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Usefulness of the detached pod test for assessment of cacao resistance to Phytophthora pod rot

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Abstract

The detached pod test by spray method (DPT-SM) was developed to facilitate the screening of cacao genotypes for resistance to Phytophthora pod rot (PPR) caused by *Phytophthora palmivora*. The test has been adopted in many cacao research institutes, and it is imperative that its validity be assessed. In this study, 40 genotypes from various cacao groups were selected and screened for resistance to PPR by DPT-SM and field observations. Significant variation was observed in the reactions of the selected accessions based on the disease rating scale for DPT-SM and the percentage pod rot from field observations. A correlation coefficient of 0.68 ($P < 0.001$) was observed between the results of year-1 and year-2 field observations. However, relatively lower correlation values were obtained between year-3 and year-1 ($r = 0.32$; $P = 0.041$) and year-3 and year-2 ($r = 0.35$; $P = 0.025$) field observations. A higher level of susceptibility was observed in the third year of field observations (63%) than in the first (15%) and second (25%) years. This suggests that the predisposing factors for PPR were unstable between the years of field observations. Data obtained from the Trinidad and Tobago Meteorological Services showed that the rainfall in November of the third year was higher than the amounts of rainfall in November of the first 2 years of field observations. This month marks the beginning of the main pod harvest season (November–February) and the high rainfall in November of the third year, and the presence of large number of mature pods may account in part for the increase in PPR in the third year of field observations than in the first 2 years. This shows that absolute reliance could not be placed on a single year of field observations in determining clonal resistance to PPR. A correlation coefficient of 0.59 ($P < 0.001$) was obtained between the result of year-2 field observations and DPT-SM. Relatively lower correlation values were observed between DPT-SM and year-1 ($r = 0.55$; $P < 0.001$) and year-3 ($r = 0.44$; $P = 0.005$) field observations. The result of DPT-SM, however, shows a higher correlation ($r = 0.66$; $P < 0.001$) with the average of years 1–3 field observations. This suggests that a stronger association may exist between the result of DPT-SM and the cumulative data on field observations for a period longer than 3 years. The correlation ($r = 0.66$; $P < 0.001$) observed in this study confirms the usefulness of DPT-SM as an effective method of assessing clonal resistance to PPR and predicting field reaction in the long term. Since field observations are labour intensive and expensive to conduct on a yearly basis, the DPT-SM offers a cheaper and effective means of assessing clonal resistance to PPR. Being a non-destructive inoculation method, the DPT-SM provides a suitable option for cacao collections in genebanks to be assessed. It is also a cost-effective method for use in cacao breeding programmes. Based on its reliability, the DPT-SM has been adopted in the CFC/ICCO/IPGRI cocoa project 'Cocoa Germplasm Utilization and Conservation: A Global Approach' for the assessment of cacao resistance to PPR in several cocoa producing countries.

Abbreviations: CFC – Common Fund for Commodities; ICCO – International Cocoa Organisation; IPGRI – International Plant Genetic Resources Institute; PPR – Phytophthora pod rot (Black pod disease); DPT-SM – Detached pod test – Spray method

Introduction

Phytophthora pod rot (PPR) caused by *Phytophthora palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora* is one of the most prevalent and destructive diseases of cacao (*Theobroma cacao*) (Iwaro et al., 1998). Global losses from PPR are enormous and were estimated by Opeke and Gorenz (1974) at about 20–30% of annual cocoa production. However, losses may be as high as 90% at some locations depending on the susceptibility of the cultivated varieties and the prevailing environmental conditions (Adegbola, 1981). The disease therefore has a large economic impact on cocoa production and consequently farmers' income. Although chemical control methods have been developed to reduce yield losses from PPR, they are expensive and often beyond the reach of average cacao farmers in developing countries (Tan and Tan, 1990). The development of high yielding, resistant material is generally agreed to be a more effective and economic control method (Rocha, 1974; Soria, 1974; Iwaro et al., 2000a), but progress in this direction has been very slow, probably due to the narrow genetic base of most cacao breeding programmes, a low level of resistance in base parents, and poor screening methods.

In order to provide cacao breeders with a wide array of resistance genes, consistent efforts have been made to collect cacao germplasm from a variety of locations. The International Cocoa Genebank, Trinidad (ICG,T) and the collection at the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica (CATIE) are now recognised as 'Universal Collection Depositories' (IBPGR, 1981). These collections provide sources of genes for the improvement of various traits in cacao including resistance to PPR. However, identification of major sources of resistance to PPR remains a challenge as regulations prohibit inoculation of plants in cacao genebanks as a precautionary measure to avoid losing susceptible accessions which might be useful for other purposes. Consequently, a non-destructive screening method is needed to enable accessions in cacao genbanks to be assessed. One such test is the detached pod test – spray method (DPT-SM).

The DPT-SM was developed in the Cocoa Research Unit, the University of the West Indies, Trinidad and Tobago (Iwaro, 1997) and proposed for use in the CFC/ICCO/IPGRI cocoa project 'Cocoa Germplasm Utilization and Conservation: A Global Approach' (Iwaro et al., 2000b) which involved research institutions in 10 cocoa producing countries. The DPT-SM allows the pathogen to penetrate the pod naturally and discriminates between the different levels of resistance to PPR in various cacao accessions (Iwaro et al., 2003). For breeding purposes, it is imperative that this method be validated with field resistance. Such information will help to determine the reliability of DPT-SM and the effectiveness of selection based on this method. For this reason, this study was conducted to compare the results of DPT-SM with field observations for resistance to PPR.

Materials and methods

Cacao germplasm

Forty genotypes in the ICG,T (Table 1) were selected for this study. These accessions are planted at the University Cocoa Research Station, Centeno, Trinidad at an altitude of 15 m above sea level. Shade is provided by trees of *Erythrina* sp., which are planted 6 m apart. The cacao trees are planted 1.8 m apart with up to 16 trees per plot for each accession; however the plots are not replicated since this is a cacao genebank. The soil type is Cunupia fine sandy clay with restricted internal drainage. Over a 30-year period from 1961, the mean annual rainfall in this location was 2392 mm, and the average temperature 26 °C. The plants are irrigated as required during the dry season (January–June) each year.

Table 1. Mean values of disease rating scores for DPT-SM and percentage PPR in the field for 40 cacao genotypes

Detached Pod test – Spray method (DPT-SM)				Field observations for Phytophthora pod rot (% PPR)			
Genotypes	Mean value	SE	Rank	Mean value	Transformedvalue[a]	SE	Rank
IMC 47	1.00	0.00	1.5	4.0	0.20	0.06	5
SCA 6	1.00	0.00	1.5	10.4	0.33	0.07	14
LX 31	1.57	0.43	3	1.2	0.11	0.08	2
PA 218 [PER]	2.00	0.58	4	5.7	0.24	0.06	8
AMAZ 12 [CHA]	2.50	0.43	5	16.4	0.42	0.07	25
PA 70 [PER]	2.83	0.48	6	15.1	0.40	0.06	23
CL 19/10	3.38	0.32	7	13.1	0.37	0.07	20
PA 120 [PER]	3.43	0.30	8	0.7	0.09	0.07	1
PA 169 [PER]	3.50	0.29	9	6.7	0.26	0.07	9
LP 1/45 [POU]	3.60	0.24	10	14.1	0.39	0.07	22
B 5/3 [POU]	3.80	0.13	11	12.6	0.36	0.07	19
CRU 101	4.00	0.00	13.5	6.9	0.27	0.10	10
NA 342	4.00	0.00	13.5	9.0	0.31	0.06	13
NA 680	4.00	0.00	13.5	4.1	0.20	0.07	6
PA 195 [PER]	4.00	0.00	13.5	3.2	0.18	0.06	4
PA 34 [PER]	5.00	0.00	16	5.2	0.23	0.06	7
AM 1/73 [POU]	5.33	0.21	17	7.2	0.27	0.08	11
B 13/5 [POU]	5.38	0.18	18	10.5	0.33	0.06	15
CL 10/10	5.56	0.18	19	17.0	0.43	0.08	26
ICS 84	5.60	0.24	20	12.4	0.36	0.06	17
NA 387	5.70	0.21	21	13.4	0.37	0.07	21
CRU 119	5.71	0.36	22	51.2	0.80	0.08	39
IMC 6	6.00	0.38	24	15.9	0.41	0.07	24
NA 149	6.00	0.32	24	38.3	0.67	0.06	36
PA 303 [PER]	6.00	0.41	24	2.1	0.15	0.06	3
UF 11	6.08	0.19	26	30.6	0.59	0.06	32
IMC 16	6.17	0.31	27	11.0	0.34	0.06	16
ICS 95	6.25	0.25	28.5	25.2	0.53	0.07	30
IMC 103	6.25	0.49	28.5	43.1	0.72	0.06	38
ICS 80	6.29	0.18	30	35.1	0.63	0.10	34
EET 58 [ECU]	6.33	0.33	31.5	12.5	0.36	0.06	18
LP 4/24 [POU]	6.33	0.21	31.5	8.7	0.30	0.06	12
JA 5/25 [POU]	6.50	0.19	33.5	19.3	0.46	0.06	27
NA 178	6.50	0.33	33.5	35.7	0.64	0.06	35
MOQ 5/5	6.63	0.32	35	26.6	0.54	0.06	31
ICS 1	6.73	0.14	36	32.9	0.61	0.06	33
NA 756	7.13	0.23	37	20.4	0.47	0.06	29
NA 45	7.20	0.20	38	41.1	0.70	0.06	37
IMC 57	7.25	0.25	39	20.1	0.46	0.06	28
CRU 96	7.38	0.18	40	74.2	1.04	0.06	40

[a] Transformed values were based on arcsin square-root transformation.

Isolation of P. palmivora *and preparation of inoculum*

An isolate of *Phytophthora* was obtained from a naturally infected cacao pod in the field. Based on the characteristic 'seaweed' odour of the infected pod, growth of the isolate on 20% V8 juice – calcium carbonate agar medium, sporangial shape, size and pedicel length (Sreenivasan and Quesnel, 1977; Zentmyer, 1988), this organism was confirmed as *P. palmivora*. Zoospore suspensions were prepared from 10 day-old cultures of the isolate following the method of Lawrence (1978). The concentration of zoospores was determined using a haemocytometer and adjusted to 100,000 ml^{-1} for DPT-SM inoculation experiments (Iwaro et al., 2000b). For long-term preservation of the isolate, cultures were grown on agar slants in McCartney bottles and covered to a depth of 1 cm above the top of the slant with sterile mineral oil

(British Pharmacopoeia quality). The McCartney bottles containing the cultures were stored at 25 °C. This method of preservation is particularly useful in tropical climates as it prevents drying out of the cultures and does not allow penetration of mites (Johnston and Booth, 1983). Since some isolates could lose their virulence after a prolonged stay on media, it is recommended that isolates of *Phytophthora* preserved on defined media be passed through a cacao pod before use in future experiments.

Detached pod test (spray method)

DPT-SM was used to assess the reaction of fully grown, unripe detached pods (about 4–5 months old) to *P. palmivora* (Iwaro, 1997; Iwaro et al., 2000b). As recommended by Iwaro et al. (2000b), 2–4 pods were tested per genotype in each of 2 experiments conducted to confirm their reaction to *P. palmivora*. Pods were harvested between 07.00 and 10.00 h, rinsed in two changes of sterile distilled water (SDW), and arranged with labels in plastic trays lined with moist tissue in a completely randomised design. Inoculation was performed by spraying the exposed surface area of each pod at a distance of 30 cm using a Chromist atomiser (Cat. No 51901 Spray Unit, Gelman Sciences, ANN ARBOR, MICHIGAN), to deliver an average of 1 ml of zoospore suspension on about 150 cm^2 of pod surface area. Control pods were sprayed with SDW in place of the zoospore suspension. Two genotypes, SCA 6 (resistant) and ICS 1 (susceptible) with consistent results in previous DPT-SM experiments were used as standards in the experiment. Each tray containing the pods was covered with another tray as a lid and enclosed in a clear transparent polythene bag to maintain a high relative humidity. The trays were incubated in open shelves at 25 °C for 4 days, after which the levels of resistance of the inoculated pods were assessed using the disease rating scale in Table 5.

Field observations for resistance to Phytophthora pod rot

Field observations were conducted in the ICG,T on the same set of genotypes assessed with DPT-SM (Table 1). Five trees were selected for field observations of 32 genotypes, while observations were conducted on at least 2 trees for 8 accessions with less than 5 trees per plot. An average of 24 pods per tree were assessed in each year of field observations. Each tree was observed monthly and the following variables were recorded:

- number of healthy pods (i.e. not showing any symptoms of PPR).
- number of pods with PPR symptoms.

Field observations were carried out for 3 years between November 1990 and October 2001. The percentage of pods affected by PPR was expressed by:

$$\frac{\text{Number of pods with PPR symptoms}}{\text{Total number of pods produced}}$$

Data analysis

Mean and standard errors were calculated from the detached pod test data for clonal reactions to inoculation with *P. palmivora*. A distribution of scores was plotted to assess the pattern of variation among the 40 accessions evaluated for resistance to PPR using the DPT-SM. The data collected for each year of field observations were transformed using an arcsin square-root transformation and subjected to analysis of variance to determine the significance of the differences among clones and the years of field observations. Data were also subjected to Pearson correlation analysis to determine the relationship between the years of field observations. A distribution of scores was also plotted to assess the patterns of clonal reactions in each year of field observations. Subsequently, the data for each year of field observations and the results of DPT-SM were subjected to a Spearman rank correlation analysis. Correlation analyses were further performed between the result of DPT-SM and averages of the first 2 and 3 years of field observations. All the analyses were performed using the 'Number Cruncher Statistical System NCSS 2001'.

Results

Assessment of 40 accessions for resistance to Phytophthora pod rot using the detached pod test

The mean values and standard errors for 40 accessions evaluated by DPT-SM are presented in

Table 1. The distribution of scores for resistance among the 40 accessions tested showed skewness towards the susceptible end of the disease rating scale (Figure 1). A large proportion of the accessions tested were susceptible to the isolate of *P. palmivora* used in the experiment. However, eight accessions were found to be resistant (disease rating 1–3), two of which showed no visible lesions (disease rating 1). Among the other six resistant accessions, two had 1–15 localised lesions (disease rating 2), while 4 had 6–15 localised lesions (disease rating 3). Seven more accessions were resistant to the spread of lesions (disease rating 4), since many localised lesions (> 15) were observed on these. In contrast, a few expanding lesions (1–5 lesions) were formed on three accessions (disease rating 5). Fourteen accessions had 6–15 expanding lesions (disease rating 6), while the remaining eight accessions had above 15 expanding lesions (disease rating 7).

Results of field observations for resistance to Phytophthora pod rot on 40 accessions

Significant differences ($P < 0.001$) were observed among the 40 genotypes assessed for resistance to PPR by field observations (Table 2). Thirteen of the 40 accessions were found resistant (< 10% pod rot), while 16 accessions were moderately resistant (10–25% pod rot) (Table 1). Eleven accessions were classified as susceptible (>25% pod rot) (Table 1). Significant differences ($P < 0.001$) were observed among the years of field observations and a significant interaction between clones and years (Table 2). A higher level of susceptibility was observed in the third year of field observations (63%)

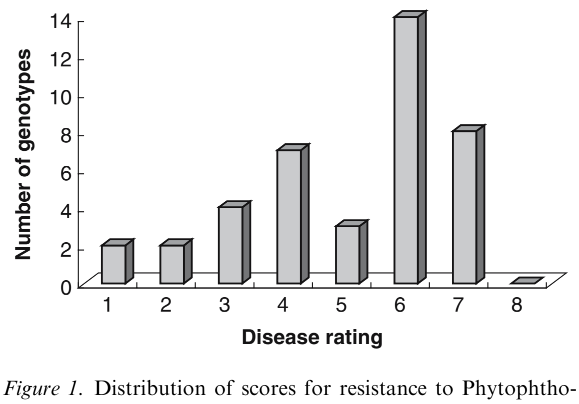

Figure 1. Distribution of scores for resistance to Phytophthora pod rot among 40 accessions from the detached pod test by spray method.

than in the first (15%) and second (25%) years (Figure 2). The results of field observations for year-1 and year-2 were correlated ($r = 0.68$; $P < 0.001$) (Table 3). However, the correlation coefficients between both year-3 and year-1 ($r = 0.32$; $P = 0.041$) and year-3 and year-2 ($r = 0.35$; $P = 0.025$) were lower.

Relationship between field observations and the reaction of detached pods to Phytophthora pod rot

Significant correlation coefficients were obtained between the rankings of DPT-SM and each year of field observations (Table 4). The correlation between the results of DPT-SM and year-1, as well as year-2 field observations were significant at $P < 0.001$, while the correlation with year-3 field observations was only significant at $P < 0.01$. The correlation coefficients ranged between 0.44 and 0.59 (Table 4). The differences in the correlation coefficients show the extent to which pod resistance varied from year to year under field conditions. A higher correlation coefficient ($r = 0.66$; $P < 0.001$) was obtained between the DPT-SM rankings and the rankings of the average of the 3 years of field observations (Figure 3).

Discussion

Varing levels of resistance to PPR were observed among the 40 accessions evaluated by DPT-SM. The accessions were categorised into 7 groups (Figure 1) based on the frequency and spread of the established lesions. According to Iwaro et al. (1997a), the frequency and spread of lesions following infection of the pod by *Phytophthora* are likely to be governed by different resistance mechanisms. Such information will facilitate recombination of genes for the two forms of resistance in new cacao varieties. The DPT-SM accounts for both lesion frequency and spread, thus reinforcing its potential for effective screening.

To determine the value of DPT-SM, it is important to assess its relationship with field observations for PPR. However, the results of field observations are usually influenced by several environmental factors (Amponsah, 1988). According to Blencowe (1962), selection for resistance is less reliable when disease incidence is low, as it becomes difficult to be sure that all pods

Table 2. Analysis of variance of clonal resistance to Phytophthora pod rot from field observations

Source term	DF	Sum of squares	Mean square	*F*-ratio	*P*-value
Clone	39	20.54	0.53	9.54	< 0.001
Year	2	9.36	4.68	84.70	< 0.001
Clone × Year	78	12.56	0.16	2.92	< 0.001
Error	403	22.26			
Total (Adjusted)	522	65.59			
Total	523				

and all trees in a given area are subjected to the same intensity of infection (Thorold, 1953). Blencowe (1962) and Lockwood (1971) also advised that absolute reliance should not be placed upon field evaluation as it distinguishes useful resistance in a given location only and does not necessarily indicate intrinsic resistance. This is because some susceptible genotypes may escape infection and thus be classified wrongly as resistant. In spite of these limitations, Amponsah and Dakwa (1969) and Amponsah (1981) indicated that true resistance to black pod infection could be observed under field conditions over a number of years.

In this study, significant variation was observed among the 40 accessions assessed for resistance to PPR, based on percentage pod rot, under field conditions. Significant differences also were observed between the years of field observations, which would have been caused by variations in the environmental conditions influencing disease incidence among the different years of field observations. A correlation value of 0.68 ($P < 0.001$) was obtained between field observations in years 1 and 2 suggesting that field conditions were quite similar in these 2 years. However, low correlation values were obtained between field observations in years 1 and 3 as well as in years 2 and 3 field observations (Table 3). This indicated that field conditions were quite different in year-3 compared to the first 2 years of field observations. The data obtained from the Trinidad and Tobago Meteorological Services on monthly rainfall, mean of monthly minimum and maximum temperatures did not show any significant differences between the yearly mean values for the 3 years of field observations. However, Figure 4 shows that rainfall in the month of November of the third year (387.6 mm) was twice the amount of rainfall in November of the first year (151.8 mm) and higher than the total rainfall in November of the second year (248.3 mm). The high rainfall in November of the third year of field observations coincided with the beginning of the main pod harvest season (November–February) in Trinidad (Purseglove, 1988). This heavy rainfall in November of the third year, and the presence of large number of mature pods provide favourable conditions for *Phytophthora* infection. These factors may account in part for the increase in PPR in the third year of field observations over the first 2 years (Figure 2). This result shows the impact of the changes in field conditions on the percentage pod rot in the field. It further emphasises the need to exercise caution in interpreting the results of any single year of field observations in terms of disease resistance, which may be strongly influenced by a range of environmental factors in the field.

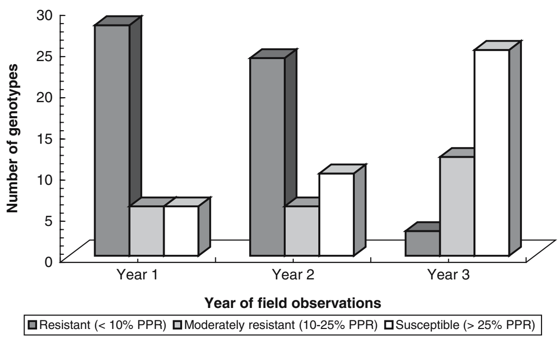

Figure 2. Distribution of categories for resistance to Phytophthora from on yearly field observations.

In this study, the correlation between the results of DPT-SM and annual field observations varied from 0.44 to 0.59 (Table 4). The highest correlation coefficient ($r = 0.59$; $P < 0.001$) was obtained between the results of the year-2 field observations and DPT-SM, while the lowest was observed between the year-3 field observations and the results of DPT-SM. This shows that the association between DPT-SM and field observations is largely influenced by field conditions. However, higher

Table 3. Pearson correlation coefficients between yearly field observations on percentage pod rot in cacao

Parameters	Correlation coefficients (r)	P-value
Year-1 vs. Year-2 field observations	0.68	< 0.001
Year-1 vs. Year-3 field observations	0.32	0.041
Year-2 vs. Year-3 field observations	0.35	0.025

correlation values were obtained between the results of DPT-SM and the average of data for years 1–2 ($r = 0.63$; $P < 0.001$) and years 1–3 ($r = 0.66$; $P < 0.001$) field observations. This demonstrates that a stronger association could be obtained between the results of DPT-SM and the average of several years of field observations. This is in agreement with earlier observations that true resistance to black pod infection could be observed under field conditions over a number of years (Amponsah and Dakwa, 1969; Amponsah, 1981).

Using the spray inoculation method on attached pods, Iwaro et al. (2000b), demonstrated that there was a strong correlation ($r = 0.73$; $P \leq 0.001$) between the results of DPT-SM and the levels of resistance of attached pods to PPR. Detached pods are generally more susceptible than the attached pods (Medeiros and Rocha, 1964; Blaha, 1974; Iwaro et al., 1997b; Iwaro et al., 2000b) and Lellis and Peixoto (1960) and Blaha (1974) noted that a degree of resistance of detached pods under laboratory conditions could be used as an indication of the higher resistance to be expected from attached pods. This suggests that the DPT-SM is an effective screening method that could be used to predict the reactions of attached pods in the field. A high correlation value ($r = 0.89$; $P \leq 0.001$) also was obtained between reactions of attached and detached pods on 10 cacao genotypes inoculated by the stab method and assessed for lesion size (Iwaro et al., 1997b). This provides further evidence that the reaction of attached pods could be reliably predicted by the reaction of the detached pods. The advantage of using detached pods lies with the opportunity to standardise the conditions under which experiments are conducted, thus reducing variation due to the physical

Table 4. Spearman rank correlation coefficients between detached pod test and field observations on Phytophthora pod rot in cacao

Parameters	Correlation coefficients (r)	P-value
Year-1 field observations vs. detached pod test	0.55	< 0.001
Year-2 field observations vs. detached pod test	0.59	< 0.001
Year-3 field observations vs. detached pod test	0.44	0.005
Average of years 1–2 field observations vs. detached pod test	0.63	< 0.001
Average of years 1–3 field observations vs. detached pod test	0.66	< 0.001

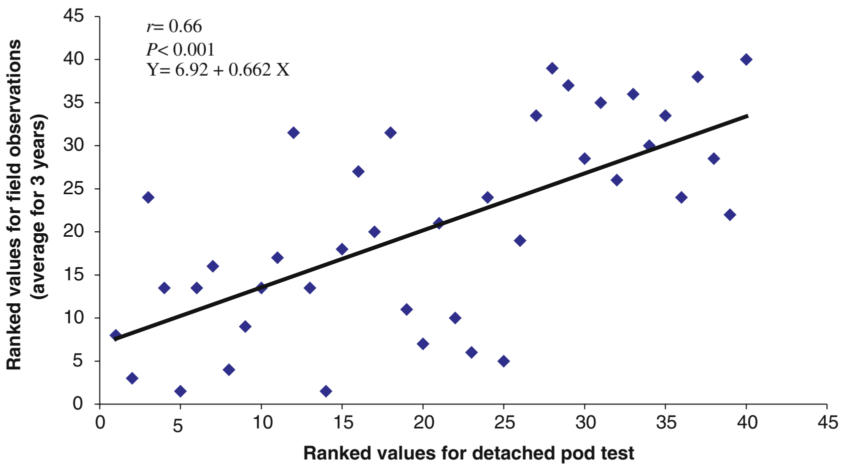

Figure 3. Relationship between the average of 3 years of field observations and detached pod test for resistance to Phytophthora pod rot.

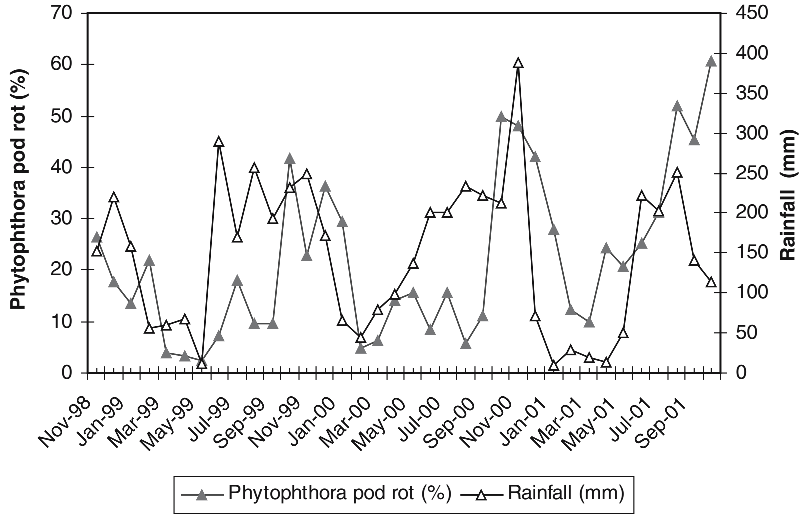

Figure 4. Relationship between rainfall pattern and the percentage of Phytophthora pod rot for 3 years of field observations.

environment and improving the precision of reactions due to genotypic effects. In addition, it is more convenient to conduct experiments under laboratory conditions than to inoculate attached pods on tree trunks/branches or to conduct field observations. Moreover, inoculations conducted on attached pods by artificial inoculation or field observations are vulnerable to changes in weather conditions as well as attack by insects and animals within the field. Where restrictions are imposed on inoculation of attached pods, as often the case in cacao genebanks, the DPT-SM offers a reliable option for the assessment of pod resistance to PPR.

The results of this study show that the DPT-SM is an effective screening method for the assessment of cacao resistance to PPR, and the results are correlated with field observations. As demonstrated in previous studies, DPT-SM is simple, reliable and cost effective (Iwaro et al., 2000b) and it discriminates effectively among genotypes with different levels of resistance (Iwaro et al., 2003). Based on the effectiveness of this method, it was adopted in the CFC/ICCO/IPGRI project 'Cocoa Germplasm Utilization and Conservation: A Global Approach' for the assessment of cacao resistance to PPR in several cocoa producing countries (Iwaro et al., 2000b). Several cocoa research institutions have adopted the DPT-SM in their breeding programmes based on its reliability and cost effectiveness. At the Cocoa Research Unit, University of The West Indies, St. Augustine, over 1000 cacao accessions have been evaluated from the International Cocoa Genebank, Trinidad (ICG,T) using the DPT-SM (Iwaro et al., 2002). The results contributed to the selection of the CFC Project Collection (Sounigo et al., 2000) and for the selection of base parents for a germplasm enhancement programme aimed at accumulating genes for resistance to PPR. It is hoped that this method will facilitate the screening of more cacao genotypes, the selection of promising resistant genotypes, and their use in breeding for

Table 5. Assessment of resistance of inoculated pods using the disease rating scale

[a]Rating	Infection level	[b]Rating	Infection level
1	No visible lesion	5	1–5 expanding lesions
2	1–5 localised lesions	6	6–15 expanding lesions
3	6–15 localised lesions	7	> 15 expanding lesions
4	> 15 localised lesions	8	Fast expanding coalesced lesions

[a]Based on the absence of visible lesions (rating 1) and the number of non-expanding (localised) lesions (rating 2–4).
[b]Based on the number of expanding, countable lesions (rating 5–7) and expanding coalesced lesions (rating 8).

the development of improved high yielding, resistant cacao varieties for commercial use.

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References

Adegbola MOK (1981) Cocoa diseases of West Africa. In: Proceedings of the 7th International Cocoa Research Conference, Douala, Cameroun, November 4–12, 1979 (pp 243–250)

Amponsah JD (1981) Field resistance of the first generation progenies of selected cocoa types to black pod disease caused by *Phytophthora palmivora* (Butler) Butler, Quatrième réunion du S/Groupe du Travail Afrique Sur *P. palmivora*; Lome, 1981

Amponsah JD (1988) Studies on field resistance of cocoa varieties to *P. palmivora* (Butler) Butler. Infection. In: Proceedings of the 10th International Cocoa Research Conference, Santo Domingo, Dominican Republic 17–23 May, 1987 (pp 557–567)

Amponsah JD and Dakwa JT (1969) Preliminary genetic assessment of black pod resistance trial in Ghana. In: 2nd International Cocoa Research Conference, 1967 Salvador, Itabuna (Bahia) (pp 162–165)

Blaha G (1974). Methods of testing for resistance. In: Gregory PH (ed.) Phytophthora Disease of Cocoa (pp 179–195) Longman/Aberdeen University Press, London, United Kingdom

Blencowe JW (1962) Future plans for cocoa breeding in Ghana. In: Report Cocoa Conference, London, 1961 (pp 141–145) Cocoa, Chocolate and Confectionary Alliance

IBPGR (1981) Genetic resources of cocoa (AGP:IBPGR/80/56). IBPGR Working Group on Genetic Resources of Cocoa, IBPGR Secretariat, Rome, Italy. 25 pp

Iwaro AD (1997) Cocoa germplasm enhancement for resistance to Black Pod and Witches' Broom diseases. In: Proceedings of the International Workshop on the Utilisation of the Genetic Resources of the International Cocoa Genebank, Trinidad (ICG,T), The Cocoa Research Unit, The University of The West Indies, Trinidad and Tobago, June 23–28, 1996 (pp 79–85)

Iwaro AD, Sreenivasan TN and Umaharan P (1997a) *Phytophthora* resistance in cacao (*Theobroma cacao*): Influence of pod morphological characteristics. Plant Pathology 46: 557–565

Iwaro AD, Sreenivasan TN and Umaharan P (1997b) Foliar resistance to *Phytophthora plamivora* as an indicator of pod resistance in *Theobroma cacao*. Plant Disease 81(6): 619–624

Iwaro AD, Sreenivasan TN and Umaharan P (1998) Cacao resistance to *Phytophthora*: Effect of pathogen species, inoculation depths and pod maturity. European Journal of Plant Pathology 104: 11–15

Iwaro AD, Sreenivasan TN, Butler DR, Thévenin JM, Mooleedhar V, Bekele FL, Sounigo O, Umaharan P and Eskes AB (2000a) General Project Strategies and Approaches: Strategy for germplasm enhancement at CRU, Trinidad. In: Eskes AB, Engels JMM and Lass RA (eds.) Working Procedures for Cocoa Germplasm Evaluation and Selection. Proceedings of the CFC/ICCO/IPGRI Project Workshop, Montpellier, France, Feb. 1–6, 1998 (pp 22–28)

Iwaro AD, Sreenivasan TN, Butler DR and Umaharan P (2000b) Rapid screening for Phytophthora pod rot resistance by means of detached pod inoculation. In: Eskes AB, Engels JMM and Lass RA (eds.) Working Procedures for Cocoa Germplasm Evaluation and Selection. Proceedings of the CFC/ICCO/IPGRI Project Workshop, Montpellier, France, Feb. 1–6, 1998 (pp 109–113)

Iwaro AD, Singh V and Jugmohan NK (2002) Evaluation of accessions in the International Cocoa Genebank, Trinidad (ICG,T) for resistance to Phytophthora pod rot. In: Annual Report 2001. Cocoa Research Unit, The University of the West Indies, St. Augustine, Trinidad (pp 35–37)

Iwaro AD, Bekele FL and Butler DR (2003) Evaluation and utilisation of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. Euphytica 130: 207–221

Johnston A and Booth C (1983) Plant Pathologist's Pocketbook, 2nd edn. (439 pp) Commonwealth Mycological Institute. Commonwealth Agricultural Bureaux, Farnham Royal, England

Lawrence JS (1978) Evaluation of methods for assessing resistance of cacao (*Theobroma cacao* L.) cultivars and hybrids to *Phytophthora palmivora* (Butler) Butler, CEPLAC, CEPEC, Itabuna, Brazil. Bioletin Tecnico 62

Lockwood G (1971) Incidence of cocoa black pod disease *Phytophthora palmivora* in relation to time of crop maturity in the Eastern Region of Ghana. Journal of Horticultural Science 46: 185–193

Lellis WT and Peixoto FO (1960) Comunicação preliminar sobre a resistancia do cacau 'Catongo' a Phytophthora palmivora Butler. *Instituto de Cacau da Bahia, Boletim Inform.* No. 61, 26–31

Medeiros AG and Rocha HM (1964) Programação dos trabalhos de selecão de cacaueiros resistantes a 'podridão parda' no Estado da Bahia-Brazil. Phytopathology (Abstract) 55: 125–126

Opeke LK and Gorenz AM (1974). *Phytophthora* pod rot: Symptoms and economic importance. In: Gregory PH (ed.) Phytophthora Disease of Cocoa (pp 117–124) Longman/Aberdeen University Press, London, UK

Purseglove JW (1988) Tropical Crops: Dicotyledons, Longman, Singapore

Rocha HM (1974). Breeding for resistance to *Phytophthora palmivora*. In: Gregory PH (ed.) Phytophthora Disease of

Cocoa (pp 211–218) Longman/Aberdeen University Press, London, United Kingdom

Sounigo O, Mooleedhar V, Iwaro AD, Bekele F, Sreenivasan TN, Thevenin JM, Khan N and Butler DR (2000) Strategy to establish a CFC Project collection. In: Eskes AB, Engels JMM and Lass RA (eds.) Working Procedures for Cocoa Germplasm Evaluation and Selection. Proceedings of the CFC/ICCO/IPGRI Project Workshop, Montpellier, France, Feb. 1–6, 1998 (pp 29–37)

Soria VJ (1974). Sources of resistance to *Phytophthora palmivora*. In: Gregory PH (ed.) Phytophthora Disease of Cocoa (pp 197–202) Longman/Aberdeen University Press, London, United Kingdom

Sreenivasan TN and Quesnel VC (1977) Field differentiation of black pod from other cocoa pod diseases. Tropical Agriculture (Trinidad) 54: 371–372

Tan GY and Tan WK (1990) Additive inheritance of resistance to pod rot caused by *P. palmivora* in cocoa. Theoretical and Applied Genetics 80: 258–264

Thorold CA (1953) The control of black pod disease of cocoa in the Western Region of Nigeria. In: Cacao Conference (pp 108–115) London 153, London Cocoa and Chocolate and Confectionery Alliance

Zentmyer GA (1988) Taxonomic relationships and distribution of species of Phytophthora causing Black Pod of cacao. In: Proceedings of the 10th International Cocoa Research Conference, Santo Domingo, Dominican Republic, May 17–23, 1987 (pp 391–395)