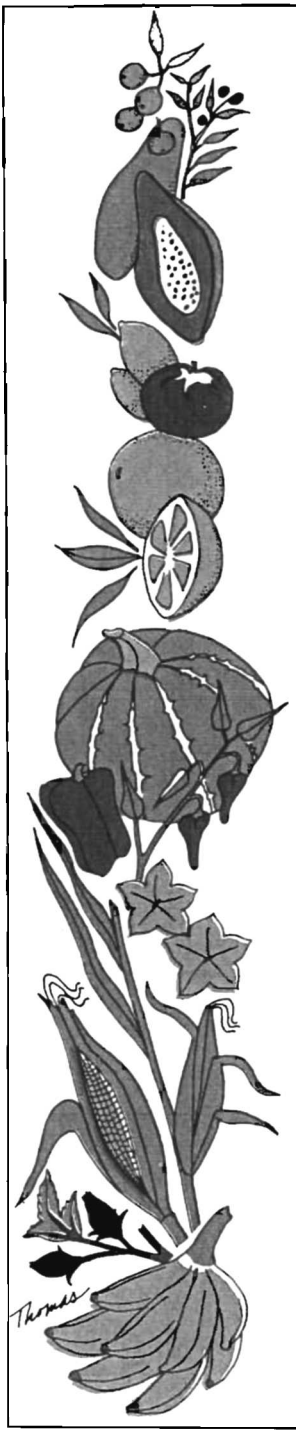

CARIBBEAN FOOD CROPS SOCIETY

30

THIRTIETH ANNUAL MEETING 1994

ST. THOMAS, U.S.V.I.

Vol. XXX

INDICATORS OF RESISTANCE IN COCOA (*THEOBROMA CACAO*) TO BLACK POD DISEASE CAUSED BY *PHYTOPHTHORA PALMIVORA*

D. A. Iwaro[1], T. N. Sreenivasan, [1] P. Umaharan[2] and G. Sirju-Charran[2]

[1]Cocoa Research Unit, [2]Department of Plant Science
The University of The West Indies
St. Augustine, Trinidad, W.I.

ABSTRACT

The relationship between morphological characteristics: stomatal frequency, stomatal pore length, surface wax, hardness of pod wall and pod susceptibility to *P.palmivora* infection was assessed in twelve selected cocoa clones of the Forastero and Trinitario types. Among these clones, significant variation was observed with respect to pod morphological characteristics and their responses to infection. A high positive correlation was obtained between the combined effect of stomatal frequency and pore length, and clonal susceptibility. This suggests that these two morphological characteristics together could be used as a selection criterion for the identification of resistant genotypes.

INTRODUCTION

Black pod disease of cocoa caused by *Phytophthora palmivora* occurs in all cocoa-growing areas and accounts for more loss of crop than any other single disease of cocoa (Spence, 1961; Prendergast and Spence, 1967). Globally, about 20 to 30 per cent of the world crop is lost annually due to *Phytophthora* infection (Opeke and Gorenz, 1974). Losses at some locations could, however, increase to about 95 per cent, depending on weather conditions and the inherent resistance of cocoa cultivars (Tollenaar, 1958; Opeke and Gorenz, 1974; Lass, 1985). Several copper-based fungicides are being used by farmers to control the disease, but this method is expensive and not completely effective (Lass, 1985). The use of genetic resistance seems cheaper in the long run, but this will require effective selection criteria for the identification of resistant genotypes.

At present, the search for varieties with desirable resistance has been difficult due to lack of selection criteria. Although, some useful information could be obtained from the incidence of diseased pods, which is often used for selection; such results are often influenced by changes in weather conditions, the amount of inoculum within the field, number of pods per tree, and possible escape from infection (Amponsah, 1987). Similarly, the identification of resistant genotypes using certain inoculation techniques have been inconsistent, thus limiting the chances of selecting the most resistant variety. To overcome these problems, this study investigates the relationship between varietal resistance and pod morphological characteristics with the intention of using those characters with high correlation as selection criteria against the black pod disease of cocoa.

MATERIALS AND METHODS

Test plants and selection of sample

The clones selected were SCA 6, SCA 12, CATONGO, PA 30, PA 47, IMC 67, (Forasteros), ICS 1, ICS 6, ICS 8, ICS 40, ICS 84, and ICS 95 (Trinitarios). Mature unripe pods (same size

as ripe pods of the same clone) were used in the experiments.

Preparation of inoculum

Zoospore suspension was prepared from a ten day old culture of *P.palmivora* following the method of Lawrence (1978). The concentration of zoospore was determined using a haemocytometer and adjusted to 30,000 zoospores/ml. This concentration was found to discriminate best among clones of varying degrees of resistance in a preliminary experiment and was used for the inoculation experiment.

Inoculation and assessment of resistance

The multiple point inoculation technique was adopted, in which inoculation was effected along the two upper ridges on pod surface by placing a drop of inoculum (4il) at 10 points using a micro-pipette. A distance of about 3cm was maintained between inoculated points (5 points per ridge) to avoid merging of lesions. Inoculated pods were incubated at 100% relative humidity and 25°C in trays lined with moist tissue paper and covered with polythene film.

After 72 hours of incubation, the number of established infection points were recorded as a measure of pod resistance. An average of 17 pods were assessed per clone to determine the degree of resistance among the selected varieties.

Evaluation of pod morphological characteristics

The following morphological characteristics were evaluated to assess the variability among clones.

Stomatal frequency and pore length

By applying nail polish to the surface of cocoa pods, stomatal impressions were obtained and examined under an Olympus microscope. Stomata were counted within the field of view using x40 objective and pore lengths measured with an ocular micrometer.

Five points per pod in 15 pods were examined for each clone and the frequency of stomata was estimated as the number of stomata/mm^2 of pod area.

Pod surface wax

Surface wax was extracted by dipping the base of pod into chloroform for 20 seconds (Martin and Batt, 1958; Balasimha, et al. 1985). The extract was evaporated to dryness in a fume cupboard and the wax content determined gravimetrically per cm^2 of pod surface area. Ten pods per clone were assessed for their wax content.

Hardness of pod wall

The relative hardness of pod wall was determined using the Instron Universal Testing Instrument (Model 1130). A brass needle (3.1mm diameter) with a piercing angle of 30^0 and a load range of 0-20kg were used for the test. Data was converted to newtons (N) per mm^2. Ten pods per clone were assessed.

Data analysis

The data collected from the above experiments were subjected to the analysis of variance to assess the differences between genotypes. In addition, a correlation analysis was performed to determine the relationship between varietal resistance and pod morphological characteristics.

RESULTS AND DISCUSSION

Assessment of pod resistance

The mean number of infection points following laboratory inoculation of the selected clones are shown in Table 1. The analysis of variance showed a significant difference among the tested clones. However, the mean number of infection points produced on IMC 67, ICS 95, ICS 6, SCA 12 and CATONGO were not significantly different from each other. These varieties were noted to be more susceptible than the other clones. SCA 6, ICS 84 and ICS 1, on the other hand, showed a marked difference from other clones. The mean number of infection points were significantly lower in these clones. Other clones including PA 47, PA 30, ICS 8 and ICS 40 were moderately susceptible to infection.

The clones SCA 6, ICS 84 and ICS 1 showed a substantial degree of resistance to infection which could be exploited for the improvement of cocoa resistance to *P.palmivora*.

Assessment of pod morphological characteristics

The mean values for stomatal frequency and pore length, surface wax and relative hardness of pod wall for the selected clones are presented in Table 2.

The analysis of variance showed a significant difference among clones with respect to the four morphological characteristics that were assessed. The frequency of stomata, ranged among the clones tested, from 13.63/mm^2 in ICS 1 to 46.00/mm^2 in CATONGO. Fewer stomata were found in ICS 1, SCA 6, ICS 84 and ICS 8 (13.63 - 19.48/mm^2). In contrast, high stomatal frequency was observed among the other clones examined (24.52 - 46.00/mm^2). This variation in stomatal frequency, suggests that selection would be effective for this character among the clones.

Among the clones investigated, the stomatal pore lengths of ICS 95 and IMC 67 (13.70μm, 13.00μm) were greater than those of PA 30, SCA 6, PA 47 and ICS 48 (10.08μm, 10.25μm, 10.65μm and 10.97μm). The differences between CATONGO, ICS 1, ICS 6 and SCA 12 were not significant.

The differences in pod surface wax, ranged from 27.81μg/cm^2 in ICS 84 to 7.70μg/cm^2 in ICS 40 among the clones that were tested. The amount of wax extracted from CATONGO, IMC 67, SCA 12, SCA 6, ICS 40 and PA 30 were not significantly different from each other.

The relative hardness of pod wall also varied among the clones. A higher level of hardness was observed in IMC 67, ICS 40, CATONGO, ICS 84 and PA 30 in comparison to the other c ones with a lower level of hardness.

Correlation between the frequency of infection and pod morphological characteristics

High correlation values were obtained for lesion frequency vs stomatal frequency, and lesion frequency vs pore length (Table 3). However, a stronger relationship was obtained between the infection frequency and the combined effect of stomatal frequency and pore length ($r = 0.89$, P 0.05). These two factors complemented each other and their relationship with resistance appeared most outstanding (Table 3). The inclusion of the other parameters (surface wax and pod hardness) did not improve the correlation value ($r = 0.90$).

The clones SCA 6, ICS 84 and ICS 1 were found to be more resistant than the other clones and they possess the lowest stomatal frequency along with relatively shorter pore length. CATONGO, IMC 67, ICS 6, ICS 95 and SCA 12, on the other hand, were found to be susceptible and they showed a higher stomatal frequency and longer pore length. This result suggests that pod reaction to *P.palmivora* could be influenced by the number of stomata, as well as the size of the pore. Consequently, these two morphological characteristics can be used together as selection criterion for the identification of resistant genotypes for use in breeding programs.

ACKNOWLEDGEMENTS

The financial support of the EEC through the Cocoa Research Unit, U.W.I, Trinidad, is hereby acknowledged. We also thank Mr D. Sookram of the faculty of Engineering, U.W.I, for his assistance.

REFERENCES

Amponsah, J.D. 1988. Studies of field resistance of cocoa varieties to *P.palmivora* infection. Proc. 10th Inter. Cocoa Res. Conf., Santo Domingo. 557-567.

Balasimha, D., Subramonian, C. and Chenchu, S.C. 1985. Leaf characteristics in cocoa accessions, Cafe Cacao The, xxix, 2: 95-98.

Lass, R.A. 1985. Diseases. In: Cocoa. Wood, G.R, Lass, R.A. Eds. London, U.K: London. 265-365.

Lawrence, J.S. 1978. Evaluation of methods for assessing resistance to cacao cultivars and hybrids to *P.palmivora*, Boletim Tecnico 62, CEPLAC, CEPEL, Itabuna, Brazil. 1-47.

Martin, J.T. and Batt, R.F. 1958. Studies on plant cuticle: 1. The wax covering of leaves. Ann. appl. Biol. 46 (3),375-387.

Opeke, L.K. and Gorenz, A.M. 1974. *Phytophthora* pod rod: symptoms and economic importance. In: *Phytophthora* Diseases of Cocoa. Ed. Gregory, P.H. Longman. 117-124.

Prendergast, N.W. and Spence, J.A. 1967. A contribution to the study of the resistance of *Theobroma cacao* L. to *P.palmivora* (Bult.) Bult. Conf. Inter. Recherches Agron. Cacaoyeres, Abidjan, 1965. Paris. 212-216.

Spence, J.A. 1961. Black pod of cocoa: II. A study of host-parasite relations. Ann. appl. Biol. 49: 723-734.

Tollenaar, D. 1958. *Phytophthora palmivora* of cocoa and its control, Neth. J. Agric. Sci. 6:24-38.

Table 1. Mean number of lesions (transformed values) produced on pods of 12 cocoa clones following inoculation with *P.palmivora*.

Clones	Mean number of lesions (transformed values)
CATONGO	2.95 (*7.79)
IMC 67	3.03 (*8.20)
SCA 6	2.53 (*5.45)
ICS 1	2.58 (*5.72)
ICS 6	3.01 (*8.11)
ICS 95	2.99 (*8.00)
PA 47	2.68 (*6.19)
PA 30	2.69 (*6.30)
ICS 40	2.64 (*6.00)
ICS 84	2.57 (*5.65)
ICS 8	2.64 (*6.00)
SCA 12	3.00 (*8.00)
LSD (0.05)	0.06

Data was transformed using, (x+1)
* Actual values

Table 2. Pod morphological characteristics of 12 cocoa clones.

Clones	Stomatal Frequency/ mm^2	Stomatal pore length (um)	Surface wax (ug/cm^2	Hardness of pod wall N/mm^2)
CATONGO	46.00	11.57	7.76	5.26
IMC 67	33.78	13.00	8.26	7.52
SCA 6	15.93	10.25	8.82	4.37
ICS 1	13.63	11.40	10.06	4.25
ICS 6	36.30	11.50	13.60	4.55
ICS 95	29.63	13.70	10.65	4.70
PA 47	39.63	10.65	23.72	4.76
PA 30	32.67	10.08	8.12	5.21
ICS 40	24.52	12.23	7.70	5.41
ICS 84	19.19	10.97	27.81	5.22
ICS 8	19.48	12.30	10.04	4.31
SCA 12	38.00	11.87	8.22	4.20
LSD (0.05)	6.22	0.60	2.05	0.49

Table 3. Correlation between morphological characteristics and the frequency of lesions produced on pod surface.

Characters	r
Lesion frequency vs stomatal frequency	+0.73
Lesion frequency vs stomatal pore length	+0.58
Lesion frequency vs surface wax	- 0.31
Lesion frequency vs hardness of pod wall	+0.31
Lesion frequency vs stomatal frequency and pore length	+0.89
Lesion frequency vs stomatal frequency, stomatal pore length and surface wax	+0.90
Lesion frequency vs stomatal frequency, stomatal pore length, surface wax and hardness of pod wall	+0.90