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Agriculture Intensive dans les Iles de la Caraibe : enjeux, contraintes et perspectives
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SUSTAINING COCOA PRODUCTION THROUGH GENETIC RESISTANCE TO BLACK POD AND LEAF BLIGHT

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ABSTRACT

The resistance of cocoa to *Phytophthora palmivora* and its mechanism(s) were investigated in leaves and pods of twelve clones. Anatomical and morphological characteristics including ; cuticle thickness, stomatal frequency and pore length were assessed on leaf and correlated with foliar resistance at penetration stage. Post-penetration resistance was also determined on leaf and pod, and their relationship assessed. Results show a significant difference in leaf susceptibility between surfaces. The adaxial surface appeared less susceptible possibly due to lack of stomata and/or the presence of a thick cuticle. Leaf susceptibility differed significantly at penetration and post-penetration stages suggesting different mechanisms. Correlation analysis shows a strong relationship between the joint effect of cuticle thickness, stomatal frequency, stomatal size and leaf resistance at penetration stage. A close relationship in the rate of spread of lesion in leaf and pod indicates that a reliable assessment of clonal reaction can be based on leaf observation. The results suggest that there are two mechanisms of resistance; one at the point of penetration and the other at post-penetration stage. The resistance mechanism(s) at post-penetration stage seems to be common for both leaf and pod, and possibly controlled by biochemical factor(s). This suggest that pod resistance to *P.palmivora* can be reliably assessed at the seedling stage using leaf resistance to spread as an indicator. Both pathological and genetic implications of these findings are discussed for the improvement of cocoa resistance to *P.palmivora* infections.

INTRODUCTION

Following the utilization of high yielding hybrids and improved husbandry, cocoa production has increased during the last two decades. Records show a considerable increase in yield from about 1.5 million tons of dried beans in 1974 (Opeke and Gorenz,1974) to over 2 million in 1991 (Wintgens,1991). However, the economic benefit of this yield increase is hardly realised due to the high incidence of black pod caused by *Phytophthora spp.* and the high cost of fungicide application. Several control methods are in use, but none could be considered as an effective economic measure with the exception of genetic resistance that minimises the cost of crop protection.

Presently, black pod disease caused by *Phytophthora spp.* accounts for more than 50 percent of total annual loss due to diseases. Pod at all stages of development are attacked. The first symptom of infection is seen as a circular brown spot which enlarges concentrically and evenly to involve the whole pod surface. Infected pods turned dark brown in about 12-17 days and become unusable. Annual loss ranged between 20-30 percent of total pod produced and disease severity varied between localities, seasons and years (Opeke and Gorenz,1974). Reports on disease epidemic showed a significant positive correlation between number of black pods and total number of pods per acre (regression coefficient, b=0.252 _ 0.013) indicating an increase in disease severity as yield increases (Blencowe and Wharton, 1961 as cited by Gregory,1969).

Infection of the leaf is considered of less importance but, Manco (1974) noted that this could be additional sources of inoculum, increasing pod infection, in addition to the damage they do in reducing photosynthesis and decreasing pod production.

A few breeding programmes pursued to date have achieved little success owing to inadequate information on host pathogen interaction, nature of resistance, components of resistance and their genetics. Current research activities are geared towards these objectives and this work investigates the nature of resistance, and related morphological and anatomical characteristics.

MATERIALS AND METHODS

Test plants and selection of samples

Twelve cocoa clones were selected for the experiments. The clones include representatives of the major types of cocoa; MXC67 (Criollo), SCAVINA6, SCAVINA12, SPEC-138-8, CATONGO, PA30, IMC67 (Forasteros), ICS1, ICS6, ICS8, ICS40 and ICS95 (Trinitarios). The experiment was conducted with ten replications.

Fully expanded green leaves of new flush (Interflush 2) and green mature pods were used as test samples.

Assessment of resistance at penetration and post-penetration stages were carried out on the test samples as follows:

Preparation of inoculum

Ten days old culture of *P.palmivora* in 20% V.8 juice-calcium carbonate agar medium was used as the source of inoculum. Zoospore suspension was prepared following the method of Lawrence (1978). The concentration of zoospore was determined using a haemocytometer and adjusted to the required inoculum strength for each experiment.

Assessment of foliar resistance at penetration stage

Both adaxial and abaxial leaf surfaces were inoculated using 400,000 and 150,000 zoospores ml^{-1} inocula respectively. The 'tissue-paper-mount' inoculation method was adopted. A drop of zoospore suspension ($0.30ml^{-1}$) was placed on the leaf surface using a micro pipette. A piece of tissue paper ($1.25cm^2$) was then placed on the inoculum to allow uniform spread of zoospores per unit area of leaf surface. Inoculated leaves were arranged randomly in trays containing moist tissue paper and incubated under 100 percent humidity at 25^0C. The number of lesions formed were recorded 72 hours after inoculation.

Assessment of leaf and pod resistance at post-penetration stage

Inoculation was performed on leaves and pods using 'punch' and 'stab' inoculation techniques, respectively. A zoospore suspension (200,000 zoospores ml^{-1}) was used in both inoculations described below:

Punch inoculation : A standard injury was created on the leaf lamina by punching a hole ($0.30cm^2$) with a leather punch. The hole was covered with a plaster at the adaxial surface and inoculated at the abaxial surface by placing a filter paper disc ($0.30cm^2$) saturated with the zoospore suspension in the punched hole.

Stab inoculation : A standard injury was made on pod using rings of pins arranged within an area of $0.30cm^2$ on a rubber stopper. Inoculation was effected over the injured surface using a filter paper disc $0.30cm^2$ saturated with the zoospore suspension.

Inoculated leaves and pods were incubated in trays under 100 percent humid condition at 25°C. The rate of spread of lesion was recorded at 24 hour intervals, by tracing lesion on a transparent plastic which was later transcribed onto a brown paper. This was used to determine lesion size at each time using a leaf area meter.

Evaluation of leaf morphological and anatomical characteristics

The following morphological and anatomical characteristics were assessed on leaf to determine their relationship with foliar resistance at penetration stage.

Stomatal density and Pore length : Assessment of stomatal density and pore length was based on stomatal impression examined under the phase contrast microscope. Stomata were counted within the grid micrometer area and pore length determined with an ocular micrometer.

Cuticle thickness : Assessment was based on hand sections of fresh leaf tissue examined on an Olympus binocular microscope. Cuticle thickness including the outer epidermal wall of the adaxial and abaxial surfaces were measured using an ocular micrometer.

RESULTS AND DISCUSSION

Assessment of foliar resistance to *P.palmivora* and its mechanisms at penetration stage of infection

The results of laboratory inoculations performed on leaf surfaces of the selected clones are presented in Table 1.

Table 1 : Lesion frequency following laboratory inoculations of leaves of cocoa clones with *P. palmivora*

	lesion frequency on leaf surfaces per 1.25 cm^2 leaf area	
Clones	Adaxial	Abaxial
Catongo	1.95	3.10
IMC67	1.93	3.81
SCA12	2.03	3.45
SPEC-138-8	1.48	2.34
SCA6	2.2	3.76
ICS1	2.51	5.21
PA30	1.59	3.75
ICS6	2.34	5.13
ICS40	1.62	4.33
MXC67	1.43	4.51
ICS95	1.82	4.90
ICS8	2.40	5.02
L.S.D.	0.43	0.51

Data was transformed using square root, adding 1 as constant.

The selected clones differed significantly ($P<0.05$) in their reaction to *P.palmivora* infection at penetration stage estimated as a function of the number of lesions observed. There was a marked difference in the number of lesions that were observed on the adaxial and abaxial leaf surfaces. The adaxial surface appeared less susceptible to infection possibly due to absence of stomata and/or the presence of a thick cuticle which may reduce the level of infection. A higher level of infection was recorded at the abaxial surface. Clonal reactions were consistent on both surfaces in most clones; ICS1, ICS6, ICS8

(susceptible), PA30, IMC67, SCAVINA12, SCAVINA6 (moderately resistant), SPEC-138-8 and CATONGO(resistant). However, the results on the upper and lower surfaces were quite different in MXC67, ICS40 and ICS95. The interaction between leaf surfaces and clonal reaction may require that both leaf surfaces be tested in selecting resistant materials for breeding purposes. SPEC-138-8 and CATONGO appear promising, in that their reaction to infection on both leaf surfaces were consistently low.

Figure 1 shows the relationship between cuticle thickness, stomata frequency and pore length, on lesion frequency at the abaxial leaf surface.

A multiple regression analysis showed a high correlation r=0.78 for the combined effect of cuticle thickness, stomatal frequency and pore length. The relationship of these morphological characteristics on number of lesion can be explained by the prediction equation:

Number of lesion (abaxial) = 0.39 -2.76 cuticle(abaxial) + 0.019 stomatal frequency + 0.306 stomatal pore length

The close association between leaf resistance and the parameters included in the model, indicates that effective screening and selection for foliar resistance can be made using the above prediction equation. Also, the relationship suggests that the observed differences in leaf resistance between clones could be attributed to cuticular thickness, stomatal frequency and pore length. These factors were reported as having strong influence on leaf infections in sweet potato, poplar and water yam (Bajit and Gapasin, 1987; Spiers and Hopcroft, 1984, and Nwankiti and Okpala (1984).

Assessment of post-penetration resistance to P.Palmivora in leaf and pod of cocoa clones

Lesion sizes following laboratory inoculation of leaves and pods of the selected clones are shown in Table 2.

Table 2 : Lesion size following laboratory inoculations of pods and leaves of cocoa clones with *P. Palmivora*

	Lesion size (cm2)	
Clones	Leaf	Pod
Catongo	2.25	8.86
IMC67	2.27	8.92
SCA12	1.68	7.46
SPEC-138-8	2.70	*
SCA6	1.44	5.88
ICS1	2.45	8.26
PA30	1.84	8.47
ICS6	2.16	7.37
ICS40	1.72	7.46
MXC67	2.19	*
ICS95	1.80	5.98
ICS8	2.50	8.59
L.S.D.	0.16	0.48

* Pods were not available for inoculation.
Data was transformed using squaare root, adding 1 as constant.

Lesion size varied significantly between clones in both pod and leaf. Spread of lesion was faster on the pod than in leaf of both resistant and susceptible clones as shown in Figure 2. A correlation analysis between spread of lesion in both leaf and pod shows a strong relationship (r=0.73) suggesting a biochemical mechanism that is nonspecific for both organs. A similar trend was observed between stem and root reaction to *P.palmivora* by Zentmyer, Mircetich and Mitchell (1986) as reported by Gregory (1969). Asomaning (1964), also indicated that the mechanism conferring resistance to black pod is systemic within the whole plant, and not restricted to the pod tissue alone. However, the poor relationship (r=0.13) between lesion frequency and spread on leaf (Figure 3), indicates that the mechanism(s) of resistance are different at penetration and post-penetration stages of *P.palmivora* infection.

CONCLUSION

This study reveals that resistance is governed by different mechanisms at penetration and post-penetration stages of infection in cocoa leaf. This conforms with earlier findings on pod in which epidermal and internal resistance were differentiated. In addition, the differences in leaf surface reaction suggest that structural defence mechanisms might vary between organs of cocoa and tissues in direct contact with the pathogen at penetration point. The relationship between pod and leaf internal resistance indicates that pod resistance at post-penetration stage can be reliably assessed based on estimates of lesion size or spread on leaves of seedlings. Consequently, a large population of plants can be assessed for their internal resistance at seedling stage.

From these findings, a reliable selection criteria can be developed to enhance the success of the current and future breeding programmes aimed at producing resistant cultivars with high yielding potential.

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