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Role of the insect vector, *Pseudotheraptus devastans*, in cassava anthracnose disease development

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Abstract

The aim of this study was to investigate the role of *Pseudotheraptus devastans* in cassava anthracnose disease transmission and development. *P. devastans*, Dist (Het. Coriedae) insects were collected from cassava (*Manihot esculenta* Crantz) field plots at the International Institute of Tropical Agriculture, Ibadan, Nigeria and reared in large cages. The insects were separated at different developmental growth stages of eggs, first to fifth instar nymph, and adults. The different stages of *P. devastans* showed the presence of *Colletotrichum gloeosporioides* both externally and internally. Isolates of *C. gloeosporioides* derived from the insects produced cassava anthracnose disease symptoms (necrotic lesions, wilt and defoliation) 8 weeks after inoculation on two cassava clones. Re-infectivity of cassava plants by the insect-derived fungus established that *P. devastans* was a potential vector in anthracnose transmission. Except for the first and second instar nymphs, all nymph stages and adult insects produced significant anthracnose symptoms on cassava plants. Defoliation and lesion diameters were greatest using fifth instar nymphs and adult insects. The association between *P. devastans* feeding and *C. gloeosporioides* f.sp. *manihotis*, showed that feeding by *P. devastans* followed by fungal inoculation and vice versa resulted in more severe anthracnose symptoms than insect feeding or fungal inoculation alone. It was also observed that the influence of *P. devastans* damage/infection on the development of anthracnose depended on cassava cultivar resistance to both the fungus and the insect feeding.

Introduction

Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* f.sp. *manihotis* Henn (Penn.) is an epidemic disease of cassava (*Manihot esculenta* Crantz) characterized by cankers on stems and branches, leaf spots and tip die-back (Muimba, 1982; Theberge, 1985; IITA, 1990). Infection also led to a significant loss in planting materials and crop failure when infected cuttings were used (Makambila and Bakala-Koumouno, 1982; Ikotun and Hahn, 1992). CAD has been reported in many countries but has received less attention than two other important diseases – African cassava mosaic virus (ACMV) and cassava bacterial blight (CBB) (IITA, 1987; Hahn et al., 1989).

The insect *P. devastans*, is associated with cassava anthracnose disease (Muimba, 1982; Boher et al., 1983). *P. devastans* is a coreid bug with a life cycle consisting principally of egg, five instar nymph stages and adult. They are common in young cassava fields found in most parts of the tropics where intensive cassava farming is practised. These insects usually appear in the fields during the cassava planting seasons, between May and August, when wet and high relative humidity conditions favour their growth and reproduction (IITA, 1990). The population of *P. devastans* insects around the necrotic areas plays an important role in the

dissemination of CAD. *P. devastans* is known to deposit conidia on healthy stems and introduce them into the stem tissues during feeding (Dubois and Mostade, 1973). Lodos (1967) found *P. devastans* feeding on tender cassava stems, but did not describe the type of damage it caused to the plants. Dubois and Mostade (1973) also collected three nymphs of the insect on young cassava shoots in Zaire, which were showing stem puncture and gum exudate. Muimba (1982) reported that *P. devastans* carries sufficient inoculum to induce CAD infection during the feeding process either internally or by crude mechanical means.

This study was designed to investigate the effect of *P. devastans* on the development of anthracnose disease on cassava and to assess the host response to insect damage after feeding. This knowledge should influence disease management recommendations within a framework of integrated pest and disease management systems in cassava production.

Materials and methods

Insect rearing

P. devastans was collected from young cassava plants showing extensive CAD growing in field plots of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria between May and October, when environmental conditions (rainfall, relative humidity and temperature) were favourable for reproduction. Insect collection was done between 07.30 and 10.00 a.m each day when the insects were less active in the field due to high humidity conditions. The insects were reared in large cages ($135 \times 84 \times 65$ cm) made of wooden frames with fine plastic mesh screen covers on the sides. The temperature in the cages ranged from 22–27 °C and relative humidity varied from 75–90%. One-month-old cassava plants growing in pots served as food for the insects.

Collection, isolation and identification of the pathogen

Cassava stems showing anthracnose symptoms (dark brown lesions, deep cankers) were collected from infected fields. Small pieces of infected material were cut from advancing edges of the canker, surface-sterilized for 3 min in 10% sodium hypochlorite solution and rinsed in five successive changes of sterile distilled water. The stem pieces were dried on sterilized filter papers and placed on Potato Dextrose Agar (PDA) containing 100 mg/l sodium novobiocin to inhibit bacterial growth. The material was incubated at 25 °C until there was fungal growth. Isolation of the fungus was made on PDA and 9 cm Pyrex petri dishes were used throughout. Identification of the fungus was done under the microscope and confirmation made using the procedure described by Barnett and Hunter (1972).

Preparation of spore suspension

Spore suspensions were prepared from 8-day-old axenic culture of *C. gloeosporioides* f.sp. *manihotis* by flooding with sterile distilled water and dislodging the spores with a small brush. The suspension was centrifuged at 2000 rpm for 3 min and the supernatant was filtered through 2 layers of sterile muslin cheesecloth. The spore concentration was adjusted with a haemocytometer to 10^6 spores/ml of sterile distilled water. A drop of Tween 80 per 100 ml inoculum was added as a wetting agent.

Insects were washed in sterile distilled water and separated into 2 parts. One part was placed on PDA media while the other was surface-sterilized for 3 min in 20% sodium hypochlorite solution and rinsed in five successive changes of sterile distilled water. After drying on sterilized filter papers, the insects were crushed and mixed with 20 ml of sterile distilled water in a small mortar. The extracts were filtered through muslin cheesecloth and the supernatant placed on PDA. There were 10 plates of five insects per plate. The plates were incubated at 25 ± 2 °C for 8 days. Plates were observed microscopically for fungal growth (Barnett and Hunter, 1972) and the incidence of *C. gloeosporioides* on the plates was recorded.

Assessment of pathogenicity

The cassava stems were punctured 3–5 times in the nodal and internodal regions with a sterile hypodermic needle. This induced narrow cavities into which a plug from a growing colony of *C. gloeosporioides* f.sp. *manihotis* on PDA, cut with a corkborer of 1–2 cm diameter, was inserted with the aid of the hypodermic needle. Spray inoculation was done by spraying test plants with fungal spore suspension (3.2×10^6). The leaflets were sprayed until runoff with a master hand sprayer. Plants were covered for 48 h before the insects

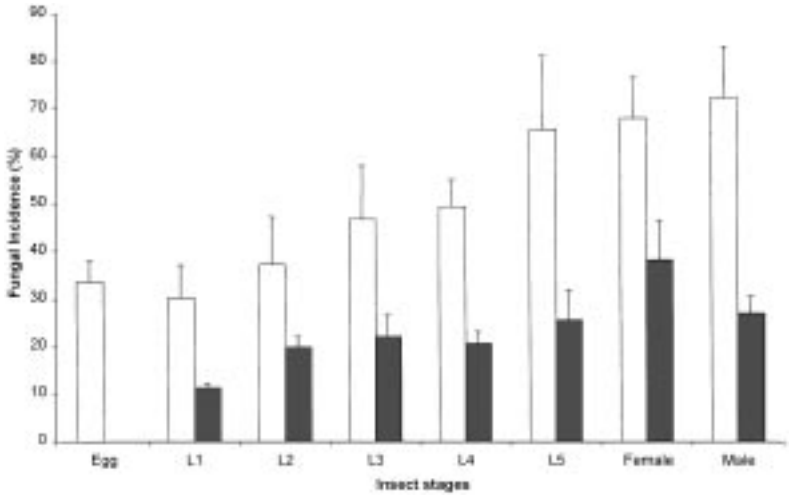

Figure 1. Incidence of *C. gloeosporioides* from insect external □ and internal ■ parts plated on potato dextrose agar media.

were introduced. Control plants were treated with sterile distilled water. The treatments were arranged in a randomized complete block design in a split plot arrangement and replicated five times. Cassava clones were the main plots and fungal suspensions from external and internal cultures formed the subplots.

Statistical analysis

Data on lesion numbers, defoliation and lesion diameters were subjected to analysis of variance (ANOVA) using the SAS statistical package (SAS, 1989). Fischer-protected least significant differences (LSD) and Duncan's multiple range tests of mean separation were performed when the ANOVA showed significance.

Results

Detection of C. gloeosporioides *in insects*

One hundred insects at each developmental growth stage of egg, the instar nymph stages of 1–5, (L1, L2, L3, L4, L5), adult male and female, were separated in small cages containing young cassava plants inoculated with a fungal suspension of *C. gloeosporioides* f.sp. *manihotis* which served as food. The insects were allowed to feed on the plants for 10 days and then each lot was collected in plastic bags and killed by suffocation.

All the insect stages except the egg stage showed the presence of *C. gloeosporioides* from external and internal parts of insects cultured on PDA media. Fungal incidence of above 60% was recorded from the external parts of L5 stage and adult female and male insects (Figure 1). The maximum fungal incidence for the internal parts (30%) was recorded from the female adult stage.

Assessment of pathogenicity

Insect-derived fungal suspensions from L4, L5, and female and male adult insects were used to inoculate one-month-old cassava clones 30572 and 91/00313, in a glasshouse. Sixty plants per cassava clone were planted in plastic pots filled with a sterilized mixture of sand and soil (2 : 2 v/v). After 8 weeks disease symptoms (defoliation, lesion size and wilt) were recorded.

Insect-derived fungal suspensions caused CAD symptoms. Lesion numbers, defoliation and wilt were greater in cultures of the fungal suspensions derived from external parts of insects than from the internal parts. (Figure 2). The adult female and male insects consistently caused higher disease infection in the cassava clones than the L4 and L5 nymphal stages.

Effect of P. devastans *developmental stages on cassava host damage/infection*

Cassava cuttings (10 cm long) from cultivar TMS 30572 were planted in plastic pots filled with steam-sterilized soil in the screenhouse. Two months later the plants were separated into two lots. The first lot was

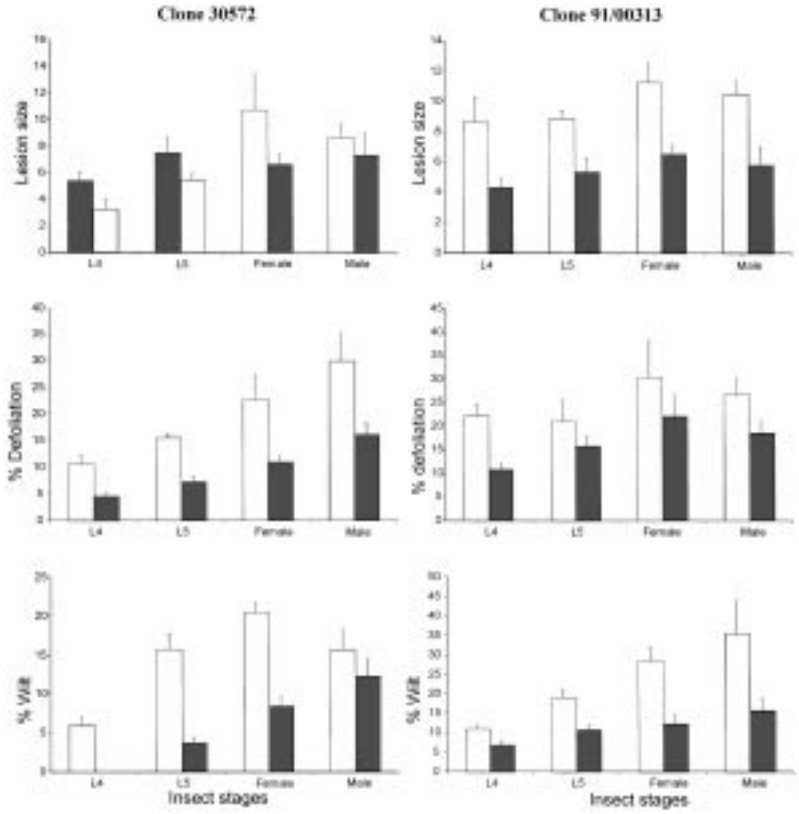

Figure 2. Anthracnose symptoms on cassava clones inoculated with fungal suspensions derived from external □ and internal ■ parts of insects.

inoculated with *C. gloeosporioides* f.sp. *manihotis* and the insects were allowed to feed on infected plants for five days to become contaminated, then introduced to feed on the healthy plants. The second lot of 2-month old healthy plants were caged and subjected to feeding by five insects each, at different stages of development L1, L2, L3, L4, L5 and adult. The insects were allowed to feed for five days. The control plants were kept insect-free. A randomized complete block design with three replications was used. Plant damage parameters (lesion number, lesion diameter and defoliation) were recorded on the leaf and stem plant parts.

The nymphal L1–L2 stages showed no significant damage based on lesion number, defoliation and lesion diameter. Adult males, on the other hand, caused the most leaf and stem lesions (Table 1). There were significant differences in stem lesion diameters among the developmental stages of the insect, with a mean maximum value of 9.13 mm caused by the adult female. The adults caused greater defoliation (24.0% by females and 19.7% by males) than that caused by any of the nymphal stages. In most cases, the L5 nymphal stages and adult insects caused the maximum damage.

Effect of sex of insect on cassava host damage/infection

Cuttings from fifteen cassava cultivars were planted in small plastic pots. Two months after planting, one potted plant per cultivar was caged in a small fine plastic mesh screen cage of 20 × 20 × 15 cm size. Five adult male or female insects contaminated by the CAD fungus were separately introduced 48 h after contamination into each cage and allowed to feed for 7 days. A randomized complete block design, in a split plot arrangement, was used and replicated four times. The cultivars represented the main plots and the sexes of insects were the subplots. Records of lesion number, lesion diameter and defoliation were taken.

Table 1. Effect of *P. devastans* developmental stages on cassava hosts damage

Insect developmental stages	Damage/infection parameters after insect feeding			
	Leaf lesion number	Stem lesion number	Lesion diameter (mm)	Percentage defoliation
Adult male	15.93a	13.73a	7.00b	19.67b
Adult female	14.07ab	10.27b	9.13a	24.00a
L5 nymph	13.07b	8.13c	5.20c	13.47c
L4 nymph	9.45c	4.80c	4.07d	7.53d
L3 nymph	6.20d	2.13e	2.33e	2.53e
L2 nymph	1.60e	0.33f	0.40f	0.00e
L1 nymph	0.00e	0.00f	0.00f	0.00e
Control	0.00e	0.00f	0.00f	0.00e
Mean	7.54	4.93	3.52	8.40
CV	43.00	40.14	29.97	34.36

Values in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range Test. L1–L5 represents identification codes for the first to the fifth instar nymph growth stages.

There were significant differences ($P \leq 0.05$) in lesion numbers, defoliation and lesion diameters among the cassava cultivars and within the sex of the insects (Figure 3). Adult male insects caused more lesions and greater defoliation among most of the cultivars. However, lesion diameters caused by adult females were larger on all cassava cultivars than those caused by adult males.

Effect of insect–pathogen interaction in anthracnose disease development

This study was designed to show the contribution of *P. devastans* in the presence of *C. gloeosporioides* f.sp. *manihotis* to the development of CAD. Cuttings from five cassava cultivars (TME1, 30211, 91/00313, 30572, and 3001) were planted as described above. The potted plants were transferred when 2 months old to insect rearing cages. They were subjected to the following treatments: five adult insects feeding for 48 h; five adult insects feeding for 48 h before spraying of plants with fungal suspensions; inoculation with fungal suspensions 48 h before feeding by five adult insects; fungal inoculation alone and the control with no insect feeding and no fungal inoculation. A randomized complete block design of five replications in a split plot arrangement was used, with the cassava cultivars representing the main plots and the treatments representing the sub-plots. Each treatment was incubated for 5 days at $25 \pm 2\,^{\circ}$C, 90–98% relative humidity in the insect rearing chamber. Records of lesion numbers, diameters and defoliation were taken.

When insects were allowed to feed on cassava followed by fungal inoculation, and vice versa, the damage (lesion number, lesion diameter and defoliation) was consistently greater than inoculation or feeding alone (Figure 4). Maximum lesion numbers and diameters were caused by *P. devastans* feeding followed by fungal inoculation in all the cultivars. Fungal inoculation prior to *P. devastans* feeding caused the greatest defoliation. Apart from the control plants which showed no significant damage, insect feeding or fungal inoculation alone showed the least damage in all the treatments.

Lesion damage caused by insects infected with *C. gloeosporioides* f.sp. *manihotis* was distinguished from the damage caused by non-infected insects, on the basis that the lesions produced by non-infected insects were thin, dry, dark spots, which did not expand further in the duration of the study, as compared to the infected lesions that were very distinct wet, dark spots which expanded and coalesced with other spots. The culture of thin sections from lesion damage on PDA caused by non-infected insects showed none of the fungal growth that was observed on cultures from insect-infected lesions.

There were significant differences ($P \leq 0.05$) in damage among the five cassava cultivars tested. There was variation in the overall response of the test cultivars after treatment with insect and fungal inoculations (Figure 5). Cultivars 30001 and 30572 were most resistant to the disease. Cultivar TME1 was most susceptible to insect feeding, with the highest mean lesion number, lesion diameter and defoliation.

Discussion

This study shows that the insect *P. devastans* carries *C. gloeosporioides* f.sp. *manihotis*, both externally and internally. Apart from the eggs, which showed no incidence of the fungus, inoculum obtained from all insect stages was capable of infecting young, healthy cassava plants.

The fungus derived from internal parts of insects showed lower infectivity of cassava plants than those from the external parts. This could be either due to low inoculum concentration of the fungus contained in the insect, or some physiological changes within the insect

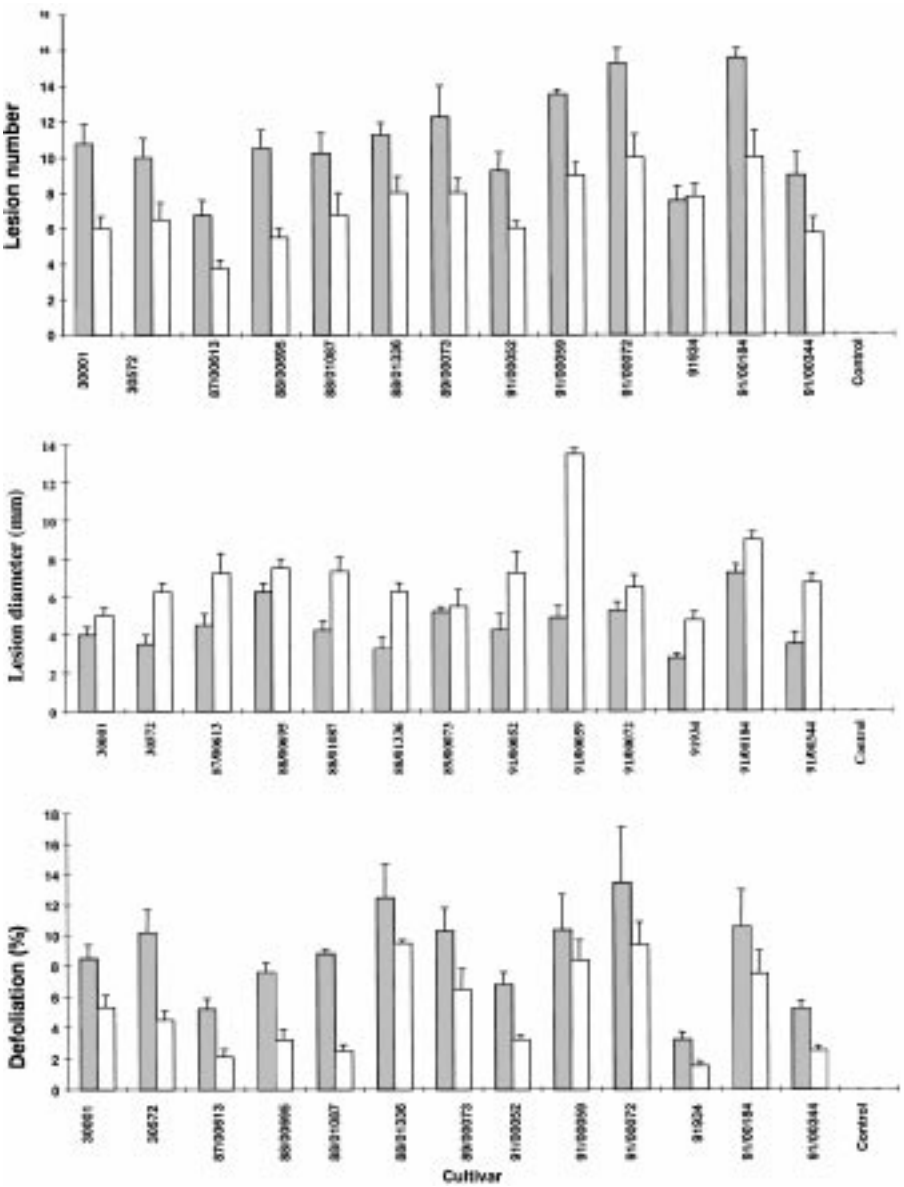

Figure 3. Effect of male ▒ and female □ adult insects on cassava cultivar damage. Values represent mean ± standard error.

that tends to reduce the fungal activity. The presence of the *C. gloeosporioides* f.sp. *manihotis* on and inside *P. devastans* suggests that the insect poses a potential threat in the spread of CAD, especially in areas of intensive cassava production. In CAD control programmes increased *P. devastans* population in farmer's and research field needs to be brought under control through an integrated pest management strategy.

The importance of stem punctures caused by *P. devastans* feeding and in the spread and development of CAD, has been reported (Makambila and Bakala-Koumouno, 1982; Boher et al., 1983). The CAD fungus attacks tissues influenced by the insect puncture and the lytic action of their saliva. This induces dark-brown lesions which can either remain limited or become deep-brown cankers with lacerated epidermis. The

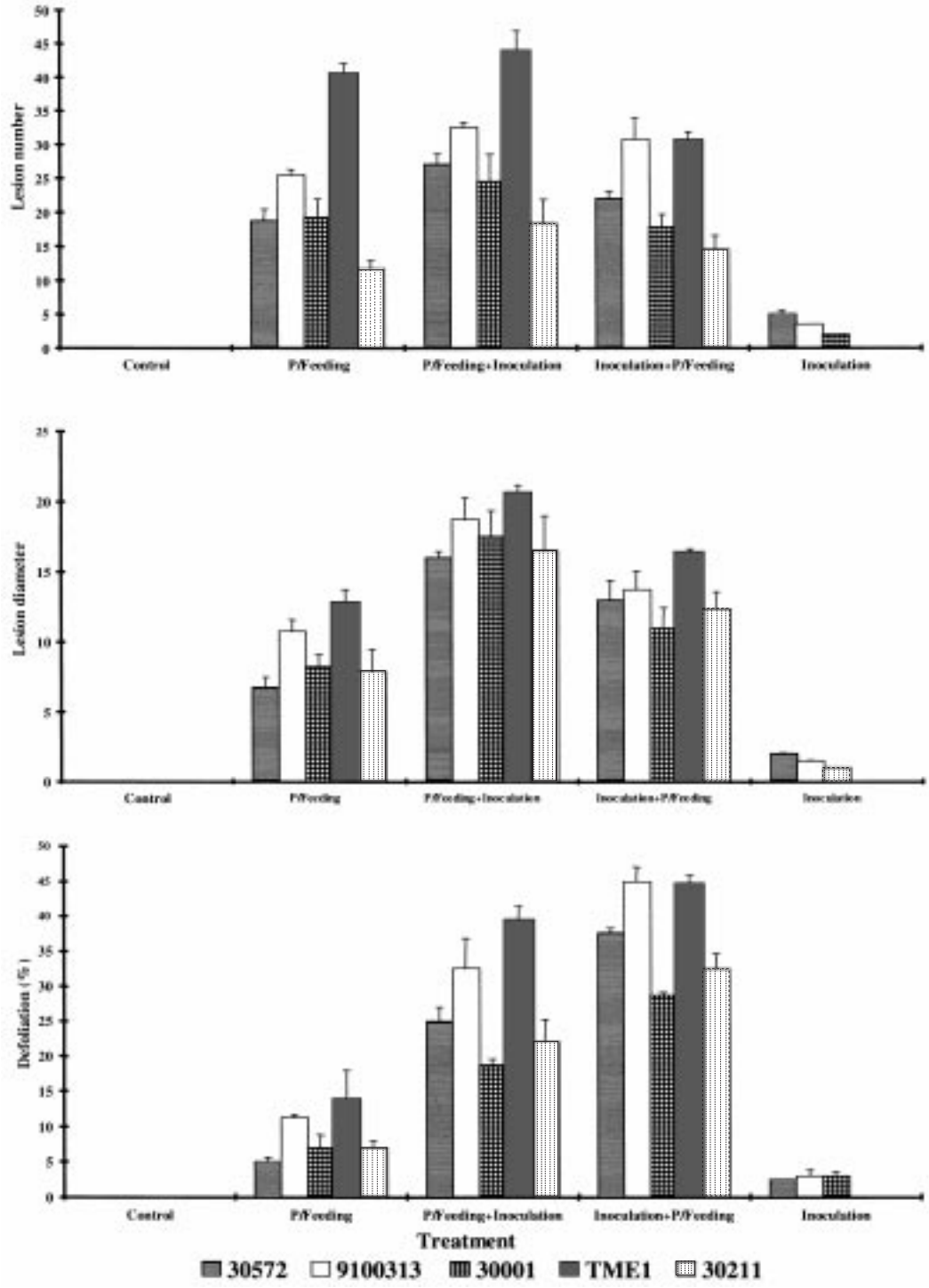

Figure 4. Effect of interaction of *C. gloeosporioides* f.sp. *manihotis* with *P. devastans* on cassava host damage. *P. devastans* feeding (P/feeding).

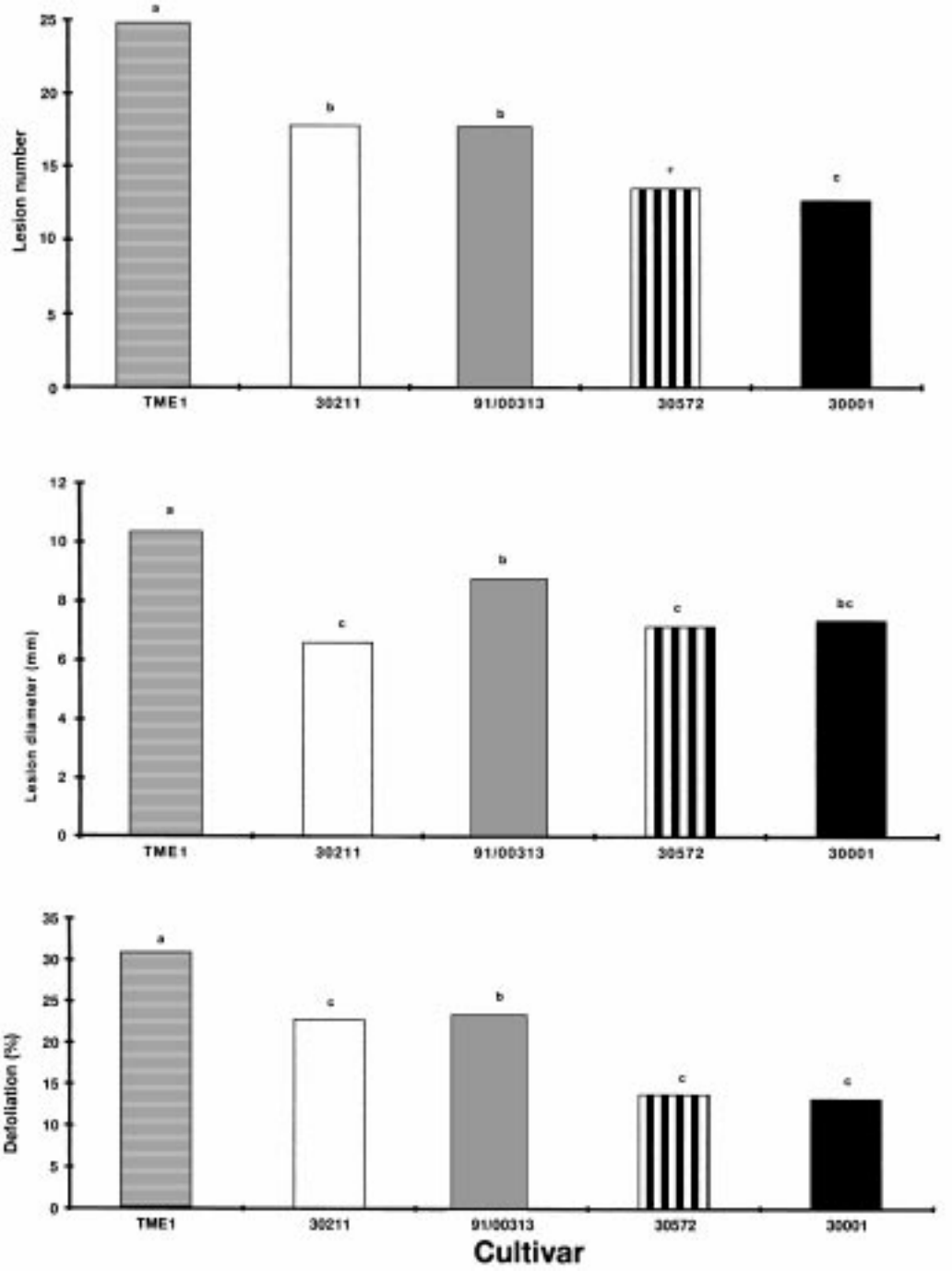

Figure 5. Cultivar response to *C. gloeosporioides* f.sp. *manihotis* and *P. devastans* feeding.

fungus penetrates through insect puncture wounds on young cassava stems, and extends through the cork layer or the epidermis up to the underside of the sclerenchyma, then immediately extends tangentially under lignified fibres (Van der Bruggen et al., 1987).

Adult insects caused higher levels of disease severity than the nymph developmental stages. This could be due to the fact that they are fully developed and have active feeding and mating habits, which requires greater mobility from one infected plant to another. This active movement exposes the insects to more inoculum sources. There is also a possibility that the adult insects have longer retentivity of the inoculum than the nymphs.

Dubois and Mostade (1973) reported that first instar nymphs of *P. devastans* did not induce significant damage on cassava host plants, whereas damage caused by the second instar nymph did not differ significantly from that caused by the adults. In contrast, in this study the first and second instar nymphs consistently failed to cause any significant damage on the cassava cultivars.

Fungal inoculation or insect feeding alone were not sufficient to explain the severe damage on the cassava host, since only the combination of insect feeding and inoculation showed severe disease reaction. The high level of anthracnose disease severity according to Boher et al. (1983) was associated to some physiological alteration of the tissues that might play an important role in pre-disposing the host cassava plant to anthracnose. The differential host reaction among five cultivars (TME1, 30211, 91/00313, 30572 and 30001) to insect feeding and fungal inoculation suggests that the disease might be managed with resistant cultivars. This study has also shown that for CAD management to be achieved under field conditions there is a need to control the insects at an early stage of their development. The use of cultivars resistant to both the insect and the pathogen could also serve as a control option to reduce CAD pressure in fields with high populations of *P. devastans*.

This study has increased the understanding of the interactions between the insect and the fungus that causes CAD, an economically important disease of cassava. This knowledge should directly influence disease management recommendations for integrated pest and disease management systems in cassava production.

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