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Synergistic Relationship of Bacterial Blight and Anthracnose Disease Pathogen in Cassava Multiple Infection

C.N. Fokunang, [1]A.G.O. Dixon and T. Ikotun
Department of Crop Protection and Environmental Biology
University of Ibadan, Oyo State, Nigeria
[1]International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria

Abstract: A study was conducted at the greenhouse of the International Institute of Tropical Agriculture, Ibadan, Nigeria to investigate the synergistic relationship of *Xanthomonas campestris* pv. *manihotis* (causal agent of cassava bacterial blight CBB), and *Colletotrichum gloeosporioides* f.sp. *manihotis* (causal agent of cassava anthracnose disease CAD), in cassava multiple infection. There were statistical differences ($P \leq 0.05$) in disease symptom expression among the cassava genotypes following sequential inoculation of the pathogens and single pathogen inoculation alone. The fastest occurrence and the highest overall mean severity of the disease symptoms (necrotic lesion, shoot die-back, gum exudate release and wilt symptoms) were observed in cassava genotypes sequentially inoculated with bacterial, followed by the fungus one week later (BFW) or in mixed combination prior to inoculation. Defoliation was high in genotypes inoculated with bacterium first followed by the fungus on the same day, with a low disease symptom expression in plants inoculated with the fungus or bacterium alone.

Key words: Cassava, bacterial blight, anthracnose, multiple infections

Introduction

Cassava is one of the least risky food crops in Africa because of its enormous ability to recover from drought, disease and pest attacks when favourable conditions returns. Yields are reasonable under marginal soil conditions and both storage roots and leaves are available all the year round. Since research began on cassava in the early 1970's, it has been found that the crop is susceptible to at least thirty different diseases of fungal, bacterial, viral and mycoplasma origin (Lozano ***et al.***, 1981 and Theberge, 1985). Of all the cassava diseases, African cassava mosaic (ACMD), bacterial blight (CBB) and anthracnose (CAD) are of major economic importance.

The disease is host specific and restricted to cassava host (Ikotun, 1981 and Muyolo, 1984). CBB has been reported in many countries throughout Africa, and result in a complete yield loss under conditions favourable for its development and spread (Lozano, 1986 and Fokunang ***et al.***, 2000). The major means of spread of CBB is by movement of infected planting materials. In the field, rain-splash is also important in spreading the disease (Elango and Lozano, 1980).

In Africa, where there are two distinct rainy and dry seasons, the disease cycle of CBB consist of two phases, an angular leaf spot phase and an epiphytic phase. The angular leaf spot phase begins soon after the first rains and continues during the rainy season. Wilting and defoliation of infected leaves follow this, tip dieback and death of the plant in susceptible varieties. The epiphytic phase begins with the onset of the dry season when the pathogen survives the dry season of 5-6 months as an epiphyte, and increases in number when moisture becomes available (Van den Mooters ***et al.***, 1987 and Hahn ***et al.***, 1989).

The disease caused by ***Colletotrichum gloeosporioides*** f.sp. ***manihotis*** is an epidemic disease characterized by particular symptoms (cankers on stems, branches and fruits, leaf spots and tip die-back) on aerial parts of the diseased plants (Muimba, 1982 and Makambila, 1987). The appearance of the disease depends on the cassava variety and the infected plant parts.

In older stems CAD infection usually occurs as round and stringy lesions which develop into deep cankers. Stem deformation occurs in some cultivars, causing the stems to become brittle and easy to break by wind action (Ikotun and Hahn, 1992 and Fokunang ***et al.***, 1999a). The deeper cankers sometimes affect the pith of the plant thus blocking translocation of vital elements to point of utilization or storage (Van der Bruggen and Maraite, 1987). CAD severity could lead to a significant loss of planting materials. Severely infected stems and seeds in some cases result in a decrease of 20-45% germination (IITA, 1987; Fokunang ***et al.***, 1999b).

Information to assess the synergistic relationship between CBB and CAD is lacking. This is partly due to the fact that the two diseases have been studied in isolation. CBB and CAD is a threat to cassava growers especially in the high rainfall belts of Africa where the two diseases had attained an epidemic level, and causing total crop failure. There is therefore the need to establish to what extend the two pathogens in combination could severely affect plants.

The present study was conducted to assess the synergistic relationship *C. **gloeosporioides*** f. sp. ***manihotis and X. campestris*** pv. ***manihotis*** in cassava multiple infection.

Materials and Methods

Experimental site

This study was conducted at the greenhouse of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The planting materials were collected from the cassava germplasm selection of the Tuber and Root Crop Improvement Programme (TRIP) IITA.

Isolation of pathogens

Cassava stems showing symptoms of anthracnose infection (dark brown lesions, deep cankers) and small portions from developing leaf spots and internal tissues from stem lesions showing bacterial exudates were collected from cassava field plots at IITA and used for isolation of *C. **gloeosporioides*** f. sp. ***manihotis and X. manihotis***, respectively. Small pieces of infected materials were cut from the advancing infected edges, surface-sterilized for approximately 3 min in 10% sodium hypochlorite solution, and rinsed in five successive changes of sterile distilled water. The stem pieces (anthracnose infected) were dried on sterilized filter papers and placed on Potato Dextrose Agar containing 100 mg $dm3^{-1}$ sodium novobiocin (PDA+N), while bacteria-infected plants

were plated on nutrient agar (NA). The material was incubated at 25°C under intermittent mixed irradiation for 12 h day^{-1}. To obtained single bacterial colonies, NA plates were opened and dried in an inverted position at 50°C for 5 min. These were then inoculated by drawing four perpendicular sets of three streaks each at the edge of each NA plate. 72 h after incubation at 25±2°C bacterial cultures were purified three times by single colony-transfer on fresh dried NA plates until an axenic culture was obtained.

In vivo assessment of the interaction between _C. gloeosporioides_ f. sp. _manihotis_ and _X. campestris_ pv. _manihotis_ in cassava seedling genotypes

The interaction between the two pathogen was assessed by sequential inoculation in the greenhouse on three cassava seedling genotypes (91/00684, 91/00051, and 30001). The fungal (F) and bacterial (B) inoculations alone were standing as single checks, and the sterile distilled water as uninoculated control. Inoculum suspensions of bacterium and fungus were prepared using the method of Kiraly ***et al.*** (1974). The concentrations of the fungal and bacterial suspensions were respectively adjusted to 10^6 and 10^8 colony forming units (CFU) ml^{-1} of sterile distilled water with the aid of a haemocytometer. The mixed inoculum (F+B) was prepared by mixing v/v the fungal and bacterial cell suspensions before inoculation.
Three sequences of inoculations were used as follows:

The sequential inoculation with the two pathogens inoculated on the same day;
The single pathogen inoculated alone
The sequential inoculations with the second pathogen inoculated after the disease caused by the first was apparent.

In the sequential inoculations the second pathogen was introduced at the same inoculation point as the former. Unless otherwise stated, on each of the three cassava seedling genotypes the study consisted of eight treatments as follows;

i = Single pathogen inoculation with the fungus (F) alone;
ii = single pathogen inoculation with the bacterium (B) alone;
iii = double pathogen inoculation with the mixture of the fungal and bacterial (F+B) cell suspension prior to inoculation;
iv = double pathogen inoculation with the bacterium first followed by the fungus inoculated sequentially on the same day (BFS);
v = double pathogen inoculation with the fungus first followed by the bacterium inoculated sequentially on the same day (FBS);
vi = double pathogen inoculation with the bacterium first followed by the fungus inoculated sequentially one week later (BFW);
vii = double pathogen inoculation with the fungus first followed by the bacterium inoculated sequentially one week later (FBW);
viii = control (sterile distilled water inoculated plants).

One month old cassava seedling genotypes planted in plastic pots filled with sterilized mixture of soils and sand (2:2 v/v) in the greenhouse, were inoculated by spraying and stem puncture technique (Muimba, 1982). The potted plants were placed close to each other on benches to obtain the thick canopy needed for disease development. Seedling pots were arranged in a randomized complete block design with split plot arrangement, in four replications, with the genotypes as the main plot and the bacterium and or fungus as subplots. The plants were watered daily throughout the growth period. Temperature and relative humidity were monitored with a recording hygrothermograph. The greenhouse temperature ranged from 26-32°C in the day and 22-26°C at night, and relative humidity averaged 80-98%.

The appearance of symptoms and development of the diseases were observed and described at regular intervals, starting from the fourth day after inoculation. Records of leaf infection (%), Defoliation (%) wilts (%), lesion size, gum exudate and shoot die-back were recorded at 2 weeks intervals for 10 weeks.

Statistical analysis

The data for leaf infection, defoliation, wilt, lesion size, gum exudate and shoot die-back were subjected to analysis of variance ANOVA (SAS Institute, 1989). Fischer-protected LSD of mean separation was performed where necessary when the analysis of variance showed significance.

Results

Effect of CBB and CAD pathogen interaction on disease symptom expression in cassava genotypes

The effect of inoculation of CAD and CBB pathogens showed an increase in shoot die back and disease severity with bacterial inoculation followed by the fungus on the same day, and also the two pathogens in mixed combination before inoculation (B+F) (Fig. 1). Lesion size and wilt were more expressed in bacterial inoculation followed by the fungus on the same day (BFS) and bacterial inoculation followed by the fungus a week later (BFW) in all the genotypes (Fig. 2). Expression of defoliation and gum exudates release were maximum in bacterial and fungal suspension combinations before inoculation (F+B) and in bacterial inoculation followed by the fungus on the same day (BFS), (Fig. 3).

Pathogen inoculum treatment and disease symptom expression

Spraying cassava leaves with bacterial inoculum first followed by fungal spray gave the highest leaf infection of 4.03% (Table 1), while fungal inoculation alone showed the least leaf infection with mean value of 2.46%. Significant variations were observed with the pathogen inoculum treatments on defoliation, wilt, necrotic lesion, gum exudate and shoot dieback symptoms. Defoliation was low among the inoculation treatments with mean maximum value of 28.75% recorded in the bacterial inoculation alone, followed by the bacterial with fungal inoculation on the same day with mean value of 17.58%.

Wilt symptom reactions was highest (56.92%) for bacterial with fungal inoculation a week later, followed by bacterial and fungal inoculation on the same day, with a mean wilt of 52% (Table 1).

Table 1: Effect of sequential inoculation of anthracnose and bacterial blight pathogens on disease symptom expression

Pathogen inoculation sequence	Leaf infection (%)	Defoliation (%)	Wilt (%)	Necrotic Lesion (mm)	Gum exudate (%)	Shoot die-back (%)
die-back						
Bacterium (B) alone	3.75a	28.75a	45.25c	28.17c	88.75a	51.08c
Fungus (F) alone	2.64c	12.58e	13.67e	18.92e	0.00e	20.03e
B+F before inoculation	3.08b	15.67bc	47.00c	26.50d	83.08b	56.42b
B+F same day	3.32b	17.58b	52.00b	32.42b	87.83a	56.00b
F+B same day	3.05b	15.33bcd	42.58d	26.08d	78.67c	42.67d
F+B 1 week later	4.03a	13.75cde	56.92a	37.33a	88.25a	68.68a
F+B 1 week later	3.10b	13.00de	42.25d	26.33a	64.17d	50.67c
Control	0.00d	0.00f	0.00f	0.28f	0.00e	.00f

Means in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 2: Cassava clone reaction to sequence of inoculation with CAD and CBB pathogens at different periods after inoculation

Cassava clones	Leaf infection(%)		Defoliation (%)		Wilt (%)		Lesion size/mm		Gum exudate (%)		Shoot die-back (%)	
	6 WAI	8WAI	6 WAI	8 WAI	6 WAI	8 WAI	6 WAI	8 WAI	6 WAI	8 WAI	6 WAI	8 WAI
91/00052	2.59a	3.25a	33.44a	19.81a	44.03a	56.22a	21.00a	27.59a	44.66b	55.59b	35.16b	43.75b
91/00684	2.39b	2.72b	13.28c	13.16b	15.16c	10.42c	16.6c	22.16c	62.91a	71.78a	38.40a	45.34a
30001	2.33b	2.66b	25.84b	10.78c	36.38b	46.50b	18.59b	24.69b	42.93c	56.66b	31.09c	40.50c

WAI= Weeks after inoculation, Means in the same column followed by the same letter is not signigicantly different ($P \leq 0.05$)

The size of necrotic lesions was highest with mean value of 37.33 mm in BFW, with the least value of 18.92 mm in fungal inoculations alone. For plant exudate release, bacterial inoculation recorded the highest mean value of 88.75% and the fungal inoculation alone showed no exudate. Dieback symptoms were least expressed in fungal inoculations with a mean value of 20.08%, while BFW and mixed inoculum of bacterium and fungus recorded mean maximum shoot dieback symptoms of 68.68 and 56.42%, respectively.

Reaction of cassava seedling genotypes to pathogen inoculation treatments

Overall reactions cassava genotypes to leaf infection were generally low at six and eight weeks after inoculation (WAI). The highest leaf infection of 3.25% was recorded on genotype 91/00052 at 8 WAI (Table 2). Percentage defoliation at 6WAI was highest in genotype 91/00052 (33.44%), while low defoliation of 13.28% was recorded in genotype 91/00684. Wilt symptoms at 8WAI was highest in genotype 91/00052 with a mean value of 56.22%, while genotype 91/00684 recorded the lowest wilt with a mean value of 10.42%. The maximum dieback symptom was recorded on genotype 91/00052 (45.34%) while genotype 30001 recorded the least dieback symptom of 40.50%.

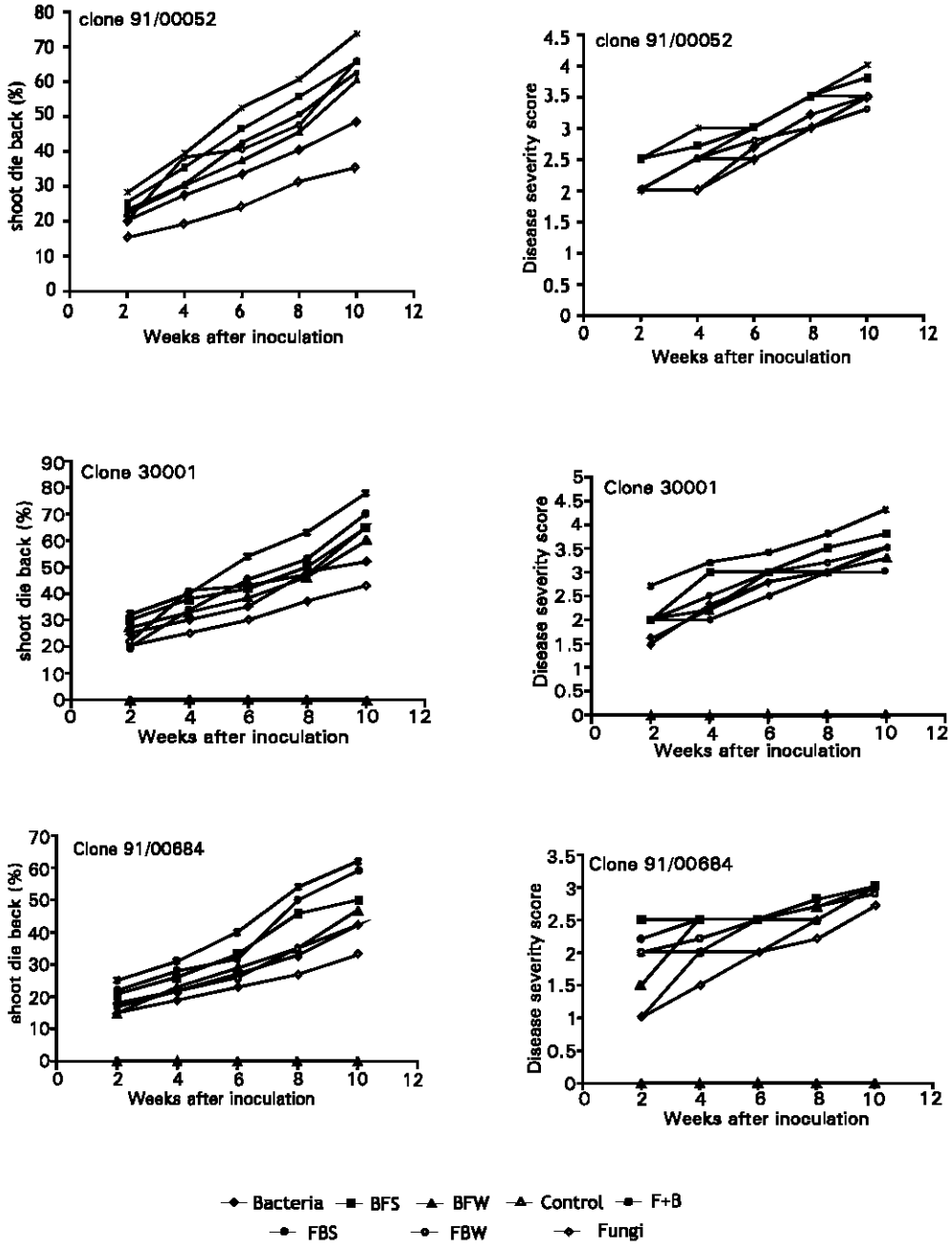

Fig. 1: Effects of interaction of CAD and CBB pathogens on shoot die back and disease

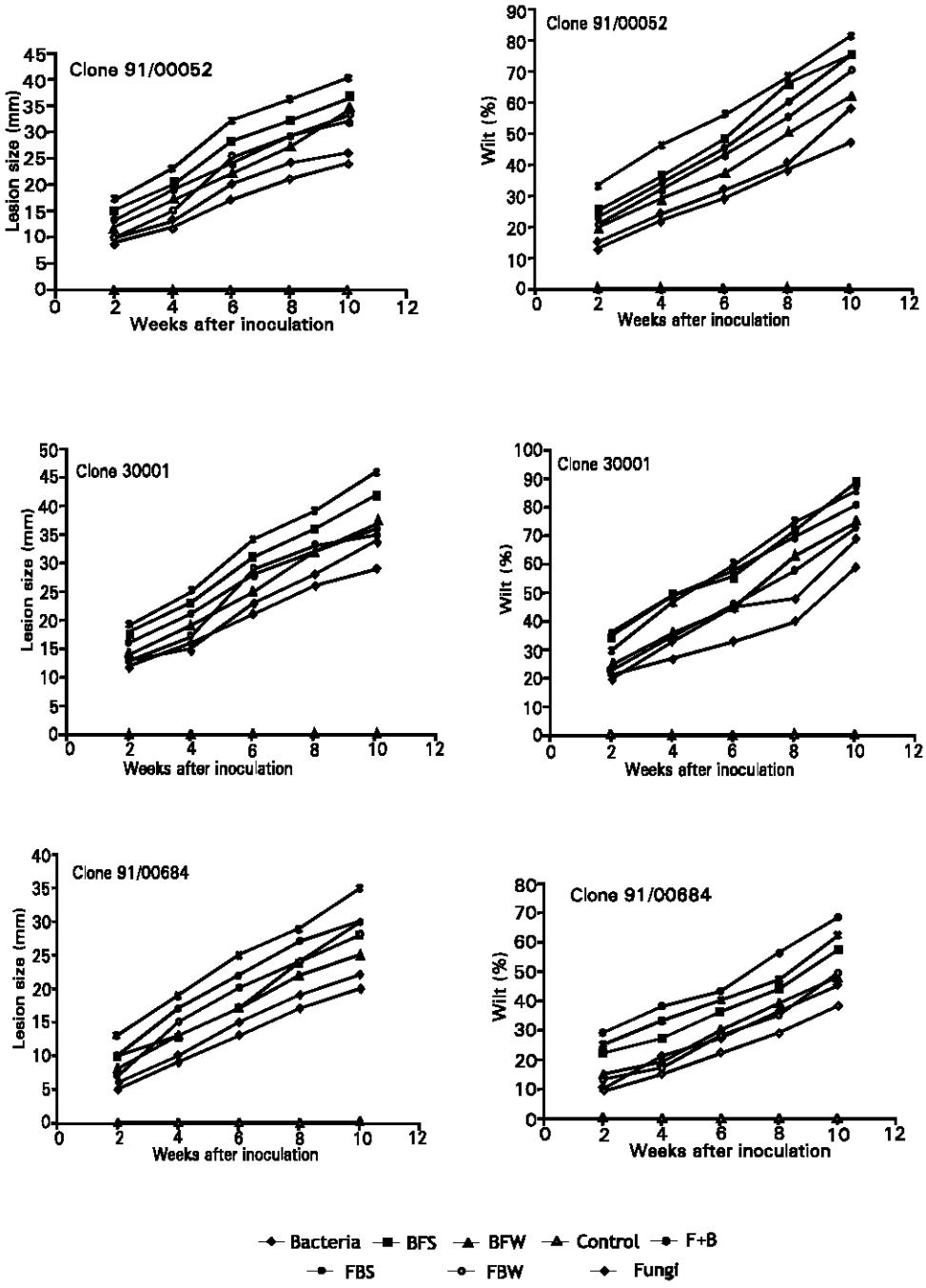

Fig. 2: Effects of interaction of CAD and CBB pathogens on lession size and wilt symptoms

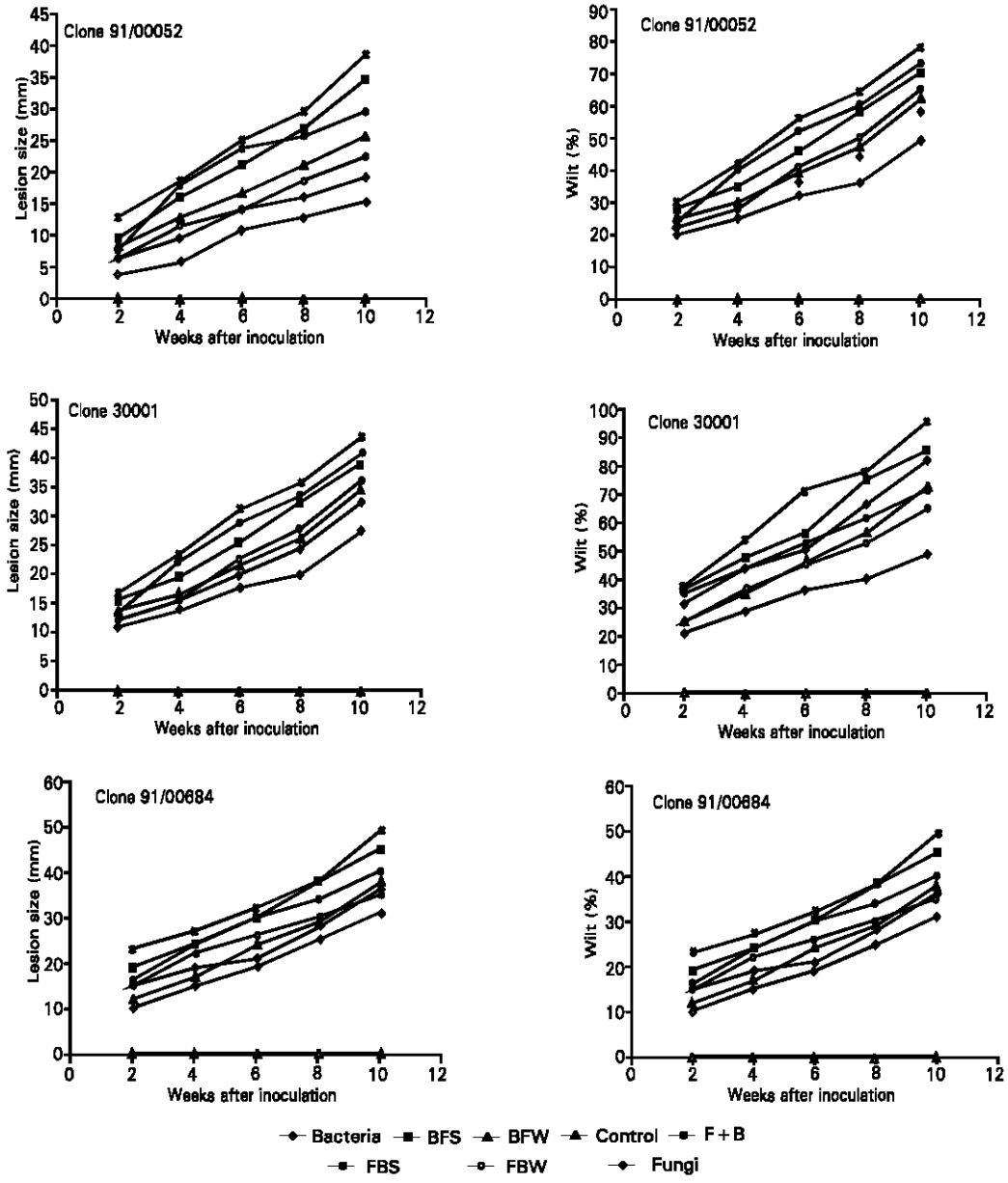

Fig. 3: Effects of interaction of CAD and CBB pathogens on defoliation and gum exudates symptoms

Discussion

In vivo studies on the interaction between *X. campestris* pv. *manihotis* and *C. gloeosporioides* f. sp *manihotis* showed that the fastest occurrence and the highest overall mean severity of the disease symptoms were observed in cassava genotypes sequentially inoculated with the bacterium first then the fungus one week later (BFW), except for defoliation which was higher in plants inoculated with the bacterium first then the fungus on the same day. Disease symptoms were less severe in plants inoculated with the fungus alone.

This study shows that the bacterium has the higher significant effect on the severity of the disease symptoms, since the fungus alone did not induce shoot dieback symptoms as induced by the bacterium. However, there was a synergistic relationship between *X. campestris* pv. *manihotis* and *C. gloeosporioides* f. sp. *manihotis*. This relationship in severe disease infection conditions during wet conditions could lead to a disease complex, which creates difficulty in the distinction between the two diseases in the field. The development of shoot tip dieback from infected cuttings have been reported to have an important implications for the epidemiology of the disease, because cassava is normally propagated vegetatively (Muimba ***et al.***, 1989).

The absence of systemic infection in cassava plant doubly inoculated with the fungus first then the bacterium one week later may have been brought about through the plants reaction as a result of lignification. Lignification can restrict the development of the plant pathogen by several possible mechanism such as the increase in the mechanical resistance of the host cell walls (Vance ***et al.***, 1980), reduction in the susceptibility of host cell walls through degradation of extracellular enzymes (Van der plank, 1984), the restriction of the diffusion of pathotoxins and nutrients, and inhibition of growth of the pathogens by the action of toxic lignin precursors (Singh and Chand, 1971; Kuc, 1982; Stephens ***et al.***, 1992).

Lozano (1986) also reported that cultivar resistance to *X. campestris* pv. *manihotis* is probably not due to a hypersensitive reaction but rather to a restriction of bacterial multiplication and a slow action of the bacterium. The fungus might have induced a plant defence response which restricted the growth of the bacterium, since lignification of plant tissues around the inoculation points may be considered as a type of hypersensitive reaction as described by Furuichi ***et al.*** (1980) and Goodman and Novacky (1994).

Multiple infection caused by CBB and CAD has become a serious epidemiological problem, in field disease management (Fokunang ***et al.***, 2000). Breeding for resistance to the two diseases and intense epidemiological studies of the disease could provide a good disease management strategies.

In conclusion the synergistic relationship between *X. campestris* p.v. *manihotis* and *C. gloeosporioides* f. sp. *manihotis* showed that the disease symptom expressions were severe when the two pathogens were inoculated simultaneously or on the same day as opposed to the fungal or bacterial inoculation in isolation.

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