72.7% in the dry savanna zone. CMD field incidences were nearly 100% in all the ecozones and high plant incidences up to 86.9% were found. *Cercospora* leaf diseases – brown leaf spot (BLS), blight leaf spot (BlLS) and white leaf spot (WLS) - occurred in all the ecozones with incidences ranging from 68% to 100%. Negative correlations between CBB and CMD, and between CMD and WLS were found, while BLS and BlLS, BLS and WLS, and BlLS and WLS were positively correlated. Field incidence of CBB was positively correlated with plant age, ecozones - higher severity in dryer ecozones - ($p < 0.01$), and weed density ($p < 0.05$). Further significant, but negative correlations occurred between CBB and vegetation type in the surroundings of the field (number of trees) ($p < 0.05$). *Cercospora* brown leaf spot (BLS) was significantly negatively associated with the number of trees in surroundings of a field and the number of crops in a field (intercropping) ($p < 0.05$), and *Cercospora* white leaf spot with more sandy soils ($p < 0.01$).

Key words: Cassava bacterial blight, mosaic disease, cercosporiose, incidence.

1.1 Introduction

Cassava (*Manihot esculenta*) is a major staple crop in the tropics. Its production is largely reduced by biotic constraints (Hahn et al., 1989) among which diseases are of high importance. Major cassava diseases in Africa include cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava root and stem rots, cassava anthracnose disease (CAD) and *Cercospora* leaf diseases (Hillocks and Wydra, 2002).

Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Arthaud-Berthet & Bondar) Dye, is worldwide distributed (Lozano, 1986; Maraite, 1993). CBB was first recorded in Brazil in 1912 but has since been reported in several countries in South America (Lozano, 1973; Lozano and Sequeira, 1974), Africa (Hahn and Williams, 1973; Maraite and Meyer, 1975; Persley, 1977) and Asia (Leu and Chen, 1972; PANS, 1978; Booth and Lozano, 1978). CBB distribution was recently established in Ghana, Benin, Nigeria and Cameroon, with variable incidence and severity according to ecozones (Wydra and Msikita, 1998). Severe CBB incidence and severity were observed in all ecozones in Benin, but the disease was rarely found in Ghana (Wydra and Verdier, 2002). The disease was reported for the first time in Togo by Olympio (1977). Later investigations on the distribution of CBB in Togo revealed that the disease was prevalent and more severe in the forest savanna transition zone and was sporadically recorded in the wet savanna zone, while it was not found in the forest zone. However, in the region of Kara in the South part of the dry savanna zone, CBB was most frequently found with variable severities (Boher and Agbobli, 1992).

Typical symptoms of CBB include water-soaked angular leaf spots, blighting, wilting, defoliation, vascular necrosis of the stem, production of exudates on leaves, petioles or stems, and stem dieback (Leuschner et al., 1980; Lozano, 1986; Maraite, 1993). Root yield losses exceeding 50% to 75% depending on the severity of the disease (Maraite, 1993; Wydra and Rudolph, 1999; Wydra, 2002; Wydra et al., 2003), or complete loss of yield and planting material in case of severe infections (Lozano and Booth, 1976; Ezelio, 1977) were reported. Yield losses due to CBB in Africa were estimated up to 7.5 million tons (CIAT, 1996). The vascular disease affects the quality and quantity of planting material (Boher and Verdier, 1994).

Cassava mosaic disease (CMD) is the most widespread cassava disease and commonly found in Africa and Southern India causing important yield losses (Lozano et al., 1981; Geddes, 1990; Thresh et al., 1994). The disease is caused by Begomoviruses [Geminiviridae: Geminivirus Sub-group III] (Leuschner et al., 1980; Théberge, 1985; Otim-Nape et al., 1997) transmitted by the whitefly *Bemisia tabaci* Genn (Agrios, 1997; Legg et al., 2001). CMD occurred frequently in all ecozones of Ghana and Benin, with higher incidence in all ecozones of Ghana (Wydra and Verdier, 2002). During the 1990s, East African cassava mosaic virus Uganda variant (EAMV-Ug) spread through Uganda and into the neighbouring countries of Kenya, Rwanda and Tanzania, causing a devastating pandemic of unusually severe cassava mosaic disease (Otim-Nape et al., 1997; Legg, 1999; Legg et al., 2001). Cassava mosaic disease was most prevalent with field and plant incidence near 100% in all ecozones of Benin, Cameroon and Ghana (Wydra and Msikita, 1998). Nevertheless, low average plant incidence was regionally observed in different ecozones in Benin and Cameroon, ranging from 29% in the moist savanna of Cameroon to 46% in the transition forest of Benin, whereas in most other regions and ecozones plant incidence was between 64% and 97% (Wydra and Msikita, 1998). The outbreaks of CMD curbed cassava production in the Democratic Republic of Congo, the second largest producer in the region (FAO/GIEWS, 2001). The average annual yield loss caused by cassava mosaic disease to cassava production in Africa is estimated to 50% of the total (Agrios, 1997). CMD is characterized by a mosaic pattern of chlorotic areas of the leaves which vary in size depending on the severity of the disease, and stunting of the plant when severely infected. The most promising methods of controlling CMD is by using resistant cultivars (Leuschner et al., 1980; Agrios, 1997; Legg et al., 2001).

Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* f. sp. *manihotis* Henn. (Penz) Sacc. is characterized by development of cankers on stems, branches and fruits, leaf spots and tip dieback (Théberge, 1985; IITA, 1990). The disease is favoured by humid, wet conditions (Fokunang et al., 1999). The importance of the insect *Pseudotheraptus devastans*, facilitating the infection by the fungus, in the occurrence and spread of CAD has been established (Muimba-Kankolongo et al., 1984; Boher et al., 1983; Fokunang et al., 2000b). The disease has been reported from cassava in many countries of Latin America, Africa and Asia (CIAT, 1972; Chadrasekharan-Nair et al., 1979; Makambila, 1994). Makambila (1979) found anthracnose disease in all cassava-growing regions in the People's Republic of Congo, but disease severity varied across regions. Field and plant incidence of cassava anthracnose disease up to 90% and 64%, respectively, in the rainforest,

and 56% and 26%, respectively, in the transition forest zones were recorded in Ghana, Benin, Nigeria and Cameroon, while in the savanna zones the disease was less important (Wydra and Msikita, 1998). Recently, CAD distribution was established in all ecozones in Benin and Ghana in up to one third of inspected fields, but disease severity was generally low (Wydra and Verdier, 2002). Also, cassava anthracnose disease was generally estimated to be of minor importance (Lozano and Booth, 1976; Wydra and Verdier, 2002). To reduce the incidence of CAD, use of disease-free planting material and planting during the late season is recommended (Leuschner et al., 1980).

Cercospora leaf diseases are essentially confined to the foliage where they cause spots and blight: brown leaf spot (BLS) caused by *C.* [*Mycosphaerella*] *henningsii* Allesch, white leaf spot (WLS) caused by *C. caribaea* Cif. [*Phaeoramularia manihotis*] and blight leaf spot (BlLS) by *C.* [*Mycosphaerella*] *vicosae* Muler & Chupp (Lozano and Booth, 1976). Cercosporioses are widely distributed in all cassava-growing areas (Théberge, 1985), but are mostly of minor importance (Lozano and Booth, 1974; Silva et al., 1988; Frison and Feliu, 1991).

While surveys on the status of cassava diseases were recently carried out in all ecozones of several African countries (Wydra and Msikita, 1998; Wydra and Verdier, 2002), the distribution of cassava diseases has never been established in all agroecological zones of Togo. Since the surveys on CBB by Boher and Agbobli (1992) covering some ecozones, no suitable control measures have been used in Togo. The present studies aimed at determining the incidence, severity and geographic distribution of cassava diseases in the major ecological zones of Togo, including a systems approach to elucidate conditions that could influence and determine disease outbreaks.

1.2 Materials and Methods

Cassava is grown in four main agroecological zones in Togo: in the forest savanna transition zone in the South part of Togo, which is characterized by a shrubby vegetation with few trees, the forest zone in the South-West with a rainforest vegetation, the wet savanna in the Center part, characterized by more shrubby vegetation, and the dry savanna zone in the North part with herbaceous vegetation. The savanna transition and the forest zones are characterized by a sub-equatorial climate with one long rainy season (March – June), one short dry season

(July – August), one short rainy season (September – October) and one long dry season (November – March); whereas the wet savanna and the dry savanna zones are characterized by a tropical climate with one long rainy season (April – September) and one long dry season (October – March) (Lamouroux, 1979). The average annual rainfall is about 1,200 mm in the forest savanna transition zone, 1,400 mm in the forest and wet savanna zones, and 1,300 mm in the dry savanna zone, with the average temparature of 28 °C, 24 °C, 27 °C and 28 °C, respectively. Annual rainfall up to 2,027 mm in the forest, 1,810 mm in the wet savanna and 1,651 mm in the dry savanna zones were recorded (DMN, 2001).

A country-wide survey was carried out shortly after the rainy season in the first two weeks of November 1998. Eighty-five fields covering the four ecozones were visited: 20 fields in the forest savanna transition zone, 19 fields in the forest zone, 25 fields in the wet savanna zone, and 21 fields in the dry savanna zone. Fields of about 1/16 ha minimum size were selected from the cassava-growing areas at a minimum of 10 km intervals (rarely less than 10 km) along the main practicable roads, and CBB symptoms were evaluated on plants following two diagonals across the field. Fifteen plants randomly selected within the two diagonals were assessed for CBB incidence and severity by scoring the expression of symptoms in five severity classes: class 1 - no symptom, class 2 - angular leaf spots, class 3 - angular leaf spots, blighting, wilting, defoliation, and sometimes exudates on stems, petioles or leaves, class 4 - blighting of leaves, wilting, defoliation, exudates and tip dieback, class 5 - blighting of leaves, wilting, defoliation, exudates, abortive lateral shoot formation, stunting, complete dieback. The 15 plants were also assessed for cassava mosaic disease (CMD), anthracnose disease (CAD) and *Cercospora* diseases (brown leaf spot, blight leaf spot and white leaf spot). Cassava mosaic disease symptoms were scored in five severity classes, 1 = no symptom, 2 = mild chlorotic patterns and slight distortion of only the base of leaves, 3 = mosaic patterns on all leaves, leaf distortion, 4 = mosaic patterns on all leaves, leaf distortion, and general reduction in leaf size, 5 = leaves twisted/misshapen, and stunting of the whole plant. For anthracnose disease and *Cercospora* diseases, one severity score was given for all the 15 plants: 1 = not present, 2 = symptoms of low severity on plants, 3 = symptoms of medium severity on many plants, 4 = severe symptoms on all the plants. Additionally, agronomic, varietal and ecological characteristics were recorded in each field, and coded for statistical analysis following the method of Cardwell et al. (1997), modified by Wydra and Verdier (2002):

Vegetation type in surroundings

1 = herbaceous savanna

2 = herbaceous savanna with few trees

3 = forest savanna

4 = forest

Soil moisture

0 = dry

1 = humid

2 = temporary waterlogged

3 = waterlogged

Soil texture

1 = clay

2 = sandy loam

3 = loamy sand

4 = sand

5 = lateritic soil

Type of branching

0 = no branching

1 = late branching

2 = profusely branching

Plant age in months

Crop system

1 = monoculture

2 = 2 to 3 associated cultures

3 = more than 3 associated cultures

4 = cassava plants as field border only

Variety mixture

1 = one cassava variety

2 = mixture of cassava varieties

Field zise

Estimated in ha.

Ecozones

1 = forest

2 = forest savanna transition

3 = wet savanna

4 = dry savanna

Weed score

0 = no weeds

1 = very few weeds

2 = few weeds

3 = medium abundant weeds

4 = abundant weeds

For each disease surveyed, field incidence was calculated as the percent of infected fields in an ecozone, and plant incidence as the percent of plants showing disease symptoms.

Statistical analysis

Field incidence, plant incidence and severity were determined using SAS software system (SAS, 1990; 1997). The relationship among cassava diseases and their interactions with the agronomic, ecological and varietal characteristics was established. Using the SAS program,

canonical correlations analysis (CANCORR) was performed between disease variables (Y-variables) on the one hand and agronomic, ecological and varietal variables (X-variables) on the other hand, to determine the extent of the association between these two sets of variables. CANCORR is a powerful multivariate statistical tool useful in exploring association between two sets of related variables. The technique consists of finding several linear combinations of the disease variables and the same number of linear combination of the agronomic, ecological and varietal variables in such a way that these linear combinations best express the correlations between the two sets. CANCORR finds a linear combination from each set, called canonical variables, such that the correlation between the two canonical variables is maximized (SAS, 1990; Afifi and Clark, 1990). The resulting canonical correlations are tested for significance using F-statistic approximation.

For the stepwise regression, the level of significance was set to 5%. However, higher probability of 6% levels were used in the preliminary analyses in order to check for and further examine any marginal variables and interactions that might be lost at the restrictive probability level of 5%. The frequency of diseased plants in severity classes was determined for CBB and CMD. Analysis of variance (ANOVA) of disease incidences was performed to compare ecozones.

1.3 Results

Field incidence, plant incidence and severity of cassava diseases

Cassava bacterial blight was observed, in 70% of the fields visited, and occurred in all the four agroecological zones of Togo, but with variable severity between ecozones (**Fig. 1**). In the forest savanna transition zone, the disease was observed in 70% of fields visited, partly with severe symptoms. Highest symptom severities were scored at Davié, Kpogamé, Ahépé, Tabligbo and Tokpli. In the forest zone, the disease was found in 52.6% of fields, however with lower severity than in the other zones. In the wet savanna, where cassava is one of the main crops, CBB occurred in 64% of fields, though with less severity than in the forest savanna transition zone. Nevertheless, a high disease severity was recorded in Blitta, Sotouboua, Bassar, and in the region of Sokodé, where some fields were scored with the highest CBB symptom class 5. In the dry savanna zone, where cassava production is less important, CBB was observed in 90.5% of fields, with highest disease severities in the region

of Kara. From this region to the extreme North of the country, CBB was rarely found, with only low incidence in the region of Dapaong. The plant incidence of CBB by field was high in all ecozones except in the forest zone. A plant incidence of 100% was observed in six fields in the wet savanna zone, 5 fields in the dry savanna zone and one field in the forest savanna transition zone. The highest ecozonal plant incidence (percent plants infected in an ecozone) of CBB was recorded in the dry savanna zone (72.7%), while the lowest was recorded in the forest zone, with 27.4% of plants infected. The field and plant incidences were significantly higher in the dry savanna zone than in the other ecozones ($p < 0.01$), but no significant differences among the forest savanna transition, the forest and the wet savanna zones were found (**Table 1**). In all the ecozones, the frequency of diseased plants in severity classes decreased from class 2 to class 5. Systemic infection of CBB - classes 3 to 5, with most of the plants in class 3 - occurred in the four ecozones including the forest zone. Plants with the highest symptom severity of class 5 - corresponding to dieback of the plant - were recorded in the savanna and forest savanna transition zones - mainly in the region of Sokodé (**Fig. 1**), but no plant with dieback symptoms was observed in the forest zone.

Table 1: Field and plant incidence (%), and frequency distribution in four severity classes of **bacterial-blight** infected plants (%) in four ecozones of Togo

Ecozone	No. of fields	Field incidence [%]	Plant incidence [%]	Plants in severity classes[1] [%]			
				2	3	4	5
FST	20[2]	70[3] b	42.7[4] b	19.3	14.7	7	1.7
Forest	19	52.6 b	27.4 b	20.4	6.3	0.7	0
WS	25	64 b	45.3 b	17.6	16	8	3.7
DS	21	90.5 a	72.7 a	27.9	24.4	17.8	2.5

FST = forest savanna transition; WS = wet savanna; DS = dry savanna; [1]Severity classes: class 2: angular leaf spots; class 3: angular leaf spots, blighting, wilting, defoliation, and sometimes exudates on stems/petioles/leaves; class 4: blighting of leaves, wilting, defoliation, exudates and tip dieback, class 5: blighting of leaves, wilting, defoliation, exudates, abortive lateral shoot formation, stunting, complete dieback; [2]Total number of fields visited in the ecozone; [3]Percentage of infected fields in the ecozone; [4]Percentage of infected plants from all plants sampled in the ecozone.

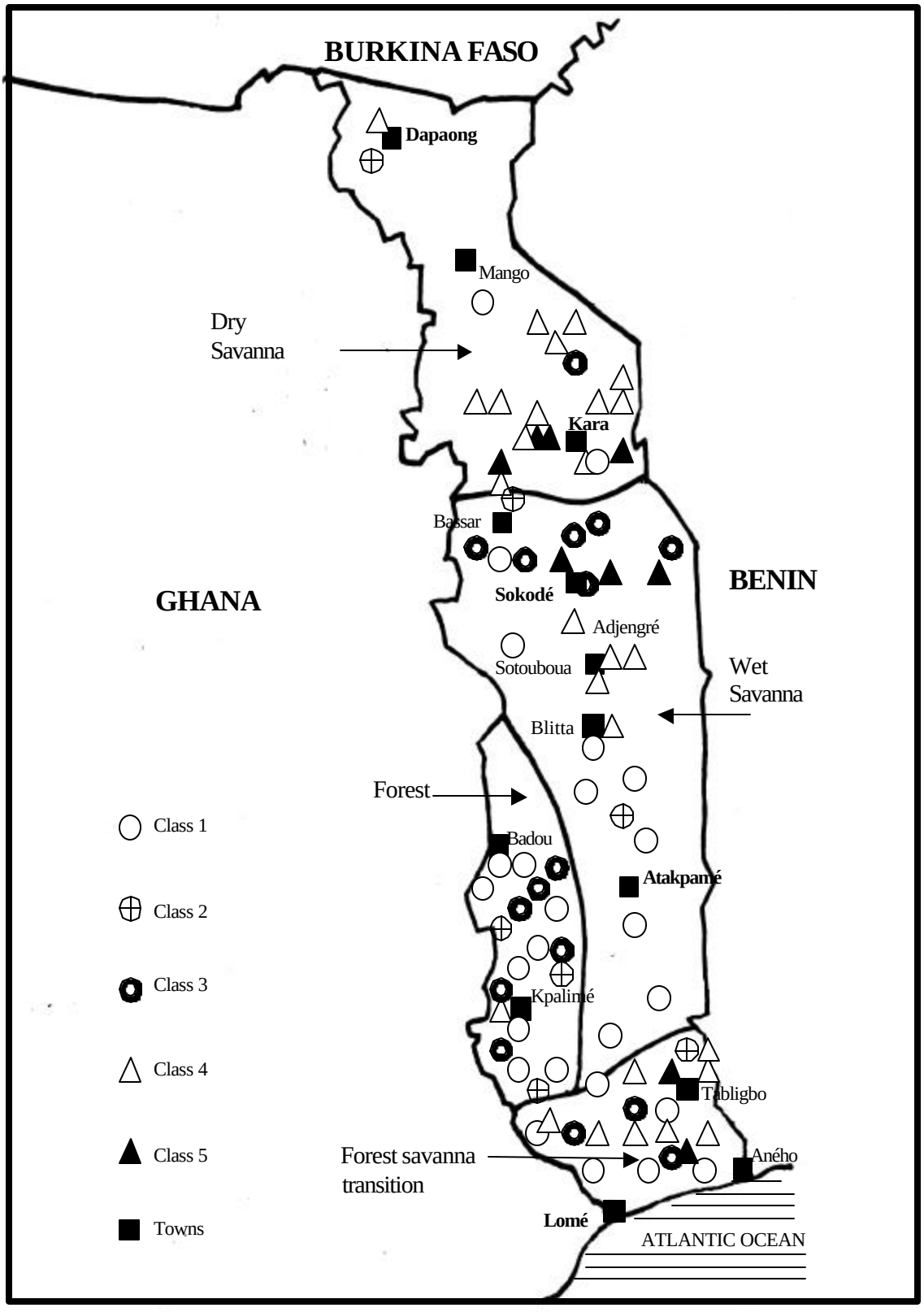
BURKINA FASO
Dapaong
Mango
Dry
Savanna
Kara
Bassar
GHANA
BENIN
Sokodé
Adjengré
Sotouboua
Wet
Savanna
Blitta
Forest
Class 1
Badou
Atakpamé
Class 2
Class 3
Kpalimé
Class 4
Tabligbo
Class 5
Forest savanna
transition
Aného
Towns
Lomé
ATLANTIC OCEAN

Fig. 1: Distribution of cassava bacterial blight in Togo across ecozones in severity classes
Symptom classes: class 1 - no symptom, class 2 - angular leaf spots, class 3 - angular leaf spots, blight, wilt, defoliation, and sometimes exudates on stems, petioles or leaves, class 4 – blight on leaves, leaf wilt, defoliation, exudates and tip dieback, class 5 - blight on leaves, leaf wilt, defoliation, exudates, abortive lateral shoot formation, stunting, complete dieback.

Cassava mosaic disease (CMD) was widely observed across all ecozones surveyed with an ecozonal field incidence of about 100%. A higher plant incidence of 86.9% was recorded in the wet savanna zone, while it was slightly lower (63.7%) in the forest savanna transition zone than in the other zones. A significant difference was only observed between the wet savanna zone and the forest savanna transition zone ($p < 0.05$) (**Table 2**). Severe CMD symptoms (classes 3-5) were observed in all the zones with higher severities of 44% and 37.9% recorded in the wet savanna and forest zones, respectively.

Table 2: Field and plant incidence (%), and frequency distribution in four severity classes of **cassava mosaic disease** infected plants (%) in four ecozones of Togo

Ecozone	No. of fields	Field incidence [%]	Plant incidence [%]	Plants in severity classes[1] [%]			
				2	3	4	5
FST	20[2]	95[3] a	63.7[4] b	36.0	16.3	10.0	1.3
Forest	19	94.7 a	78.9 ab	41.1	17.5	13.0	7.4
WS	25	100 a	86.9 a	42.9	22.4	18.7	2.9
DS	21	100 a	75.2 ab	52.1	18.4	4.4	0.3

FST = forest savanna transition; WS = wet savanna; DS = dry savanna; [1]Severity classes: class 2 = mild chlorotic patterns and slight distortion of only the base of leaves; class 3 = mosaic patterns on all leaves, leaf distortion; class 4 = mosaic patterns on all leaves, leaf distortion, and general reduction in leaf size; class 5 = leaves twisted/misshapen, and stunting of whole plant; [2]Total number of fields visited in the ecozone; [3]Percentage of infected fields in the ecozone; [4]Percentage of infected plants from all plants sampled in the ecozone.

Cercospora leaf diseases occurred in all the ecozones with high field incidences (**Table 3**). BLS and BlLS were observed in all the fields visited across ecozones with an incidence between 90-100%. WLS was significantly lower in the forest and the wet savanna zones than in the other ecozones ($p < 0.01$), while BLS was significantly higher in the wet savanna zone than in the other ecozones ($p < 0.05$), and BlLS was significantly lower in the dry and wet savanna zones than in the forest and forest savanna transition zones ($p < 0.001$).

Table 3: Field incidence (%) of *Cercospora* leaf diseases in four ecozones of Togo

Ecozone	No. of fields	BLS	BlLS	WLS
FST	20[1]	95[2] b	100[2] a	95[2] a
Forest	19	89.5c	100a	73.7b
WS	25	100a	96b	68c
DS	21	95.2b	95.2b	95.2a

FST = forest savanna transition; WS = wet savanna; DS = dry savanna; BLS = brown leaf spot; BlLS = blight leaf spot; WLS = white leaf spot; [1]Total number of fields visited in the ecozone; [2]Percentage of infected fields in the ecozone.

Relationship between cassava disease variables

Negative correlations were observed between CBB and CMD as well as between CMD and WLS field incidences ($p < 0.05$) (**Table 4**). The severities of Cercospora leaf diseases were all positively correlated (BLS/BlLS: $p < 0.001$; BLS/WLS: $p < 0.01$; BlLS/WLS $p < 0.05$).

Table 4: Correlation matrix (Pearson correlation coefficients) between the severity scores of cassava diseases in 85 fields

	CBB	CMD	BLS	BlLS	WLS
CBB	1	-0.220*	-0.074	-0.072	-0.004
CMD		1	-0.099	0.055	-0.237*
BLS			1	0.403***	0.281**
BlLS				1	0.226*
WLS					1

CBB = cassava bacterial blight; CMD = cassava mosaic disease; BLS = brown leaf spot; BlLS = blight leaf spot; WLS = white leaf spot; *probability level = 0.05 ; **probability level = 0.01; ***probability level = 0.001.

Relationship between cassava diseases and the agronomic, ecological and varietal characteristics

In the Pearson correlation analysis (**Table 5**), significant positive correlations occurred between severity of CBB and plant age, ecozones - higher severity in dryer ecozones - ($p < 0.01$), and weed density ($p < 0.05$). Further significant, but negative correlations occurred between CBB and soil moisture, field size and vegetation type in surroundings of the field ($p < 0.05$). Cassava bacterial blight was more severe in savanna zones than in the forest zone. The highest CBB incidence and severity were recorded in the herbaceous savanna without trees (dry savanna zone) followed by the herbaceous savanna with few trees (wet savanna zone) and the forest savanna, while severity was lowest in the forest. Cassava plant age and ecozones had highest influence on CBB occurrence. The highest severities of CBB were observed in two fields of 16 years monoculture cassava in the wet savanna zone and one field of 18 years monoculture cassava in the dry savanna zone. For CMD a significant positive correlation was observed only with soil moisture ($p < 0.05$). *Cercospora* brown leaf spot (BLS) was significantly negatively associated with the number of trees in surroundings of a field and intercropping cassava with other crops ($p < 0.05$), and *Cercospora* white leaf spot with more sandy soils ($p < 0.01$), while no significant correlation occurred between *Cercospora* blight leaf spot and agronomic, ecological and varietal characteristics (**Table 5**).

The stepwise regression analyses of cassava diseases on each of agronomic, ecological and varietal characteristics revealed for CBB significant positive regression coefficients for ecozones and plant age ($p < 0.0001$), indicating an increase in CBB severity in older plantations and in dryer ecozones (**Table 6**). All other variables did not meet the significance criterion for entering the model. The variation in CBB was largely unaccounted for by those two variables as the model R^2 was only 25%. Cassava mosaic disease (CMD) was significantly related to soil moisture, vegetation type in surroundings of the fields, branching type ($p < 0.01$) and mixture of cassava varieties in a field ($p < 0.05$). CMD occurred more frequently on more profusely branching cultivars. The stepwise analysis showed a significant regression coefficient for variety mixture (growing of more than one cassava genotype in a field). The disease was favored by soil moisture, whereas trees in surroundings of a field seemed to have a suppressive effect on its occurrence. No other variables measured met the significance criterion of the model. Three variables affected significantly *Cercospora* brown leaf spot (BLS). The disease was significantly reduced with more trees in surroundings of a

field ($p < 0.01$), intercropping cassava with other crops ($p < 0.05$) and in sandy soils ($p < 0.05$). *Cercospora* brown and white leaf spots were more severe on loamy sand and sandy loam soils than on sandy soils ($p < 0.05$ and $p < 0.01$, respectively). Among the cassava diseases, CBB variation was more affected by the agronomic, ecological and varietal variables left in the model ($R^2 = 0.25$) than CMD ($R^2 = 0.23$), whereas BLS ($R^2 = 0.16$) and WLS ($R^2 = 0.12$) were less influenced, though significant, by these characteristics, indicating a significant contribution of these characteristics to the variation of CBB, CMD, BLS and WLS (**Table 6**).

Table 5: Pearson correlation between severities of cassava diseases and the agronomic, ecological and varietal characteristics in 85 fields

	CBB	CMD	BLS	BlLS	WLS
Ecozones	0.28**	0.02	-0.15	-0.16	-0.17
Field size	-0.23*	0.12	0.04	-0.03	0.09
Vegetation type	-0.23*	-0.18	-0.23*	-0.06	0.14
Soil texture	0.13	0.15	-0.12	-0.19	-0.28**
Soil moisture	-0.27*	0.22*	0.01	-0.16	-0.12
Weed score	0.26*	0.07	0.11	0.12	0.07
Crop system	0.04	0.02	-0.22*	0.02	-0.17
Variety mixture	-0.16	0.19	-0.12	0.1	-0.21
Branching type	-0.12	0.19	-0.01	0.15	0.04
Plant age	0.29**	-0.14	0.09	0.14	0.09

CBB = cassava bacterial blight; CMD = cassava mosaic disease; BLS = brown leaf spot; BlLS = blight leaf spot; WLS = white leaf spot; *probability level = 0.05; **probability level = 0.01.

Table 6: Stepwise regression analysis of severities of cassava diseases on agronomic, ecological and varietal characteristics

Disease	Variable	Parameter estimate	Standard error	F	Probability
CBB	Intercept	0.08	0.34	0.05	0.8228
(R^2 = 0.25)	Plant age	0.10	0.02	18.91	0.0001
	Ecozones	0.33	0.08	18.20	0.0001
	Intercept	1.34	0.45	8.82	0.0039
CMD	Soil moisture	0.26	0.09	7.62	0.0072
(R^2 = 0.23)	Vegetation type	-0.38	0.11	10.99	0.0014
	Branching type	0.66	0.22	9.01	0.0036
	Variety mixture	0.47	0.19	5.74	0.0189
	Intercept	5.22	0.62	70.22	0.0001
BLS	Vegetation type	-0.36	0.11	9.76	0.0025
(R^2 = 0.16)	Crop system	-0.54	0.23	5.64	0.0199
	Soil texture	-0.19	0.09	4.58	0.0354
	Intercept	3.77	0.47	64.47	0.0001
WLS	Soil texture	-0.24	0.09	7.06	0.0095
(R^2 = 0.12)	Variety mixture	-0.42	0.22	3.75	0.0561

CBB = cassava bacterial blight; CMD = cassava mosaic disease; BLS = brown leaf spot; BlLS = blight leaf spot; WLS = white leaf spot. R^2 is approximately the percentage of the total variance (or variation) in the dependent variable (each of the diseases measurements: CBB, CMD, WLS or WLS) explained by the independent variables entered (agronomic, ecological or varietal variables).

Canonical correlations between cassava disease variables and agronomic, ecological and varietal characteristics

The canonical correlation analysis revealed further relations between the groups of variables.

The first three canonical correlations between disease variables and agronomic, ecological and varietal variables were significant ($p = 0.0001$, $p = 0.001$ and $p = 0.03$, respectively) using the approximate likelihood ratio significance test (**Table 7**). In the first canonical variate of the disease variables, CMD had the highest weight of 0.86, while coefficients of BLS and CBB were lower. WLS had a negative coefficient of -0.25, indicating a moderately reversed influence on the relationship between disease and agronomic, ecological and varietal variables. BlLS did not considerably contribute to the relationship (coeff. = 0.001). Vegetation in the surroundings of a field (canonical coefficient of -0.84) had a reverse influence on the first canonical variate of the non-disease variables that is a disease-decreasing effect, while branching varieties, abundance of weeds in a field, high soil moisture and a mixture of cassava varieties in a field had a positive influence, that is, increasing effect on the severity of cassava diseases (**Table 7**).

The second canonical correlation between the two groups of variables were significant ($p = 0.001$), and CBB had the greatest influence (coeff. = 0.88) for the disease variables, while plant age (coeff. = 0.76) and ecozones (coeff. = 0.46) had the highest weight for the non-disease variables. In the third canonical variates of the disease variables, WLS and BlLS had the highest reverse direction weight (coeff. = -0.62 and coeff. = -0.47, respectively), whereas for the non-disease variables ecozones and soil texture had the highest positive influence (coeff. = 0.62 and coeff. = 0.63, respectively). These canonical correlations confirmed the relation between WLS and soil texture (**Table 6**) and newly revealed an importance of the variables ecozones, soil moisture, vegetation type in surroundings of a field (was shown for BLS also by Pearson correlation analysis) and abundance of weeds for the *Cercospora* diseases, especially WLS.

Table 7: Canonical correlations between cassava disease variables and agronomic, ecological and varietal variables, and standardized canonical coefficients for these variables

	1st canvar	2nd canvar	3rd canvar
CBB	0.41	0.88	0.12
CMD	0.86	-0.30	-0.06
BLS	0.52	0.19	-0.26
BlLS	0.001	0.05	-0.47
WLS	-0.25	0.003	-0.62
Ecozones	0.23	0.46	0.62
Field size	0.10	-0.12	-0.25
Vegetation type	-0.84	-0.17	0.39
Soil texture	0.08	-0.12	0.63
Soil moisture	0.41	-0.29	0.47
Weed score	0.43	-0.12	-0.34
Crop system	-0.21	-0.11	0.06
Variety mixture	0.29	-0.30	0.15
Branching type	0.65	-0.26	-0.30
Plant age	-0.02	0.76	0.24

	Canonical correlation	Standard Error	F	Probability
1	0.603	0.069	2.188	0.0001
2	0.560	0.075	1.969	0.0014
3	0.483	0.084	1.655	0.0328
4	0.405	0.091	1.319	0.2027
5	0.239	0.103	0.747	0.6139

CBB = cassava bacterial blight; CMD = cassava mosaic disease; BLS = brown leaf spot; BlLS = blight leaf spot; WLS = white leaf spot; 1st canvar = first canonical variate; 2nd canvar = second canonical variate; 3rd canvar = third canonical variate.

1.4 Discussion

A country-wide survey for cassava diseases in Togo revealed the occurrence of cassava diseases across ecozones. Statistical analyses indicated the relationship among these diseases, and between the diseases and agronomic, ecological and varietal variables.

Cassava bacterial blight was observed in all the major agroecological zones in Togo. The incidence and severity varied across ecozones, with higher severity in the dry and wet savanna and in the forest savanna transition ecozones than in the forest zone. However, significant differences in field and plant incidences were observed only between the dry savanna and the other ecozones. Earlier observations in Togo reported a higher severity of the disease in the forest savanna transition zone than in the wet savanna zone, where it was rarely found, and the absence of the disease in the forest zone (Boher and Agbobli, 1992). These authors also reported the frequent occurrence of the disease in the region of Kara in the dry savanna zone which confirms our observations. Cassava bacterial blight was found in various ecozones across four West African countries, with generally higher incidences in the savanna than in the transition forest zones, and rarely or not described in the forest zones (Wydra and Msikita, 1998; Wydra and Verdier, 2002).

In the present data, differences in CBB incidences were not significant between the forest, the wet savanna and the forest savanna transition zones. This may be due to the generally high variability of survey data influenced by factors such as field history, plant age and weed density which are avoided in well planned field trials. The significant correlations observed among some of these factors and the incidence of CBB may confirm their influence on the disease occurrence. The low severity of the disease in the forest zone compared to the savanna zones may be due the vegetation type (forest) that could not provide optimal environmental development conditions to the disease, since great differences in night versus day temperatures were reported to promote the disease (Takatsu et al., 1978). Nevertheless, it has to be considered, that conclusions based on data from a survey of one year should be confirmed by studies covering several years. CBB was not observed in the rainforest of Cameroon (Wydra and Msikita, 1998) and in the rainforest - and the Sudan savanna - zones of Ghana in 1993 (Wydra and Verdier, 2002) and hardly found in the rainforest of Ghana and Benin in 1994, with disease incidence of 2% and 4%, respectively. Low CBB incidences of

8.3% and 9.8% were reported from the rainforest zones of Nigeria-West and Nigeria-East, respectively (Wydra and Msikita, 1998).

Our results showed a high CBB incidence of 27.4% of plants as never reported before from the forest zones in Africa. Although the lowest average disease severity of 1.7 (data not shown) was recorded from this zone, systemic infections (classes 3-5) were also observed. Glasshouse experiments revealed that *Xam* survived longer under dry than moist conditions (Fanou et al., 2001) which may contribute to the lower disease severity observed in the forest zone than in the savanna zones. The increase of the disease incidence and severity observed in the forest zone of Togo may be due to the continuous introduction of infected planting material deriving from the epidemic areas, especially the forest savanna transition zone, high rainfall which provides high relative humidity and the deforestation due to human activities. These factors may offer favorable development conditions for the disease. Similar observations of high CBB incidence (up to 100%) and severities were reported from deforested high rainfall areas in Nigeria (Wydra and Verdier, 2002). Also, CBB was reported to occur more frequently in warm and wet weather (Leu, 1978).

Higher field incidence and severity of CBB in the savanna zones than in the forest transition and rainforest zones was reported earlier in Congo and in Central Africa (Daniel et al., 1979, 1981; Persley, 1979). In Benin, CBB incidence of 85% in the dry savanna zone (Wydra and Msikita, 1998) and 86% in the Sudan savanna zone (Wydra and Verdier, 2002) were reported.
The incidence of the disease in the savanna transition zone may be favored by two rainfall seasons alternated by two dry seasons (Lamouroux, 1979) supporting a better survival of the pathogen (Fanou et al., 2001), and the old establishment of the pathogen in this area (Olympio, 1977). The fact that cassava fields were in close neighbourhood may have increased the transmission of the pathogen by insect vectors from infected to healthy plants and from diseased to healthy fields (Terry, 1974; Daniel and Boher, 1985; Fanou et al., 2001; Zandjanakou et al., 2001). Additionally, the possibility that the pathogen spread from one area to another by the use of infected planting material or cuttings (Lozano and Sequeira, 1974; Otim-Nape, 1976) coupled with the easy exchange of planting material between farmers may contribute to the dissemination of the disease. In Togo, the disease may have been introduced in the wet savanna and in the dry savanna by infected planting material deriving from the littoral zone (forest savanna transition) from which the cultivation of cassava spread all over the country and where the disease was reported for the first time (Olympio, 1977). Epiphytic

and systemic survival of the causal agent of CBB in the cuttings and plants was frequently demonstrated (Lozano and Sequeira, 1974; Terry et al., 1979; Fanou, 1999; Banito, this thesis). Nevertheless, no rigorous and suitable quarantine measures against CBB were introduced after the disease was reported from the last survey of Boher and Agbobli (1992).

Cassava bacterial blight was positively correlated with ecozones, with decreasing incidence and severity from the herbaceous savanna without trees (dry savanna zone) to the herbaceous savanna with few trees (wet savanna zone), the forest savanna transition and to the forest zone. The suppressive effect of vegetation and soil moisture was confirmed by Pearson correlation and canonical correlations analyses. A vegetation with many trees may offer high humidity and shade, and low temperature fluctuations between day and night which may be unfavorable for the development of the disease. The role of day and night temperatures in CBB occurrence was established by Lozano (1986) who reported the increase of the disease severity by wide fluctuations in night/day temperatures during the rainy season. Also, Wydra and Verdier (2002) observed higher severity of CBB in old than in young plantations in Benin and Ghana. A long vegetative period of an infected cassava plant may provide enough time to *Xam* for its multiplication and systemic colonization, and for infection of the whole plant, especially in susceptible varieties. CBB was more important in weedy plantations, indicating that weeds could play a role in the spread of the disease. The epiphytical survival and multiplication of *Xam* on weeds have been reported (Daniel and Boher, 1985; Fanou et al., 2001). The survival of *Xam* up to 60 days on some African weeds has been established (Fanou, 1999). Thus, weeds may constitute an inoculum source that can be transferred to cassava plants by insects such as *Zonocerus variegatus* (Terry, 1974; Fanou, 1999; Zandjanakou et al., 2001) and by rain splash. However, no weed has been identified as alternative hosts of *Xam* (Ikotun, 1981; Amusa et al., 1992; Fanou, 1999).

Our results revealed that cassava mosaic disease incidence and severity were more prevalent than CBB in all ecozones of Togo as it was also reported from several countries in West and Central Africa (Wydra and Msikita, 1998; Wydra and Verdier, 2002). Cassava mosaic disease occurred in all ecozones with high incidences ranging from 63.7% to 86.9%. For CMD and the other cassava diseases, except CBB, no ecozonal differentiation was found. Similar observations were made for CMD in Benin and Ghana by Wydra and Verdier (2002) and in Rwanda by Legg et al. (2001), who did not find clear differences between ecozones. However, CMD was reported to be prevalent in the wet coastal areas in Kenya (Bock, 1994)

and in the rainforest of Côte d'Ivoire (Fauquet et al., 1988), and Legg et al. (2001) observed that CMD symptoms were more severe in the North-East administrative region than in the other areas surveyed in Rwanda. Our results revealed an average CMD incidence of 76%, while Wydra and Verdier (2002) observed incidences of 31% and 80% in Benin and Ghana, respectively. The differences in CMD incidence observed between Benin, Ghana and Togo may be due to population differences of the whitefly (*B. tabaci*), the vector insect of the disease. Legg (1999) and Legg et al. (2001) reported that the spread into Rwanda of the EACMV-Ug associated pandemic of severe CMD, was evident through migration of viruliferous whitefly populations from the neighbouring countries of Uganda and/or Tanzania, which had been affected in previous years. Our results revealed that CMD severity increased when several cassava varieties were grown in mixture in a field and in fields with abundant weeds as confirmed by the observations of Wydra and Verdier (2002) in Benin and Ghana. Fargette et al. (1994) found that CMD was less severe in old cassava plantations, while the present analysis did not reveal this relationship.

Cercospora leaf diseases occurred in all the ecozones of Togo with high field incidences. An increasing susceptibility to the disease on sandy loam and loamy sand soils was observed. Also, few trees in the surroundings of a field and intercropping systems favored the infection by *C. henningsii*, while a mixture of cassava varieties in a field had a suppressive effect on white leaf spots. Lozano and Booth (1974) and Boher et al. (1978) observed that *Cercospora* brown leaf spots occurred more in dryer areas, while Wydra and Verdier (2002) found BLS associated with trees in the surroundings of a field. In Congo, *Cercospora* white leaf spots occurrence showed no ecological preference (Boher et al., 1978), but Lozano and Booth (1974) found that the disease was associated to more humid and cooler ecozones in Latin America.

Correlation analysis on a field basis revealed significant negative correlations between CBB and CMD incidences and between CMD and *Cercospora* white leaf spots, while no significant correlation was found between CBB and *Cercospora* leaf diseases. Evaluating cassava genotypes for reaction to major diseases, Fokunang et al. (2000c) found that CBB and CMD incidence were not significantly correlated. However, significant correlation was observed between CBB and CMD severity in a cassava germplasm collection (Fokunang et al., 2000a).

Conclusions

The cassava disease survey conducted in farmers' fields in four agroecological zones of Togo, provided country-wide and detailed data on cassava bacterial blight compared to the previous studies on the disease, and on two other cassava diseases, cassava mosaic disease and cercosporioses, never reported at this level before in Togo. The present studies revealed high field incidences of the three diseases in all the ecozones surveyed and found correlations between the diseases and between these and the agronomic, ecological and varietal characteristics. A further survey is recommended to confirm the present data.

Thus, bacterial blight is becoming more severe in all the ecozones, including the forest zone, where the disease was not found some years before. Therefore, measures to control cassava diseases must be taken to avoid possible epidemics and prevent losses of yields in farmers' productions.

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2 Pathological characterization of *Xanthomonas axonopodis* pv. *manihotis* strains from Togo

Abstract

The selection of resistant genotypes is a major element in the development of an integrated control system of CBB. Therefore, knowledge on the virulence and diversity of pathogen strains is important. Fourty-seven strains of *Xanthomonas axonopodis* pv. *manihotis* were isolated from leaf samples collected during the disease survey from the forest savanna transition, forest, wet savanna and dry savanna zones of Togo and tested for virulence by stem-inoculation of the susceptible cassava genotype Ben86052. Most (94%) strains were highly virulent, and generally only slight differences in virulence among strains were observed. Differences in virulence were independent of their origin in agroecological zones.

2.1 Introduction

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is the basic staple crop for 500 million people in tropical and sub-tropical parts of the world (FAO and FIAD, 2000), one of the major carbohydrate sources throughout Asia's and Africa's lowland tropics (Nilmanee, 1986), and one of the most important crops in Africa (FAO/GIEWS, 1995). Cassava provides smallholder households with cash income and low-income urban consumers with a low-cost carbohydrate supply (Nweke, 1998). It is mainly produced in the forest savanna and wet savanna zones (DESA, 1998; DSID, 1999). Global production reached 167.7 million tons in 1999 (FAO and FIAD, 2000), but generally stagnated over the last years (FAO, 1997; 1998).

Cassava production is largely reduced due to the attack by pests and diseases (Hahn et al., 1989; Hillocks and Wydra, 2002) among which cassava bacterial blight is of major importance (Wydra and Msikita, 1998). The disease is characterized by angular leaf spots developping into blight areas, and by a systemic infection of the stem leading to necrosis of vascular tissues, exudation of bacterial ooze, wilt, and tip die-back (Lozano and Sequeira, 1974). Causal agent is *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Bondar, 1915). Cassava is propagated by planting cuttings of stems, which are a primary source of dissemination of the pathogen (Lozano, 1986; Boher and Verdier, 1994). Cassava bacterial blight can be reduced through the use of *X. axonopodis* pv. *manihotis*-free planting material and by growing resistant genotypes (Cooper et al., 1997; Wydra et al., 2001; Zinsou et al., 2001; Wydra, 2002). However, selection of resistant genotypes needs information on the diversity and geographical distribution of the pathogen.

Considerable variation has been described among African *X. axonopodis* pv. *manihotis* strains in relation to biochemical and physiological (Fessehaie, 1997; Grousson et al., 1990), serological (Wydra et al., 1999) and genetic characters (Verdier et al., 1998; Assigbétsé et al., 1999). Differences in virulence among *X. axonopodis* pv. *manihotis* strains first described by Robbs et al. (1972), were also observed among strains from Brazil (Takatsu et al., 1978; Alves and Takatsu, 1984), and Africa (Maraite and Meyer, 1975; Wydra et al., 1999), but were never established for strains from different ecozones of Togo. The variability of aggressiveness among *X. axonopodis* pv. *manihotis* strains led to a wide range of classifications of strains into different groups of virulence, but generally no

correlation between aggressiveness and geographic origin have been found (Alves and Takatsu, 1984; Fessehaie, 1997). However, Restrepo and Verdier (1997) reported that the pathogen showed high levels of diversity and geographic differentiation in Colombia. Knowledge on the virulence of strains is important for screening for resistance, and the most virulent strains occurring in an area concerned should be used for inoculation (CIAT, 1978). Therefore, the pathological characterization of strains from Togo is the prerequisite to select resistant cassava genotypes and recommend suitable genotypes to farmers.

2.2 Materials and Methods

During a field survey covering the ecozones of Togo – forest savanna transition, forest, wet savanna and dry savanna zones -, leaves showing early symptoms of the disease were sampled for isolation of bacteria from each field, where cassava bacterial blight was observed.

Forty-seven *X. axonopodis* pv. *manihotis* strains – ten from the forest savanna transition, 6 from the forest, 13 from the wet savanna and 18 from the dry savanna zones – were isolated on GYCA medium (glucose 5 g/l, yeast 5 g/l, $CaCO_3$ 10 g/l, agar 15 g/l) (Dye, 1962) and incubated at 30 °C for 48 to 72 hours. Isolated *X. axonopodis* pv. *manihotis* strains were conserved on GYCA medium modified with 20 g calcium carbonate in test tube slants at 16 °C until further utilization.

The virulence test was conducted in an air-conditioned glasshouse with temperatures from 25 to 30 °C at the International Institute of Tropical Agriculture (IITA) station in Benin. The highly susceptible cassava genotype Ben86052 was used. Cuttings from apparently healthy plants were planted in pots of 16 cm diameter filled with field soil. Normal watering was applied during the whole experiment. One-month old vigorous plants were stem-inoculated with 48-hour old bacterial cultures of *X. axonopodis* pv. *manihotis* strains by stem puncture in the upper third of the stem using a sterile toothpick with inoculum taken directly from the agar plate (Maraite et al., 1981). Five plants were inoculated with each strain. Five control plants were stem-punctured using sterile toothpicks without inoculum. Symptoms were evaluated from 5 dpi every five up to 30 days on a 1 to 5 scale: class 1 - no symptoms, class 2 - wilting of 1 leaf, class 3 - wilting of 2 to 4 leaves, class 4 - wilting of more than 4 leaves, class 5 - dieback of the plant. Plant height was measured on the day of inoculation.

Statistical analysis

Means of symptom classes of 5 plants were calculated for each date of evaluation for each strain. For the discrimination of the bacterial strains on basis of their virulence (Andrivon, 1993), the area under disease progress curve (AUDPC) for the whole evaluation period was calculated for each replication as follows (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001):

$AUDPC = \sum_i [(DS_i + DS_{i-1})*(t_i - t_{i-1})]/2$

where "i" $\in$ {5; 10; 15; 20; 25; 30} are the days after inoculation, "DS" is the disease score using the severity scale of 1 to 5 as described above, and "t" represents the days post- inoculation. To avoid the area due to the note 1 (class1) which is supposed to be "zero", each "DS" value was transformed by subtracting "one" before integrating into the above formula. AUDPC values were log-transformed to stabilize variances and the analysis of variance was performed using the General Linear Model (GLM) of SAS software (SAS, 1990; 1997). The Tukey test was performed to compare the means of AUDPC values (Danielie, 1975). Pearson correlation analysis between AUDPC and plant height at inoculation time was performed to analyse a possible relationship between plant height at time of inoculation and symptom development.

2.3 Results

Fourty-seven strains of *Xanthomonas axonopodis* pv. *manihotis* were isolated from leaf samples from the forest savanna transition, forest, wet savanna and dry savanna zones and tested for virulence by stem-inoculation of the susceptible cassava genotype Ben86052. Symptoms commenced about three to five days post inoculation (dpi) with an olive-green colored water-soaked spot developed first at the inoculation point, followed by the appearance of yellowish to yellow brown exudates on the inoculation point and along the stem in case of severe infections. Subsequently, wilting of leaves and defoliation occurred - before 10 dpi with highly virulent strains -, and, finally, dieback of the apex and plant death in case of highly virulent strains.

Only slight differences in virulence among strains were observed. At 15 dpi, 41 strains were scored higher than a mean symptom note of 2.2 (**Fig. 1 A**), of which 16 strains were recorded with a note $\geq$ 3, at 20 dpi 37 strains were scored higher than note 3 and 14 strains

were recorded with a note ≥ 4 (**Fig. 1 B**). At 25 dpi, 19 strains had caused dieback of at least 2 plants, while 20 strains had caused dieback of 5 plants (**Fig. 1 C**). At 30 dpi, 44 strains were scored higher than 4, of which 8 strains had caused dieback of at least 4 plants and 31 strains had caused dieback of all 5 plants (**Fig. 1 D**).

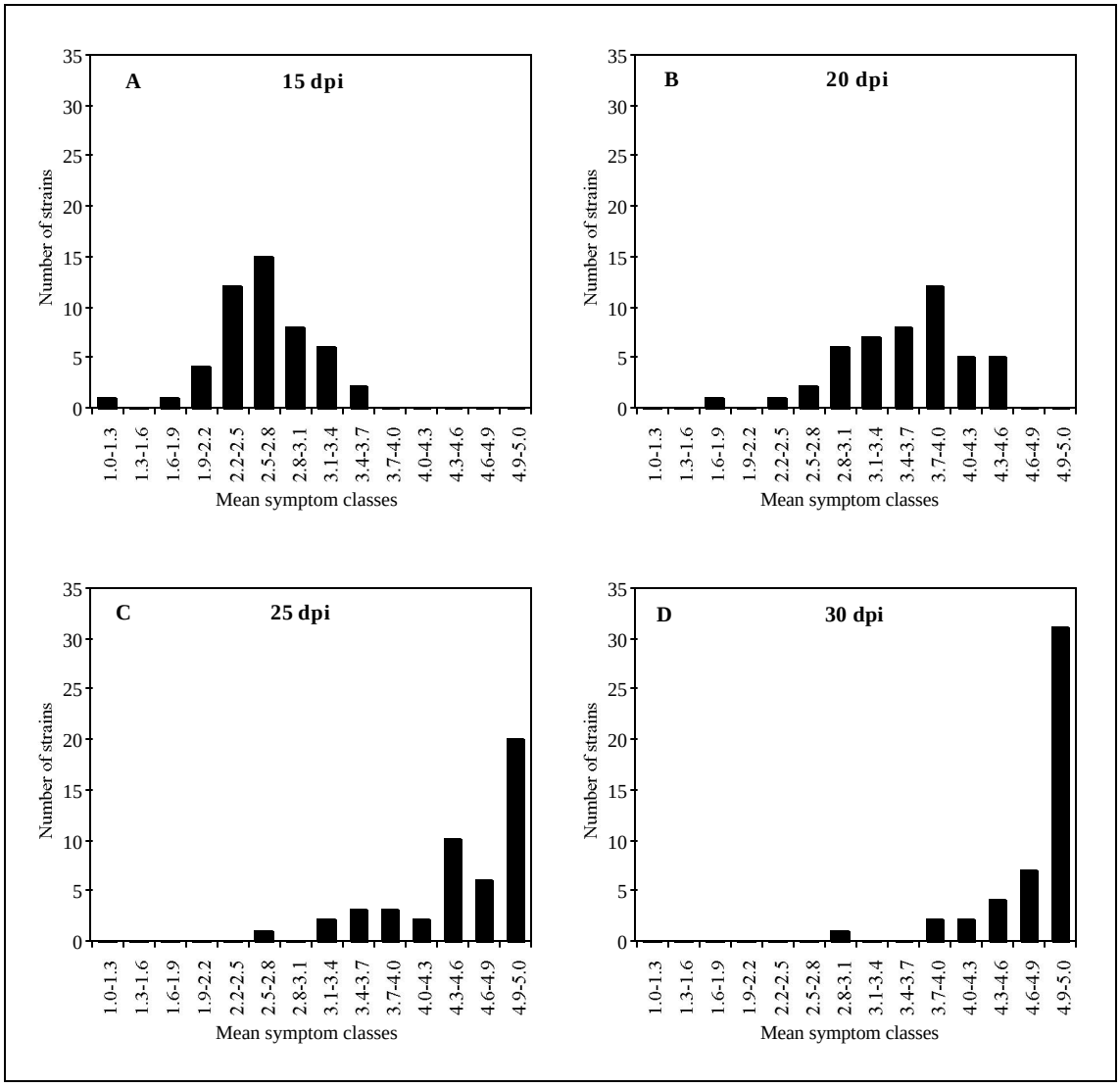

Fig. 1: Frequency distribution of strains from different origin in Togo over symptom classes at 15, 20, 25 and 30 days after stem inoculation under glasshouse conditions

Symptom classes: class 1: no symptoms, class 2: wilting of 1 leaf, class 3: wilting of 2 to 4 leaves, class 4: wilting of more than 4 leaves, class 5: dieback of the plant.

Statistic Analysis Of Variance (ANOVA) also showed slight significant differences among strains ($p < 0.05$) (**Table 1**). Five virulence classes were formed according to the statistical analysis – class 5 = very highly virulent ("a", "ab", AUDPC 55.0–62.0), with 32% of strains; class 4 = highly virulent ("abc", AUDPC 35.5-54.9) with 62% of strains; class 3 = virulent ("bc", AUDPC 26.4-35.4) with 2% of strains; class 2 = lowly virulent ("c", AUDPC 23.8–26.3) with 4% of strains; and class 1 = without symptoms (control).

Table 1: Virulence expressed as area under disease progress curve (AUDPC) of *X. axonopodis* pv. *manihotis* strains on cassava genotype Ben86052 in the glasshouse after stem inoculation

Code of strain	Ecozone	AUDPC	Tukey test	Virulence class
TGXAM/98/57	Wet savanna	62.0±4.1[1]	a	5
TGXAM/98/3	FST	60.0±4.7	ab	5
TGXAM/98/12	FST	59.0±3.7	ab	5
TGXAM/98/38	Dry savanna	58.8±4.7	ab	5
TGXAM/98/11	FST	57.5±4.8	ab	5
TGXAM/98/13	FST	57.5±4.8	ab	5
TGXAM/98/39	Dry savanna	57.5±6.0	ab	5
TGXAM/98/9	FST	56.9±8.7	ab	5
TGXAM/98/41	Dry savanna	56.7±7.3	ab	5
TGXAM/98/14	Forest	55.0±3.2	ab	5
TGXAM/98/15	Forest	55.0±3.5	ab	5
TGXAM/98/23	Forest	55.0±4.2	ab	5
TGXAM/98/35	Dry savanna	55.0±5.0	ab	5
TGXAM/98/40	Dry savanna	55.0±2.7	ab	5
TGXAM/98/59	FST	55.0±5.0	ab	5
TGXAM/98/28	Wet savanna	53.3±4.4	abc	4
TGXAM/98/42	Dry savanna	53.3±4.4	abc	4
TGXAM/98/44	Dry savanna	53.0±3.7	abc	4
TGXAM/98/48	Dry savanna	53.0±5.0	abc	4
TGXAM/98/16	Forest	52.5±2.5	abc	4
TGXAM/98/29	Wet savanna	52.5±8.3	abc	4
TGXAM/98/31	Wet savanna	52.5±2.5	abc	4
TGXAM/98/34	Wet savanna	52.5±4.8	abc	4
TGXAM/98/51	Dry savanna	51.7±6.7	abc	4
TGXAM/98/56	Wet savanna	51.0±2.4	abc	4
TGXAM/98/49	Dry savanna	50.0±5.2	abc	4
TGXAM/98/54	Wet savanna	50.0±4.5	abc	4
TGXAM/98/1	FST	49.0±3.7	abc	4
TGXAM/98/47	Dry savanna	48.8±5.5	abc	4
TGXAM/98/18	Forest	48.1±7.7	abc	4
TGXAM/98/26	Wet savanna	48.1±6.9	abc	4
TGXAM/98/8	FST	48.0±3.4	abc	4
TGXAM/98/27	Wet savanna	48.0±5.1	abc	4
TGXAM/98/37	Dry savanna	48.0±3.0	abc	4
TGXAM/98/6	FST	47.5±7.5	abc	4
TGXAM/98/45	Dry savanna	47.5±5.5	abc	4
TGXAM/98/25	Wet savanna	44.4±4.6	abc	4
TGXAM/98/53	Dry savanna	44.4±8.6	abc	4
TGXAM/98/36	Dry savanna	42.5±4.3	abc	4
TGXAM/98/19	Forest	40.0±3.4	abc	4
TGXAM/98/30	Wet savanna	39.4±7.8	abc	4
TGXAM/98/50	Dry savanna	39.2±8.2	abc	4
TGXAM/98/24	Wet savanna	36.9±2.4	abc	4
TGXAM/98/46	Dry savanna	35.5±6.7	abc	4
TGXAM/98/43	Dry savanna	31.9±4.5	bc	3
TGXAM/98/55	Wet savanna	26.3±4.1	c	2
TGXAM/98/7	FST	23.8±5.9	c	2
Control		0.0	d	1

[1]Standard error; AUDPC = area under disease progress curve (mean of five plants and standard error); FST = forest savanna transition; Class 1 = without symptom (control); class 2 = lowly virulent; class 3 = virulent; class 4 = highly virulent; class 5 = very highly virulent.

Regarding ecozonal distribution, 94% of the strains across ecozones were highly or very highly virulent (classes 4 and 5) (**Table 2**), with 100% of the strains from the forest zone belonging to these classes. No correlation was found between plant height on the day of inoculation and virulence of *X. axonopodis* pv. *manihotis* strains (coeff. –0.11, p = 0.12).

Table 2: Frequency distribution of *X. axonopodis* pv. *manihotis* strains from four ecozones in virulence classes

Ecozone	Class 1	Class 2	Class 3	Class 4	Class 5
FST	0	1	0	3	6
Forest	0	0	0	3	3
Wet savanna	0	1	0	11	1
Dry savanna	0	0	1	12	5
	0%	4%	2%	62%	32%

Class 1 = without symptom (control); class 2 = lowly virulent; class 3 = virulent; class 4 = highly virulent; class 5 = very highly virulent; FST = forest savanna transition.

2.4 Discussion

The virulence test of *X. axonopodis* pv. *manihotis* strains on the susceptible cassava genotype Ben86052 revealed that most of the strains were highly virulent. Only slight differences in virulence among the strains were observed in all ecozones, indicating low variability in their virulence characters. Although the lowest cassava bacterial blight severity and field incidence were recorded in the forest ecozone (Banito et al., 2002), all strains collected in this zone were highly virulent. The virulence of the pathogen may be masked under field conditions in the forest zone due to favorable growing conditions for the plant. These strains were probably newly introduced to the forest zone, since cassava bacterial blight had not been reported from the last disease survey in this zone (Boher and Agbobli, 1992). Although the pathogen did not cause high disease severity in the forest zone, it had maintained its virulence. Thus, the virulence of strains was independent of ecozones, although differences in disease incidence and severities had been observed during a survey (Banito et al., 2002). Genetic studies using restriction fragment length polymorphism (RFLP) on 218 *X. axonopodis* pv. *manihotis* strains from Togo including strains used in the present studies revealed genetic diversities among strains and nine different haplotypes were defined. Cluster analysis on genetic characteristics of strains revealed the existence of 7 groups at 70% similarity (Mosquera et al., unpublished).

An ecozonal differentiation in the occurrence of highly virulent strains was also not observed among strains from Ghana, Benin, Nigeria, Cameroon and Uganda by Wydra et al. (1999). Verdier et al. (1993, 1994) reported differences in the speed of symptom development among *X. axonopodis* pv. *manihotis* strains, suggesting variations in aggressiveness. The use of five cultivars allowed them to define 10 pathotypes among 91 *X. axonopodis* pv. *manihotis* strains in Venezuela (Verdier et al., 1998). Variation in virulence has also been found among *X. axonopodis* pv. *manihotis* strains from Brazil (Takatsu et al., 1978; Alves and Takatsu, 1984), from Africa (Maraite and Meyer, 1975; Grousson et al., 1990; Fessehaie, 1997), and from Africa, Asia and South America (Maraite et al., 1981). *X. axonopodis* pv. *manihotis* strains collected from different geographic regions in Africa revealed great differences in virulence as well as in physiological, biochemical and serological features (Wydra et al., 1999), but the latter characteristics were not correlated with virulence. Mutations changing virulence have been considered to occur readily among *Xanthomonas* species (Stolp et al., 1965), and may explain not only the high virulence of the strains from the dry and wet savanna zones, but also the recent epidemics

in these ecozones as reported in several cassava-growing regions of West Africa (Wydra and Verdier, 2002).

Our studies aimed to select virulent strains by testing their aggressiveness on one susceptible genotype. Nevertheless, the pathogenic variability found among the highly virulent strains has to be tested with various cultivars to investigate the presence of strain x genotype interactions, which were observed with strains from other African origin (Zinsou et al., 2002) and, thus, to provide a representative set of strains for the selection of resistant genotypes (Verdier et al., 1998).

The stem puncture inoculation allowed the discrimination among *X. axonopodis* pv. *manihotis* strains. Stem puncture inoculation and leaf inoculation methods were used for pathogenicity tests of *X. axonopodis* pv. *manihotis* strains (Maraite et al., 1981; Restrepo et al., 2000). The stem inoculation technique was reported as a suitable method for resistance screening of cassava cultivars for bacterial blight resistance (Restrepo et al., 2000), and for clear differentiation among cultivars and *X. axonopodis* pv. *manihotis* strains (Maraite et al., 1981). Nevertheless, additional leaf inoculation experiments may reveal more pathogenic diversity and mechanisms of resistance of the plant (Zinsou et al., 2002).

The virulence classification of the strains based on the statistical analysis of the area under the disease progress curve (AUDPC) provided similar results as the classification based on the time of symptom development on the plants (Banito, 2001), developped by Wydra et al. (1999). A similar, but less exact classification method, with evaluation of disease symptom classes 1, 2 and 4 weeks after inoculation was used by Restrepo and Verdier (1997) to evaluate the virulence of *X. axonopodis* pv. *manihotis* strains from Latin America. Thus, the inoculation of few strains – from different ecozones, in case that virulence determinants might differ - selected among the highly virulent group is recommended to test genotypes for resistance to cassava bacterial blight.

During a cassava diseases survey across ecozones in Togo (Banito et al., 2001), generally, higher cassava bacterial blight incidences and severities were observed over all ecozones compared to the results of a previous survey conducted by Boher and Agbobli (1992). Besides the possible spread of the pathogen by the exchange and use of infected planting material all over the cassava growing areas and possible changes in environmental conditions due to the deforestation, the present results suggest that an increase of pathogen aggressiveness over years could be responsible for the increase of disease severity.

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3 Characterization of resistance and tolerance of cassava to bacterial blight based on genotype x environment interaction studies

Abstract

To monitor the resistance characteristics of cassava genotypes to CBB infection under field conditions, 22 improved and local genotypes from Togo were screened under natural infection and after spray-inoculation with *Xanthomonas axonopodis* pv. *manihotis* in the forest and forest savanna transition zones in years 1998 and 1999, and in the wet savanna zone in year 1999. High genotype x environment interactions were observed, and no genotype with disease resistance in the three sites in the forest savanna transition and forest zones over a two year-experiments, and wet savanna zone in a one-season trial, was found. However, genotypes CVTM4, Main27, TMS30572 and TMS92/0429 were resistant in at least one environment and medium resistant in other environments, and TMS91/02316 was medium resistant across environments, while Lagos, Toma289 and Toma378 were over all susceptible. Cassava bacterial blight severity was significantly negatively correlated to cassava root yield in inoculated plots in the site in the forest zone in 1998 and 1999, and in non-inoculated plots in the forest savanna transition zone and the wet savanna zone in 1998 and 1999, respectively.

Analysing the development of the different symptom types by genotypes, generally spot and blight symptom development was positively correlated, while there was no relation, or a negative correlation between leaf symptoms and the wilt symptom development. The same oberservation was made, when data were analysed across genotypes and environments. Significant negative correlations were observed between blight and wilt symptoms, and root yield in each of the three ecozones, and between spots and root yield in the forest zone.

3.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a major food crop in sub-Saharan Africa (Nweke, 1996) and also serves as raw material for local industries (Onabolu and Bokanga, 1998; Sanni et al., 1998). Cassava was the first crop in terms of production among the major staple crops in 1999 in Togo, followed by yam and maize (DSID, 1999). However, the yield was constantly below the African average yield of 8.2-8.3 t/ha (FAO, 1997; 1998), and the production generally stagnated over the last years (DESA, 1998; DSID, 1999).

Cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *X. campestris* pv. *manihotis* (Bondar, 1915) is one of the most severe diseases of cassava in South America and Africa (Lozano, 1986) and an epidemic disease distributed by infected cuttings (Lozano, 1986; Boher and Verdier, 1994). The disease is characterized by symptoms comprising water-soaked angular leaf spots, leaf blight and wilt, defoliation, exudation on stems, petioles and leaves, vascular necrosis and dieback. Cassava yield losses of more than 50% due to CBB were reported (Fanou, 1999; Wydra and Rudolph, 1999).

Due to the long growth cycle and the vegetative propagation of cassava, the most appropriate approach to control CBB is by growing resistant cultivars (Lozano, 1973; Wydra et al., 2001), as element of an integrated control system (Wydra and Rudolph, 1999). Careful selection of cassava bacterial blight-free cassava stakes (Lozano and Laberry, 1982; Lozano, 1986), and the use of planting material derived from tissue cultures to establish cassava plantations for production of propagation material may support disease control by resistant genotypes and reduce the occurrence of CBB (Kpémoua et al., 2001). But, since most cassava growers are small farmers (Phillips, 1974) with traditional technical know-how and few economic resources (Lozano and Laberry, 1982), there is usually no alternative to the production of own planting material. In this situation, use of resistant genotypes is the most important control measure. Additionally, quarantine regulations to avoid the introduction of the pathogen into bacterial-blight free areas are indispensable (Elango and Lozano, 1981; Lozano, 1986). The movement of planting material should be controlled and cuttings for distribution be tested by available pathogen isolation and molecular detection techniques (Verdier et al., 1998; Ojeda and Verdier, 2000).

Genotypes with different levels of resistance were reported (Fanou, 1999; Fokunang et al., 2000b; Restrepo et al., 2000b; Zinsou et al., 2003a). Defense mechanisms against *X. axonopodis* pv. *manihotis* were observed in the vascular system of stems of infected cassava plants by Kpémoua et al. (1996), with differential reactions comparing susceptible and resistant cultivars. In the leaf mesophyll of resistant cultivars no mechanisms limiting bacterial multiplication were found by some authors (Boher and Verdier, 1994), and after leaf inoculation by wounding of four cassava genotypes, Restrepo et al. (2000a) could not correlate leaf reactions with resistance. Also, after leaf infiltration with *Xam* suspensions at higher concentrations (10^8 cells·ml^{-1}), no significant differences in bacterial populations at leaf level were observed between resistant and susceptible genotypes (Flood et al., 1995). However, Zinsou et al. (2001) clearly demonstrated differences in symptom development and bacterial multiplication on leaf level comparing resistant, medium resistant and susceptible cultivars, using lower inoculum levels. Also Wydra et al. (2003b) observed differences in cell wall pectins of leaves with different levels of resistance.

Resistance in *M. esculenta* introgressed from a wild relative, *M. glaziovii*, is polygenic and additively inherited. Accessions for genetic diversity and resistance to cassava bacterial blight revealed a high level of polymorphism among cassava genotypes (Sãnchez et al., 1999). Jorge et al. (2000) identified six regions of the cassava genome controlling resistance to *X. axonopodis* pv. *manihotis* strains, confirming the polygenic character of the resistance. A specific interaction between the cassava plant and the pathogen was suggested, and resistance markers specific for African strains were recently identified (Wydra et al., 2003b).

Screening cassava genotypes for resistance to cassava bacterial blight was performed by observing symptom development in the field under strong disease pressure over several crop cycles (Boher and Verdier, 1994). Under controlled conditions, differentiation between susceptible and resistant genotypes after leaf infiltration was observed only at an incoculum concentration of lower than 10^2 cfu ml^{-1} (Flood et al., 1995). On the contrary, Zinsou et al. (2003b) identified an inoculum concentration of 10^5 cfu ml^{-1} as most differentiating for screening of genotypes for resistance. Zinsou (2002) described differences in spot symptom development comparing genotypes of different resistance after leaf inoculation under controlled conditions. Genotypes also differed in symptom development with respect to leaf- and stem inoculation, suggesting different mechanisms of resistance in different parts of the cassava plant. Differences in symptom type development

of genotypes have not been investigated unter field conditions in various environments.

Evaluation of a cassava germplasm collection for reaction to three major diseases under natural infection revealed that cassava tuber number and tuber dry matter were significantly negatively correlated with cassava bacterial blight, African cassava mosaic disease and cassava anthracnose disease severity (Fokunang, 2000a). Among twenty-three cassava genotypes screened for resistance to cassava bacterial blight in three ecozones, no cassava genotype with complete field resistance was found, however, differences in reaction to the disease allowed to classify the cultivars into susceptible, moderately resistant and resistant groups (Fanou, 1999). The genotypes Ben86052 and I91/02322 were among the susceptible ones, while I89/02078 and TMS30572 were overall resistant. The latter author observed that the CBB-susceptible genotypes TME1 and Ben86052 yielded high root weight, whereas the most resistant genotype I89/02078 had the lowest root weight. It was suggested that a high genotype x environment interaction and tolerance characteristics in some cultivars play a role in disease development and root formation (Wydra, 2002). Also Zinsou et al. (2003a) observed high genotype x environment interactions among local and improved genotypes from Benin.

Improved and local genotypes from Togo have never been characterized for their reaction to bacterial blight infection in various agroecological zones of Togo. Therefore, in the present studies selected cassava genotypes from Togo and from an international collection were evaluated for their reaction to cassava bacterial blight under field conditions in the forest savanna transition, forest and wet savanna zones to select resistant, high yielding genotypes suitable for farmers.

3.2 Materials and methods

Experimental sites

Field experiments were conducted at two sites in the forest savanna transition and forest zones in 1998-1999, at Davié and Adéta [Institut Togolais de Recherche Agronomique (ITRA) stations], respectively, and in three sites in the forest savanna transition, forest and wet savanna zones in 1999-2000, at Davié, Adéta and Sotouboua (ITRA stations), respectively, in Togo. The trials were not conducted in year 1998 at Sotouboua in the wet savanna zone due to the lack of planting material. The sites are typical for their respective ecozones. The forest savanna transition zone (littoral zone) in the South part of the country,

is characterized by a shrubby vegetation with few trees, the forest zone in the South-West is predominated by rainforest vegetation, and the wet savanna in the Center part of the country is characterized by dominance of a shrubby vegetation. The savanna transition and forest zones are characterized by a sub-equatorial climate with one long rainy season (March – June), one short dry season (July-August), one short rainy season (September – October) and one long dry season (November – March). The wet savanna is characterized by a tropical climate with one long rainy season (April – September) and one long dry season (October – March) (Lamouroux, 1979). The average annual rainfall is about 1200 mm in the forest savanna transition zone and 1400 mm in the forest and wet savanna zones, with average temperatures of 28 °C, 24 °C and 27 °C, respectively. However, annual rainfall up to 2027 mm in the forest zone and 1810 mm in the wet savanna zone were recorded (DMN, 2001). In years 1998, 1999 and 2000, the average rainfall was 855.4 mm, 1,204.8 and 713.0 mm in the forest savanna transition zone, 1,018.2 mm, 864.5 mm and 1,483.1 mm in the forest zone and 1,371.0 mm, 1,309.1 mm and 1,309.8 mm in the wet savanna zone, spread over 9 months in the first two ecozones and over 7 months in the latter one.

Planting materials

Cuttings from the 27 local, Togolese and improved cassava genotypes Fétonégbodji, Nakoko Lagos, Cameroon, Tuaka, Gbazékouté, Ankra (local), and Toma378, Ben86052, TMS92/0057, Toma219, TMS30572, Toma289, TMS92/0343, 312-524, TMS91/02316, TMS4(2)1425, CVTM4, TMS92/0326, Toma159, TMS92/0067, Main27, TMS91/02322, TMSCBS10(80411), Boram, Sorad and TMS92/0429 (improved by IITA) derived from plants apparently free of CBB symptoms were received from ITRA Lomé/Togo, or farmers fields, and Ben86052 and TMS30572, the susceptible and resistant standard genotypes, respectively, from IITA (International Institute of Tropical Agriculture) Benin-Station. Due to insufficient of planting material 22 of the 27 genotypes were tested in all ecozones, genotype TMSCBS10(80411) was used instead of Toma159 in the wet savannazone, and genotype Toma219 was replaced by genotypes Boram and Sorad in the forest and wet savanna zones, respectively.

Experimental design, planting and maintenance

The trial set up was an augmented complete randomized block design with three replications of ten plots. This design is used for the assessment of a large number of

genotypes when a randomised complete block design is not possible due to availability of land and lack of planting material. The concept is to establish a standard replication design using check genotypes. Each replicate forms a complete block of the standard design. Additional unassigned plots are created within each replicate, and non-replicated genotypes are assigned to these plots in the form of an incomplete block design (Scott and Milliken, 1993; Wolfinger et al., 1997).

The augmented design avoids the space-consuming repetition of all the 24 genotypes. Cassava genotypes Ben86052, Gbazékouté and TMS30572 were used as checks because of their susceptible and resistant - the latter genotype - reaction to cassava bacterial blight, and their general good performance (Boher and Agbobli, 1992; Akparobi et al., 1998). These were replicated throughout the blocks, with each block consisting of the three checks and 7 other genotypes (non-replicated). Each plot (20 m^2) representing one genotype consisted of two rows of 10 m, at a spacing of 1 m, with 1.5 m between plots. Cassava stem cuttings of 20 cm length of each genotype were single planted at a spacing of 1 x 1 m on well prepared flat ground in June. Each plot with an area of 20 m^2 (10 m length and 2 m width) consisted of 2 rows of 10 plants. The control plots were separated by a screen of maize plants of 5 m from the inoculated plots. Weeding was conducted, when necessary, and no additional watering was applied.

Bacterial suspension and spray inoculation

A 48-hour old culture of *X. axonopodis* pv. *manihotis* strain X27 from Togo produced on GYCA (glucose 5 g/l, yeast 5 g/l $CaCO_3$ 10 g/l, agar 15 g/l) medium (Dye, 1962) was harvested from agar plates using 0.01 M $MgSO_4$ solution, diluted to 10^7 cfu/ml and used for inoculation. One-month old cassava plants were inoculated with the bacterial suspension by spraying the abaxial surface of leaves using a motorized sprayer. A total of three inoculations were performed at 3-weekly intervals.

Symptom assessment

Disease symptoms were assessed 3 weeks after each of the 3 inoculations and during the six and the twelve months harvests on ten plants (five plants at harvesting) randomly selected in each plot by counting leaves bearing angular leaf spots or blight or wilted/dropped leaves and the number of shoot tips with dieback among the total shoot tips.

When leaves showed more than one symptom type, they were recorded under the more severe symptom type. The percentages of leaves with spots, with blight, wilted/dropped leaves and shoots with dieback were calculated for each plant. The severity index (Si) was calculated for each plant at each evaluation date as follows:

$Si = (1xS + 2xB + 1xW + 2xD)/6$

where S, B, W and D represent the percentage of leaves with spots, blight, wilt and shoots with dieback, respectively. The weight attributed to the symptoms blight and dieback is an estimation resulting from regression analysis of symptom and plant growth data, revealing blight as most important factor influencing root yield, and dieback with highest influence on overall plant growth (leaf and stem weight) (unpublished data). The standardized area under the severity index progress curve (AUSiPC) was calculated for each plant at six evaluation dates, by the trapezoidal integration (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001) according to ecozones. In the forest and forest savanna transition zones:

$AUSiPC = [(Si1+Si2)x21/2 + (Si2+Si3)x21/2 + (Si3+Si4)x60/2 + (Si5+Si6)x120/2]/275$

In the wet savanna zone:

$AUSiPC = [(Si1+Si2)x21/2 + (Si2+Si3)x21/2 + (Si3+Si4)x30/2 + (Si5+Si6)x90/2]/215$

where Si1, Si2, Si3, Si4, Si5 and Si6 represent the severity index at the evaluation dates 1, 2, 3, 4, 5 and 6, respectively. Si4 and Si5 correspond to severity index during the dry season and are equal to zero. The area under the severity index progress curve in days over the growing period was divided by the evaluation period of 275 or 215 days, corresponding to 365 days minus the dry season period of 90 days in the forest and forest savanna transition zones and 150 days in the wet savanna zone, respectively, in order to receive the standardized AUSiPC comparable between ecozones. The AUSiPC values of each symptom type were also calculated.

Harvest

Cassava roots were harvested at 12 months after planting by uprooting 5 plants randomly selected per plot. All the roots of each plot were mixed and a sub-sample was taken, cut into small pieces, weighed and dried in an oven at 105 °C for 72 hours for dry weight determination.

Statistical analysis

Standardized area under severity index progress curve (AUSiPC) and dry root weight values were log-transformed to stabilize variances and the analysis was performed using the Linear Mixed Model ANOVA (Harville, 1988; Bernardo, 1994; Tempelman and Gianola, 1996). Values and standard errors in tables are the real, non-transformed values. The analytical procedures for augmented design using mixed models as implemented in the SAS software (SAS, 1990; 1997) were performed as described by Korie and Okechukwu (2000). The analysis involves estimation of block effects and plot error using replicated checks. In each environment, the percentages of mean AUSiPCs of genotypes were calculated considering the highest AUSiPC value as 100%. The genotypes were classified into resistant (R, AUSiPC < 50%), medium resistant (MR, AUSiPC 50%-74.9%) and susceptible (S, AUSiPC 75%-100%) groups. The two-dimensional biplot of principal component analysis on genotypes was performed to show grouping of genotypes and genotype x environment interactions based on symptom severity, severity of symptom types and root yield data. Pearson correlation analysis using 22 genotypes grown in all environments including inoculated and non-inoculated treatments was performed to establish the relationship between symptom severity (AUSiPC) and cassava dry root weight, between severity values of different symptom types, and between severity of symptom types and dry root weight. Regression analysis of symptom severity (AUSiPC) and root dry weight was performed with of 4 genotypes selected for their high yield in spite of high disease severity.

3.3 Results

The disease developed during the rainy season, with a peak at 4 months after planting (**Fig. 1**). Symptoms disappeared during the dry season and reappeared in the rainy season of the following year. In the inoculated plots, the severity index was high from the second month after planting, while it was close to zero during the first 3 months after planting in non-inoculated plots. Genotype Ben86052 developped a higher severity index than genotypes Gbazékouté and TMS30572 in the inoculated plots, while the first 2 genotypes did not differ in the non-inoculated plots.

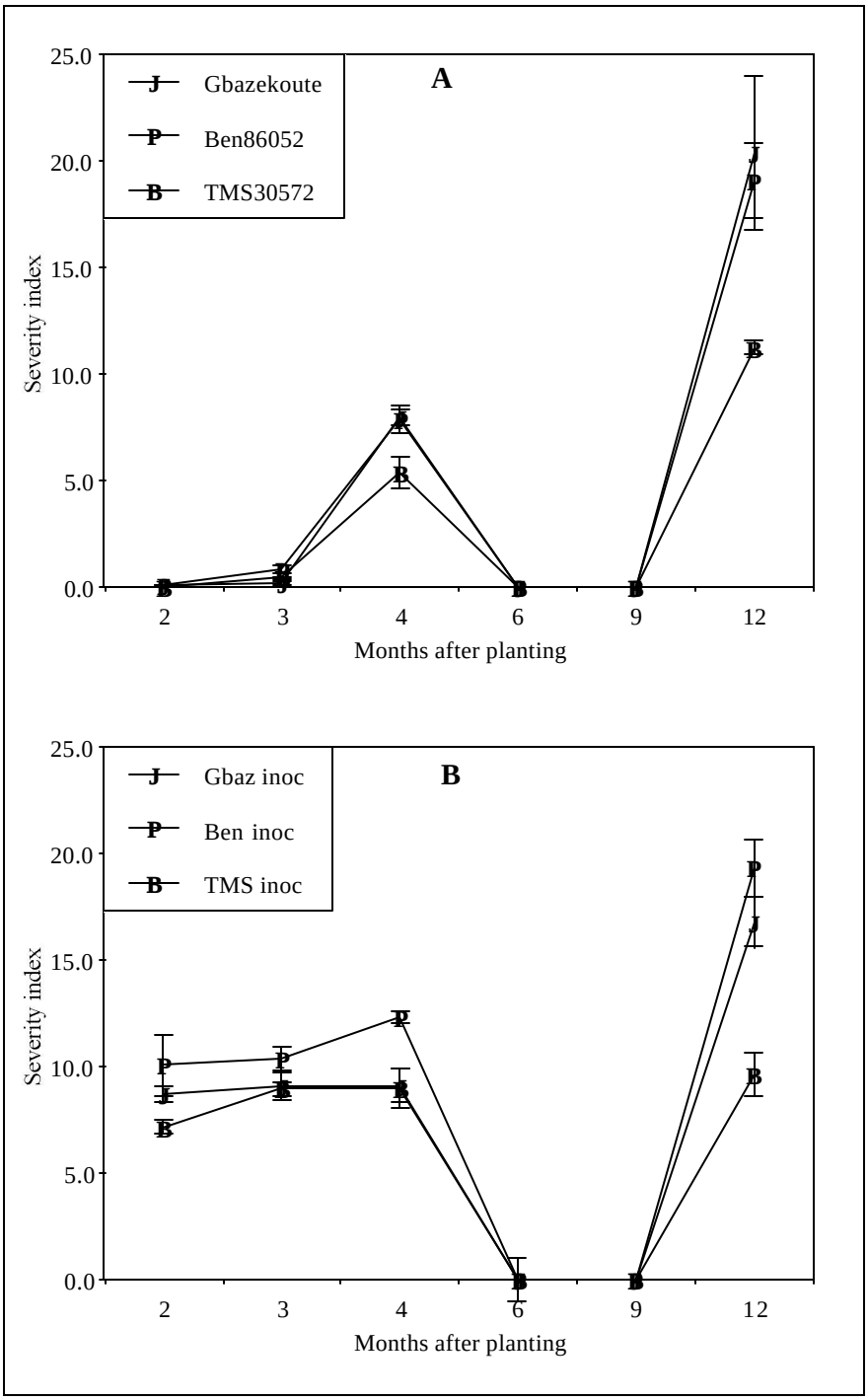

Fig. 1: Development of severity index in the susceptible genotypes Ben86052 and Gbazékouté, and the resistant genotype TMS30572 in non-inoculated (**A**) and inoculated (**B**) treatments in the forest zone in year 1998 (dates of inoculation: 30, 51 and 72 days after planting)

Comparing the reaction of 27 genotypes, of which 22 were repeated in each environment in inoculated plots in three sites in three ecozones over two years the highest disease severity expressed as standardized area under severity index progress curve (AUSiPC) was recorded in the forest zone (total AUSiPC 166.8), with a range of 4.1-9.1 in year 1999 and the lowest in the forest savanna transition zone (total AUSiPC 139.3), with a range of 4.4-8.0 in 1998 (**Table 1**). In the non-inoculated plots, the highest total AUSiPC of 146.5 was recorded in the forest zone, with a range of 3.9-8.2 (data not shown, **Annex 1**). However, generally, the AUSiPC values in the non-inoculated plots did not differ sufficiently to classify the genotypes. Therefore, the classification was based on the AUSiPC values of the inoculated plots.

The disease severity of the total of 27 genotypes varied between ecozones and years, with lowest AUSiPC for genotypes CVTM4 and TMS30572 in 1998, and Main27 and TMS30572 in 1999 in the forest zone, TMS30572 and TMS92/0429 in 1998, and TMS92/0429 and TMS91/02316 in 1999 in the forest savanna transition zone, and genotypes TMS92/0429 and Main27 in the wet savanna zone. The highest disease severities were recorded in genotypes Lagos and Toma289 in 1998 and Nakoko and TMS4(2)1425 in 1999 in the forest zone, Lagos and Gbazékouté in 1998 and Ankra and Toma289 in 1999 in the forest savanna transition zone, and Tuaka and Lagos in the wet savanna zone.

No genotype was found with a resistant reaction in more than two environments (**Table 2**). Most of the genotypes were medium resistant and/or susceptible across ecozones over the two years. The local genotype Lagos and the improved genotypes Toma289 and Toma378 were susceptible in all environments, and the local genotypes Ankra, Gbazékouté and Nakoko, and the improved Ben86052 were susceptible in four of the five environments. The four genotypes TMS30572, CVTM4, Main27 and TMS92/0429 were resistant in at least one environment and were never susceptible in the 3 sites over the 2 years, with genotype Main27 showing resistance in one year in two ecozones. Genotype TMS91/02316 was medium resistant over all environments, while TMS4(2)1425 showed high variability between years across ecozones, being among the medium resistant, extremely susceptible and susceptible ones.

Table 1: Disease severity expressed as standardized area under the severity index curve (AUSiPC) of cassava genotypes spray-inoculated with *X. axonopodis* pv. *manihotis* in three sites in the forest, forest savanna transition and wet savanna zones in two years in decreasing order of the total mean of AUSiPC

	Forest		Forest savanna transition		Wet savanna
	1998	1999	1998	1999	1999
Genotypes	AUSiPC	AUSiPC	AUSiPC	AUSiPC	AUSiPC
Lagos	8.4	8.5	8.0	6.6	8.5
Sorad	nd	nd	nd	nd	7.8
Boram	7.7	7.8	nd	nd	nd
Toma289	8.2	6.9	6.5	7.2	7.6
Ankra	6.4	8.8	7.1	7.6	6.3
Nakoko	6.9	9.1	5.4	6.1	8.0
Toma378	6.8	7.7	7.1	6.1	7.6
Gbazékouté "C"	6.0	7.7	7.2	6.6	7.3
Ben86052 "C"	7.2	7.9	5.6	6.0	7.4
Fétonégbodji	6.0	8.3	5.1	6.1	7.5
312-524	5.9	7.3	5.9	5.5	8.2
Cameroon	6.9	7.7	5.5	6.1	6.5
Toma219	nd	nd	6.3	6.5	nd
TMS92/0057	6.1	6.8	5.8	6.3	6.5
TMS92/0067	6.6	7.3	5.3	6.4	5.4
Tuaka	5.9	5.1	4.7	6.2	8.9
TMS92/0343	7.2	5.7	5.7	5.6	6.6
TMS92/0326	6.5	6.3	5.6	5.9	6.1
TMS4(2)1425	4.9	9.0	6.4	4.7	4.7
TMS91/02322	5.4	6.5	5.7	6.0	5.6
TMS91/02316	6.3	6.3	5.3	4.6	6.6
Toma159	6.1	6.4	5.1	5.1	nd
TMSCBS10(80411)	nd	nd	nd	nd	5.3
CVTM4	3.7	6.0	5.9	5.1	5.1
Main27	4.8	4.1	5.1	5.6	4.3
TMS30572 "C"	4.4	4.5	4.4	5.1	5.3
TMS92/0429	4.9	4.9	4.6	4.0	3.9
Total AUDPC	149.4	166.8	139.3	141.2	156.8
Range	3.7 - 8.4	4.1 - 9.1	4.4 - 8.0	4.0 - 7.6	3.9 - 8.9
SE	C 0.27	C 0.23	C 0.66	C 0.26	C 0.66
SE	X 0.48	X 0.40	X 1.12	X 0.45	X 1.15

"C" = check genotype;"X" = non-replicated genotypes; nd = not determined; SE = standard error.

Table 2: Reaction of 25 cassava genotypes, of which 22 were tested in all environments, to spray-inoculation with *X. axonopodis* pv. *manihotis* in three sites in three ecozones of Togo

	Forest		Forest savanna transition		Wet savanna
Genotypes	1998	1999	1998	1999	1999
Boram	S	S	nd	nd	nd
Toma219	nd	nd	S	S	nd
Lagos	S	S	S	S	S
Toma289	S	S	S	S	S
Toma378	S	S	S	S	S
Gbazékouté "C"	MR	S	S	S	S
Ankra	S	S	S	S	MR
Ben86052 "C"	S	S	MR	S	S
Nakoko	S	S	MR	S	S
Cameroon	S	S	MR	S	MR
Fétonégbodji	MR	S	MR	S	S
TMS92/0067	S	S	MR	S	MR
TMS92/0326	S	MR	MR	S	MR
312-524	MR	S	MR	MR	S
Tuaka	MR	MR	MR	S	S
TMS4(2)1425	MR	S	S	MR	MR
TMS91/02322	MR	MR	MR	S	MR
TMS92/0057	MR	MR	MR	S	MR
TMS92/0343	S	MR	MR	MR	MR
Toma159	MR	MR	MR	MR	nd
TMS91/02316	MR	MR	MR	MR	MR
CVTM4	R	MR	MR	MR	MR
TMS30572 "C"	MR	R	MR	MR	MR
TMS92/0429	MR	MR	MR	MR	R
Main27	MR	R	MR	MR	R

"C" = check genotype; R = resistant (0-50%); MR = medium resistant (50-74.9%); S = susceptible (75-100%); nd = not determined; genotypes Sorad and TMSCBS10(80411) were tested only in the wet savanna zone and reacted with S and MR, repsectively.

The frequency distribution of 27 genotypes, of which 22 were repeated in each site (ecozone), in inoculated treatments across disease severity values varied between ecozones and years (**Fig. 2**). The disease developped tendenciously more in the forest and the wet savanna zones than in the forest savanna transition zone, where most of the genotypes were found in the severity index classes 7.

Comparing genotypes in non-inoculated and inoculated plots, differences in reaction of genotypes to inoculation were observed across environments (data not shown, **Annex 1**). Some genotypes, such as Boram, Lagos, TMS92/0067 and Toma289 in 1998 and TMS4(2)1425 in 1999 in the forest zone and Tuaka in the wet savanna zone in 1999 reacted strongly to inoculation, with a considerable difference in AUSiPC compared to the non-inoculated treatment, while others reacted not or only slightly. Among the resistant genotypes, genotypes TMS30572, Main27 and TMS92/0429 reacted generally less on inoculation. In the forest savanna transition zone all 24 genotypes did not react or only slightly to inoculation over the two year experiments.

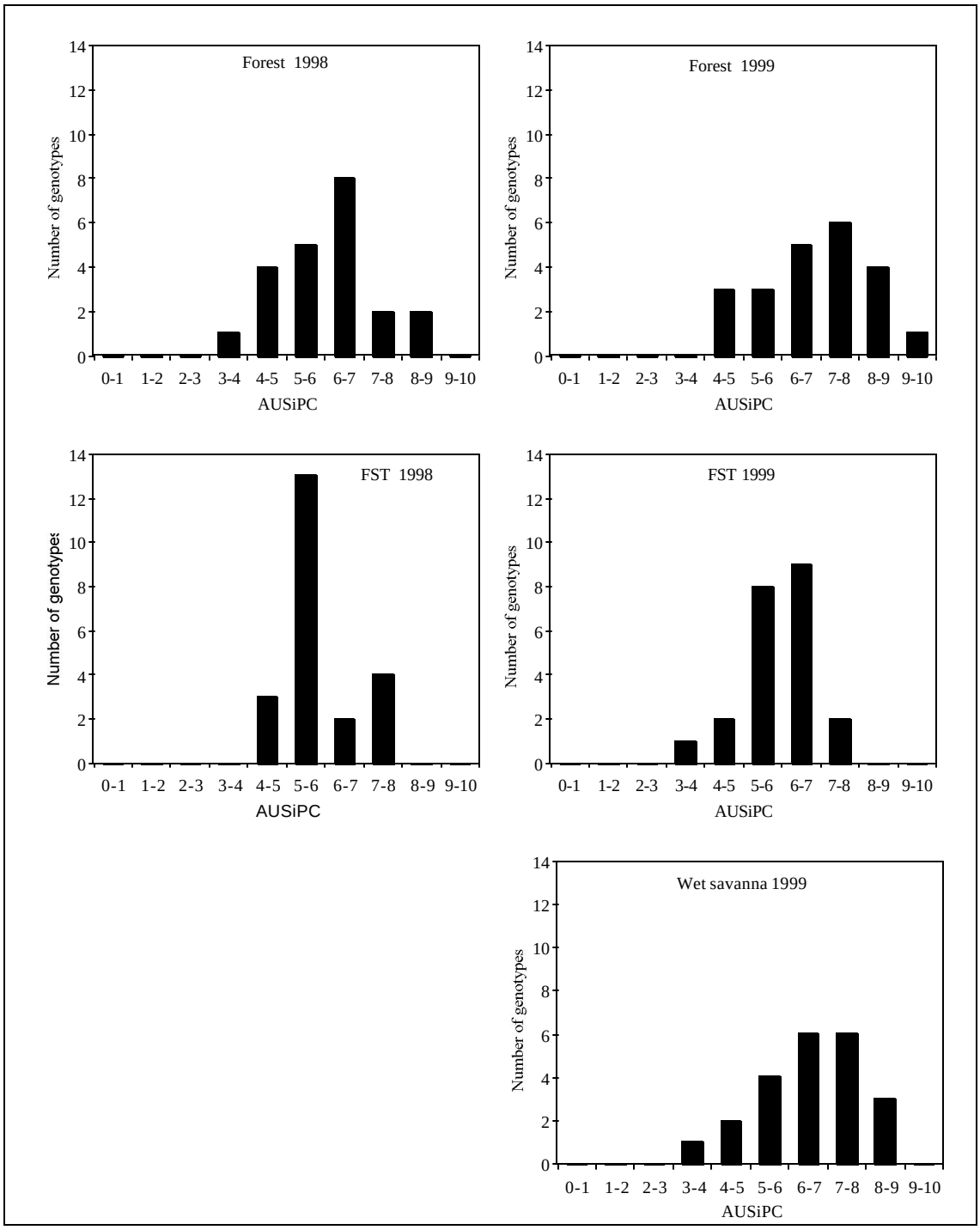

Fig. 2: Frequency distribution of 22 genotypes repeated in each site (ecozone) and year according to their disease development expressed as standardized area under severity index progress curve in inoculated treatments in the forest, forest savanna transition (FST) and wet savanna zones in years 1998 and 1999

The principal component analysis of AUSiPC of 22 genotypes of inoculated plots across all environments revealed six genotypes with low disease severity (genotypes left of the midpoint), with genotypes TMS92/0429 (19), TMS30572 (11) and Main27 (9) being most resistant, and 9 highly susceptible genotypes (genotypes right of the midpoint), with genotypes Lagos (8) and Toma289 (20) being highest susceptible (**Fig. 3**). Among the 22 genotypes, only the genotypes CVTM4 (5), TMS92/0057 (15) and Nakoko (10) with low IPCA2 scores showed negligible interactions with environments and were stable in their disease reaction, while most of the genotypes had high positive or negative IPCA2 scores and revealed medium or high genotype x environment interactions.

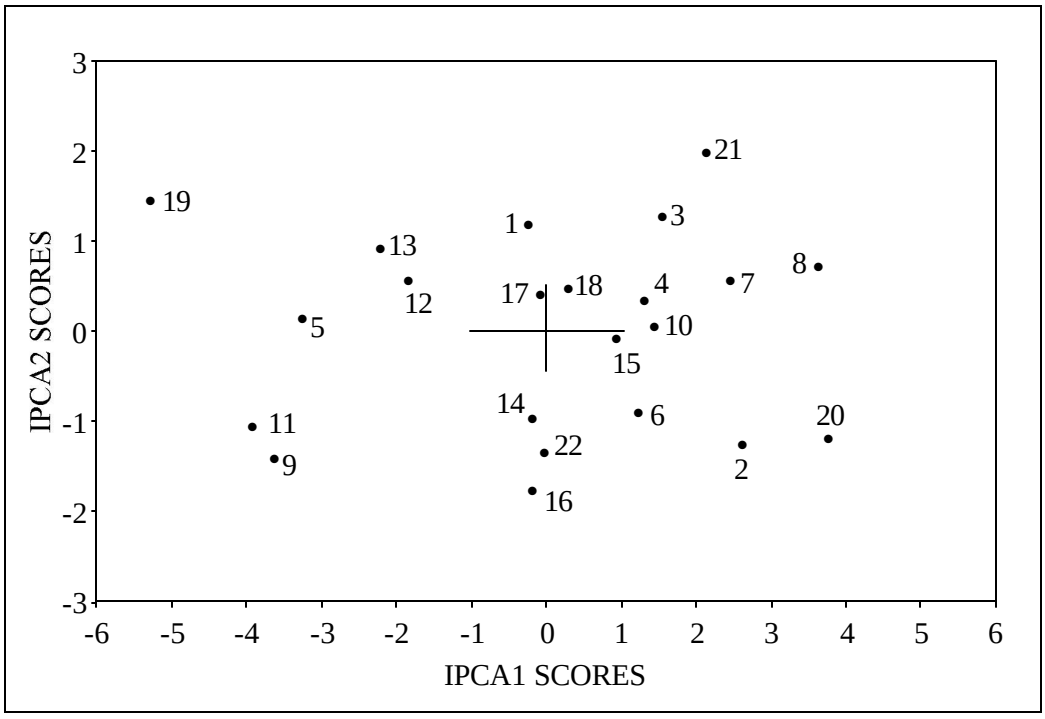

Fig. 3: Relation between IPCA1 and IPCA2 scores of standardized area under severity progress curve of 22 genotypes grown in 10 environments

Genotype identification 1: 312-524, 2: Ankra, 3: Ben86052, 4: Cameroon, 5: CVTM4, 6: Fétonégbodji, 7: Gbazékouté, 8: Lagos, 9: Main27, 10: Nakoko, 11: TMS30572, 12: TMS4(2)1425, 13: TMS91/02316, 14: TMS91/02322, 15: TMS92/0057, 16: TMS92/0067, 17: TMS92/0326, 18: TMS92/0343, 19: TMS92/0429, 20: Toma289, 21: Toma378, 22: Tuaka.

Development of spot, blight and wilt symptoms

The principal component analysis of area under symptom progress curve of percentage of leaves with spot symptoms of 24 genotypes showed six genotypes with lower spot symptom development, with genotypes Main27 (9), TMS30572 (11) and Nakoko (10) being the most resistant, and seven genotypes with higher spot symptom development, with genotypes Lagos (8), TMS92/0057 (15) and Toma378 (21) being the most susceptible (**Fig. 4A**). Analysing blight symptom development, genotypes Main27 (9), TMS92/0429 (19) and TMS92/0067 (16) showed less leaves with blight symptoms, whereas genotypes Toma289 (20), Lagos (8), Gbazékouté (7) and Ankra (2) revealed high susceptibility to the blight symptom (**Fig. 4B**). Comparing wilt symptom development, six genotypes had a lower percentage of leaves showing wilt, with genotypes TMS92/0429 (19), TMS30572 (11), CVTM4 (5) and Main27 (9) being the most resistant, while genotypes Ankra (2), Toma289 (20) and Lagos (8) revealed high susceptibility (**Fig. 4C**). Considering the three symptom types, genotypes Main27 (9), TMS92/0429 (19) and TMS30572 (11) were over all resistant, while genotypes 8, 7, 20, 21, 2 and 3 were susceptible. Genotypes 19, 11, 5 and 9 with low wilt symptom development also showed low spot and blight symptom development, while not all genotypes with low spot and/or blight symptom development revealed a low pecentage of systemic symptoms (wilt), e.g. genotype 1 with resistance against spot and blight was susceptible to wilt, genotype 10 showed only lower spot symptom development, while genotype 16 developed only less blight symptoms. The number of genotypes with low wilt symptom development was lower than the number of genotypes, which showed low spot and blight symptom development. Genotypes 11, 13 and 7 for spot, 9, 17 and 15 for blight, and 6, 7 and 20 for wilt symptoms showed negligible interactions with environments, and were stable in their reaction to the respective symptom types, while genotypes 12 and 15 for spot, 21, 7, 6 and 20 for blight, and 19 and 9 for wilt revealed high genotype x environment interactions. In susceptible genotypes the influence of the environment on leaf symptom development was generally higher than in the more resistant genotypes.

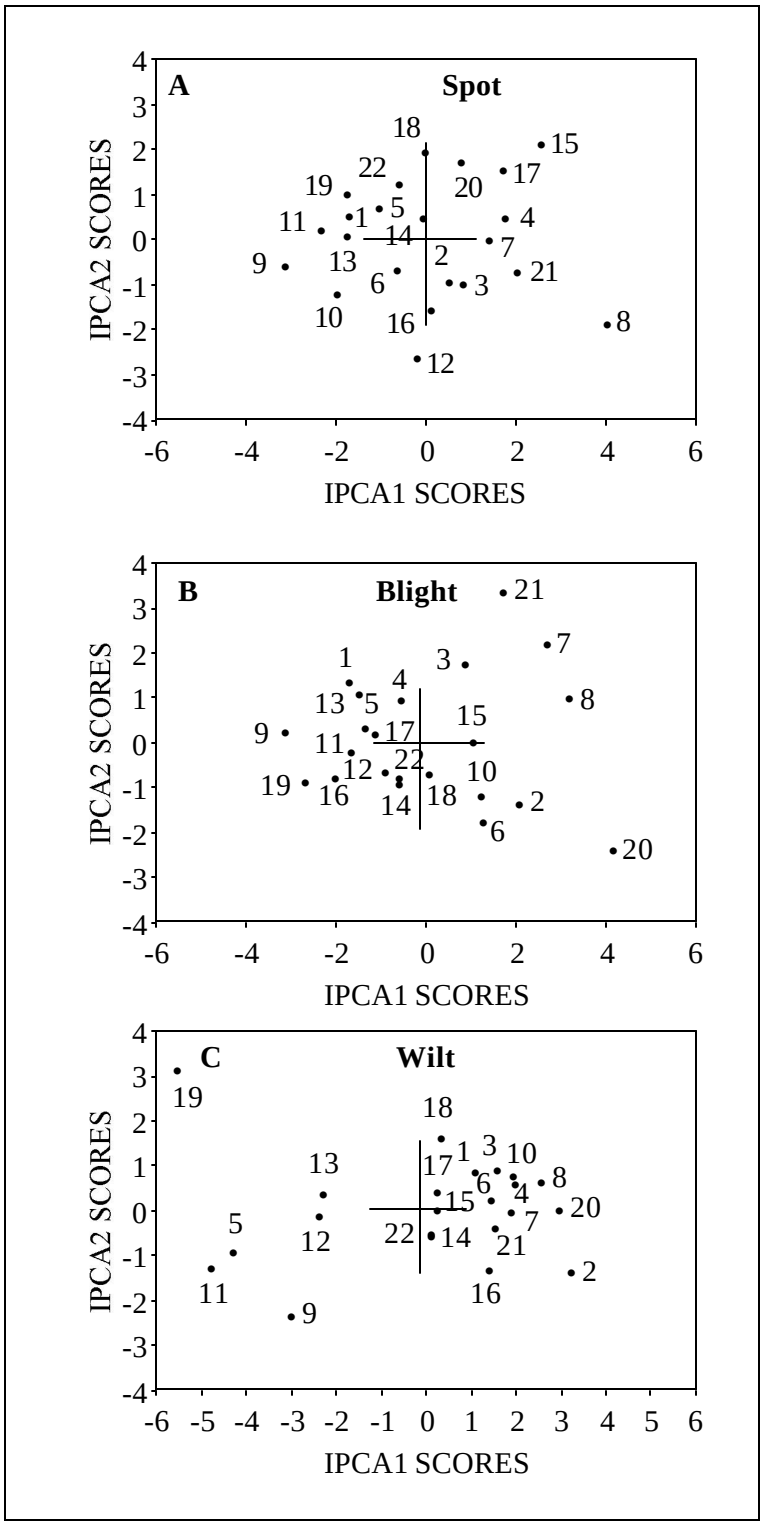

Fig. 4: Relation between IPCA1 and IPCA2 scores of standardized area under severity progress curve of spot (**A**), blight (**B**) and wilt (**C**) of 22 genotypes grown in 10 environments

Genotype identification 1: 312-524, 2: Ankra, 3: Ben86052, 4: Cameroon, 5: CVTM4, 6: Fétonégbodji, 7: Gbazékouté, 8: Lagos, 9: Main27, 10: Nakoko, 11: TMS30572, 12: TMS4(2)1425, 13: TMS91/02316, 14: TMS91/02322, 15: TMS92/0057, 16: TMS92/0067, 17: TMS92/0326, 18: TMS92/0343, 19: TMS92/0429, 20: Toma289, 21: Toma378, 22: Tuaka.

Relationship between symptom types

The relationship between the number of leaves with symptom types expressed as area under symptom type progress curve was analysed. Spot symptoms were significantly positively correlated with blight symptoms in the three sites (ecozones) over the two years, except in the forest zone in 1998 (**Table 3**). In some environments, spot and blight symptoms were positively correlated with wilt symptoms. When data were analysed irrespective of the inoculation treatment, spot, blight and wilt symptoms were generally significantly correlated across sites (ecozones) (**Table 4**).

Table 3: Correlation coefficients between different bacterial blight symptom types (spot, blight and wilt) expressed as area under curve of percentage of leaves with symptom type calculated for 22 genotypes in 10 environments

		Non-inoculated genotypes			Inoculated genotypes		
		spot	blight	wilt	spot	blight	wilt
	spot	1	0.06	0.38*	1	0.01	0.49**
Forest 1998	blight		1	0.25		1	0.32
	wilt			1			1
	spot	1	0.43*	0.51**	1	0.74***	0.57**
Forest 1999	blight		1	0.42*		1	0.62***
	wilt			1			1
	spot	1	0.54**	0.34	1	0.71***	0.38*
FST 1998	blight		1	0.18		1	0.27
	wilt			1			1
	spot	1	0.58***	0.09	1	0.62***	0.21
FST 1999	blight		1	0.28		1	0.44*
	wilt			1			1
	spot	1	0.57***	0.47**	1	0.43*	0.15
Wet savanna 1999	blight		1	0.70***		1	0.39*
	wilt			1			1

* = significant at probability level of p < 0.05; * * = significant at probability level of p < 0.01; ** * = significant at probability level of p < 0.001; FST = forest savanna transition.

Table 4: Correlation coefficients between different bacterial blight symptom types (spot, blight and wilt) expressed as area under curve of percentage of leaves with symptom type calculated for 22 genotypes in 10 environments, irrespective of inoculation, but by ecozone and year

		spot	blight	wilt
	spot	1	0.36**	0.54***
Forest 1998	blight		1	0.41**
	wilt			1
	spot	1	0.71***	0.49***
Forest 1999	blight		1	0.44***
	wilt			1
	spot	1	0.66***	0.36**
FST 1998	blight		1	0.23
	wilt			1
	spot	1	0.60***	0.15
FST 1999	blight		1	0.36**
	wilt			1
	spot	1	0.61***	0.29*
Wet savanna 1999	blight		1	0.51***
	wilt			1

* * = significant at probability level of p < 0.01; ** * = significant at probability level of p < 0.001; FST = forest savanna transition.

When the relationship between severity of symptom types expressed as area under curve of percentage of leaves with symptoms was determined irrespective of genotype, inoculation treatment, ecozone and year, spot and blight symptoms were highly significantly correlated ($r = 0.63$, $p < 0.001$) (**Table 5**). Analysing the relationship between symptom types for each genotype across treatments, sites and years revealed significant positive correlations between spot and blight for 15 genotypes, whereas generally no relationship between spot and wilt, and blight and wilt symptom development was observed (**Table 6**). Only in genotypes TMS92/0057 and TMS92/0326 spot, and spot and blight, respectively, were significantly negatively correlated to wilt symptom development.

Table 5: Correlation coefficients between severity of symptom types expressed as area under curve of percentage of leaves with symptom type, and between them and root yield calculated for 21[1] genotypes in 10 environments, irrespective of inoculation, ecozone and year

	spot	blight	wilt	root DW
spot	1	0.63***	0.02	-0.03
blight		1	0.02	-0.07
wilt			1	0.03
root DW				1

** * = significant at probability level of $p < 0.001$; root DW = root dry weight. [1]Genotype Tuaka not included due to a missing root weight value in the forest savanna transition zone in the inoculated plot in year 1999.

Cassava yield

In the forest zone, significant differences in root yield were found between check genotypes over the two years. Genotype TMS30572 recorded significantly higher root yield (20.2 t/ha and 33.2 t/ha in 1998 and 1999, respectively) than Ben86052 (14.6 t/ha and 22.9 t/ha, respectively) over the two years in inoculated plots ($p = 0.01$), but no significant differences were observed between these and the local check genotype Gbazékouté (data not shown, **Annex 2**). Genotypes TMS91/02322 and TMS30572 in 1998 and TMS30572 and TMM92/0429 in 1999 recorded the highest root yield of 22.6 t/ha and 20.2 t/ha, and 33.2 t/ha and 29.2 t/ha, respectively, while Toma289 and Fétonégbodji were the lowest yielding genotypes with 4.4 t/ha and 4.3 t/ha in 1998 and 1999, respectively. In the forest savanna transition zone, a significant difference was observed between check genotypes in non-inoculated plots in 1999, with Ben86052 (18.2 t/ha) yielding higher than TMS30572 (11.9 t/ha) and Gbazékouté (9.4 t/ha). Genotypes TMS92/0057 (29.1 t/ha), CVTM4 (28.1 t/ha) and Lagos (25.3 t/ha) recorded a high dry root weight, and the local genotypes Fétonégbodji (0.3 t/ha) and Tuaka (1 t/ha) the lowest yield. In the wet savanna zone, the highest yield was obtained by genotype TMS92/0057 (23.1 t/ha and 21.1 t/ha in non-inoculated and inoculated plots, respectively), however, no significant difference was observed between check genotypes (**Annex 2**).

Table 6: Correlation coefficients of severity of symptom types expressed as area under curve of percentage of leaves with symptom type calculated for 22 genotypes in 10 environments, irrespective of inoculation, ecozone and year, but per genotype (genotypes in decreasing order from susceptible to resistant, according to the total mean of AUSiPC, see Table 10)

Genotype		spot	blight	wilt	Genotype		spot	blight	wilt
Lagos	spot	1	0.66*	0.36	312-524	spot	1	0.96***	-0.28
	blight		1	-0.12		blight		1	-0.27
	wilt			1		wilt			1
Toma289	spot	1	0.46	0.004	TMS92/0326	spot	1	0.93***	-0.78**
	blight		1	-0.17		blight		1	-0.72*
	wilt			1		wilt			1
Gbazékouté	spot	1	0.47	-0.48	Tuaka	spot	1	0.96***	-0.21
	blight		1	-0.53		blight		1	-0.09
	wilt			1		wilt			1
Toma378	spot	1	0.49	-0.28	TMS92/0067	spot	1	0.71*	0.17
	blight		1	-0.58		blight		1	0.16
	wilt			1		wilt			1
Ankra	spot	1	0.41	0.25	TMS91/02322	spot	1	0.75*	-0.27
	blight		1	0.23		blight		1	-0.48
	wilt			1		wilt			1
Ben86052	spot	1	0.83**	0.01	TMS4(2)1425	spot	1	0.83**	0.28
	blight		1	-0.46		blight		1	0.25
	wilt			1		wilt			1
Nakoko	spot	1	0.85**	0.15	TMS91/02316	spot	1	0.95***	-0.36
	blight		1	-0.08		blight		1	-0.41
	wilt			1		wilt			1
Cameroon	spot	1	0.52	-0.57	CVTM4	spot	1	0.49	-0.45
	blight		1	-0.18		blight		1	0.09
	wilt			1		wilt			1
Fétonégbodji	spot	1	0.83**	0.21	Main27	spot	1	0.91***	-0.42
	blight		1	0.27		blight		1	-0.53
	wilt			1		wilt			1
TMS92/0057	spot	1	0.28	-0.70*	TMS30572	spot	1	0.90***	-0.4
	blight		1	-0.31		blight		1	-0.47
	wilt			1		wilt			1
TMS92/0343	spot	1	0.29	-0.34	TMS92/0429	spot	1	0.87***	-0.01
	blight		1	-0.51		blight		1	-0.04
	wilt			1		wilt			1

* = significant at probability level of p < 0.05; * * = significant at probability level of p < 0.01; ** * = significant at probability level of p < 0.001.

The principal component analysis of dry root yield of 21 genotypes across 10 environments revealed four genotypes with low root yield, with genotypes Fétonégbodji (6) and Toma289 (20) being the lowest yielding, and one high yielding genotype, TMS92/0057 (15). Seven genotypes with low IPCA2 scores showed negligible interactions with environments (**Fig. 5**).

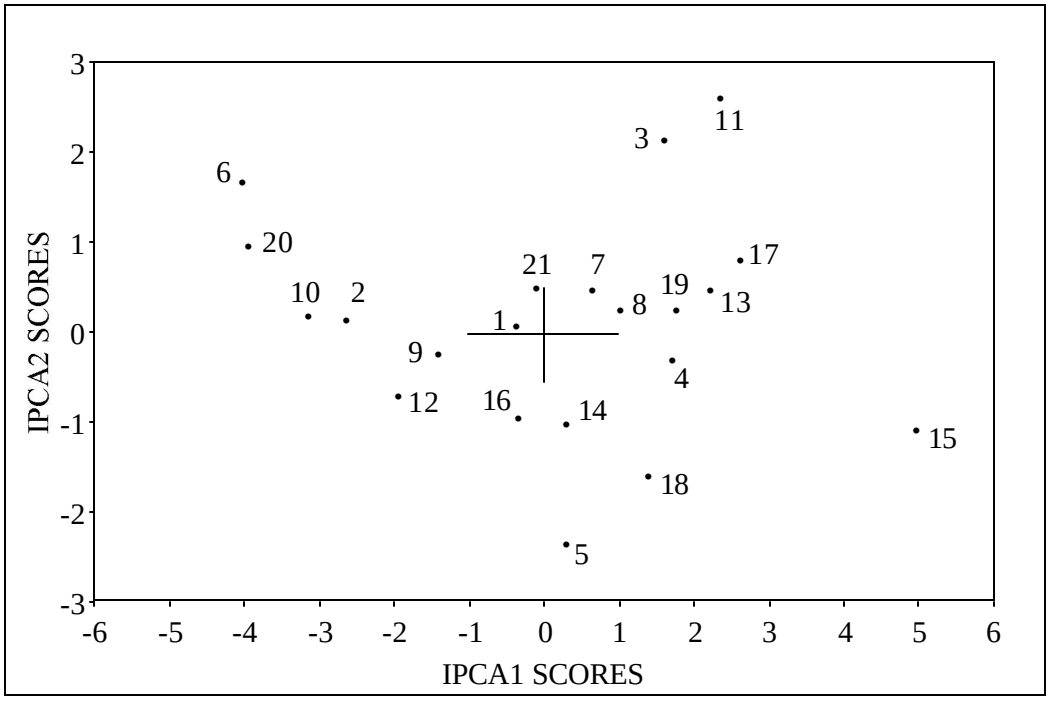

Fig. 5: Relation between IPCA1 and IPCA2 scores of standardized root dry weight of 21 genotypes grown in 10 environments

Genotype identification 1: 312-524, 2: Ankra, 3: Ben86052, 4: Cameroon, 5: CVTM4, 6: Fétonégbodji, 7: Gbazékouté, 8: Lagos, 9: Main27, 10: Nakoko, 11: TMS30572, 12: TMS4(2)1425, 13: TMS91/02316, 14: TMS91/02322, 15: TMS92/0057, 16: TMS92/0067, 17: TMS92/0326, 18: TMS92/0343, 19: TMS92/0429, 20: Toma289, 21: Toma378.

Relationship between symptom severity and root yield

Pearson correlation analysis of disease severity (AUSiPC) and dry root yield revealed a significant negative correlation between AUSiPC and root yield in inoculated plots in the forest zone in 1998 and 1999 (r = -0.48, p = 0.006 and r = -0.50, p = 0.004, respectively), and in non-inoculated plots in the forest savanna transition zone in 1998 (r = -0.43, p = 0.017) and in the wet savanna zone in 1999 (r = -0.51, p = 0.003) (**Table 7**).

Table 7: Correlation coefficients between disease development expressed as area under severity index progress curve (AUSiPC) and root yield calculated with 21 genotypes grown in 10 environments

		Non-inoculated genotypes	Inoculated genotypes
Forest	1998	0.34	-0.48**
Forest	1999	-0.21	-0.50**
Forest savanna	1998	-0.43*	-0.03
transition	1999	-0.32	-0.22
Wet savanna	1999	-0.51**	-0.07

** = significant at probability level of $p < 0.01$; * = significant at probability level of $p < 0.05$.

Significant correlations were observed between spot, blight and wilt symptom development and dry root weight in some environments (**Table 8**). In non-inoculated plots the blight symptom was generally negatively correlated to yield, while in inoculated plots wilt symptoms were generally negatively correlated to yield.

Table 8: Correlation coefficients between bacterial blight symptom types expressed as area under curve of percentage of leaves with symptom type and root yield calculated for 21 genotypes grown in 10 environments

		Non-inoculated genotypes			Inoculated genotypes		
		spot	blight	wilt	spot	blight	wilt
Forest	1998	0.33	0.40*	-0.004	-0.25	-0.40*	-0.34
Forest	1999	0.07	-0.23	-0.21	-0.40*	-0.46*	-0.43*
Forest savanna	1998	-0.27	-0.38*	-0.29	0.14	0.24	-0.43*
transition	1999	0.01	-0.41*	-0.23	0.19	0.01	-0.37*
Wet savanna	1999	-0.15	-0.39*	-0.58***	0.08	0.08	-0.31

** * = significant at probability level of $p < 0.001$; * = significant at probability level of $p < 0.05$.

Analysing the relationship between area under curve of symptom types and root yield irrespective to inoculation treatment, ecozone and year, no significant relationship was observed (**Table 5**). When the same data were analysed by genotype, a significant relationship was generally not found (**Table 9**). Only in genotype Cameroon the blight symptom was related to a significant yield loss, while in genotypes Ben86052 and in TMS92/0067 the wilt symptom and in Main27 the spot and blight symptoms were positively correlated to yield.

Table 9: Correlation coefficients between severity of symptom types expressed as area under curve of percentage of leaves with symptom type and root yield calculated with 21 genotypes in 10 environments, irrespective of inoculation, ecozone and year, but per genotype

Genotype	spot	blight	wilt
312-524	0.08	-0.09	0.26
Ankra	0.04	0.59	0.52
Ben86052	0.08	-0.25	0.81**
Cameroon	-0.47	-0.86**	0.46
CVTM4	-0.06	0.49	0.39
Fétonégbodji	-0.17	-0.11	-0.05
Gbazékouté	0.21	0.28	0.18
Lagos	-0.14	-0.27	0.58
Main27	0.65*	0.71*	-0.57
Nakoko	-0.05	0.22	-0.41
TMS30572	-0.41	-0.10	0.04
TMS4(2)1425	0.37	0.23	0.50
TMS91/02316	-0.25	-0.38	0.35
TMS91/02322	0.08	0.12	-0.16
TMS92/0057	-0.38	0.29	0.22
TMS92/0067	0.07	0.18	0.66*
TMS92/0326	-0.36	-0.49	0.34
TMS92/0343	-0.03	-0.21	0.37
TMS92/0429	-0.30	-0.24	0.33
Toma289	-0.17	-0.24	0.50
TOMA378	0.16	-0.28	0.62

* = significant at probability level of $p < 0.05$; * * = wsignificant at probability level of $p < 0.01$.

Considering the mean AUSiPC and root yield across 10 environments, 22 genotypes were ranked in decreasing order (**Table 10**). Genotypes TMS91/02316, TMS30572 and TMS92/0429 belonged to the more resistant group (AUSiPC lower than 5.4) and had a high root yield (14 t/ha), while Lagos, Ben86052, TMS92/0057 and TMS92/0343 had a higher disease severity (AUSiPC > 5.9), but also a high root yield. To identify a possible tolerant reaction of the latter genotypes, a regression analysis between disease severity (AUSiPC) and root dry weight was performed. No significant relationship between severity and root yield was found ($p > 0.05$, $r^2 =$ 0.07, 0.02, 0.04 and 0.004, respectively). With increasing disease severity, root yield was not affected (**Fig. 6**).

Table 10: Ranking of 22 genotypes according to means for AUSiPC and dry root yield (t/ha) in 10 environments

Genotypes	Mean AUSiPC	Genotypes	Dry root yield (t/ha)
Lagos	7.1	TMS92/0057	21.3
Toma289	6.9	TMS92/0326	17.1
Gbazékouté "C"	6.6	TMS91/02316	16.6
Toma378	6.6	TMS30572 "C"	16.6
Ankra	6.6	Cameroon	16.1
Ben86052 "C"	6.4	TMS92/0429	15.8
Nakoko	6.4	TMS92/0343	15.5
Cameroon	6.3	Ben86052 "C"	15.0
Fétonégbodji	6.2	Lagos	14.7
TMS92/0057	6.1	Gbazékouté "C"	13.8
TMS92/0343	5.9	CVTM4	13.8
312-524	5.9	TMS91/02322	13.3
TMS92/0326	5.8	TMS92/0067	12.2
Tuaka	5.8	Toma378	11.9
TMS92/0067	5.7	312-524	11.9
TMS91/02322	5.7	Main27	8.6
TMS4(2)1425	5.4	Tuaka	8.5[1]
TMS91/02316	5.2	TMS4(2)1425	8.4
CVTM4	4.8	Ankra	7.5
Main27	4.5	Nakoko	6.4
TMS30572 "C"	4.5	Toma289	5.3
TMS92/0429	4.2	Fétonégbodji	4.5

"C" = check genotype; AUSiPC = area under severity index progress curve; [1]Mean value of 9 environments instead of 10 due to missing value of root dry weight in the inoculated plots in the forest savanna transition zone in year 1999.

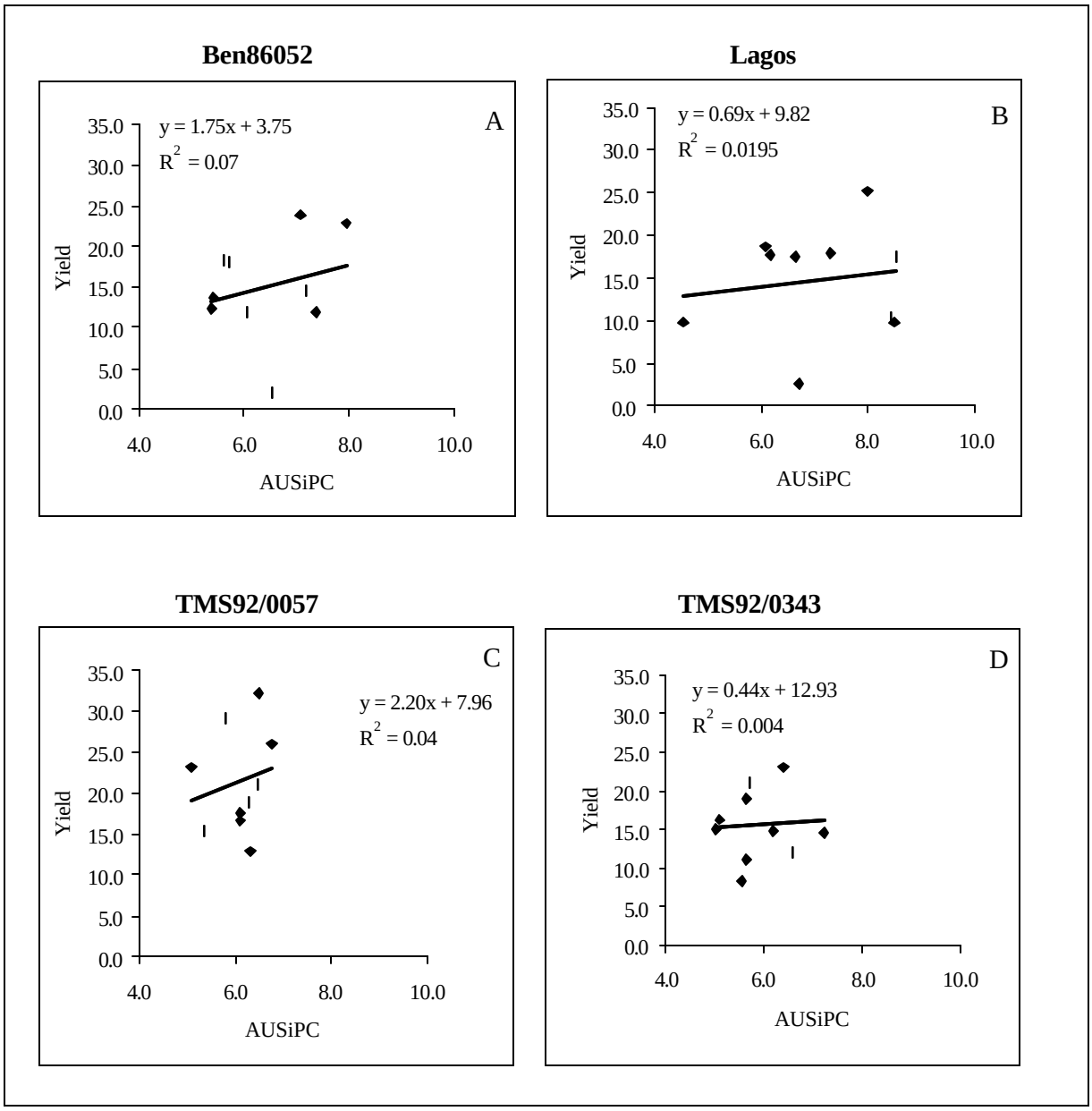

Fig. 6: Regression analysis between disease severity (AUSiPC) and root dry weight of genotypes Ben86052, Lagos, TMS92/0057 and TMS92/0343

3.4 Discussion

Cassava has a long vegetative cycle and severe epidemics such as cassava bacterial blight develop on susceptible genotypes under favorable environmental conditions. Since chemical control is not possible, and the systemic infection by *X. axonopodis* pv. *manihotis* facilitates the distribution of the pathogen by vegetative propagation of cassava, host plant resistance is the best means for a long term control of cassava bacterial blight (Wydra and Rudolph, 1999). In the present study, 24 cassava genotypes were screened for resistance to cassava bacterial blight after spray-inoculation in three sites in three agroecological zones of Togo over two years, except in the wet savanna site with one trial year, to identify high yielding genotypes with stable resistance to cassava bacterial blight. No genotype with resistance across the sites (ecozones) was found, but genotypes TMS92/0429, TMS30572, CVTM4 and TMS91/02316 had a lower disease severity combined with high yield. The difference in rainfall quantity and distribution between the sites in the forest and the forest savanna transition ecozones in the trial years were marginal, since in both sites a rainy season of 7 months was recorded.

No cultivar with complete disease resistance was reported from field evaluation of cassava cultivars in Latin America (CIAT, 1995; 1998). Also, Fanou (1999) and Zinsou et al. (2003a) did not find completely resistant genotypes among twenty-three and sixteen genotypes, respectively, tested in ecozones of Nigeria and Benin, respectively.

High variability in disease expression was observed. The three genotypes Lagos, Toma289 and Toma378 showed susceptible reactions to CBB across ecozones and over years, while the other genotypes revealed genotype x environment interactions, with variability between and/or within ecozones over the two years. Genotype x environment interactions in reaction to CBB as well as to other cassava diseases among cassava cultivars were reported from Benin and Nigeria (Fanou, 1999; Dixon and Nukenine, 2000; Zinsou et al., 2003a). Field evaluation of CBB of Togolese cassava cultivars in the forest savanna transition zone had revealed Fétonégbodji and Tuaka as highly susceptible genotypes (Boher and Agbobli, 1992), which, in our

studies, showed genotype x environment interactions, with medium resistant or susceptible reactions depending on the environments.

Genotype TMS30572, a widely distributed improved genotype in West Africa, disseminated by the International Institute of Tropical Agriculture (IITA) as resistant to CBB, revealed medium resistance in four of the five environments and resistance in one environment, the forest zone, while it was relatively resistant to cassava bacterial blight in former field trials in the forest savanna transition and the humid forest zones (Boher and Agbobli, 1992; Akparobi et al., 1998; Fanou, 1999). Genotype TMS91/02322 showed medium resistance in four environments and was susceptible in one environment, while Fanou (1999) identified this genotype as susceptible in two environments. The evaluation of genotype Ben86052 as susceptible to bacterial blight in four of five environments confirmed former observations (Fanou, 1999).

The highest cassava bacterial blight severities were recorded in the forest zone. This could be due to the high rainfall and relative humidity in this ecozone, and the possible effect of the progressive degradation of the forest to a forest savanna allowing higher fluctuations of day/night temperatures. Disease severity was enhanced by wide fluctuations in night/day temperatures during the rainy season, especially in the range of 15 to 30 °C (Lozano, 1986). The importance of rainfall and high relative humidity in the development of cassava diseases was emphasized by Terry (1976), and by Fanou (1999) for cassava bacterial blight, who observed highest cassava bacterial blight severity in the humid forest in 1996. On the contrary, during a cassava bacterial blight survey in Togo conducted by Boher and Agbobli (1992), and in surveys in Ghana and Cameroon (Wydra and Verdier, 2002; Wydra and Msikita, 1998), the disease symptoms were not found in the forest zones and only rarely observed in neighbouring areas. However, a recent cassava disease survey in Togo revealed high cassava bacterial blight incidence and severity also in the forest zone (Banito et al., 2000, 2001), suggesting that conditions for epidemics are becoming more favorable in this ecozone.

Genotypes TMS92/0429, TMS92/0343, TMS92/0326, TMS92/0057, TMS91/02316 and CVTM4 were medium resistant in at least three of the five environments, when

spray-inoculated under field conditions, but resistant in greenhouse trials after stem-inoculation with *X. axonopodis* pv. *manihotis* strains from Benin, Nigeria and Uganda (Banito et al. 2002). On the other hand, genotypes Toma289 and Toma378, susceptible under field conditions, were resistant after stem-inoculation. The local genotype Gbazékouté was highly resistant to stem-inoculation with strains not originating from Togo under controlled conditions, but showed high suceptibility under field conditions in four environments. However, some genotypes such as Ben86052, Lagos, Ankra, Nakoko, and Fétonégbodji, which were susceptible after stem-inoculation, revealed susceptibility in at least three environments under field conditions. On the other hand, the highest susceptible genotype Toma159 after stem-inoculation was medium resistant in the four environments in which it was tested, and genotype Main27, also susceptible after stem-inoculation, reacted medium resistant in three environments and resistant in two environments. These observations point to a high genotype x pathotype interaction. Pathotypes overcoming the resistance of genotype Gbazékouté may have developed in the region, while in Togo also some genotypes may have been selected which are adapted to local conditions (strains) and, thus, are medium resistant or resistant. Also, Banito et al. (2002) and Wydra et al. (2003b) in Africa and Restrepo et al. (2000a) and Restrepo and Verdier (1997) in Colombia recently described pathotypes of *X. axonopodis* pv. *manihotis*, which caused disease in only some genotypes.

Lozano and Laberry (1982) observed that the plant reaction to cassava bacterial blight under controlled conditions and during the first cycle of field testing were similar, however, some genotypes that were rated resistant under controlled conditions showed susceptibility under field evaluation. Resistance in cassava to *X. axonopodis* pv. *manihotis* is polygenic (Sãnchez et al., 1999) and pathotype-specific (Wydra et al., 2003b). Mew and Natural (1993) suggested that multigenic resistance in *Xanthomonas* diseases of bean and cotton may be ineffective in some areas due to influences of the environment. Cassava genotypes selected for resistance to cassava bacterial blight in areas of high disease pressure in Colombia were susceptible after artificial leaf-inoculation under conditions optimal for disease development (Flood et al., 1995). Comparing the screening of cassava genotypes under greenhouse and field conditions revealed that genotypes resistant under controlled conditions were also resistant in the field, but genotypes susceptible in the greenhouse trial could be resistant

under field conditions (Restrepo et al., 2000b).

Thus, the pathotype as well as the environment influence the reaction of the cassava plant and determine the high genotype x environment interaction. Genotypes also showed genotype x environment interactions in their reaction to the different cassava bacterial blight symptom types, spot, blight and wilt. Thus, the observations of genotypes with some resistance to spot and/or blight symptoms combined with a suseptibility to wilt symptoms suggest the existence of an independent resistance mechanism on stem level. Also, the number of genotypes with some resistance against the leaf symptoms was higher than the number of genotypes showing resistance against systemic symptoms. Nevertheless, it has to be considered that the reactions in some genotypes were highly influenced by the environment, which may have an increasing or inhibiting effect on the development of various symptom types. Generally, the influence of the environment on the development of the wilt symptom in the more resistant genotypes was higher compared to the development of leaf symptoms in the more resistant genotypes. Thus, some genotypes (e.g. 9, 11, 19) with resistance against wilt showed highest interactions with the environment, indicating that a possible mechanism inhibiting the development of the wilt symptom is depending on the ecological condititions.

The analysis of the relationship between symptom types comparing genotypes revealed that generally, neither the spot nor the blight symptom development were significantly correlated to the development of systemic symptoms and, therefore, supports the hypothesis that leaf and stem symptom development in the more resistant genotypes are regulated by different resistance mechanisms. Especially the reaction of genotypes with a strong negative correlation between spot and blight symptom development on the one hand and wilt symptom developoment on the other hand (e.g. in genotypes TMS92/0057 and TMS92/0326) indicate independent mechanisms of resistance on leaf and stem level, depending on the cassava genotype.

Also Zinsou et al. (2002; 2003a) observed differential reactions of genotypes to leaf- compared to stem-inoculation under controlled conditions. Wydra et al. (2003a) suggested the involvement of cell wall characterstics, specifically pectic

polysaccharides, in the resistance reaction of cassava leaves to *X. axonopodis* pv. *manihotis*. Thus, in breeding for resistance, genotypes with different types of resistance or a combination of both types should be considered.

High variability in cassava dry root yield was observed across and within ecozones over the two years, denoting high genotype x environment interactions as was also reported by other authors for cassava yield (Otoo et al., 1994) and yield components (Dixon and Nukenine, 2000), irrespective of disease factors. Widely grown local genotypess such as Fétonégbodji, Nakoko, Ankra, Tuaka and Main27 revealed a generally low yield potential across ecozones, even when symptom severity was low (e.g. gentoype Main27), while most of the improved genotypes had higher root production, among them TMS92/0057, with highest production across ecozones, and TMS30572 with the highest root yield of 33.2 t/ha in the forest zone in 1999 (data not shown) recorded during the trials.

A significant negative correlation between disease severity and root yield was found in some environments. The principal component analysis results were in accordance with the grouping based on the mean AUSiPC values, thus each of both methods can be used for ranking. Although cassava bacterial blight severity generally was higher in the forest zone than in other ecozones, root yield was highest in this ecozone (data not shown), and, even higher than in the forest savanna transition zone where the lowest disease severity was recorded.

Considering the influence of symptom types on root yield, blight and wilt were found to significantly decrease root yield. Nevertheless, analyzing this relationship by genotypes, few significant decreases were generally not observed with one exception, but also significant increases were observed in three genotypes. This may be due to the growth habit of some genotypes, which quickly formed new sprouts resulting in high increases in photosynthetic area, when one stem was heaviliy infected (own observations).

Thus, depending on the environment and the genotype, cassava may easily recover from the disease under favorable growing conditions, or suffer losses in leaf, stem and root material (data not shown) under harsh conditions. These observations indicate a

high genotype x environment interaction in disease expression as well as root formation, influencing the interaction between plant and pathogen, but also with high impact on root yield, independent of the disease. Additionally, pathogenic specialization in form of pathotype prevalence in different ecozones resulting in pathotpye x genotype interactions has to be considered (Banito, this thesis). Therefore, a prediction of yield loss due to bacterial blight and the determination of a threshhold for loss seems impossible.

Fokunang et al. (2000b) reported that cassava bacterial blight incidence was significantly negatively correlated with storage root weight and fresh tuber number of cassava, respectively. Also, the percentage of dry matter content of yield was significantly positively correlated with cassava bacterial blight severity (Fokunang et al., 2000a). But, they only evaluated trials of one year and used only disease data of a geveral scale of 1 to 5 at 3 and 6 months after planting for their analysis, while our disease evaluation considered the development of different symptom types over the whole growing period. However, Fanou (1999), using a similar disease evaluation method, found no relation between disease severity and dry root yield.

Some genotypes including Lagos, TMS92/0057, TMS92/0343 and Ben86052, though highly susceptible, had a high root yield, and could be identified as tolerant, since they did not react with a yield decrease on an increasing disease level. A tolerance effect of gentoype Ben86052 in reaction to cassava bacterial blight, observed across ecozones and years, was also described by Wydra (2002).

In conclusion, differences in reaction of genotypes to cassava bacterial blight and in yield production across and/or within ecozones were observed. Genotypes differ in the development of different symptom types, indicating the existence of independent mechanisms of resistance in different plant parts. Also, the relationship between symptom severity and between different symptom types and yield depends on the genotypes, but also on the environment. Thus, tolerance reactions were observed in some genotypes.

Gentoypes TMS92/0429, TMS30572 and TMS91/02316 with low disease severity and high root yield could be recommended to famers. Genotypes TMS92/0326,

TMS92/0057, Cameroon and Ben86052, tolerant to the disease, may be high yielding, but should be avoided by farmers due to the risk of dissemination of inoculum. Genotypes Main27 and CVTM4, resistant, but with low root yield could be recommended to breeders to introduce their resistance characteristics into the breeding materials. Additionally, genotypes TMS30572 and TMS92/0429 genotypes should be used to introgress their high resistance to the wilt symptom into genotypes with susceptibility to systemic symptoms.

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4 Strain x genotype interactions of cassava genotypes and African *Xanthomonas axonopodis* pv. *manihotis* strains

Abstract

Twenty-four improved and local genotypes from Togo were screened for resistance to cassava bacterial blight by stem-inoculation with four highly virulent *Xanthomonas axonopodis* pv. *manihotis* strains from different geographic origins in Africa under controlled conditions. The local genotypes Nakoko and Toma159 were most susceptible against the four strains, while most other genotypes including the reference genotype Ben86052, with susceptible reaction against at least two strains were resistant to at least one strain. Six genotypes showed a resistant reaction against the four strains. Among them, the local genotype Gbazékouté and the improved CVTM4 were the most resistant ones. Six groups of genotypes, with differential reactions to the strains were formed, and the strains were defined as pathotypes.

Key words: CBB, varieties, resistance, *X. axonopodis* pv. *manihotis*, pathotypes.

4.1 Introduction

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is one of the major sources of carbohydrate throughout Asia's and Africa's lowland tropics (Nilmanee, 1986) and one of the most important crops in Africa (FAO/GIEWS, 1995). It provides smallholder households with cash income and low-income urban consumers with a low-cost carbohydrate source (Nweke, 1994). Cassava production is reduced due to many abiotic and biotic constraints, with the attack by pests and diseases being among the major ones (Nilmanee, 1986; Hahn et al., 1989). Among the pathological constraints of cassava production, bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Bondar, 1915), is one of the most severe diseases in South America and Africa (Lozano, 1986). Typical symptoms of cassava bacterial blight (CBB) include water-soaked angular leaf spots, leaf blight and wilt, defoliation, exudation on stems, petioles and leaves, vascular necrosis and dieback. Cassava yield losses of more than 50% due to CBB were reported (Fanou, 1999; Wydra and Rudolph, 1999).

Most cassava growers are small farmers (Phillips, 1974) with traditional technical know-how and few economic resources (Lozano and Laberry, 1982), and usually produce their own planting material. Since chemical control of the disease does not exist, growing resistant cultivars as element of an integrated control system (Wydra and Rudolph, 1999; Wydra et al., 2003a) is an important control measure.

Defense mechanisms against *X. axonopodis* pv. *manihotis* were observed in the vascular system of stems of infected cassava plants (Kpémoua et al., 1996), with differences in reactions comparing susceptible and resistant cultivars. Resistance in *M. esculenta* introgressed from a wild relative, *M. glaziovii* is polygenic and additively inherited. Genetic diversity and resistance to cassava bacterial blight revealed a high level of polymorphism among cassava varieties (Sãnchez et al., 1999). Jorge et al. (2000) identified six regions of the cassava genome controlling resistance to *X. axonopodis* pv. *manihotis* strains, confirming the polygenic character of the resistance. A specific interaction between the cassava plant and the pathogen was suggested, and resistance markers specific for African strains were recently identified (Wydra et al., 2003a).

Variation has been observed among *X. axonopodis* pv. *manihotis* strains in biochemical and physiological (Fessehaie, 1997), serological (Wydra et al., 1999) and genomic characteristics (Restrepo et al., 1999; Assigbétsé et al., 1999). Strain x genotype interactions were reported after stem-inoculation (Restrepo and Verdier, 1997), and pathotypes were defined among *X. axonopodis* pv. *manihotis* strains in Colombia (Restrepo et al., 2000) and Africa (Wydra et al., 2003b).

Local and improved cultivars from Togo were not characterized for their reaction to strains of *X. axonopodis* pv. *manihotis*. Therefore, in the present studies selected cassava genotypes from Togo and from an international collection were inoculated with strains from various geographic origin and their reaction evaluated.

4.2 Materials and methods

Planting materials and bacterial strains

Cuttings from the 24 local, Togolese and improved cassava genotypes Ankra, Cameroon, Fétonégbodji, Gbazékouté, Lagos, Nakoko, Tuaka (local), and 312-524, Ben86052, CVTM4, Main27, TMS30572, TMS4(2)1425, TMS91/02316, TMS92/0057, TMS92/0067, TMS91/02322, TMS92/0326, TMS92/0343, TMS92/0429, Toma159, Toma219, Toma289 and Toma378 (improved by IITA), derived from plants apparently free of cassava bacterial blight symptoms, were received from ITRA Lomé/Togo, or farmers fields, and Ben86052 and TMS30572, the susceptible and resistant standard genotypes, respectively, from IITA (International Institute of Tropical Agriculture), Benin-Station. The three highly virulent strains of *X. axonopodis* pv. *manihotis* from different geographic origins GSPB2506, GSPB2507 and GSPB2511 (Göttinger Sammlung phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität Göttingen, Germany) isolated by K. Wydra (IITA, Cotonou, Benin), in Cotonou, Benin and in Ibadan and Onne, Nigeria, respectively, and the strain Uganda12, isolated by B. Boher (IRD, France) in Uganda were used.

Planting, maintenance and inoculation

Cuttings were planted in pots with field soil in a glasshouse (25 to 30 °C) at the IITA Benin-Station. Water was provided to plants when necessary during the experimental period.

X. axonopodis pv. *manihotis* strains were grown for 48 hours on glucose yeast calcium carbonate agar medium (glucose 5g/l, yeast extract 5g/l, calcium carbonate 10g/l, agar 15g/l) (Dye, 1962). One-month old, vigorous plants were stem-inoculated with bacterial cultures of the four *X. axonopodis* pv. *manihotis* strains by stem puncture using a sterile toothpick with inoculum taken directly from the agar plate (Maraite et al., 1981; Restrepo and Verdier, 1997). Each cassava genotype was inoculated with each of the four *X. axonopodis* pv. *manihotis* strains in four replications. Control plants were stem-punctured using sterile toothpicks without inoculum. Plant height was measured on the day of inoculation.

Symptom assessment

Symptoms were evaluated from 5 days post inoculation every five up to 30 days on a 1 to 5 scale: class 1 - no symptoms, class 2 - wilting of 1 leaf, class 3 - wilting of 2 to 4 leaves, class 4 - wilting of more than 4 leaves, class 5 - dieback of the plant. The area under the disease progress curve (AUDPC) was calculated on a single plant basis by the trapezoidal integration over the whole observation period as follows (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001):

$AUDPC = \sum_i [(DS_i + DS_{i-1}) \times (t_i - t_{i-1})]/2$

where "i" $\in$ {5; 10; 15; 20; 25; 30} are the days of evaluation, "DS" is the disease score using the severity scale of 1 to 5 as described above, and "t" represents the days post- inoculation. To avoid the area due to the note 1 (class 1) which is supposed to be "zero", each "DS" value was transformed by subtracting "one" before integrating into the above formula. AUDPC values were log-transformed to stabilize variances and the analysis of variance was performed using the General Linear Model (GLM) of SAS software (SAS, 1990; 1997). Based on the percentage of AUDPC of each strain, - means of highest AUDPC values of the 4 strains taken as 100% -, groups of resistant (0-33.2%), medium resistant (33.3-49.9%) and susceptible genotypes (50-100%), were formed. After adding the AUDPC values of the 4 strains (total AUDPC), groups of resistant, medium resistant and susceptible genotypes were defined using the same percentage ranges as above. Principal component analysis of disease severity expressed as area under disease progress curve (AUDPC) of 4 strains was performed to confirm the grouping of differential genotypes. Pearson correlation analysis between AUDPC and plant height at inoculation time was performed to examine the relationship between plant height and virulence of *X. axonopodis* pv. *manihotis* strains.

4.3 Results

A continuum of genotype reactions to the four strains from susceptible to resistant was observed (Table 1, 2). Nevetheless, strong differential reactions with one or two of the strains occurred with some genotypes, such as Ankra, Ben86052, Cameroon, Fétonégbodji, Main27 and Tuaka.

Eleven of the 24 varieties revealed a resistant, four a medium resistant and nine a susceptible reaction against the four strains (**Table 1**). The reference genotype Ben86052 was susceptible, with the highest total AUDPC of 104.5, while the standard CBB-resistant genotype TMS30572 was medium resistant. Analysing strain x genotype interactions, six groups of differential genotypes which could be useful for pathotype identification, were identified among the 24 genotypes (**Table 2**). Strains Uganda12 and GSPB2507 were significantly higher virulent than GSPB2511 and GSPB2506 ($p < 0.0001$). The four strains represented four different pathotypes (**Table 2**).

No correlation was found between plant height at inoculation time and virulence of strains (coeff. –0.04, $p = 0.40$). Principal component analysis of disease severity expressed as area under disease progress curve (AUDPC) of 24 genotypes revealed high variation among the genotypes (**Fig. 1**). Nine genotypes on the right side of the midpoint with high AUDPC were generally susceptible, while 11 genotypes revealed low AUDPC, with lowest AUDPC for CVTM4 (24) and Gbazékouté (23). Genotypes with high differential reactions fell under high or low IPCA2 score. Additionally, Toma159 (2) and TMS92/0057 (18) were revealed as genotypes with highly variable reaction. But, differential genotypes Main27 (4), 312-524 (11), TMS91/02322 (13), TMS92/0343 (14), TMS92/0429 (15) and TMS92/0067 (17) were identified to have low genotype x strain interactions in the principal component analysis.

Table 1: Reaction of 24 local and improved genotypes to stem-inoculation by four highly virulent *X. axonopodis* pv. *manihotis* strains under controlled conditions expressed as area under disease progress curve (AUDPC) over 30 days

	Genotypes	Uganda12[7]	GSPB2507	GSPB2511	GSPB2506	Total AUDPC	Total reaction
1	Ben86052[4, 8]	10.6±1.6	28.8±9.8	16.3±7.4	48.8[6]±1.2	104.5[5]	S[1]
2	Toma159	28.8[6]±5.2	27.5±2.7	27.5±2.5	15.0±5.3	98.8	S
3	Nakoko	15.0±1.8	23.8±1.3	20.0±2.7	13.1±1.9	71.9	S
4	Main27	19.4±7.1	8.8±5.2	18.8±3.3	23.1±9.1	70.1	S
5	Tuaka[4]	27.5±2.5	18.8±5.3	16.3±9.9	5.0±0.0	67.6	S
6	Ankra[4]	20.6±4.8	15±2.3	28.1[6]±5.9	0.0±0.0	63.7	S
7	Cameroon[4]	8.8±3.6	32.5[6]±3.2	9.4±2.6	8.1±5.9	58.8	S
8	Fétonégbodji[4]	24.4±0.6	18.1±4.5	7.5±4.8	5.0±2.3	55	S
9	Lagos	11.9±4.3	16.9±6.7	13.1±1.2	12.5±4.6	54.4	S
10	Toma219	13.1±4.3	12.5±7.5	15.6±5.8	4.4±2.1	45.6	MR[2]
11	312-524	17.5±6.8	9.4±3.6	5.6±3.6	5.6±4.1	38.1	MR
12	TMS30572[8]	16.3±1.6	8.8±5.6	3.8±2.2	8.1±4.9	37	MR
13	TMS91/02322	15.0±6.1	18.1±4.3	3.1±0.6	0.6±0.6	36.8	MR
14	TMS92/0343	18.1±1.2	12.5±2.3	1.3±1.3	1.3±0.7	33.2	R[3]
15	TMS92/0429	10.6±6.2	18.8±6.8	0.6±0.6	2.5±2.5	32.5	R
16	TMS92/0326	18.8±5.9	1.9±1.2	6.9±4.0	1.9±1.2	29.5	R
17	TMS92/0067	18.8±1.3	3.8±2.2	1.9±1.2	4.4±1.9	28.9	R
18	TMS92/0057	5.6±3.6	8.3±5.8	0.0±0.0	7.5±4.4	21.4	R
19	Toma289	13.8±3.9	2.5±1.4	2.5±1.4	0.0±0.0	18.8	R
20	Toma378	8.1±3.6	8.12.4	1.3±0.7	1.3±1.3	18.8	R
21	TMS4(2)1425	5.6±2.6	6.3±3.3	1.9±1.2	0.0±0.0	13.8	R
22	TMS91/02316	6.3±3.3	0.6±0.6	5.6±3.3	0.0±0.0	12.5	R
23	Gbazékouté	5.6±2.8	0±0.0	0.6±0.6	0.0±0.0	6.2	R
24	CVTM4	0.6±0.6[10]	0.0±0.0	5.0±1.8	0.0±0.0	5.6	R
	Total AUDPC[9]	**340.8a**	**301.8a**	**212.7b**	**168.2b**		

[1]S = susceptible 50-100% T (total)-AUDPC 52.3-104.5; [2]MR = medium resistant 33.3-49.9% T-AUDPC 34.8-52.2; [3]R = resistant 0-33.2% T-AUDPC 0-34.7; [4]genotypes with differential reaction between strains; [5]Highest AUDPC value used as 100% to determine the reaction group according to the total AUDPC; [6]The mean of the highest AUDPC values of the 4 strains was used as 100% value in order to determine the reaction group for each of the strains; [7]*X. axonopodis* pv. *manihotis* strains from Cotonou, Benin (GSPB2506), Ibadan, Nigeria (GSPB2507), Onne, Nigeria (GSPB2511) and Kampala, Uganda (Uganda12); [8]Ben86052 and TMS30572 as susceptible and resistant standard, respectively; [9]Sum of AUDPC values of 24 genotypes; [10]Standard error.

Table 2: Reaction of cassava genotypes stem-inoculated with four *X. axonopodis* pv. m*anihotis* strains under controlled conditions, and differential genotypes for pathotypes identification

		Pathotypes				
	Genotypes[1]	Uganda12[1]	GSPB2507	GSPB2511	GSPB2506	Groups of diff. gt.[2]
1	Ben86052	R[3]	S	MR[4]	S[5]	1[6]
2	Toma159	S	S	S	MR	-
3	Nakoko	MR	S	S	MR	-
4	Main27	S	R	S	S	2
5	Tuaka	S	S	MR	R	3
6	Ankra	S	MR	S	R	3
7	Cameroon	R	S	R	R	5
8	Fétonégbodji	S	S	R	R	4
9	Lagos	MR	MR	MR	MR	-
10	Toma219	MR	MR	MR	R	-
11	312-524	S	R	R	R	6
12	TMS30572	MR	R	R	R	-
13	TMS91/02322	MR	S	R	R	4
14	TMS92/0343	S	MR	R	R	4
15	TMS92/0429	R	S	R	R	5
16	TMS92/0326	S	R	R	R	6
17	TMS92/0067	S	R	R	R	6
18	TMS92/0057	R	R	R	R	-
19	Toma289	MR	R	R	R	-
20	Toma378	R	R	R	R	-
21	TMS4(2)1425	R	R	R	R	-
22	TMS91/02316	R	R	R	R	-
23	Gbazékouté	R	R	R	R	-
24	CVTM4	R	R	R	R	-

[1]*X. axonopodis* pv. *manihotis* from Cotonou, Benin (GSPB2506), Ibadan, Nigeria (GSPB2507), Onne, Nigeria (GSPB2511) and Kampala, Uganda (Uganda12); [2]diff. gt. = differential genotypes; [3]R = resistant 0-33.2%, with AUDPC 34.6 (mean of highest value of the 4 strains) set as 100%, AUDPC 0-11.4;. 4MR = medium resistant 33.3-49.9%, AUDPC 11.5-17.2; [5]S = susceptible 50-141.0%, , AUDPC 17.3-48.8; [6]The same numbers indicate that the corresponding genotypes belong to one differential group.

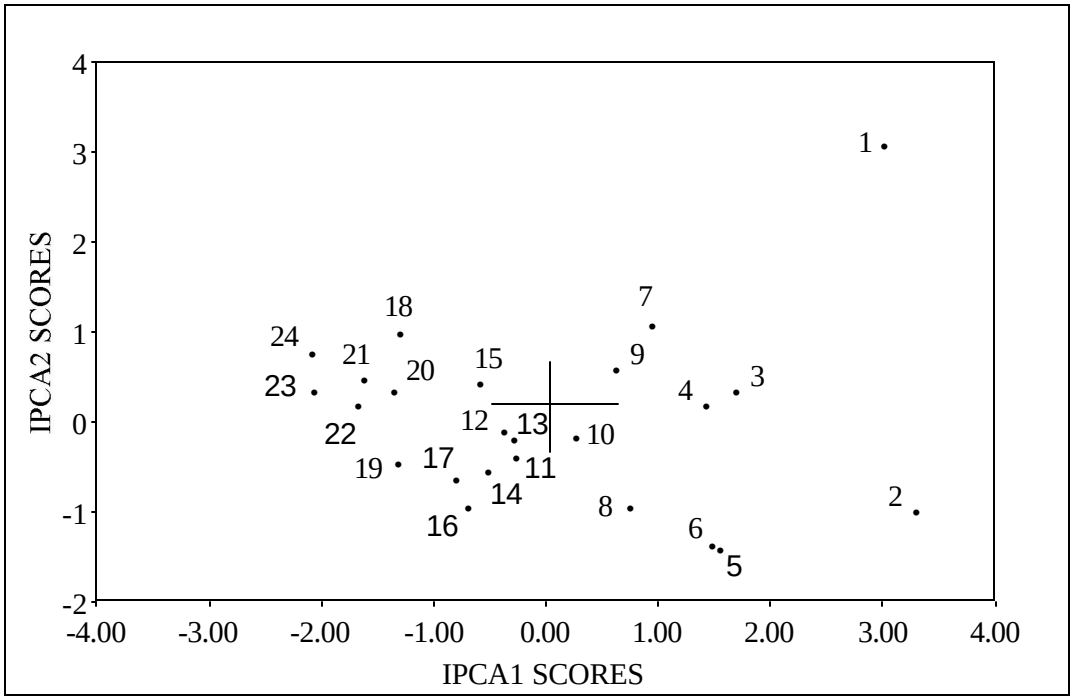

Fig. 1: Principal component analysis of area under disease progress curve (AUDPC) after stem-inoculation of 24 genotypes with four *X. axonopodis* pv. *manihotis* strains, with genotypes 1-9 (susceptible), 10-13 (medium resistant) and 14-24 (resistant)

Genotype identification 1: Ben86052, 2: Toma159, 3: Nakoko, 4: Main27, 5: Tuaka, 6: Ankra, 7: Cameroon, 8: Fétonégbodji, 9: Lagos, 10: Toma219, 11: 312-524, 12: TMS30572, 13: TMS91/02322, 14: TMS92/0343, 15: TMS92/0429, 16: TMS92/0326, 17: TMS92/0067, 18: TMS92/0057, 19: Toma289, 20: Toma378, 21: TMS4(2)1425, 22: TMS91/02316, 23: Gbazékouté, 24: CVTM4.

4.4 Discussion

Host-plant resistance is an important element in the integrated control of cassava bacterial blight. In the present study the reaction of 24 cassava genotypes from Togo and from an international collection to cassava bacterial blight (CBB) to stem-inoculation with four *X. axonopodis* pv. *manihotis* strains from various geographic origins was evaluated, and genotype x strain interactions were analysed.

The genotypes such as Ankra, Cameroon, Fétonégbodji, Lagos, Nakoko and the reference genotype Ben86052, which were susceptible after stem-inoculation revealed susceptibility in field trials in Togo (Banito, this thesis), while genotypes 312-524, TMS91/02322 and the standard CBB-resistant genotype TMS30572 were medium resistant after inoculation of the four strains as well as in field experiments. The susceptibility of genotypes Tuaka and Fétonégbodji to the disease in a stem-inoculation trial under field conditions (Boher and Agbobli, 1992) was confirmed by the present studies with 4 strains under glasshouse conditions. Genotypes CVTM4 and TMS91/02316 were resistant after stem inoculation and also belonged to the more resistant group in the general ranking across ecozones in field trials, while genotypes Gbazékouté, Toma289 and Toma378 were resistant to CBB after stem-inoculation, but were among the most susceptible genotypes in field trials (Banito, this thesis).

The stem-inoculation method was reported as a suitable method to screen cassava cultivars for resistance to CBB (Maraite et al., 1981; Restrepo et al., 2000). Defense mechanisms in cassava stems were described and an important role of phloem and xylem parenchyma cells in resistance was found. During the systemic infection of the vascular tissue, barriers such as gels and tyloses block the xylem vessels and reduce water movement in the xylem, and antimicrobial compounds accumulate to inhibitory concentrations in the infected vessels (Deshappriya, 1992; Kpémoua, 1995). Differences in these structural features, physiological activities, and morphological modifications between resistant and susceptible cultivars were observed (Kpémoua et al., 1996), and could contribute to the susceptibily or resistance of genotypes in the present data. Also, mechanisms of resistance of cassava on leaf level were suggested by Zinsou et al. (2002; 2003) and Wydra et al. (2003a).

Strain x genotype interactions were observed, and the four *X. axonopodis* pv. *manihotis* strains GSPB2506, GSPB2507, GSPB2511 and Uganda12 were identified as different pathotypes according to their reactions on six groups of differential genotypes. Genotypes

deriving from a backcross of five F_1 individuals with female parent TMS30572 varied in their reaction on leaf and stem levels against the four *X. axonopodis* pv. *manihotis* strains, which were also defined as different pathotypes according to their reaction by Zinsou et al. (2002; 2003). A specific interaction between the cassava plant and the pathogen was suggested, and resistance markers specific for African strains were recently identified (Wydra et al., 2003b). Restrepo and Verdier (1997) reported strain x genotype interactions on stem level and pathotypes were identified among *X. axonopodis* pv. *manihotis* strains in Colombia (Restrepo et al., 2000). Wydra et al. (2003b) and Zinsou (2002) reported on differences between genotypes in reaction towards leaf- compared to stem-inoculation and suggested the existence of independent mechanisms of resistance on leaf level. This may contribute to the differences observed between field evaluation and reaction to stem-inoculation in the glasshouse.

The principal component analysis was generally in accordance with the grouping based on the AUDPC differences. However, genotype Toma159 which was not among the differential genotypes based on the AUDPC differences, was revealed as a highly variable genotype in the principal component analysis. But, this variability did not include a resistant reaction. On the other hand, some differential genotypes were identified to have low genotype x strain interactions in the principal component analysis. This position in the two-dimensional biplot is due to the fact that in the principal component analysis the real AUDPC values were used, while the grouping of differential genotypes was based on a grouping of a percentage of AUDPC. Considering the differences in reaction of genotypes to stem- and leaf-inoculation, and between stem-inoculation and field evaluation, field-testing in various ecozones and leaf-inoculation are recommended for selection of resistant genotypes.

Genotypes, TMS30572 and TMS91/02316, with resistant, medium resistant and resistant reaction, respectively, after stem-inoculation, showed low disease severity and high root yield in field trials, while TMS92/0326 and TMS92/0057, and Cameroon and Ben86052, resistant and susceptible, respectively, after stem-inoculation, revealed tolerance to the disease under field conditions (Banito, this thesis). Thus, genotypes, TMS30572 and TMS91/02316 can be recommended to famers. Stem-inoculation with a set of pathotypes under controlled conditions revealed important to analyse genotype x pathotype interactions, and is, therefore, recommended to breeders to select resistant genotypes.

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5 Distribution of *Xanthomonas axonopodis* pv. *manihotis* in stems of cassava genotypes and the impact on new sprouts

Abstract

A prerequisite for a healthy cassava plantation is the use of non-infected planting material. Therefore, the distribution of *X. axonopodis* pv. *manihotis* in cassava stems was studied with the aim to develop recommendations for the selection of healthy stem material. *X. axonopodis* pv. *manihotis* was detected in stems of the susceptible varieties Ben86052 and Fétonégbodji, in a discontinuous colonization pattern and not restricted to any part of the stem. *X. axonopodis* pv. *manihotis* numbers were higher in the upper parts, with about 10^7 cfu/g in Ben86052 and 10^6 cfu/g in Fétonégbodji, including plants without systemic symptoms, than in the middle and basal parts, where the lowest numbers were found. Although 90-100% and 50-90% of cuttings of varieties Ben86052 and Fétonégbodji, respectively, harboured the pathogen, only 40-50% and 20-40%, respectively, of emerging sprouts were infected. From most of the cuttings in which *X. axonopodis* pv. *manihotis* was not detected, healthy sprouts emerged. No bacterial blight symptoms occurred on genotypes TMS30572 and Ggazékouté in the field, and the pathogen was not found in any part of the plants, nor did any of the new shoots from the planted cuttings show bacterial blight symptoms. Thus, symptomless plants of the latter genotypes could be considered free of *X. axonopodis* pv. *manihotis*. The selection of bacterial-blight-free cassava planting material from symptomless, resistant varieties is recommended to farmers to reduce disease incidence.

Key words: Bacterial blight, planting material, *Xanthomonas campestris* pv. *manihotis*.

5.1 Introduction

Cassava is an important staple crop in the tropics, and Africa produces more cassava than the rest of the world (FAO, 1998). However, most of the increases in cassava production in Africa have been due to increases in area under cultivation, rather than increases in yield per hectare (Hillocks, 2002). Cassava is affected by a wide range of virus, bacterial, fungal, and nematode diseases, among which cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Bondar, 1915), is the second most important disease of cassava in Africa (Hillocks and Wydra, 2002). Annual yield losses due to cassava bacterial blight in Africa is estimated up to 7.5 million tons (CIAT, 1996).

Cassava is propagated vegetatively, and cassava stem cuttings are used by farmers to establish a new plantation. Dissemination of CBB from one area to another and the carry-over of the pathogen from one growing season to the next are largely due to the use of infected planting materials or cuttings (Lozano, 1986; Boher et al., 1996). Symptoms of cassava bacterial blight include angular leaf spots, blighting, wilting, vascular necrosis of the stem, production of exudates and dieback (Maraite, 1993). In a later stage of infection, the pathogen invades the plant systemically resulting in often symptomless stems where it can survive for over one year (Lozano and Laberry, 1982; Dinesen, 1990; Boher and Verdier, 1994). As part of an integrated control of cassava bacterial blight (Wydra et al., 2001, 2002) careful selection of CBB-free planting material is important (Lozano, 1986; Pacumbaba, 1987). For instance, after use of control measures including careful selection of planting material from only the most lignified – basal – portion of the stem, CBB severity was reduced, and cassava production in Cuba increased from 7-8 t/ha to 20 t/ha (Cock, 1985). The basal stem part was suggested to be the most resistant to cassava bacterial blight, because of lignification and high accumulation of pectin and cellulose (Cock, 1985). During the systemic infection of the vascular tissue, barriers such as gels and tyloses block the xylem vessels and reduce water movement in the xylem, and antimicrobial compounds accumulate to inhibitory concentrations in the infected vessels (Deshappriya, 1992; Kpémoua, 1995). Differences in these structural features, physiological activities, and morphological modifications between resistant and susceptible cultivars were observed, and dead *X. axonopodis* pv. *manihotis* cells were found close to tyloses in tissues of infected, CBB-resistant cassava plants (Kpémoua et al., 1996). Bactericidal activity of phenolics in Xanthomonad-infected plants such as cotton, rice, and

cabbage was reported (Jalali et al., 1976; Horino and Kaku, 1989; Reimers and Leach, 1991; Nmasivayam et al., 1971).

However, *X. axonopodis* pv. *manihotis* was found to invade the cassava stem down to the basal part above ground level also in resistant and intermediate-resistant genotypes after inoculation of leaves (Lozano and Laberry, 1982; Fanou, 1999). Pruning most of the above ground portion of infected plants (Lozano, 1986) or cutting off diseased leaves (Fanou, 1999) to delay spread of the disease and secondary infections was reported to reduce CBB severity. Also heat-treated plantlets derived from meristem cultures were reported as a successful means of producing bacteria-free cuttings for propagation (Lozano, 1986).

The distribution of *X. axonopodis* pv. *manihotis* in infected stems of field plants was reported for some varieties (Fanou, 1999), but never established in detail for varieties frequently grown in Togo. Also the incidence of infected sprouts deriving from infected cuttings has not been studied. To develop sanitation measures in areas with a high pressure of cassava bacterial blight, the role of infected cuttings in disease dissemination has to be known. Therefore, the aim of the present studies was to determine (i) the distribution of *X. axonopodis* pv. *manihotis* in different parts of stems of cassava varieties from Togo, and (ii) the incidence of infected sprouts, in order to develop recommendations for the selection of stem cuttings.

5.2 Materials and methods

Cuttings of the local, susceptible varieties Fétonégbodji and Gbazékouté and the improved highly susceptible and resistant varieties Ben86052 and TMS30572, respectively, (Boher and Agbobli, 1992; Banito et al., 2001) were planted in the field in the forest savanna transition zone at the Institut Togolais de Recherche Agronomique (ITRA) station, Lomé, Togo. Plants were inoculated three times with a bacterial suspension of 10^7 cfu/ml of a 48-hour old culture of a virulent *X. axonopodis* pv. *manihotis* strain from Togo (X27) at intervals of three weeks. Ten stems per variety from 14 months old plants were sampled at random in the field and CBB symptoms described on each selected plant. Detection followed the method described by Fanou (1999) with few modifications. The plants were divided into upper, middle and basal part, and surface-disinfected with 70% ethanol. For each part, a cutting of 40 cm length was used and 10 cm of both sides were cut off, weighed, cut into small pieces, crushed using a mixer blender and suspended in 0.01 M $MgSO_4$ for one hour. The suspension was filtered through cheesecloth and centrifuged for 20 min at 4,000 x g. The pellet was suspended in 5 ml

of sterile 0.01 M $MgSO_4$ and serial dilutions were performed. Fifty ìl of each dilution were plated on GYCA (glucose 5 g/l, yeast 5 g/l, CaCO3 10 g/l, agar 15 g/l) (Dye, 1962) medium supplemented with Cycloheximide (Sigma, Germany) (250 mg/l GYCA medium) and incubated at 30 °C. After 48 to 72 hours, *X. axonopodis* pv. *manihotis* colonies were counted.

To check symptom development on sprouts, cassava stem cuttings of 20 cm length from the 40 cm sample of each stem part were planted at a spacing of 0.5 x 0.5 m on well prepared flat ground in the field. Weeding and watering were applied when necessary. Evaluation of CBB symptom development started five days after planting and was followed up every five days up to 40 days.

5.3 Results

X. axonopodis pv. *manihotis* was detected in stem cuttings of the local, improved variety Ben86052 from Benin and the local, susceptible variety Fétonégbodji from Togo (**Table 1**), while no bacteria were found in stems of the improved variety TMS30572, and the local variety Gbazékouté from Togo, which also did not show CBB symptoms on the selected plants in the field.

All the plants of the susceptible variety Ben86052 selected for *X. axonopodis* pv. *manihotis* detection showed CBB symptoms in the field, but no dieback. Exudates were observed on the tips of four plants and five plants showed wilt symptoms, while three plants showed no systemic symptoms. Ninety percent of the cuttings from the upper and basal parts, and all the cuttings from the middle part were infected with numbers of up to 4.3×10^7 cfu/g in the upper, 6.6×10^4 cfu/g in the middle and 2.5×10^4 cfu/g in the basal part. The average bacterial number was significantly higher in the upper part than in the middle and basal parts ($p = 0.02$). Only among the 3 stems without systemic symptoms (stem numbers 1, 2, 3), two showed a discontinuous distribution of *X. axonopodis* pv. *manihotis*, while *X. axonopodis* pv. *manihotis* was detected in all 3 parts of the stems of plants with systemic symptoms. Infected sprouts developped from 40-50% of the cuttings planted. Also sprouts derived from cuttings with low numbers of bacteria or no detection of bacteria developped symptoms, while cuttings with high bacteria numbers did not always develop infected sprouts.

Seven plants of the variety Fétonégbodji selected for *X. axonopodis* pv. *manihotis* detection showed dieback in the field, exudates were observed on six plants. Only one plant did not

show systemic symptoms. Ninety percent of the cuttings from the upper, 70% from the middle and 50% from the basal part were infected with up to 6.1 x 10^6 cfu/g, 4.1 x 10^3 cfu/g, and 2.3 x 10^3 cfu/g, respectively. Differences in the average bacterial numbers between stems parts were not significant ($p = 0.1$). *X. axonopodis* pv. *manihotis* was found in all 3 parts of the stem in four, and not continuously detected in six plants, one of them being the plant without systemic symptoms. Also from a cutting without bacterial detection, a wilted sprout developped. Infected sprouts emerged from 20% of cuttings from the basal and upper parts, and from 40% of the middle part, although 50, 90 and 70% of the cuttings, respectively, harboured *X. axonopodis* pv. *manihotis*.

Stems of variety Ben86052 harboured higher average bacterial numbers in all three parts than variety Fétonégbodji. In both varieties, the bacterial concentration decreased from the upper to the basal part of the stems, though not significantly in variety Fétonégbodji, with higher numbers in the upper part of Ben86052, but similar numbers in basal parts of both varieties. Fourty-three percent of sprouts from Ben86052 and 27% from Fétonégbodji were infected. No correlation between the bacterial number in stems and symptom development on the sprouts in genotype Ben86052 (coeff. 0.09) nor in Fétonégbodji (coeff. 0.33) was found ($p > 0.05$). Symptoms on sprouts occurred generally earlier in variety Fétonégbodji than on sprouts of Ben86052.

Table 1: Detection of *Xanthomonas axonopodis* pv. *manihotis* in stems of 14-month old cassava plants and the symptoms on new sprouts

	Ben86052										
	Symptoms on 14-month old plants					Mean cfu/g		Mean cfu/g		Mean cfu/g	
Stems	spot	blight	wilt	exudates	dieback	Basal part	Symptoms	Middle part	Symptoms	Upper part	Symptoms
1	+	+	-	-	-	2.8×10^2	-	6.0×10^2	W 10	3.8×10^6	-
2	-	+	-	-	-	0	E 15, W 20	6.0×10^4	E, W 20	1.7×10^7	E 10 , W 25
3	-	+	-	-	-	1.1×10^3	E 10, W 25	1.4×10^3	-	0	-
4	+	+	+	-	-	3.5×10^2	-	4.5×10^4	W 15	4.3×10^7	W 10
5	-	+	+	+	-	3.2×10^3	-	6.6×10^4	-	3.5×10^4	-
6	+	-	-	+	-	3.6×10^2	-	1.3×10^3	-	2.2×10^7	W 20
7	+	+	+	+	-	4.1×10^2	-	1.1×10^4	-	2.8×10^5	-
8	+	+	+	-	-	2.4×10^3	E 30	4.6×10^3	E 35	4.3×10^6	E 15 , W 20
9	+	-	-	+	-	5.4×10^3	W, W 35	2.1×10^3	-	1.6×10^3	-
10	+	+	+	-	-	2.5×10^4	-	3.6×10^2	-	7.4×10^2	E, W 40
	Mean CFU/g of infected stems					4.3×10^3 b*		1.9×10^4 b		1×10^7 a	
	Infected stems/sprouts (%)					90	40	100	40	90	50
	Fétonégbodji										
	Symptoms on 14-month old plants					Mean cfu/g		Mean cfu/g		Mean cfu/g	
Stems	spot	blight	wilt	exudates	dieback	Basal part	Symptoms	Middle part	Symptoms	Upper part	Symptoms
1	+	+	-	-	-	0	-	4.4×10^2	-	2.5×10^6	B, E 10
2	-	+	+	+	+	0	-	0	-	2.1×10^5	-
3	+	+	-	+	+	2.3×10^3	-	8.0×10^2	B 10	1.5×10^5	-
4	+	-	+	-	+	6.8×10^2	W 10	4.1×10^3	-	2.5×10^6	-
5	-	-	+	+	-	5.2×10^2	-	2.6×10^3	W 10	6.1×10^6	B, E 10
6	+	+	-	-	+	7.9×10^2	-	0	-	6.6×10^4	-
7	+	+	-	-	+	0	-	0	W 10	2.1×10^5	-
8	-	+	-	+	+	0	-	1.1×10^3	-	0	-
9	+	-	+	+	-	0	W 10	1.6×10^2	-	3.8×10^5	-
10	-	+	-	+	+	1.8×10^2	-	4.8×10^2	B 10	1.5×10^4	-
	Mean CFU/g of infected stems[1]					8.9×10^2 a		1.4×10^3 a		1.4×10^6 a	
	Infected stems/sprouts (%)					50	20	70	40	90	20

[1]significant differences in means of cfu/g between stem parts at $p < 0.05$; + = present; - = absent; W = wilt; E = exudates; B = blight;
e.g. B 10 = blight symptoms observed 10 days after planting.

5.4 Discussion

Cassava bacterial blight is an important and world-wide occurring disease of cassava that is subjected to international phytosanitary quarantine. Infected cassava stems are largely responsible for the carry-over of *X. axonopodis* pv. *manihotis* from one growing season to the next, and for dissemination to different areas. Therefore, recommendations to farmers on the choice of planting material are necessary. The study was designed to investigate the colonization and distribution of *X. axonopodis* pv. *manihotis* in different parts of the stem of infected cassava plants of two locally important cassava varieties, Gbazékouté and Fétonégbodji, compared to the standard susceptible and resistant varieties Ben86052 and TMS30572, respectively.

The basal, middle and upper stem parts of the varieties Ben86052 and Fétonégbodji were colonized by *X. axonopodis* pv. *manihotis*, continuously or discontinuously, with more *X. axonopodis* pv. *manihotis*-free cuttings in variety Fétonégbodji. Also Fanou (1999) and Daniel and Boher (1985) found partly discontinuous colonization of cassava plant stems. *X. axonopodis* pv. *manihotis* invaded cassava stems downwards until five centimeters above ground level on resistant and intermediate-resistant genotypes after leaf-inoculation (Lozano and Laberry, 1982). But, these authors did not relate the bacterial number in stems to the symptoms on the plant in the field and to latent infection, nor was the relation of infected stems to infected sprouts studied. In both susceptible varieties, the average *X. axonopodis* pv. *manihotis* concentration was by a factor of 10^3 higher in the upper part than in the middle and basal parts. Although some plants of the susceptible varieties had only shown leaf symptoms when sampling, bacteria were generally found in all parts of the stems, indicating that the pathogen invaded the stem from the infected leaves, but stayed in a latent phase in stems. Nevertheless, infected sprouts emerged from stem parts in which no bacteria were detected. Bacteria still existing inside stems in low numbers might not have been detected due to methodological limits in sampling and bacterial detection, since the parts used for planting could not be used for bacterial detection, but only small portions on the ends of the planted cuttings were tested for bacteria, and low bacterial numbers may not always be detected by the agar plating method. Daniel and Boher (1981) reported that the classical techniques of isolation on agar may fail to detect low levels of pathogen populations in seeds. Nevertheless, isolation is still considered as among the most sensitive method for detection of bacteria.

X. axonopodis pv. *manihotis* was not found in the CBB-resistant genotype TMS30572 and the local genotype Gbazékouté, which also had not shown symptoms on mother plants of cuttings in spite of several inoculations, nor on emerging sprouts. The variety Gbazékouté was among the highly resistant varieties, whereas Fétonégbodji was susceptible after stem-inoculation with four highly virulent strains from different geographic origins (Banito et al., 2000, 2001). Nevertheless, symptoms had been observed in previous field trials in all the tested genotypes, and for the 4 genotypes, the following order of CBB severity calculated as area under severity index progress curve (AUSiPC) was established: Gbazékouté > Ben86052 > TMS30572 > Fétonégbodji (unpublished data). Among them, Gbazékouté showed resistance to CBB after stem-inoculation (Banito et al., 2000, 2001), but was susceptible under field conditions. Also Fanou (1999) detected no *X. axonopodis* pv. *manihotis* in stems of variety TMS30572 deriving from symptomless stems, but only in stems of plants showing CBB symptoms. Zinsou (2001) found lower *X. axonopodis* pv. *manihotis* numbers in stems and leaves of some plants of the resistant variety TMS30572 than in the suceptible Ben86052 after leaf-inoculation under greenhouse conditions, and after leaf-infiltration of low inoculum concentrations, no CBB symptoms occurred on variety TMS30572.

Multiple resistance factors are induced in resistant plants after inoculation (Mansfield, 1983; Nicholson and Hammerschmidt, 1992), and defense mechanisms in CBB-resistant cassava plants were demonstrated (Kpémoua et al., 1996). Cassava cultivars may vary in their resistance to *X. axonopodis* pv. *manihotis* due to toxin concentrations (Perreaux et al., 1982; 1985), some of which were found to be too low in susceptible cultivars (Cooper et al., 1995). In few cases bacteria were not detected although exudate had been observed on stems of susceptible genotypes. This might be due to the discontinuous distribution of *X. axonopodis* pv. *manihotis* in stems and the destructive sampling method which did not allow to detect *X. axonopodis* pv. *manihotis* in the whole stem.

Surprisingly, 50-80% of the planted cuttings from the stem parts of Ben86052 and Fétonégbodji did not develop CBB symptoms on the new sprouts, although the stems harboured the pathogen. Although sprout infection was less in Fétonégbodji, sprout symptoms generally developed faster than in Ben86052. These observations could be generally due to the discontinuity of the colonization of *X. axonopodis* pv. *manihotis* or to differences in vascular connections between the xylem of new sprouts and the one of the old cuttings (unpublished data). New shoots generate a new system of xylem apart from the old vessels of

the previous stem. Most of the new xylem was observed to be unconnected to the old xylem as demonstrated by dye uptake and standard light microscopy (unpublished data). Additionally, old xylem was heavily occluded by tylosis. Thus, *X. axonopodis* pv. *manihotis* is unlikely to cross easily between old and new xylem because living parenchyma cells separate them. However, occasionally a connection was found, and *X. axonopodis* pv. *manihotis* could cross over from the xylem of the old stem to the new shoots, but this seems to be an infrequent event (Cooper, personal communication) and may depend on storage conditions of the cuttings and environmental conditions after planting (Wydra, personal communication).

Since the pathogen was not found in any part of the 10 tested plants of varieties TMS30572 and Gbazékouté, nor did any of the new shoots from the planted cuttings show CBB symptoms, symptomless plants of both cultivars could be considered free of *X. axonopodis* pv. *manihotis*. But, in case of field plants with symptoms, also these varieties may harbour the pathogen in their cuttings (Fanou, 1999). Therefore, a careful selection of cuttings from plants of resistant varieties without symptoms is recommended to farmers to receive CBB-free cassava planting material. A latent infection of symptomless cuttings of any stem part has always to be considered when cuttings come from an infected field. Therefore, also the basal part of the stems should not, contrary to recommendations of Cock (1985), be used to receive healthy planting material. Furtheron, breeders should consider differences between varieties in restriction of systemic infection, latent infection of stems and restriction of sprout symptoms as additional characteristics in selection of varieties for resistance.

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6 Studies on intercropping and soil amendments for control of cassava bacterial blight

Abstract

The influence of intercropping cassava with common staple crops in Togo on cassava bacterial blight, and the effects of potassium (KCl) fertilizer doses of 60 and 120 kg/ha and the application of *Cassia siamea* mulch on disease development were studied under field conditions in four agro-ecological zones of Togo. Bacterial blight severity was significantly reduced compared to sole cassava in the forest highland in cassava-taro and cassava-maize intercropping at medium and high inoculum levels; in cassava-maize intercropping in the forest savanna transition zone at medium, but not at high inoculum levels (p 0.01), and in cassava-maize intercropping in the wet savanna zone at high inoculum level (p 0.05), with generally no significant negative effect on yield. Though significant, disease reductions by intercropping generally were low (6-23%). The application of potassium and mulch revealed only unclear disease reducing and increasing effects and can, thus, not be recommended as part of a disease control strategy. Since no varieties with complete resistance had been identified among local and local improved varieties across ecozones in Togo, the combination of medium resistant varieties and an intercropping system, both adapted to the respective ecozone, is recommended to farmers.

6.1 Introduction

Cassava production is largely reduced due to the attack by pests and diseases (Nilmanee, 1986; Hahn et al., 1989; IITA, 1990), with bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Bondar, 1915), being one of the major constraints (Lozano and Booth, 1974; Wydra and Msikita, 1998). Symptoms include angular leaf spots, blighting, wilting, defoliation, vascular necrosis of the stem, exudation and dieback. The vascular symptoms affect the quality and quantity of planting material (Boher and Verdier, 1994; Banito, this thesis), while root yield losses due to bacterial blight of more than 50% (Wydra and Rudolph, 1999; Wydra, 2002) and 77% in some cassava varieties in the dry savanna zone (Fanou, 1999) were reported.

Due to the instable and environmentally dependent nature of resistance to bacterial blight (Wydra, 2002), only an integrated control system combining host plant resistance with agronomic and cultural measures is promising to reduce bacterial blight epidemics (Wydra and Rudolph, 1999; Wydra et al., 2003). Intercropping and soil amendments were described to reduce diseases in several crops.

Intercropping

In the intercropping system two or more crops are grown simultaneously on the same area of land for a substantial part of their growing periods, but crops are not necessarily sown or planted at exactly the same time. Intercropping provides an efficient utilization of resources, economic stability for farmers, and is a dominant system of farming in Asia, Africa and South America, where population pressure is high and resources are limited. The increase of food production due to intercropping was reported in the United States (Murdock and Wells, 1978; Helsel and Wedin, 1981). Andrews and Kassam (1976) described various intercropping methods including mixed intercropping, row intercropping, strip intercropping, relay cropping and alley cropping. Especially in tropical areas of the world, cereals such as maize, sorghum, millet, but also common bean, cowpea, peanut and soybean are commonly used as components of intercrop combinations (Robinson, 1984). A useful strategy in areas with unreliable rainfall distribution is to intercrop a relatively short-duration crop with a long-season one for which the periods of maximum demand on water resources are different (Davis and Woolley, 1993). In Africa, more than 75% of the total area under cultivation are intercropping systems (Nweke et al., 1994). Intercropping cassava with other staple crops was

reported as the most common system of cassava production in the tropics (Olasantan et al., 1994) and more than 60% of the farmers intercrop cassava with maize (Biaou and Issaka, 1997). Nevertheless, monocropping of cassava is increasing, especially in marginal areas (unpublished data).

Intercropping can affect disease and pest incidence and severity (Van Rheenan et al., 1981; Ofuya, 1991; Trenbath, 1993). Intercropping cassava with cowpea reduced egg populations of *Aleurotrachelus socialis* and *Trialeurodes variabilis*, compared to those in monoculture (Gold et al., 1990), while intercropping cassava with maize did not reduce egg populations (Gold, 1993), indicating that the success of this technique can depend on the intercropped species. However, intercropping is a promising means of reducing pest populations for small-scale farmers (Bellotti, 2002). Thus, the combination of improved genetic resistance with the benefits of intercropping should result in a more sustainable control of diseases and pests (Davis and Woolley, 1993). Besides the decrease of bacterial blight under the cassava-maize intercropping system, Larios and Moreno (1976, 1977) and Moreno (1979) also observed a delay in the development of superelongation of cassava (*Elsinoe brasiliensis*) and of rust (*Uromyces manihotis*), and a reduced incidence and severity of mildew, superelongation, and anthracnose (*Colletotrichum sp.*) in a cassava-common bean association in Turrialba, Costa Rica, while Ghosh et al. (1986) reported a reduction of brown leaf spots (*Cercosporidium henningsii*) in cassava associated with *Eucalyptus sp.* and *Leucaena sp*. Intercropping cassava-maize has been reported to reduce weeds up to 37% compared to sole cassava (Zuofa et al., 1992). However, a reduction of incidence and severity of diseases and pests by intercropping does not always occur (Woolley and Davis, 1991). The latter authors found that intercropping maize-bean reduced most of bean insects, but among diseases, anthracnose may be increased.

Generally, intercropping has been reported as one of the measures to reduce cassava bacterial blight (Nyango, 1979; Terry, 1974). Ene (1977) reported that cassava bacterial blight was significantly reduced by providing shade or intercropping cassava with maize or melon. The use of intercropping was proposed as means to reduce cassava bacterial blight in the dry savanna (Tabot, 1995) and in the humid forest (Arene, 1976). Significant reduction of cassava bacterial blight severity in cassava intercropped with cowpea and maize compared to cassava monoculture were observed in the forest savanna transition zone of Nigeria, with the highest disease reduction of 53% in a cassava-maize intercrop, without significant yield effect due to

cropping system (Fanou, 1999). The latter author suggested that intercropping could have a barrier effect to inhibit the transport of the inoculum of *X. axonopodis* pv. *manihotis* since bacterial diseases are generally disseminated in the field by rainsplash and aerosols combined with wind. The effect of intercropping on cassava bacterial blight severity may vary with intercrops used and across ecozones. Therefore, as part of an integrated control system for cassava bacterial blight suggested by Wydra et al. (2001), an adapted intercropping system should be developed in each cassava growing area. In Togo, studies on the use of intercropping to reduce cassava bacterial blight, have never been conducted. Thus, the objective of the present studies was to determine the effectiveness of intercropping cassava with common staple crops in controlling cassava bacterial blight under field conditions in various agroecological zones in Togo.

Fertilization

In many tropical areas, cassava is grown on poorest soils and still produces a considerable yield under conditions, where other staple crops such as maize would fail. Cassava is suitable for marginal areas with adverse climatic and soil conditions because of its exceptional tolerance to drought and to acid, infertile soils (Howeler, 2002). This tolerance of cassava to marginal soils has often led to the opinion that cassava, either grown alone or intercropped, does not require high soil fertility for good yields nor responds to fertilizer application. However, cassava commonly requires some application of nitrogen and potassium for maximum growth and root yields (Obigbesan and Fayemi, 1976; Howeler, 1991). But, yield depressions have been observed at potassium rates higher than 200 kg/ha applied in the form of KCl (CIAT, 1974). The lack of potassium has been reported to affect the plant's response to nitrogen and phosphorus (CIAT, 1975). In acidic soils, a low availability of potassium, phosphorus and calcium may affect cassava production (CIAT, 1995). In Colombia, no difference in yields was observed between KCl and K_2SO_4 application at SO_4 content of 9.0 ppm in the soil, while at low SO_4 content, with K_2SO_4 application significantly higher yields than with KCl were produced (Ngongi, et al., 1976). For cassava fresh root yield of 35.7 t/ha, an average removal of 55 kg/ha nitrogen (N), 13.2 kg/ha phosphorus (K) and 112 kg/ha potassium (K) were observed (Howeler, 1991).

The application of fertilizers was suggested to reduce cassava bacterial blight (Ezumah and Terry, 1974). Studying the effect of NPK fertilization on cassava bacterial blight, Arene (1978) found, that only potassium reduced significantly the severity of the disease in

greenhouse trials. A significant reduction of cassava bacterial blight incidence and severity was also observed in field trials at potassium rates of 90 and 180 kg K_2O/ha compared to 0 kg K_2O/ha, while no difference occurred between the two doses (Arene and Odurukwe, 1979).

Rainsplashing is the most important factors of dissemination in the field or between fields over short distances (Lozano and Sequeira, 1974; Otim-Nape, 1976). Ene (1977) found that cassava bacterial blight could be controlled by the use of any means such as mulching which reduce the impact of rain splash. Additionally, a green manure is known to release nutrients for the plant, suppress weeds, support root development and increase soil moisture (Maliki et al., 1997). The application of mulch produced significantly greater corm yield, but also showed a higher incidence of corm rots of taro compared to non-mulched plots (Miyasaka et al., 2001). The use of lower potassium rates in reducing cassava bacterial blight compared to those proposed by Arene and Odurukwe (1979), could contribute to minimize the cost of fertilizer application. Therefore, the present study was designed to investigate the effects of KCl fertilizer doses of 60 and 120 kg/ha and the *Cassia siamea* mulch on cassava bacterial blight development under field conditions in different ecozones of Togo.

6.2 Materials and Methods

Experimental sites

Intercropping and fertilization trials were conducted in four sites in stations of the Institut Togolais de Recherche Agronomique (ITRA), Togo, in 1999-2000: in the forest savanna transition zone at Davié, in the forest lowland zone at Adéta, in the forest highland zone at Danyi, and in the wet savanna zone in Sotouboua. The vegetation in the forest savanna transition zone (littoral zone) in the South part of the country is characterized by a shrubby vegetation with few trees, in the forest zone in the South-West by a rainforest vegetation, and in the wet savanna in the Center part of the country by more shrubby vegetation. The forest savanna transition and the forest zones are characterized by a sub-equatorial climate with one long rainy season (March – June), one short dry season (July-August), one short rainy season (September – October) and one long dry season (November – March), whereas the wet savanna is characterized by a tropical climate with one long rainy season (April – September) and one long dry season (October – March) (Lamouroux, 1979). The average annual rainfall is about 1,200 mm in the forest savanna transition zone and 1,400 mm in the forest and wet savanna zones, with an average temperature of 28 °C, 24 °C and 27 °C, respectively

(DMN, 2001). In years 1999 and 2000, the average rainfall was 958 mm in the forest savanna transition zone, 1,403 mm in the forest zone and 1,326 mm in the wet savanna zone, spread over 9 months in the first two ecozones and over 7 months in the latter one.

Planting materials and planting

The bacterial blight-susceptible variety Ben86052 was used in both intercropping and fertilization experiments. Cassava stem cuttings of 20 cm deriving from apparently healthy field plants were single planted at a spacing of 1 x 1 m on well prepared flat ground in June 1999. Each treatment consisted of three plots (20 m^2 per plot) of 20 plants. The non-inoculated and inoculated plots were separated by a 5 m wide screen of maize plants.

Intercropping. Cassava was intercropped with maize in a row intercropping system. Additionally in the forest highland, cassava was row-intercropped with taro, a common tuber crop in this area. Maize plants within rows were 40 cm spaced apart, while cassava and taro were 1 m spaced. The three crops were planted at the same time.

Fertilization. Potassium chloride (KCl) was applied in two doses (60 kg/ha, 120 kg/ha) to cassava plants two weeks after planting. For mulching, leaves of *Cassia siamea* (Caesalpinaceae) were applied at a rate of 2 t/ha dry matter at the planting day. The control plots for both experiments did not receive potassium nor mulch.

Experimental design

In both trials, block design was not used due to accessity of land. Each treatment was in 3 plots non-replicated. Control plots were the same for all treatments. Weeding was conducted when necessary and no watering was applied.

Bacterial suspension and inoculation

A 48-hour old culture of *X. axonopodis* pv. *manihotis* strain X27 from Togo was harvested in mass from GYCA (glucose 5 g/l, yeast 5 g/l, $CaCO_3$ 10 g/l, agar 15 g/l) plates (Dye, 1962) using 0.01 M $MgSO_4$ solution. One-month old cassava plants were inoculated with a bacterial suspension of 10^7 cfu/ml by spraying the abaxial surface of cassava leaves with a motorized sprayer. All the plants of each plot in the fertilization trial were sprayed, whereas in the mono- and intercropping, only the border plants (outer rows of each plot) were inoculated. A total of three inoculations were performed at 3-weekly intervals.

Disease assessment

Disease symptoms were evaluated 3 weeks after each inoculation and after six and twelve months, by counting leaves with angular leaf spots, blight or wilt on ten randomly selected plants per plot in the fertilization trial, and selected within rows between the inoculated outer rows of each plot in the intercropping trial. When leaves showed more than one symptom type, they were recorded under the more severe symptom type. The total remaining leaves, dropped leaves (number of scarifications remaining on stem) and number of shoot tips with dieback were also recorded. The percentages of leaves with spots, blight, wilted/dropped leaves and shoots with dieback were calculated for each plant. The severity index (Si) was calculated for each plant at each evaluation date as follows:

$Si = (1xS + 2xB + 1xW + 2xD)/6$

where S, B, W and D represent the percentage of leaves with spots, blight, wilt and shoots with dieback, respectively. The weight attributed to the symptoms blight and dieback is an estimation resulting from regression analysis of symtom and plant growth data, revealing blight as most important factor influencing root yield, and dieback with highest influence on overall plant growth (leaf and stem weight) (unpublished data). The effect of fertilization and mulching as well as the effect of the cropping system on cassava bacterial blight severity were assessed by calculating the area under the severity index progress curve (AUSiPC) for each plant at six evaluation dates, by the trapezoidal integration (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001) according to ecozones. In the forest and forest savanna transition zones:

$AUSiPC = [(Si1+Si2)x21/2 + (Si2+Si3)x21/2 + (Si3+Si4)x60/2 + (Si5+Si6)x120/2]/275$

In the wet savanna zone:

$AUSiPC = [(Si1+Si2)x21/2 + (Si2+Si3)x21/2 + (Si3+Si4)x30/2 + (Si5+Si6)x90/2]/215$

where Si1, Si2, Si3, Si4, Si5 and Si6 represent the severity index at the evaluation dates 1, 2, 3, 4, 5 and 6, respectively. Si4 and Si5 correspond to severity index during the dry season and are equal to zero. The AUSiPC in days over the growing period was divided by the evaluation period of 275 or 215 days, corresponding to 365 days minus the dry season period of 90 days in the forest and forest savanna transition zones and 150 days in the wet savanna zone. To receive an average comparable between ecozones. Thus, all AUSiPC values are standardized.

Harvests

Cassava roots were harvested at 12 months after planting by uprooting ten plants randomly selected in each plot in the fertilization trial, and selected within rows between the inoculated outer rows of each plot in the intercropping trial. Plant height was measured, and roots of each plant were counted and weighed. All the roots of each plot were mixed and a sub-sample was cut into small pieces, weighed and dried in an oven at 105 °C for 72 hours for dry weight determination.

Statistical analysis

Standardized area under severity index progress curve (AUSiPC) and of dry root weight values were log-transformed to stabilize variances and the analysis was performed using the Linear Mixed Model ANOVA (Harville, 1988; Bernardo, 1994; Tempelman and Gianola, 1996). Values and standard errors in tables are the real, non-transformed values. Analysis of variance was performed on AUSiPC and root dry weight values using the General Linear Model (GLM) procedure in the SAS system (SAS, 1990; 1997). The Student-Newman-Keuls (SNK) test was used to compare the means of AUSiPC and root dry weight values (Danielie, 1975), and to discriminate between the KCl fertilizer doses and mulch as well as the intercropping patterns.

6.3 Results

Effect of intercropping on cassava bacterial blight severity

Cassava bacterial blight severity expressed as area under severity index progress curve (AUSiPC) was significantly reduced in the forest highland in cassava-taro and cassava-maize intercropping in inoculated ($p = 0.0003$) and in non-inoculated ($p < 0.0001$) treatments compared to sole cassava, in the forest savanna transition zone in cassava-maize intercropping in non-inoculated treatment ($p = 0.007$) and in the wet savanna zone in cassava-maize intercropping in the inoculated treatment ($p < 0.0001$) (**Table 1**). Though significant in some treatments, disease severity reductions by intercropping were generally low (6-23%). In two non-inoculated treatments, disease severity was significantly lower in cassava monocropping.

In the forest highland, the disease develops during the rainy season, with the highest values at 2 and 4 months after planting (**Fig. 1**). Symptoms disappear during the dry season and reappear in the rainy season of the following year.

Table 1: Effect of intercropping cassava-maize and cassava-taro on cassava bacterial blight severity expressed as area under the severity index progress curve (AUSiPC) in four ecozones of Togo

	Forest lowland		Forest highland (plateau)	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Crop system	AUSiPC	AUSiPC	AUSiPC	AUSiPC
Cassava	5.8±0.11[1]b***	7.5±0.19a	5.1±0.11a***	5.8±0.11a***
Cassava-maize	6.4±0.08a	7.5±0.17a	4.8±0.10b	5.2±0.08b
Cassava-taro	nd[2]	nd	4.3±0.07c	5.2±0.14b
	Forest savanna transition		Wet savanna	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Crop system	AUSiPC	AUSiPC	AUSiPC	AUSiPC
Cassava	6.4±0.10a**	6.3±0.05a	6.4±0.24b***	7.8±0.32a***
Cassava-maize	5.9±0.12b	6.2±0.07a	7.9±0.21a	6.0±0.08b

* = significant (SNK test) at probability level $p < 0.05$; ** = significant at probability level $p < 0.01$; *** = significant at probability level $p < 0.001$; 1 = standard error, 2 nd = not done.

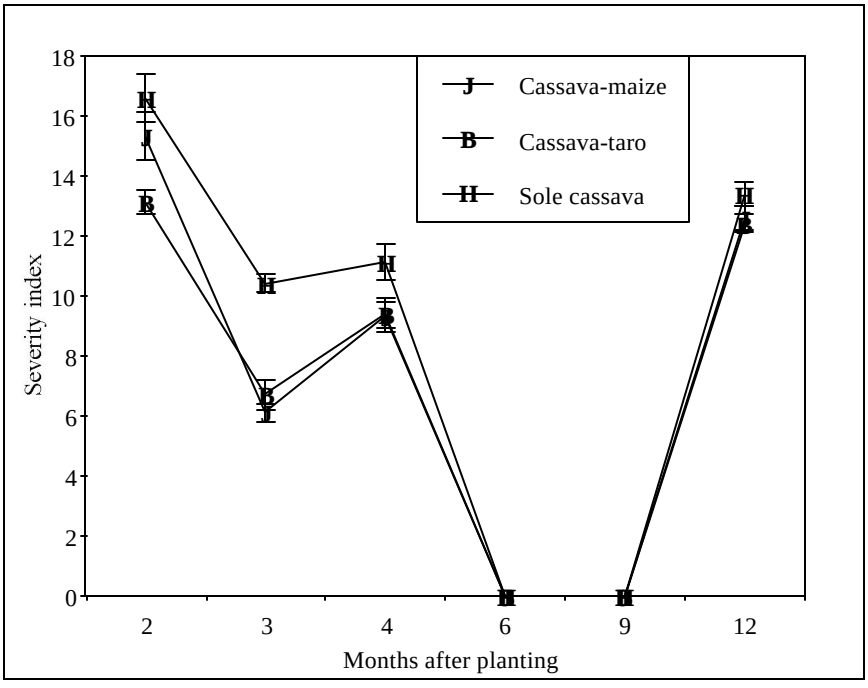

Fig. 1: Development of severity index in intercropping patterns in the susceptible genotype Ben86052 in the inoculated treatment in the forest highland in year 1999 (dates of inoculation: 30, 51 and 72 days after planting)

Effect of intercropping on cassava yield

Generally, no significant differences in yield (root dry weight) were observed between cassava monoculture and cassava intercropping plots across environments and treatments, except in the forest highland (non-inoculated plots) and the forest savanna transition zones (inoculated plots), where intercropping cassava-taro and cassava-maize, respectively, significantly reduced root yield ($p = 0.02$, $p = 0.007$, respectively) (**Table 2**).

Table 2: Effect of intercropping on cassava yield (t/ha) in four ecozones of Togo

	Forest lowland		Forest highland	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Crop system	Root DW	Root DW	Root DW	Root DW
Cassava	20.7±2.97[1]a	26.1±3.86a	20.2±1.47a*	19.5±2.23a
Cassava-maize	23.9±1.77a	23.1±1.90a	18.1±2.08ab	15.3±3.94a
Taro-Cassava	nd[2]	nd	13.1±1.31b	20.0±3.29a
	Forest savanna transition		Wet savanna	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Crop system	Root DW	Root DW	Root DW	Root DW
Cassava	17.0±2.55a	26.3±2.21**	5.6±1.36a	17.3±1.92a
Cassava-maize	15.2±1.73a	15.8±2.47b	9.5±1.71a	12.4±1.92a

* = significant (SNK test) at probability level $p < 0.05$; ** = significant at probability level $p < 0.01$; Root DW = root dry weight; 1 = standard error, 2 nd = not done.

Effect of KCl fertilizer and *Cassia siamea* mulch on cassava bacterial blight severity

The effects of potassium and mulch treatment on cassava bacterial blight severity were variable across ecozones and treatments (inoculated, non-inoculated) (**Table 3**). Significant reductions in cassava bacterial blight severity comparing control and treatments were observed in the forest-savanna transition zone in all treatments in the non-inoculated plots ($p < 0.0001$) and in the KCl 60 kg/ha treatment in inoculated plots ($p = 0.0002$), in the wet savanna zone in the KCl 120 kg/ha treatment in non-inoculated plots, and in the mulching and in the KCl 120 kg/ha treatments in inoculated plots ($p < 0.001$), and in the forest highland in the KCl 120 kg/ha in non-inoculated plots ($p < 0.001$). The disease severity reductions, though significant, were generally low (8-19%). The inverse situation was observed in all treatments in non-inoculated plots, in the forest lowland and in the KCl 60 kg/ha treatment in

the forest highland zones, where disease severity increased significantly compared to the control ($p < 0.001$).

Table 3: Effect of potassium (KCl) and mulch from *Cassia siamea* on cassava bacterial blight severity expressed as area under the severity index progress curve (AUSiPC) in four ecozones of Togo

	Forest lowland		Forest highland (plateau)	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Fertilizers	AUSiPC	AUSiPC	AUSiPC	AUSiPC
Control	5.8±0.11[1]c*	7.5±0.19ab*	5.1±0.11b***	5.8±0.11a
KCl 60	6.5±0.09b	7.9±0.26a	5.6±0.08a	5.8±0.19a
KCl 120	6.2±0.12b	7.2±0.28ab	4.6±0.09c	5.7±0.13a
Mulch	6.9±0.09a	7.0±0.12b	5.3±0.09ab	5.3±0.16a
	Forest savanna transition		Wet savanna	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Fertilizers	AUSiPC	AUSiPC	AUSiPC	AUSiPC
Control	6.4±0.10a***	6.3±0.05a***	6.4±0.24a***	7.8±0.32a***
KCl 60	5.9±0.09b	5.8±0.08b	6.6±0.16a	7.9±0.37a
KCl 120	5.9±0.05b	6.2±0.10a	5.6±0.13b	6.8±0.26b
Mulch	5.8±0.08b	6.2±0.09a	7.0±0.11a	6.3±0.28b

* = significant (SNK test) at probability level $p < 0.05$; *** = significant at probability level $p < 0.001$; 1 = standard error.

In the wet savanna, a peak of disease severity occurs in the rainy season at 2 months after planting, symptoms disappear during the dry season and symptom development starts again in the rainy season of the following year (**Fig. 2**).

Effect of fertilizer and mulch application on cassava yield

Generally, no significant differences in root yield were observed between the fertilizer and mulch applications and the control (**Table 4**). Nevertheless, in the forest highland zone, yield in the mulch plots was significantly higher than yield obtained by the KCl rate of 60 kg/ha in non-inoculated plots ($p = 0.03$). In the forest savanna transition zone, significant reduction of root weight was observed at a KCl rate of 120 kg/ha in inoculated plots ($p = 0.05$). In the wet savanna zone, mulch significantly reduced root dry yield compared to the control in the inoculated plots ($p = 0.004$), while with the KCl dose of 120 kg/ha a significantly higher root yield was recorded than with the mulch treatment under natural infection ($p = 0.007$).

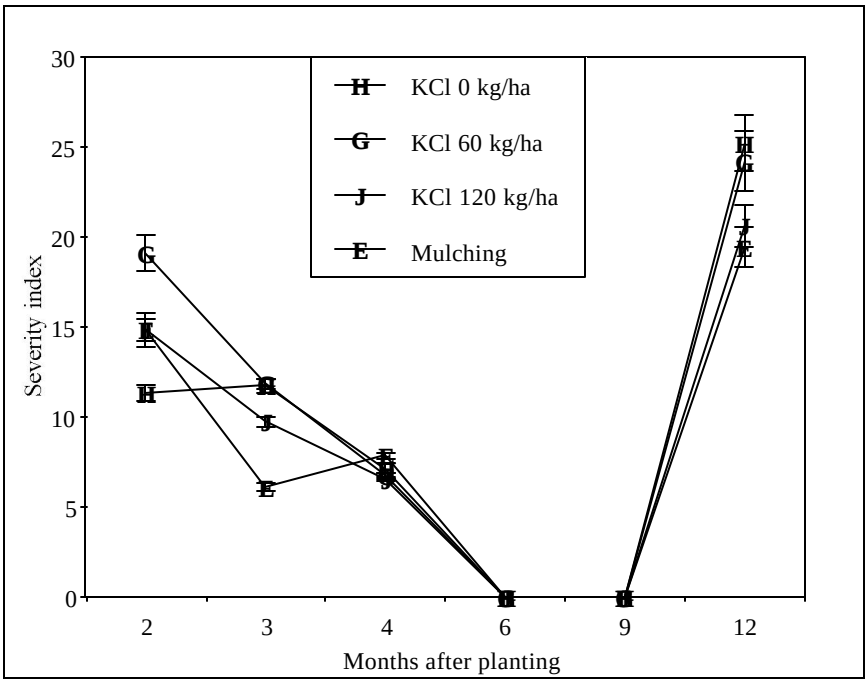

Fig. 2: Development of severity index in fertilization patterns in the susceptible genotype Ben86052 in the inoculated treatment in the wet savanna zone in year 1999 (dates of inoculation: 30, 51 and 72 days after planting)

Table 4: Effect of potassium (KCl) and mulch from *Cassia siamea* on cassava yield (t/ha) in four ecozones of Togo

	Forest lowland		Forest highland (plateau)	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Fertilizers	Root DW	Root DW	Root DW	Root DW
Control	20.7±2.97[1]a	26.1±3.86a	20.2±1.47ab*	19.5±2.23a
KCl 60	16.7±2.11a	27.2±3.84a	21.0±3.04b	18.7±1.62a
KCl 120	21.9±2.53a	32.7±4.33a	21.2±1.29ab	19.6±3.28a
Mulch	18.8±2.00a	31.2±4.24a	33.4±4.70a	13.4±1.49a
	Forest savanna transition		Wet savanna	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Fertilizers	Root DW	Root DW	Root DW	Root DW
Control	17.0±2.55a	26.3±2.21a*	5.6±1.36b**	17.3±1.92a***
KCl 60	16.0±2.76a	21.2±3.78ab	6.1±1.08ab	11.3±1.97ab
KCl 120	22.1±2.87a	15.3±2.97b	11.3±1.35a	9.4±1.43ab
Mulch	23.5±2.04a	18.4±3.07ab	4.3±1.22b	7.1±1.93b

* = significant (SNK test) at probability level $p < 0.05$; ** = significant at probability level $p < 0.01$; *** = significant at probability level $p < 0.001$; Root WD = root dry weight. 1 = standard error.

6.4 Discussion

The effect of intercropping cassava-maize and cassava-taro on cassava bacterial blight was investigated. Significant, but relatively low reductions of cassava bacterial blight severity (AUSiPC) were observed in cassava-maize intercropping in the forest savanna transition zone and in the wet savanna zone, and in cassava-maize and cassava-taro intercropping in the forest highland zone. Intercropping can influence disease and pest incidence and severity (Van Rheenan et al., 1981; Ofuya, 1991; Davis and Woolley, 1993). Cassava bacterial blight reduction due to cassava-maize and cassava-cowpea intercrops in the forest savanna transition zone and cassava-maize intercrop in the dry savanna zone in Nigeria was reported by Fanou (1999) and Tabot (1995), respectively. Intercropping was proposed to reduce *Ralstonia solanacearum* causing bacterial wilt, in potato (Autrique and Potts, 1987; Kloos et al., 1987) and tomato (Pan, 1990), while other studies only revealed a slight reduction or no reduction of bacterial wilt under intercropping of tomato with cowpea, soybean or Welsh onion (Michel et al., 1997). Also Sikirou (1999) did not observe clear effects on cowpea bacterial blight when cowpea was intercropped with maize or cassava in the forest-savanna transition zone of West Africa. On the other hand, cassava-maize intercropping increased the severity of powdery mildew, and a bean-cassava association showed no effect on scab, rust, and *Cercospora* leaf spots of cassava (Moreno, 1979). The reduction of the disease severity observed in intercropping might be due to the barriers provided by maize or taro plants, which could reduce plant-to-plant dissemination of the disease through rainsplash or drops of water carried by wind. Ene (1977) reported that cassava bacterial blight was significantly reduced by providing shade or intercropping cassava with maize or melon. Also, a reduced bacterial blight incidence and severity in cassava intercropped with maize and melon in the humid forest zone was observed by Arene (1976).

A relation between significant reduction of disease severity which occurred in few treatments and yield data was not observed. Comparing cropping systems, generally, no significant yield loss occurred in intercropped cassava compared to monocropping, except a significant yield reduction in intercropping cassava with maize in the inoculated treatment in the forest savanna transition zone and in intercropping cassava with taro in the non-inoculated treatment in the forest highland. The latter reduction may be due to a high competition between the two root crops. Intercropping cassava with maize in the forest savanna transition zone of Nigeria (Fanou, 1999), and across a wide range of environments in South Nigeria under nitrogen fertilization (Ezumah et al., 1988) did not cause a reduction in yield, while, on the contrary, a

significant cassava yield loss due to intercropping cassava with maize was reported from the rainforest zone of Nigeria (Zuofa et al., 1992). Okoli et al. (1996) reported significant cassava root yield losses up to 40% in susceptible and up to 35% in resistant cassava cultivars intercropped with cowpea, while Fanou (1999) found no significant difference in cassava root yields between cassava-maize and cassava-cowpea intercropping and monocropping cassava. In maize-soybean intercropping, Mohta and De (1980) reported increased total grain yield, whereas Crookston and Hill (1979) observed no grain yield effect. Also yields of intercropped soybean with maize were up to 32% less than yields of soybean in monoculture, however, yield of intercropped maize was increased up to 53% compared with the yield of monoculture maize and compensated for the reduced yield of soybean (Herbert et al., 1984). Thus, the present results and studies of other authors show that intercropping may cause a yield reduction of the main crop, but, even when yield reductions of the main crop occur, the additional yield gained by the intercrop has to be considered, which increases the land equivalent ratio (Sikirou, 1999). Higher cash incomes in cassava intercropped with maize and groundnut compared to cassava monoculture were reported in Togo (Marqette and Pouzet, 1988).

Since intercropping cassava-maize and cassava-taro reduced disease severity slightly, but in most cases significantly in the forest highland, the wet savanna and the forest savanna transition zones, with no significant negative yield effect due to the cropping system with two exceptions, these cropping systems can be recommended in these ecozones as part of an integrated control strategy for cassava bacterial blight. A suppressive effect of intercropping might be more obvious, when a medium resistant variety is used. Since no resistant varieties were identified among local and local improved varieties across ecozones in Togo (Banito, this thesis), the combination of medium resistant varieties and an intercropping system, both adapted to the respective ecozone, could be recommended to farmers. Besides the possible effect of disease reduction, yield stability and an additional yield would be achieved by planting the second crop.

The application of potassium and mulch revealed only unclear disease reducing and increasing effects and can, thus, not be recommended for a disease control strategy. In the present studies, no significant effect of the KCl fertilizer on cassava bacterial blight severity was observed across ecozones and environments, except a significant reduction of cassava bacterial blight severity of the susceptible genotype Ben86052 by KCl in some environments.

However, generally no obvious effect of potassium chloride was found across environments. On the contrary, the application of NPK fertilizer was reported to reduce bacterial blight of cassava under greenhouse conditions, with potassium (K) being the main component responsible for the reduction (Arene, 1978). Greenhouse trials on the effect of fertilizer based on N, P and K revealed significant reductions of cassava bacterial blight incidence and severity on a susceptible variety with the potassium dose of 90 kg/ha (Adeniji and Obigbesan, 1976) and of 90 and 180 kg/ha (Arene and Odurukwe, 1979). Also Boher and Verdier (1994) suggested that the potassium fertilization enhances cassava plant resistance to *X. axonopodis* pv. *manihotis* infection. This was not obvious in the present data. Mulching had generally no disease reducing effect, however, the use of green manure in farmers' cultivation may have other advantages, such as maintenance of soil moisture and soil fertility, improvement of soil structure, limitation of soil erosion, support of soil microfauna, and inhibition of weeds' competition. By reducing weeds in the field, an additional inoculum source in form of epiphytic populations of *X. axonopodis* pv. *manihotis* on weeds (Fanou et al., 2001) can be avoided.

Our results revealed a significant yield increase only at KCl application of 120 kg/ha under natural infection in the wet savanna, while significant depressions on root yield were recorded at the KCl dose of 120 kg/ha in the forest savanna transition zone in the inoculation treatment and with the application of mulch in the wet savanna zone. Yield depressions of cassava have been reported at potassium rates higher than 200 kg/ha applied in the form of KCl (CIAT, 1974). Similar yield depressions at high potassium rates were observed for yam (Ferguson and Haynes, 1970). Ngongi et al. (1976) found that at a high level of soil sulfur content, no differences in cassava yields occurred between potassium in the form of KCl and K_2SO_4, but at low soil sulfur content, K_2SO_4 produced significantly higher cassava root yields than KCl. In the present studies a significant yield increase was found only at KCl dose of 120 kg/ha in the wet savanna zone, but the sulfur content of the soil had not been determined. The effectiveness of KCl application on cassava yields in relation to the SO_4 content in the soil (Ngongi et al., 1976) could explain our generally variable results observed across environments.

Conclusions

Intercropping cassava with maize and taro revealed a disease reducing effect in some ecozones, with generally no effect on yield, and can thus be recommended as part of a cassava

bacterial blight control strategy. No clear effect of potassium fertilizer and mulch on cassava bacterial blight severity was observed. However, due to many other advantages of the green manure, mulching can be recommended to farmers. The results should be confirmed in a second season trial.

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General conclusions

Knowledge on the status of a disease and on the virulence of pathogen strains from different ecozones are a prerequisite to develop control strategies and important for screening varieties for resistance. Cassava diseases were assessed in the forest, forest savanna, wet savanna and dry savanna zones of Togo in relation to agronomic and environmental characteristics, and strains of cassava bacterial blight pathogen *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) were pathologically characterized. Genotype-pathogen and genotype-pathogen-environment interactions were investigated for selection of resistant cassava genotypes suitable to an integrated control system of cassava bacterial blight (CBB). Therefore, cassava genotypes were tested for their reaction to CBB by leaf-inoculation under field conditions in different ecozones of Togo. Furthermore, the genotypes were evaluated for their reaction to stem-inoculation with four highly virulent *Xam* strains from different geographic origins in a glasshouse experiment, and the existance of *Xam* pathotypes was studied. To develop sanitation measures in areas with a high pressure of cassava bacterial, the distribution of *Xam* in different parts of stems of cassava genotypes and the incidence of infected sprouts were determined. Control measures suitable to farmers' conditions and adapted to ecozones, contributing to an integrated control strategy of CBB are recommended.

1. Assessing cassava diseases, high incidences of CBB were observed in all ecozones, even in the forest zone where earlier observations revealed the absence of the disease. However, disease severities were higher in the dry savanna, wet savanna and forest savanna transition zones than in the forest zone. CBB was negatively correlated with rainfall, with decreasing incidence and severity from the herbaceous savanna without trees (dry savanna zone) to the forest zone. Suppressive effects of vegetation and soil moisture on CBB was observed. The disease was more important in weedy plantations, indicating that weeds could play a role in the spread of the disease.

Cassava mosaic disease (CMD) occurred in all ecozones with high incidences. Correlation analysis revealed an increased CMD severity when several cassava varieties were grown in mixture and in fields with abundant weeds. *Cercospora* leaf diseases occurred in all the ecozones with high incidences. The susceptibility of genotypes increased on sandy loam and loamy sand soils. Also, few trees in the surroundings of a field and intercropping systems favored *Cercospora* brown leaf spots (BLS), while a mixture of cassava varieties in a field had a suppressive effect on white leaf spots (WLS). Significant negative relationship between CBB and CMD and between CMD and WLS were found. The cassava disease survey

conducted in farmers' fields in the four agroecological zones provided country-wide and detailed data on cassava bacterial blight, and on two other cassava diseases, cassava mosaic disease and cercosporioses, never reported at this level before in Togo. CBB is becoming more severe in all the ecozones, including the forest zone, where the disease was not found some years before. A further survey is recommended to confirm the present data. Measures to control cassava diseases must be taken to avoid possible epidemics and prevent losses of yields in farmers' productions.

2. Fourty-seven *X. axonopodis* pv. *manihotis* strains from the forest, forest savanna transition, wet savanna and dry savanna zones, were isolated from leaf samples collected across ecozones. Pathological characterization conducted on the susceptible cassava genotype Ben86052 by stem-inoculation revealed, that most of the strains were highly virulent independent of their geographic origin. Only slight differences in virulence among the strains from the four ecozones were observed. Although the lowest CBB severity and field incidence were recorded in the forest zone, all strains collected in this zone were highly virulent. An increase of pathogen aggressiveness over years could be responsible for the increase of disease severity. However, the pathogenic variability of strains has to be tested with various genotypes to investigate strain x genotype interactions, and additional leaf-inoculation experiments may reveal more pathogenic diversity and mechanisms of resistance of the plant. The inoculation of few strains selected among the highly virulent ones is recommended to test genotypes for resistance to cassava bacterial blight.

3. To identify suitable high yielding genotypes with resistance to cassava bacterial blight, 23 improved and local cassava genotypes from Togo and from an international cassava collection from the International Institute of Tropical Agriculture (IITA), were screened under natural infection and after spray-inoculation with *Xam* in the forest, forest savanna transition and wet savanna zones. No genotype with stable resistance to CBB across the ecozones was found, but genotypes TMS92/0429, TMS30572, CVTM4 and TMS91/02316 had a lower disease severity combined with high yield.

High variability in disease expression was observed. The three genotypes Lagos, Toma289 and Toma378 showed susceptible reactions to CBB across all ecozones and over years, while the other genotypes revealed high genotype x environment interactions, with variability between and/or within ecozones over the two years. Genotype TMS30572, a widely distributed improved genotype in West Africa, revealed medium resistance in four of the five environments and resistance in one environment. High CBB severities expressed as area

under severity index progress curve (AUSiPC) were recorded from the forest zone, suggesting that conditions for epidemics are becoming more favorable in this ecozone. Genotypes also showed genotype x environment interactions in their reaction to the CBB symptom types, spot, blight and wilt. Genotypes Main27, TMS30572 and TMS92/0429 with partly resistance against wilt showed highest interactions with the environment in this character, indicating that a possible mechanism inhibiting the development of the wilt symptom is depending on the ecological conditions. Generally, neither the spot nor the blight symptom development were significantly correlated to the development of systemic symptoms. The reaction of genotypes with a strong negative correlation between spot and blight symptom development on the one hand and wilt symptom development on the other hand (e.g. in genotypes TMS92/0057 and TMS92/0326) indicated independent mechanisms of resistance on leaf and stem levels, depending on the cassava genotype. Thus, in breeding for resistance, genotypes with different types of resistance or a combination of both types should be considered.

High variability in cassava dry root yield was observed across and within ecozones over the two years, denoting high genotype x environment interactions. Widely grown local genotypes such as Fétonégbodji, Nakoko, Ankra, Tuaka and Main27 revealed a generally low yield across ecozones, even when symptom severity was low (e.g. genotype Main27), while most of the improved genotypes had higher root production, among them TMS92/0057, with highest production across ecozones, and TMS30572 with the highest root yield of 33.2 t/ha in the forest zone. CBB severity significantly reduced cassava root yield in some environments. Investigating the effect of symptom types on yield, blight and wilt were found to significantly decrease root yield. However, analyzing relationships between CBB symptom types and root yield by genotype, significant decreases of yield in correlation to symptom type were generally not observed.

Genotypes including Lagos, TMS92/0057, TMS92/0343 and Ben86052, though highly susceptible, had a high root yield, and could be identified as tolerant, since they did not react with a yield decrease on an increasing disease level. Genotypes TMS30572 and TMS91/02316 with low disease severity and high root yield could be recommended to farmers, whereas genotypes TMS92/0326, TMS92/0057, Cameroon and Ben86052, tolerant to the disease, in spite of their higher yield, but should be avoided by farmers due to the risk of dissemination of inoculum. Genotypes Main27 and CVTM4, resistant, but with low root yield could be recommended to breeders to introduce their resistance characteristics into the breeding materials. Additionally, genotypes TMS30572 and TMS92/0429 should be used to

introgress their high resistance to the wilt symptom into genotypes with susceptibility to systemic symptoms.

4. After stem-inoculation with 4 *Xam* strains from different geographic origins, six genotypes showed a resistant reaction against the four strains, with genotypes CVTM4 and Gbazékouté being the most resistant. Most of the genotypes tested including the reference genotype Ben86052, with susceptible reaction against at least two strains were resistant to at least one strain. Thus, strain x genotype interactions were observed, and six groups of differential genotypes which could be useful for pathotype identification, were determined, and the four strains, Uganda12, GSPB2506, GSPB2507 and GSPB2511, originating from Uganda, Cotnou, Benin and Ibadan and Onne, Nigeria, respectively, represented four different pathotypes.

Analyzing data from greenhouse experiments and field trials, genotypes Ankra, Cameroon, Fétonégbodji, Lagos, Nakoko and the reference genotype Ben86052, which were susceptible after stem-inoculation revealed susceptibility in field trials, while genotypes 312-524, TMS91/02322 and TMS30572 were identified as medium resistant after inoculation of the four strains as well as in field experiments. Genotypes CVTM4 and TMS91/02316 were resistant after stem-inoculation and also belonged to the more resistant group in the general ranking across ecozones in field trials, while genotypes Gbazékouté, Toma289 and Toma378 were resistant after stem-inoculation, but were among the most susceptible genotypes after leaf-inoculation in field trials. Field-testing in various ecozones and stem- and leaf-inoculation are recommended for selection of resistant genotypes.

5. The colonization and distribution of *Xam* in different parts of the stem of infected cassava plants of two locally important cassava genotypes, Gbazékouté and Fétonégbodji, compared to the standard susceptible and resistant genotypes Ben86052 and TMS30572, respectively, were investigated. Although some plants of the susceptible genotypes Ben86052 and Fétonégbodji had only shown leaf symptoms in the field, bacteria were generally found in all parts of the stems, indicating a latent phase of the pathogen in stems. However, most of the planted cuttings from the stem parts of Ben86052 and Fétonégbodji did not develop CBB symptoms on the new sprouts, although the stems harboured the pathogen. These observations could be generally due to the discontinuity of the colonization of *Xam* in stems or to differences in vascular connections between the xylem of new sprouts and the one of the old cuttings hindering the transfer of the pathogen. Since the pathogen was not found in any part

the tested plants of genotype TMS30572 nor did any of the new shoots from the planted cuttings show CBB symptoms, and due to its medium resistant reaction to leaf-inoculation in field trials, symptomless plants of this genotype selected from non-infected fields could be considered free of *Xam*, and, thus, TMS30572 is recommended to farmers to receive CBB-free cassava planting material. Furtheron, breeders should consider differences among varieties in restriction of systemic infection, latent infection of stems and restriction of sprout symptoms as additional characteristics in selection of varieties for resistance.

6. Intercropping cassava-maize and/or cassava-taro, according to ecozone, significantly reduced disease severity in a susceptible genotype compared to cassava monocropping in the forest highland, the wet savanna and the forest savanna transition zones, with generally no significant negative yield effect. Thus, these cropping systems can be recommended in these ecozones as part of an integrated control strategy for CBB. A suppressive effect of intercropping might be more obvious, when a medium resistant genotype is used. Therefore, the combination of medium resistant genotypes and an intercropping system, both adapted to the respective ecozone, could be recommended to farmers. Besides the possible effect of disease reduction, yield stability and an additional yield would be achieved by planting the second crop. No clear effect of potassium fertilizer and mulch on CBB severity was observed. However, due to many other advantages of the green manure, mulching can be recommended to farmers. The results should be confirmed in a second season trial.

Annex 1: Disease severity (AUSiPC) of non-inoculated and inoculated genotypes in forest, forest savanna transition and wet savanna zones in two years

	Forest zone				Forest savanna transition zone				Wet savanna zone	
	1998		1999		1998		1999		1999	
Genotypes	Non-inoc. AUSiPC	Inoc. AUSiPC	Non-inoc. AUSiPC	Inoc. AUSiPC	Non-inoc. AUSiPC	Inoc. AUSiPC	Non-inoc. AUSiPC	Inoc. AUSiPC	Non-inoc. AUSiPC	Inoc. AUSiPC
312-524	4.7	5.9	6	7.3	4.7	5.9	5.5	5.5	5.2	8.2
Ankra	4.2	6.4	7.1	8.8	6.5	7.1	6.7	7.6	5.2	6.3
Ben86052 "C"	5.4	7.2	7.1	7.9	5.4	5.6	5.7	6	6.5	7.4
Boram	4.5	7.7	6.7	7.8	nd	nd	nd	nd	nd	nd
Cameroon	4.8	6.9	6.5	7.7	5.6	5.5	6	6.1	7.2	6.5
CVTM4	4.1	3.7	4.9	6	4.9	5.9	5.1	5.1	3.1	5.1
Fétonégbodji	4.1	6	6.2	8.3	6.2	5.1	6.5	6.1	6.4	7.5
Gbazékouté "C"	5.6	6	6.8	7.7	6.4	7.2	6.3	6.6	6.2	7.3
Lagos	4.5	8.4	7.3	8.5	6.2	8	6.1	6.6	6.7	8.5
Main27	3	4.8	4.7	4.1	4.8	5.1	5.3	5.6	3.7	4.3
Nakoko	4.7	6.9	6.2	9.1	5.6	5.4	6.5	6.1	5.4	8
Sorad	nd	nd	nd	nd	nd	nd	nd	nd	5.4	7.8
TMS30572 "C"	3.3	4.4	4.5	4.5	4.9	4.4	5.2	5.1	3.5	5.3
TMS4(2)1425	3.8	4.9	5.3	9	5.4	6.4	5.2	4.7	4	4.7
TMS91/02316	4	6.3	5.1	6.3	4.6	5.3	5	4.6	4.5	6.6
TMS91/02322	4.7	5.4	5.2	6.5	6.2	5.7	6.6	6	4.8	5.6
TMS92/0057	5.3	6.1	6.5	6.8	6.1	5.8	6.3	6.3	5.1	6.5
TMS92/0067	2.9	6.6	6	7.3	5.8	5.3	6	6.4	5.3	5.4
TMS92/0326	5	6.5	5.7	6.3	5.5	5.6	5.7	5.9	5.7	6.1
TMS92/0343	5	7.2	6.4	5.7	5.1	5.7	6.2	5.6	5.5	6.6
TMS92/0429	4.5	4.9	3.9	4.9	3.2	4.6	4.7	4	3.5	3.9
TMSCBS10(80411)	nd	nd	nd	nd	nd	nd	nd	nd	4.6	5.3
Toma159	3.9	6.1	6.5	6.4	5.4	5.1	5.3	5.1	nd	nd
Toma219	nd	nd	nd	nd	6	6.3	7.3	6.5	nd	nd
Toma289	4.7	8.2	8.2	6.9	6.7	6.5	7.3	7.2	5.7	7.6
Toma378	6.1	6.8	7.6	7.7	5.8	7.1	5.6	6.1	5.6	7.6
Tuaka	3.6	5.9	5.9	5.1	5.4	4.7	6.3	6.2	5.8	8.9
Total AUSiPC	106.6	149.4	146.5	166.8	132.6	139.3	142.2	141.2	124.7	156.8
Range	2.9 - 6.1	3.7 - 8.4	3.9 - 8.2	4.1 - 9.1	3.2 - 6.7	4.4 - 8.0	4.7 - 7.3	4.0 - 7.6	3.1 - 7.2	3.9 - 8.9
SE	C 0.51	C 0.27	C 0.39	C 0.23	C 0.22	C 0.66	C 0.13	C 0.26	C 0.41	C 0.66
SE	X 0.80	X 0.48	X 0.66	X 0.40	X 0.34	X 1.12	X 0.21	X 0.45	X 0.71	X 1.15

"C" = check genotype; X = other genotypes than check;SE = standard error; Non-inoc. = non-inoculated; Inoc. = inoculated; nd = not determined.

Annex 2: Screening of 24 cassava genotypes for resistance to cassava bacterial blight (CBB) in three ecozones of Togo: root dry weight (DW) in (t/ha)

	Forest zone				Forest savanna transition zone				Wet savanna zone	
	1998		1999		1998		1999		1999	
	Non-inoc.	Inoc.**	Non-inoc.	Inoc.**	Non-inoc.	Inoc.	Non-inoc.**	Inoc.	Non-inoc.	Inoc.
Genotypes	Root DW	Root DW	Root D W	Root DW	Root DW	Root DW	Root DW	Root DW	Root DW	Root DW
312-524	17.8±3.96	7.8±1.57	19.3±1.97	17.1±2.14	10.0±1.71	9.1±1.26	10.6±1.26	9.1±2.39	6.2±2.20	11.9±3.72
Ankra	2.2±0.15	10.3±3.46	15.5±2.99	10.9±4.07	2.2±1.43	12.1±1.93	8.8±2.65	6.7±0.72	2.6±3.44	3.8±1.48
Ben86052 "C"	12.3±2.93	14.6±2.71	23.9±4.19	22.9±4.86	13.8±2.78	18.4±2.85	18.2±3.36	12.0±3.59	2.0±0.99	11.9±2.62
Boram	3.7±1.30	4.9±0.74	3.1±1.01	7.0±1.47	nd	nd	nd	nd	nd	nd
Cameroon	18.3±2.67	13.8±2.93	22.3±4.38	16.8±2 .57	21.0±6.10	18.2±3.76	20.3±2.20	21.0±2.73	0.6±0.07	8.2±2.56
CVTM4	11.9±1.49	13.5±3.24	9.2±1.59	15.8±2.96	15.8±2.56	28.1±9.58	12.9±1.60	10.5±1.50	9.7±2.02	10.5±2.98
Fétonégbodji	6.3±1.29	5.5±2.17	5.2±1.10	4.3±1.08	5.5±0.73	0.3±0.72	5.6±0.62	5.8±1.23	3.8±0.75	2.7±0.53
Gbazékouté "C"	12.7±1.98	17.8±1.66	16.8±3.35	26.9±5.22	12.6±1.53	19.4±2.59	9.4±1.97	8.0±1.33	3.1±1.08	11.6±2.88
Lagos	9.8±3.24	10.3±0.70	18.0±3.46	17.5±2.75	17.8±1.63	25.3±4.28	18.6±3.01	17.5±2.81	2.5±0.81	9.9±3.16
Main27	6.4±0.96	11.5±1.81	10.7±2.24	16.9±5.56	5.6±2.29	7.1±1.55	4.8±1.02	6.0±0.73	8.6±0.85	9.0±2.86
Nakoko	7.8±0.98	11.3±2.59	6.2±1.62	6.5±2.08	6.4±2.23	7.9±2.99	5.1±1.11	4.8±0.66	3.4±2.06	4.2±1.22
Sorad	nd	nd	nd	nd	nd	nd	nd	nd	5.0±2.98	3.9±1.21
TMS30572 "C"	14.7±3.20	20.2±3.68	21.6±4.90	33.2±7.43	15.6±2.97	21.3±4.61	11.9±1.63	8.8±1.10	9.7±3.01	8.8±1.85
TMS4(2)1425	13.4±4.29	9.7±5.31	13.1±3.60	17.4±3.83	5.9±0.27	6.2±1.52	3.3±0.49	9.4±1.95	4.0±1.44	1.2±3.75
TMS91/02316	21.4±5.43	11.8±3.95	22.5±2.32	22.8±3.75	16.6±4.67	18.7±5.73	14.0±2.56	14.0±1.97	10.9±2.20	13.3±1.92
TMS91/02322	7.1±1.38	22.6±4.70	26.5±3.15	19.2±6.32	9.6±2.15	12.9±1.47	9.2±1.91	6.5±1.03	8.1±1.64	11.2±3.42
TMS92/0057	15.4±3.44	16.5±3.62	32.1±2.57	26.1±0.95	17.7±1.45	29.1±4.81	18.9±4.65	12.9±2.07	23.1±4.42	21.1±1.99
TMS92/0067	3.8±5.08	16.5±4.92	29.0±5.74	21.6±3.93	6.9±1.63	12.3±1.19	14.2±0.77	9.8±1.35	2.5±1.07	5.8±1.55
TMS92/0326	19.8±2.55	7.3±2.53	27.3±2.47	26.9±4.88	14.0±3.77	17.2±4.46	15.8±3.16	17.0±3.25	7.7±0.99	17.7±3.42
TMS92/0343	14.9±4.13	14.7±3.40	23.0±2.78	19.0±2.87	16.3±2.52	21.1±1.56	14.9±2.10	11.2±1.28	8.3±2.01	11.9±3.81
TMS92/0429	10.8±2.13	13.7±0.94	25.4±6.86	29.2±5.14	11.1±3.27	8.5±3.41	13.3±4.23	20.9±6.07	12.8±2.42	12.2±2.41
TMSCBS10(80411)	nd	nd	nd	nd	nd	nd	nd	nd	6.3±0.51	13.9±2.31
Toma159	4.3±0.14	10.6±2.18	16.2±1.75	7.0±0.62	3.9±2.35	8.9±2.38	18.4±10.54	5.6±1.31	nd	nd
Toma219	nd	nd	nd	nd	9.6±0.39	5.6±0.37	4.6±0.30	6.1±1.58	nd	nd
Toma289	2.9±0.82	4.4±1.32	3.7±0.26	5.5±1.59	8.0±0.27	8.6±0.88	8.4±1.07	7.9±2.77	0.9±2.88	2.4±0.66
Toma378	9.6±1.96	13.6±2.36	15.0±1.57	17.2±3.24	12.9±1.18	14.0±1.66	14.3±5.08	7.9±1.28	7.2±0.13	7.6±1.41
Tuaka	4.0±0.81	12.6±3.29	17.2±1.67	25.1±7.75	1.5±1.95	8.1±2.15	1.0±0.46	-[2]	0.8±2.68	6.8±0.87

**= significance at probability level of 0.01 between check genotypes "C" (Ben86052, Gbazékouté and TMS30572); 1 standard error; 2 missing value of root dry weight in the inoculated plots in the forest savanna transition zone in year 1999; Non-inoc. = non-inoculated; Inoc. = inoculated; Root DW = root dry weight.

Annex 3: Rainfall (mm) in 12 sites of Togo

	Year	Jan.	Feb.	Mars	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Tabligbo[1]	1998	20.0	42.2	2.6	24.3	236.0	176.1	74.4	68.4	87.4	144.8	53.5	2.1	931.8
	1999	34.7	29.1	169.8	100.5	129.8	173.3	162.3	143.8	139.9	229.7	28.6	0.0	1341.5
	2000	7.1	11.5	62.0	40.7	109.4	86.2	86.7	90.1	87.9	120.8	16.4	0.0	718.8
Tsévié[1]	1998	37.1	16.3	73.9	0.0	168.6	128.0	51.6	14.3	110.9	138.2	39.4	0.7	779.0
	1999	49.0	2.5	98.3	48.8	118.9	200.5	128.9	67.7	165.4	122.7	64.9	0.4	1068.0
	2000	2.3	24.2	53.8	83.0	37.5	106.7	26.9	79.7	83.2	117.4	72.4	20.0	707.1
Kpalimé[2]	1998	33.2	52.0	33.6	162.2	194.8	254.8	117.3	52.8	141.5	238.7	14.7	19.4	1315.0
	1999	27.7	82.2	106.6	138.6	98.9	386.8	370.8	232.7	238.5	261.6	82.3	0.0	2026.7
	2000	0.0	0.0	48.3	59.4	162.6	392.6	118.7	80.0	448.0	92.0	43.9	6.5	1452.0
Adéta[2]	1998	2.5	7.4	25.8	91.3	253.2	199.6	80.2	97.4	93.1	154.1	8.8	4.8	1018.2
	1999	0.0	2.8	70.1	64.1	133.8	120.1	97.0	89.2	110.4	146.6	30.4	0.0	864.5
	2000	21.3	0.0	82.3	78.6	161.5	351.5	201.3	265.0	241.0	74.0	4.6	2.0	1483.1
Danyi[2]	1998	6.0	58.3	31.2	124.4	122.7	161.6	80.6	116.5	208.4	105.8	20.0	17.6	1053.1
	1999	15.0	58.6	25.2	74.0	82.9	140.7	188.4	104.7	188.5	196.2	90.7	0.0	1164.9
	2000	17.6	0.0	42.0	95.3	91.5	291.4	300.5	200.0	216.7	148.1	22.1	0.6	1425.8
Atakpamé[3]	1998	0.1	74.9	6.4	284.2	240.0	131.0	195.3	189.6	62.6	116.1	0.4	0.4	1301.0
	1999	170.0	47.2	54.2	153.3	165.2	156.5	309.5	350.4	221.4	168.7	13.7	0.0	1810.2
	2000	6.5	0.0	14.5	110.9	109.6	152.1	140.5	156.7	259.9	115.8	2.3	0.0	1089.6
Stouboua[3]	1998	13.3	45.2	36.7	65.1	96.7	189.8	195.7	281.8	277.7	167.0	0.0	2.0	1371.0
	1999	0.0	34.7	22.8	69.9	212.7	134.2	204.2	217.1	287.2	188.1	19.2	0.0	1309.1
	2000	0.2	0.0	17.5	175.5	117.9	242.4	161.5	322.5	175.9	96.4	0.0	0.0	1309.8
Sokodé[3]	1998	9.9	0.7	0.3	111.4	95.2	149.6	213.9	240.0	327.5	229.3	0.0	19.0	1396.8
	1999	0.0	29.0	49.1	180.5	84.3	78.5	162.3	330.3	219.8	123.7	12.5	0.0	1270.0
	2000	0.0	0.0	5.3	87.7	84.0	189.7	243.8	239.6	210.8	104.5	0.0	0.0	1165.4
Kara[4]	1998	0.0	5.6	0.0	64.7	192.2	323.5	203.3	246.4	243.0	137.3	0.0	0.0	1416.0
	1999	0.0	63.7	19.6	87.2	78.3	107.1	158.0	266.3	193.0	200.0	0.0	0.0	1173.2
	2000	7.0	0.0	24.2	54.5	93.1	185.7	182.3	260.0	292.6	90.3	13.3	0.0	1203.0
Niamtougou[4]	1998	10.0	1.0	35.6	50.9	147.0	206.1	247.1	293.3	208.3	106.7	0.0	0.0	1306.0
	1999	0.0	29.5	2.6	69.1	160.3	188.1	284.7	312.4	374.9	229.1	0.0	0.0	1650.7
	2000	38.2	0.0	3.6	89.2	89.3	166.4	237.4	305.5	267.9	133.6	0.0	0.0	1331.1
Mango[4]	1998	0.0	0.6	0.0	33.2	126.8	173.7	154.6	355.3	342.5	93.3	0.0	0.0	1280.0
	1999	0.0	1.1	17.8	86.9	84.5	115.1	227.6	400.3	306.9	169.7	0.0	0.0	1409.9
	2000	0.0	0.0	0.0	96.5	147.0	140.9	126.6	95.9	254.9	45.7	0.0	0.0	907.5
Dapaong[4]	1998	0.0	0.0	0.0	134.9	161.1	170.1	140.5	351.2	215.9	105.5	0.0	0.0	1279.2
	1999	0.0	40.0	8.6	90.6	22.5	76.8	217.3	439.4	283.3	46.2	0.0	0.0	1224.7
	2000	0.0	0.0	0.0	33.9	82.8	112.8	112.7	175.6	208.2	101.3	0.0	0.0	827.3

[1]forest savanna transition zone; [2]forest zone; [3]wet savanna zone; [4]dry savanna zone.

Acknowledgments

I express my sincere thanks to Pof. Dr. B. Hau for accepting to be my referee, and for his advices, constructive discussion and correction of the present work.

I am most grateful to Dr. K. Wydra to be my supervisor, for her guidance, correction of the work, constructive comments, and for providing me a scholarship to pursue the PhD degree.

I am specially grateful to Prof. Dr. A. von Tiedemann for his readiness to be my co-referee.

Great thanks to Prof. Dr. H. Stützel, Director of Institute of Vegetable Protection, and Dr. Elisabeth Esch, Department of Applied Genetic, University of Hannover, for accepting me for examination.

My particular thanks go to Dr. K. Kpémoua acting as my supervisor in Togo, for his guidance, advices and friendship.

I am grateful to Dr. K. Rudolph for his advice and friendly behavior.

Special thanks to Sam Korie from IITA for his readiness to help, his advices and constructive comments for statistic.

Due respect to the director of ITRA Dr. C. A. Agbobli for providing laboratory and fields for experiments, and to IITA for providing laboratory and glasshouse space for trials.

Cordial thanks to all my friends from Togo and from Benin, especially, B. Bissang, V. Zinsou, and to H. Amiton and K. Assignon at ITRA for their friendship.

Grateful thanks to all my family, especially to my parents for their prayers.

I express my heart affection to A. Badanadou and O. M. Banito for their moral support and their patience.

Thanks to the Europian Community for the financial support.

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Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation eigenständig verfasst habe und sie weder an der Universität Hannover, noch an anderen Universitäten als Diplomarbeit oder andere Prüfungsarbeit verwendet oder eingereichet habe. Die zur Erstellung der Arbeit benutzten Hilfen oder Hilfsmittel habe ich vollständig aufgeführt.

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