



## A study of genotype-isolate interaction in cacao (*Theobroma cacao* L.): resistance of cacao genotypes to isolates of *Phytophthora palmivora*

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### Summary

The resistance of cacao (*Theobroma cacao* L.) leaves to 10 isolates of *Phytophthora palmivora* (Butler) Butler was investigated in 18 genotypes of cacao in three experiments. Tissue-paper-mount and punch-inoculation methods were used to distinguish between resistance at the penetration and post-penetration levels based on lesion frequency and size, respectively. There was a 5–6 fold difference in aggressiveness among isolates collected from different locations in the islands of Trinidad and Tobago for both types of resistance. However, host genotype × isolate interaction effects were not significant for resistance at both the penetration and post-penetration levels. A strong positive relationship ( $r = 0.8$ ) was observed in the abilities of isolates to effectively breach the two types of resistance. This suggests that the same isolate can be used in screening for both types of resistance. No significant relationship was found between resistance at the penetration and post-penetration levels suggesting that the resistance mechanisms were different. Subsequent experiments using a wider range of cacao genotypes from the International Germplasm Collection, Trinidad, confirmed the initial results. These experiments indicate that resistance found using one isolate would be equally valid using other isolates of *Phytophthora palmivora*. The implications of the findings are discussed.

### Introduction

Cacao (*Theobroma cacao* L., family *Sterculiaceae*), is an understory tree species found in the tropical forests of Central and South America (Wood & Lass, 1985). Cacao is grown as a subsistence crop in the tropical Americas and Caribbean, West Africa, Asia and the Pacific. The cacao bean, used in the manufacture of chocolates, cocoa powder and cocoa butter, has become a commodity of economic importance in many of these countries which derive a major part of their foreign exchange earnings from it (Asomaning, 1972).

*Phytophthora palmivora*, a causal agent of the black pod disease, is regarded as one of the most destructive pathogens of cacao (Opeke & Gorenz, 1974; Thorold, 1975). In addition to pod infection, infections of the leaf, stem and root of cacao are important, as they have been shown to have an adverse effect on

the health of the cacao tree (Manco, 1974; Opeke & Gorenz, 1974; Prior, 1981). Although other species of *Phytophthora* (*P. megakarya*, *P. capsisi*, *P. citrophthora*, *P. megasperma*, *P. araceae* and *P. nicotiana*) have also been reported, *P. palmivora* is the only species found in all cacao growing countries (Zentmyer, 1988). Yield losses are estimated to be about 20–30% of the annual cocoa production (Opeke & Gorenz, 1974), with losses in individual plantations reaching levels as much as 90%. Although field sanitation and use of copper-based fungicides have been reasonably effective in reducing the level of infection, the high cost of fungicides often make them inaccessible to the peasant farmer, who produces over 50% of cacao worldwide (Amponsah, 1988; Tan & Tan, 1990). Breeding for resistance to black pod disease has hence been long regarded as the most economical and effective control method (Soria, 1974; Rocha, 1974; Amponsah, 1988).

Iwaro et al. (1997b) demonstrated that resistance to *P. palmivora* existed at two levels, the penetration level, which controls lesion number and the post-penetration level, which determines lesion size. The latter level of resistance has been shown to be highly correlated between pod and leaf (Iwaro et al., 1993; 1997b; Nyassé et al., 1995) and governed predominantly by additive genetic effects in both pod (Tan & Tan, 1990) and leaf (Iwaro et al., 1997a). Recently, a pre-breeding programme was established at the Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad, with the aim to develop gene pools with superior levels of resistance that can be distributed to the various cacao-growing countries.

For the pre-breeding programme to have global relevance, the genotype  $\times$  pathotype interaction should be demonstrated to be negligible. Such studies, however, are difficult to conduct due to quarantine restrictions. Simmonds (1994) considered resistance of cacao to *Phytophthora* to be horizontal. Nevertheless, some studies (conducted using isolates within a country) have shown genotype  $\times$  pathotype interactions (Chowdappa & Chandra Mohananan, 1993). However, the inoculation methods used in these studies did not take into account the different levels of resistance (penetration and post-penetration). The objective of this study was therefore to investigate genotype  $\times$  pathotype interaction between a number of genetically diverse cacao genotypes and isolates of *P. palmivora*, using leaf inoculation tests capable of discriminating between penetration and post-penetration resistance.

## Materials and methods

### *Collection and establishment of isolates of P. palmivora*

Isolates of *P. palmivora* were obtained from naturally infected cacao pods from diverse locations in Trinidad (Marper, Cedros, Gran Couva, Tamana, La Reunion and Santa Cruz) and Tobago (Mount Grace and Les Coteaux). Two isolates (CRU I & II) maintained at the Cocoa Research Unit, The University of the West Indies, Trinidad, were also included in the study. Following a preliminary characterisation based on the pattern of growth in culture, sporangial shape, size and pedicel length, the isolates were identified as *P. palmivora*. Each isolate was subsequently introduced into cacao pods of the genotype ICS 84 to activate them on the same substrate. The isolates were

re-isolated and maintained on 20% V8-juice-calcium carbonate-agar medium.

### *Cacao genotypes*

A genetically diverse set of eighteen (18) cacao clones (SCA 6, ICS 1, ICS 41, ICS 66, ICS 84, MO 81, IMC 11, IMC 30, IMC 55, IMC 67, PA 115, PA 128, B14/13, B 53, EET 338, EET 400, JA 64 and UF 29) representing various levels of resistance were selected for this study. These accessions represent Forastero, Refractario and Trinitario cultigroups maintained at the International Cacao Genebank, Trinidad.

### *Leaf inoculation tests*

Mature leaves at the interflush-2 stage, as described by Greathouse et al. (1971), were surface sterilised in 0.5% sodium hypochlorite for two minutes and rinsed three times in sterile distilled water. The surface sterilised leaves were then placed in plastic trays lined with moist paper towels, with the abaxial surface facing upwards.

Resistance at the penetration and post-penetration levels was assessed using the tissue-paper-mount and punch inoculation methods, respectively (Iwaro et al., 1997b).

The zoospore suspension was prepared following the method of Lawrence (1978) for use in the penetration and post-penetration tests. Tissue-paper-mount inoculation of the leaf samples was done by applying a 30  $\mu\text{L}$  drop of zoospore suspension ( $80,000 \text{ mL}^{-1}$ ) on the leaf surface over which a piece of tissue paper (area:  $1 \text{ cm}^2$ , thickness: 0.23 mm) was placed. This ensured a uniform spread of the inoculum throughout the tissue-paper covered area. For the punch-inoculation a 4 mm diameter filter paper disc previously immersed in a zoospore suspension was placed in a punched hole (diameter, 4 mm) from the abaxial side of the leaf. The suspension at a concentration of  $200,000 \text{ zoospores mL}^{-1}$  constituted the inoculum. A leaf from each clone was inoculated with sterile distilled water as a negative control in each experiment. Following inoculation, the trays were covered with polythene film and incubated at  $25^\circ\text{C}$ .

Following a 72-hour incubation period, the tissue-paper-mounts were carefully removed and penetration resistance was determined by counting the number of lesions. Post-penetration resistance to infection was determined based on lesion size, following incubation for a period of 144 hours. Lesion sizes were

determined by using a leaf area meter (M2K, Delta-T Services, Burnwell, Cambridge, England).

### Experimentation

Three experiments were conducted to investigate genotype  $\times$  isolate interactions. In the first experiment, five cacao clones (SCA 6, ICS 1, ICS 84, MO 81 and IMC 55) were evaluated for resistance at the penetration and post-penetration levels of infection, using ten isolates of *P. palmivora*. The fifty treatment combinations (5 clones, 10 isolates) were arranged in a completely randomised design with four replications. The leaves were inoculated at 4 points using the tissue-paper-mount method and another four points using the punch-inoculation method. This experiment was repeated to confirm the consistency of results obtained.

In subsequent experiments (Experiments 2 & 3) two isolates, Les Coteaux and Gran Couva, representing the high and moderately aggressive categories, were used in penetration and post-penetration tests to study the consistency of reaction of isolates over a genetically diverse set of cacao clones obtained from the International Cacao Genebank, Trinidad. The least aggressive isolate was not used as the reactions were barely visible and were difficult to quantify, especially in the more resistant genotypes.

In Experiment-2, selected isolates (Les Coteaux and Gran Couva) were used to inoculate 11 cacao clones (PA 115, PA 128, IMC 11, IMC 30, IMC 67, ICS 41, ICS 66, B14/13, B 53, EET 338, and UF 29) using the tissue-paper-mount method. The experimental design and replication were similar to the previous one. The experiment was repeated to confirm the consistency of results.

In Experiment-3, the same two isolates (Les Coteaux and Gran Couva) were used to inoculate twelve clones (PA 128, MO 81, IMC 11, IMC 30, IMC 67, ICS 41, B14/13, B 53, JA 64, EET 338, EET 400 and UF 29) using the punch-inoculation method. It was not possible to use an identical set of clones as in Experiment 2 because of differences in flushing times of clones. Experimental design and replications were similar to the previous experiments.

### Data analysis

The data on number of lesions and lesion size were subjected to analysis of variance to assess the significance of treatment effects and interaction between treatments. A correlation analysis was performed to assess

Table 1. Mean number of lesions and lesion size following inoculation of cacao with 10 isolates of *P. palmivora* (means are averages over 5 genotypes)

Isolate	Number of lesions	Lesion size (mm <sup>2</sup> )
Les Coteaux	26.6	12.1
Santa Cruz	22.7	13.8
Cedros	22.4	8.2
Marper	21.0	10.1
Mt. Grace	20.3	13.2
CRU II	13.5	7.4
Gran Couva	12.2	7.0
Tamana	11.5	9.3
La Reunion	9.6	8.6
CRU I	4.6	3.3
Lsd ( $p = 0.05$ )	5.27	3.3

the association between resistance at the penetration and post-penetration levels and between aggressiveness of isolates at the penetration and post-penetration levels of resistance. Joint regression analysis was carried out to test the performance of individual host genotypes against mean performances of isolates over various genotypes.

### Results

Analysis of variance of number of lesions obtained from the tissue-paper-mount test in Experiment-1 indicated that the number of lesions was significantly ( $p < 0.001$ ) influenced by cacao genotypes and *P. palmivora* isolates. The host genotype  $\times$  isolate interaction effects were however not significant ( $p < 0.05$ ). The results from the two trials were consistent.

The mean number of lesions showed a 6-fold variation (4–26) among the isolates tested (Table 1). The isolates fell into three categories of aggressiveness, based on a Duncan's Multiple Range Test (DMRT). Isolates from Marper, Cedros, Santa Cruz, Mt. Grace and Les Coteaux fell into the highly aggressive class (18–26 lesions), isolates from Gran Couva, Tamana, La Reunion and CRU II formed the moderately aggressive category (9–12 lesions), while the isolate from CRU I formed the least aggressive category (5 lesions). Similarly, the cacao genotypes tested showed significant differences at the penetration level of infection, with number of lesions varying between 9 for IMC 55 to 21 for ICS 1 (Figure 1). The clones ICS 1,

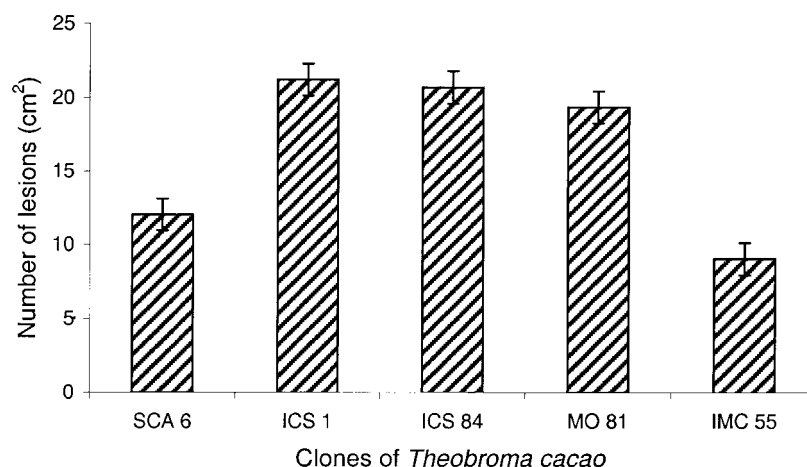


Figure 1. Variation in resistance to *P. palmivora* at the penetration level of infection in five cacao genotypes (mean number of lesions are averaged over the 10 isolates of *P. palmivora*). Bars indicate  $LSD_{(0.05)}$ .

ICS 84 and MO 81 produced mean numbers of lesions between 18 and 21 and were classified as susceptible. Clone SCA 6 gave an intermediate reaction.

Analysis of variance of lesion sizes obtained from the punch-inoculation tests in Experiment-1, indicated that lesion size was significantly ( $p < 0.001$ ) influenced by cacao genotypes and *P. palmivora* isolates (Figure 2). The host genotype  $\times$  isolate interaction effects were again not significant ( $p = 0.05$ ). The results from the two trials were consistent.

Lesion sizes showed a 5-fold variation among the isolates tested and varied between 3 and 14 mm<sup>2</sup> (Table 1). Again, the CRU I isolate was the least aggressive giving a mean lesion size of 3 mm<sup>2</sup>. Isolates from Marper, Santa Cruz, Mt. Grace and Les Coteaux fell into a highly aggressive category (10–14 mm<sup>2</sup>), while the rest formed a moderately aggressive category (7–9 mm<sup>2</sup>), based on DMRT. The cacao clones also varied significantly in their susceptibility at the post-penetration stage (Figure 2), with all genotypes showing significantly more resistance than IMC 55.

The mean number of lesions for each of the 10 isolates, averaged over all genotypes, was regressed against those for each individual genotype in the two trials, to determine the consistency of performance of genotypes over isolates. Linear regression lines were able to explain the performance of individual genotypes over mean values of aggressiveness for isolates. A joint regression analysis showed that the regression lines for SCA 6 and IMC 55 were the same, and those for ICS 1 and ICS 84 were the same. The parallelity of the lines for all the genotypes tested, indicated by the non-significance ( $p < 0.05$ ) of slopes, showed that the

relative performance of genotypes over the 10 isolates were similar (Figures 3 & 4). Based on the intercepts, the five genotypes tested fell into three categories of resistance.

Figure 4 provides a comparison of the relative consistency of reaction of the five genotypes tested in response to the 10 isolates for lesion size, using data from the two repetitions. Joint regression analysis showed that the regression lines for ICS 1 and ICS 84 and those for SCA 6 and MO 81 were the same. Again all the regression lines were parallel, indicated by non-significance of slopes, which suggested that the relative genotypic performances were consistent over the 10 isolates tested. Based on the intercepts, the genotypes can be grouped into three categories of response. These findings confirm the results of the analysis of variance, which indicated no significant genotype  $\times$  isolate interaction.

A correlation analysis between number of lesions and lesion size (Figure 5) showed no significant correlation ( $r = 0.33$ ,  $p < 0.05$ ) between resistance at the penetration and post-penetration levels of infection. Despite the lack of correlation between number of lesions and lesion size the aggressiveness of isolates at the penetration level of infection was highly correlated ( $r = 0.8$ ) to aggressiveness at the post-penetration level of infection (Figure 6), indicating that the aggressive isolates were able to breach resistance at both penetration and post-penetration levels equally effectively.

Similarly, the analysis of variance for number of lesions and lesion size in Experiments 2 & 3 respectively indicated that both penetration and post-penetration

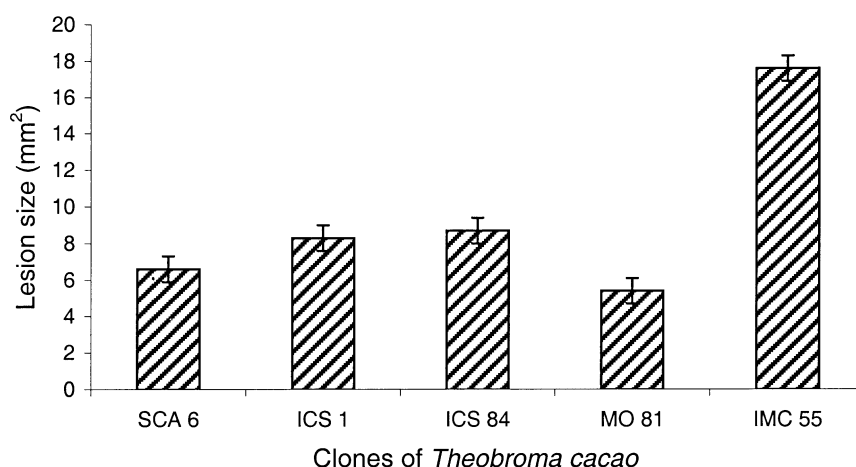


Figure 2. Variation in resistance to *P. palmivora* at the post-penetration level of infection in five cacao genotypes (mean lesion sizes are averaged over 10 isolates). Bars indicate  $LSD_{(0.05)}$ .

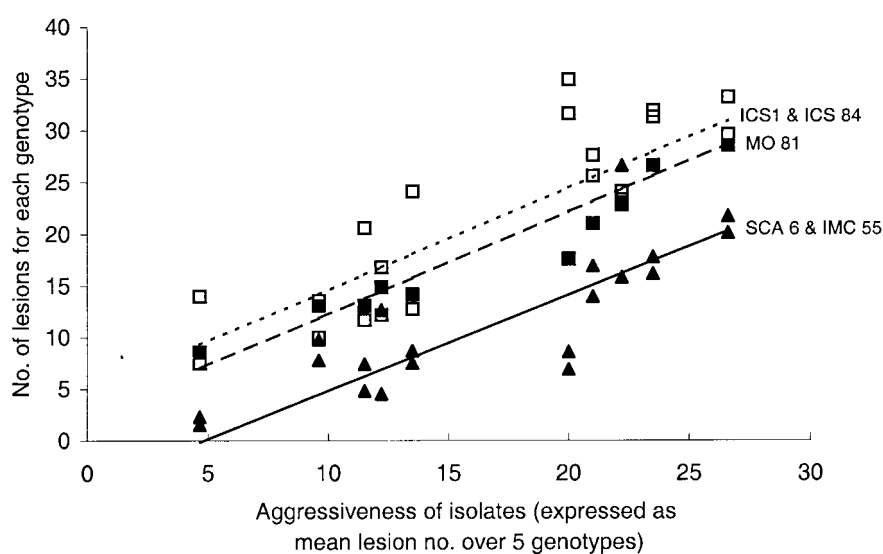


Figure 3. Relationship between aggressiveness of isolates (mean number of lesions over genotypes) and resistance of individual cacao genotypes to 10 isolates *P. palmivora*.

Fitted line equations: SCA 6 and IMC 55:  $Y = -4.5 + 0.932x$ , ICS 1 and ICS 84:  $Y = 4.75 + 0.985x$ , MO 81:  $Y = 2.44 + 0.985x$ .

responses were significantly ( $p < 0.001$ ) influenced by the respective host genotypes and *P. palmivora* isolates (Table 2). In addition, host genotype  $\times$  isolate interaction effects were again not significant ( $p < 0.05$ ) and the results over the two trials conducted were consistent. The two isolates (Les Coteaux and Gran Couva) were able to invoke a wide range of responses in the genotypes tested both at the penetration and post-penetration levels. Among these genotypes tested, PA 128 and ICS 41 were the most resistant at the penetration and post-penetration levels, respectively. Genotypes IMC 30 and EET 338 were highly

susceptible at the penetration and post-penetration levels (Table 2).

## Discussion

The study showed that there was significant variation in aggressiveness among isolates of *Phytophthora palmivora* in Trinidad and Tobago, with a 5-6 fold difference observed between the most aggressive and least aggressive isolates. Under the conditions

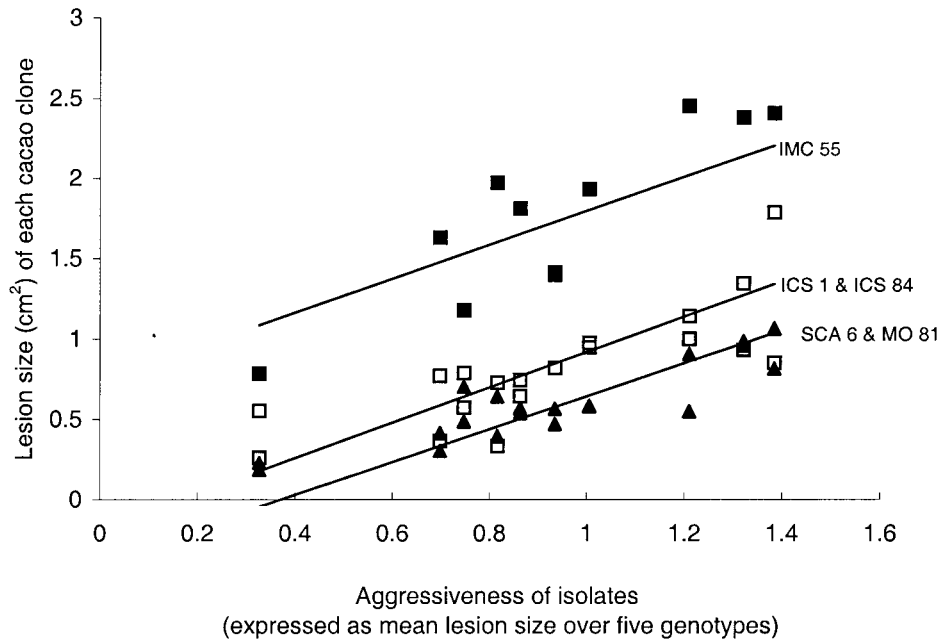


Figure 4. Relationship between aggressiveness of isolates (mean lesion size over genotypes) and resistance of individual cacao genotypes to isolates of *P. palmivora*.  
 Fitted line equations: SCA 6 and MO 81:  $Y = -0.38 + 1.024 x$ , ICS 1 and ICS 84:  $Y = -0.18 + 1.099 x$ , IMC 55:  $Y = 0.74 + 1.057 x$ .

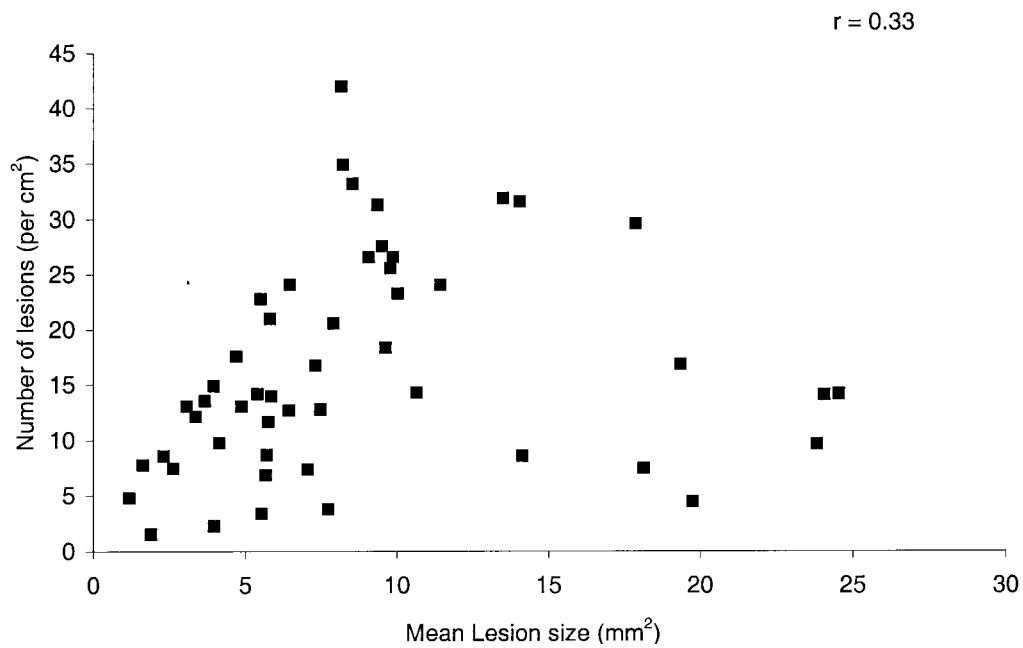


Figure 5. Relationship between number of lesions and size in five cacao genotypes (SCA 6, ICS 1, ICS 84, MO 81 and IMC 55) inoculated with 10 isolates of *P. palmivora*.

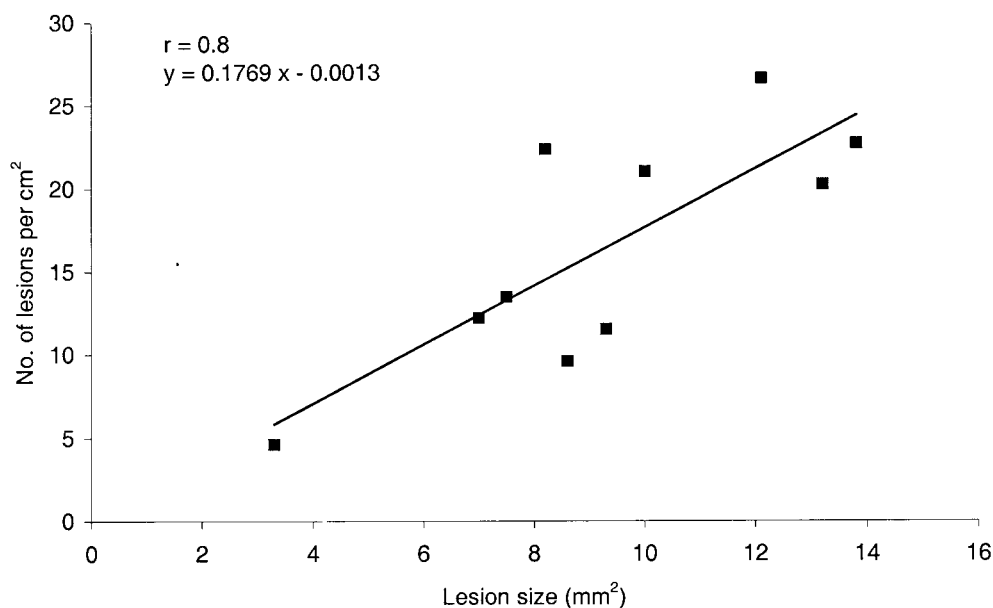


Figure 6. Relationship between aggressiveness of *P. palmivora* isolates at the penetration and post-penetration levels of infection in cacao leaves (scatter plot show means over replicates).

Table 2. Mean number of lesions and size following inoculation of cacao germplasm with two isolates of *P. palmivora* (Les Coteaux and Gran Couva)

Clone	Mean number of lesions per cm <sup>2</sup>	Mean lesion size (mm <sup>2</sup> )
IMC 30	55.4	16.9
B 14.13	43.2	12.2
EET 338	43.0	17.0
UF 29	42.5	14.8
ICS 66 <sup>a</sup>	36.3	–
IMC 11	28.2	12.1
B 53	23.5	12.8
IMC 67	19.5	11.5
PA 115 <sup>a</sup>	17.4	–
ICS 41	15.2	9.3
PA 128	3.7	12.3
MO 81 <sup>b</sup>	–	15.1
JA 64 <sup>b</sup>	–	16.9
EET 400 <sup>b</sup>	–	14.8
S.E	5.08	12.0

<sup>a</sup> Genotypes tested at penetration level only.

<sup>b</sup> Genotypes tested at post-penetration level only.

of experimentation in this study, this variation can be attributed predominantly to genetic causes.

The development of disease is a function of host, pathogen and the environment (Agrios, 1988). In this

study the selection of leaves at the same developmental stage and from plants grown under similar environmental conditions would have helped to minimise the influence of the environmental factors. Similarly, the isolates were passed through pods of the same cacao clone (ICS 84) to minimise any epigenetic influences on pathogen aggressiveness. In addition, the environment under which the experiments were carried out was kept at constant relative humidity and at 25 °C to standardise the environmental influence on pathogen aggressiveness. These point to a genetic basis for the variation observed in aggressiveness. The constancy of ranking of isolates over replicated trials and over the three experiments in this study, further provides strong evidence to this claim.

Some studies have demonstrated similar variation in aggressiveness among isolates of *P. palmivora* originating from different geographical locations based on pathogenicity tests on pods and stems (Orellana, 1959; Zentmyer, 1972; Ram & Ram, 1973). Rates of spread of lesions on pods, amount of mycelial growth and degree of sporulation have been used to quantify the aggressiveness of the isolates (Spence, 1961; Turner, 1965; Akinrefon, 1971; Fagan, 1984). Iwaro et al. (1997a) demonstrated different mechanisms and genetic bases for resistance at the penetration and post-penetration levels of infection and developed inoculation methods that can effectively distinguish

between resistance at these two levels. Results from the present study confirm and further validate the findings of Iwaro et al. (1997a) that resistances at these two levels are distinct.

There was a strong correlation between aggressiveness measured at the penetration and post-penetration levels over a range of genotypes, in this study. This indicates that although the mechanisms and genetics of resistance are different at the penetration and post-penetration levels of infection there was a general correspondence in the abilities of isolates to breach these barriers. This augers well for screening cacao genotypes for resistance to *P. palmivora*, since it eliminates the need to use two different isolates to effectively assess resistance at the penetration and post-penetration levels.

Both the analysis of variance and joint regression analysis confirmed the absence of host genotype  $\times$  isolate interactions at both the penetration and post-penetration levels of infection in the first experiment. The subsequent experiments were conducted to further validate this finding by using a genetically more diverse group of genotypes, representing a known range of resistance phenotypes selected from the International Cacao Genebank, Trinidad. These experiments together demonstrate the absence of host genotype  $\times$  isolate interactions at both the penetration and post-penetration levels of infection. These findings are contrary to those of Ram & Ram (1973) and Chowdappa & Chandra Mohananan (1993) who showed significant genotype  $\times$  isolate interactions using isolates of *P. palmivora* and *P. capsici* in cacao using a pod inoculation procedure. The method used in these studies however did not differentiate between penetration and post-penetration resistance.

The results however enhance and support the views of Tan and Tan (1990) and Simmonds (1994), who, based on the accruing evidences suggesting an additive genetic basis for resistance to *P. palmivora* (Tan & Tan, 1990; Iwaro et al., 1997b), indicated that resistance is most likely of the race-nonspecific type. Although additive genetic effects have generally been associated with race-nonspecific resistance, occasionally, they have been found to be associated with the race-specific kind of resistance (Vanderplank, 1982). This study provides direct evidence for race-nonspecific resistance using a genetically diverse group of cacao genotypes and a diverse group of *P. palmivora* isolates, from the islands of Trinidad and Tobago.

Although the present study is based on leaf resistance, the results can be extended to post-penetration resistance in the pod, since the mechanisms of post-penetration resistance in both pods and leaves as well as their genetic basis are very highly correlated (Nyassé et al., 1995; Iwaro, 1997b). Hence, it would be possible to conclude that post-penetration resistance in pod is also likely to be race-nonspecific.

The non-significance of the host genotype  $\times$  isolate interaction has important implications in cacao breeding for *Phytophthora* resistance. Firstly, the levels of resistance of cacao genotypes to *P. palmivora* can be identified using any of the isolates since resistance has been shown to be race-nonspecific in this study. However, the use of the most aggressive isolate could lead to the identification of useful levels of resistance against the pathogen. Secondly, if the relationship between genotypes and isolates observed in this study turns out to be universal for all *Phytophthora* isolates and cacao genotypes throughout the world; then the varieties bred for *P. palmivora* resistance in one location would also have resistance to *P. palmivora* in other parts of the world. Indeed some similarity in the ranking order for resistance to *P. megakarya* in Cameroon and that for *P. palmivora* in Ivory Coast (Van der Vossen, 1997) and between *P. palmivora* and *P. capsici* in Trinidad (Iwaro et al., 1998) have been observed. This would justify a pre-breeding program in one location, to accumulate genes for *Phytophthora* resistance from which genetic material can be distributed to other locations. The resistant germplasm should be incorporated into local breeding programs for adaptation to specific environments.

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