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Cacao resistance to *Phytophthora*: Effect of pathogen species, inoculation depths and pod maturity

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Abstract

Two species of *Phytophthora (P. palmivora* and *P. capsici*) and inoculations at two depths (3 mm and 9 mm) were tested each on 10 clones of *Theobroma cacao* to determine their effects on pod resistance. Ripe and unripe pods were also assessed to determine the influence of physiological status of the pod on the expression of resistance. The two pathogens tested (*P. palmivora* and *P. capsici*) differed significantly in their reactions on pods, with *P. palmivora* being more aggressive than *P. capsici*. However, the lack of interaction between clones and pathogen species and the similarity in the ranking of clones based on lesion size suggested that selection for resistant clones can be based on one of the two pathogens, preferably the more aggressive one. Pod reactions differed between inoculation depths (3 mm and 9 mm), and between pod maturity stages (ripe and unripe pods) with relatively larger lesions being recorded at 9 mm depth and on unripe pods as compared to those observed at 3 mm depth and on unripe pods, respectively. The magnitude of increase in lesion sizes, however, varied with genotypes, indicating that inoculation depth and pod maturity stage should be standardized in screening cacao germplasm for resistance to *Phytophthora*.

The black pod disease caused by *Phytophthora* species is one of the most prevalent and destructive diseases of *Theobroma cacao*. Of the three major species of *Phytophthora* causing black pod: *P. palmivora, P. megakarya, P. capsici* (Brasier et al., 1981; Zentmyer, 1988), only *P. palmivora* and *P. capsici* are present in Trinidad. Developing genetic resistance against this disease is considered to be the most cost effective and reliable method of control. Although a number of methods of assessment of resistance to black pod have been developed (Blaha, 1974), the results not always showed similarity in the ranking of genotypes of cacao (Soria, 1974; Rocha, 1974). Such discrepancies in screening results may account for the slow progress in breeding for resistance to black pod disease. While the observed differences in clonal reactions over locations can be interpreted as partly due to pathogen variability, other variables could also have resulted in such discrepancies.

In this study, the effects of two species of *Phytophthora* (*P. palmivora* and *P. capsici*), inoculations at two depths (3 mm and 9 mm) and pod maturity stages on resistance to *Phytophthora* were examined. Three experiments were conducted on thirty clones of cacao.

In the first experiment, both *P. palmivora* and *P. capsici* were tested to assess their aggressiveness on ten selected clones. Isolates of *P. palmivora* and *P. capsici* were obtained from naturally infected cacao pods at the University of the West Indies, St. Augustine, Trinidad. Since the two pathogens were obtained from different cacao clones, they were grown on pods of the same cacao clone (ICS 1) and re-isolated. The isolates were maintained on 20% V8 juice – calcium carbonate agar medium slants as stock and Petri dish cultures were prepared for zoospore extraction. Zoospore suspensions were prepared from ten-day-old cultures of *P. palmivora* and *P. capsici* by inundating each culture plates of 9 cm diameter with 10 ml of sterile distilled water (chilled to 10 °C), refrigerating for 25 min and incubating in the dark for 30 min (Lawrence, 1978). Zoospores concentration was determined using a haemocytometer and adjust-

Table 1. Mean of lesion size (a) following inoculation of fully mature unripe pods of 10 cacao clones with *P. palmivora* and *P. capsici* using stab-3 method of inoculation

Clone	Pathogen species				Clone means (a^2)
	P. palmivora		*P. capsici*		
	a^1	a^2	a^1	a^2	
SLC 18	17.83	4.17	8.08	2.82	3.50
CLM 91	32.88	5.73	24.20	4.86	5.29
MO 9	33.84	5.81	26.46	5.11	5.46
JA 539	35.98	5.98	28.77	5.31	5.64
SLC 19	38.87	6.21	32.84	5.70	5.95
SJ 222	48.39	6.94	38.65	6.20	6.57
NA 286	52.72	7.24	47.50	6.87	7.06
NA 90	54.28	7.35	43.77	6.57	6.96
JA 10.12	60.78	7.79	55.55	7.43	7.61
NA 186	63.65	7.95	54.89	7.36	7.66
Pathogen means (a^2)		6.52		5.82	
LSD (P = 0.05) clone		= 0.36			
LSD (P = 0.05) pathogen		= 0.16			

[1] Actual values (cm^2).
[2] Transformed values ($\sqrt{x}$).

ed to 200,000 ml^{-1} in each inoculation experiment. Fully mature unripe pods were harvested from the ten selected clones and inoculated using the stab-3 method (Iwaro et al., 1997). A standard injury was created on the pod surface using an apparatus that consists of 20 pins with a piercing length of 3 mm. Inoculation was effected by placing a filter paper disc (4 mm diameter) immersed in zoospore suspension on the injured area (0.30 cm^2) and covered with a spot plaster (Johnson and Johnson, New Brunswick, USA). After inoculation, the twenty-treatment-combinations (ten clones, two pathogens) were arranged in a completely randomized design with five replications. For the control treatment (two pods per clone), sterile distilled water was used instead of the zoospore suspension. The inoculated pods and the controls were incubated at 25 °C in trays lined with moist paper towels and covered with polythene film. After five days of incubation, the area of the established lesion was determined by transcribing the outline of the lesion unto brown paper. The area of the brown paper cuttings was then determined using a leaf area meter (MK2, Delta-T Devices by Burwell, Cambridge, England). The experiment was repeated twice. The data collected were subjected to square root transformation and analysis of variance to determine the significance of treatment effects.

There were significant differences in lesion size ($P \leq 0.05$) between genotypes and the two pathogens (Table 1). Relatively large lesions were formed with *P. palmivora* (av. 43.92 cm^2) as compared with *P. capsici* (av. 36.07 cm^2), indicating that the former was comparatively more aggressive. The interaction between genotypes and pathogen species was not significant ($P \leq 0.05$). The similarity in the ranking of clonal reactions to *P. palmivora* and *P. capsici* (Figure 1A) suggests that one of the two species can be used in screening for resistance to black pod. Since *P. palmivora* is the more aggressive species in this study and the most widely distributed in cocoa growing regions, it would be the species of choice for screening for resistance. Recent studies have also shown similarity in the ranking order for resistance to *P. megakarya* in Cameroon and that for *P. palmivora* in Ivory coast (Van der Vossen, 1997).

In the second experiment, two inoculation depths (3 mm and 9 mm) were tested by stab-3 and stab-9 inoculation methods to assess the effect of the depth of inoculation on clonal ranking of susceptibility. Fully mature unripe pods of ten clones were inoculated with zoospore suspension of *P. palmivora* (200,000 ml^{-1}). The inoculation procedure for stab-3 is as described in the first experiment. In the case of stab-9, a standard injury was created on the pod surface using an apparatus consisting of 20 pins with a piercing length of 9 mm. Inoculation was similar to that described in experiment-1. The control treatment (two pods per

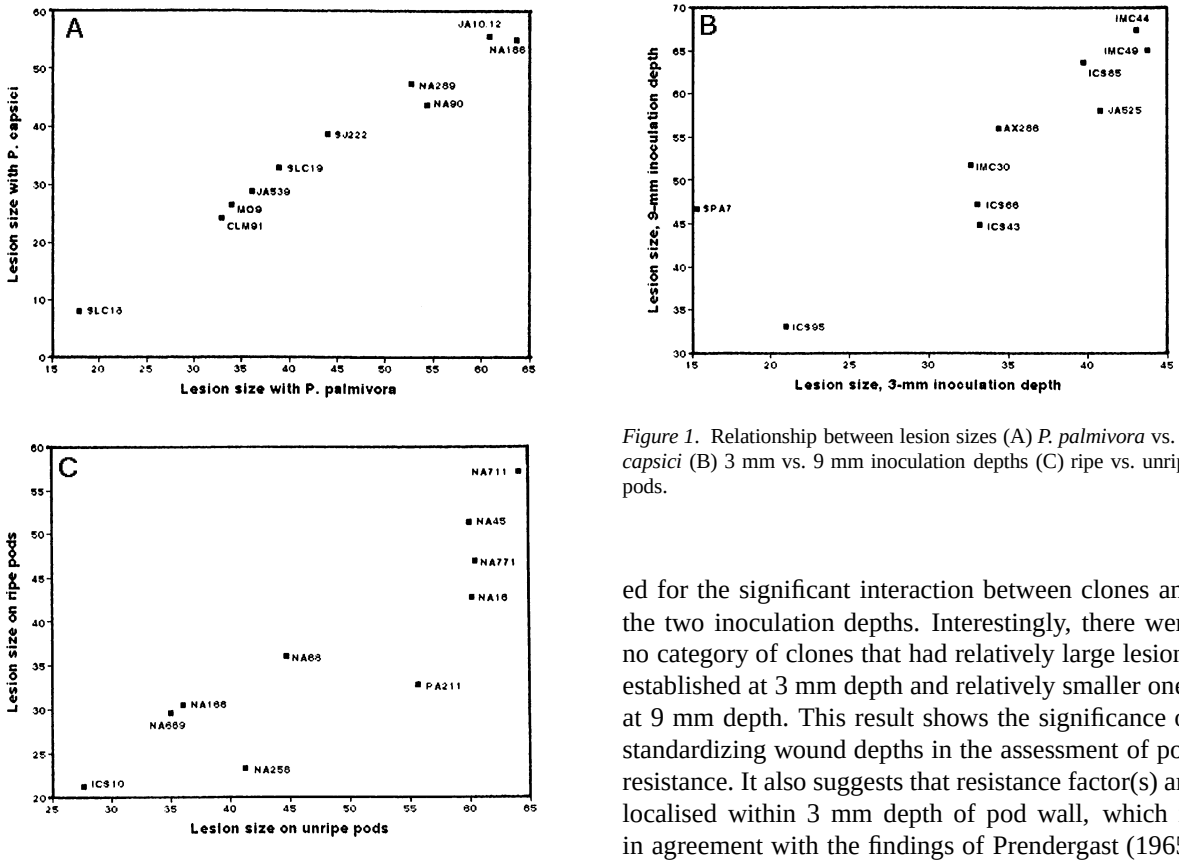

Figure 1. Relationship between lesion sizes (A) *P. palmivora* vs. *P. capsici* (B) 3 mm vs. 9 mm inoculation depths (C) ripe vs. unripe pods.

clone) received distilled water instead of the zoospore suspension. Lesion sizes were determined after five days of incubation. The experiment was carried out with twenty-treatment-combinations (ten clones, two inoculation depths) and five replications. Two trials were conducted.

Analysis of variance showed a significant difference ($P \leq 0.05$) in lesion size (Table 2) produced at the two inoculation depths (3 mm and 9 mm), with stab-9 producing relatively larger lesions (av. 53.37 cm^2) than those with stab-3 (av. 33.68 cm^2). The effects of genotype and genotype $\times$ inoculation depths were significant ($P \leq 0.05$), suggesting a differential response of genotypes to the two inoculation depths. Four reaction types could be differentiated. Lesion sizes were consistently large in IMC 44 and IMC 49 at both inoculation depths, consistently small in ICS 95 and consistently of a moderate size in ICS 43 and ICS 66 (Figure 1B). In contrast, large lesions were formed in SPA 7 and ICS 85 at 9 mm depth as compared to the relatively small lesions established at 3 mm depth (Figure 1B). The latter category of clones accounted for the significant interaction between clones and the two inoculation depths. Interestingly, there were no category of clones that had relatively large lesions established at 3 mm depth and relatively smaller ones at 9 mm depth. This result shows the significance of standardizing wound depths in the assessment of pod resistance. It also suggests that resistance factor(s) are localised within 3 mm depth of pod wall, which is in agreement with the findings of Prendergast (1965) who reported that biochemical substances related to resistance (polyphenols) were concentrated immediately below the epidermis. Another explanation could be that factor(s) associated with susceptibility may be at a higher concentration at deeper tissues of the pod. Similar differential reaction of clones to multiple-point and stab-3 inoculation methods was reported by Iwaro et al. (1997).

In a third experiment, ripe and unripe pods of ten other clones were inoculated with a zoospore suspension of *P. palmivora* (200,000 ml^{-1}) by stab-3 method to understand the effect of pod maturity on pod susceptibility. For the control treatment (two pods per clone), sterile distilled water was used instead of zoospore suspension. The experiment was conducted with twenty-treatment-combinations (ten clones, ripe and unripe pods) and five replications. Two trials were conducted.

The analysis of results showed a significant difference ($P \leq 0.05$) in established lesion sizes between genotypes and maturity stages (Table 3). The interaction between genotypes and pod maturity stages was also significant ($P \leq 0.05$). On an average, lesion size was larger on unripe pods (av. 48.52 cm^2) as compared to the ripe ones (av. 37.16 cm^2). Although the

Table 2. Mean of lesion size (a) produced on fully mature unripe pods of 10 cacao clones inoculated at 3 mm and 9 mm depths with *P. palmivora* using stab-3 and stab-9 methods of inoculation

Clone	Inoculation depths			
	3 mm		9 mm	
	a[1]	a[2]	a[1]	a[2]
SPA 7	15.30	3.88	46.76	6.82
ICS 95	20.98	4.54	33.00	5.71
IMC 30	32.63	5.70	51.68	7.18
ICS 66	33.10	5.73	47.15	6.86
ICS 43	33.18	5.74	44.87	6.69
AX 286	34.40	5.85	56.00	7.47
ICS 85	39.69	6.28	63.71	7.98
JA 525	40.73	6.35	58.10	7.61
IMC 44	43.00	6.53	67.37	8.20
IMC 49	43.76	6.61	65.10	8.06
LSD (P = 0.05) Clone × Inoculation depths = 0.41				

[1] Actual values (cm^2).
[2] Transformed values ($\sqrt{x}$).

Table 3. Mean of lesion size (a) following inoculation of ripe and unripe pods of 10 cacao clones with *P. palmivora* using stab-3 method of inoculation

Clone	Lesion size (cm^2)			
	Unripe pods		Ripe pods	
	a[1]	a[2]	a[1]	a[2]
ICS 10	27.64	5.23	21.25	4.51
NA 669	35.01	5.90	29.49	5.41
NA 168	36.07	5.99	30.44	5.50
NA 258	41.24	6.35	23.29	4.82
NA 68	44.72	6.68	36.02	5.97
PA 211	55.67	7.43	32.86	5.71
NA 45	60.00	7.74	51.35	7.16
NA 16	60.23	7.75	42.82	6.54
NA 771	60.50	7.76	46.97	6.85
NA 711	64.15	8.00	57.13	7.55
LSD (P = 0.05) Clone × pod maturity stages (ripe/unripe) = 0.48				

[1] Actual values (cm^2).
[2] Transformed values ($\sqrt{x}$).

established lesion sizes showed a consistent decrease (significant at $P \leq 0.05$) with ripening in all genotypes (Figure 1C), the magnitude of the difference between ripe and unripe pods, varied among clones. Clonal reaction to *Phytophthora* in response to ripening was relatively small in ICS 10, NA 669, NA 168, NA 711, NA 86 and NA 45, moderate in NA 771, NA 16, NA 258 and high in PA 211 (Figure 1C). Such differential responses accounted for the interaction between clones and pod maturity stages (ripe and unripe). This suggests that the factor(s) responsible for resistance accumulate with ripening, or alternatively, factors that predispose pods to infection may be decreasing. The results suggest that pod maturity stage has to be standardized so that results obtained from different clones are comparable. Since pods infected at ripening would not represent a cost to the farmers, assessment at the mature unripe stage is recommended.

In conclusion, this study underscores the importance of inoculation depth and pod maturity stage in the assessment of cacao resistance to black pod disease. The results of this study along with those of Iwaro et al. (1997) may explain the discrepancies in clonal ranking observed in previous reports. Further, the similarity in clonal ranking to *Phytophthora* species observed in this study and in studies cited by Van der Vossen (1997) suggest that a unified effort of germplasm enhancement for resistance to *P. palmivora* at one location would be both a cost effective and a practical approach to protecting cacao from *Phytophthora* diseases.

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