Plant Pathology (2007) **56**, 219–226 Doi: 10.1111/j.1365-3059.2006.01547.x

Foliar resistance of cacao (*Theobroma cacao*) to *Phytophthora palmivora* as an indicator of pod resistance in the field: the effect of light intensity and time of day of leaf collection

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Resistance of cacao leaves to *Phytophthora palmivora* was studied with regard to the time of leaf collection (morning, afternoon) and the degree of exposure of the leaves to light in the field (low, medium and high). The efficiency of leaf disc inoculations in predicting field resistance of nine clones was compared with that of detached and attached pod inoculations. Significant effects were observed, with leaves exposed to high light intensity and collected early in the afternoon showing highest susceptibility. The effect of time of leaf collection was reduced when leaves were stored overnight and leaf discs prepared and inoculated the following day, as compared to inoculations on the day of collection. Interactions between the main factors were significant, though less substantial than the clone effects. The most significant correlations with pod resistance (r = 0·70 to 0·97) were obtained for leaves collected early in the morning and exposed to intermediate shade conditions in the canopy. For other treatments, the correlations with pod resistance were still positive (r = 0·23 to 0·83) but often not significant. Pod inoculations in the laboratory were better correlated with field resistance (r = 0·92) than pod inoculations in the field (r = 0·72). Detached pod inoculations were also better correlated with leaf disc inoculations than those of attached pods. The results confirm the validity of laboratory inoculations of leaves and pods to assess field resistance to *Phytophthora*. Standardization of the leaf disc test is essential to obtain reliable results.

Keywords: black pod, cocoa, correlations, field resistance, leaf-disk and pod inoculations, phytophthora pod rot

Introduction

Loss of cacao to phytophthora pod rot (PPR, also called black pod) is estimated to account for 30% of world production (Renard, 1999). At least four species of the pathogen have been identified: *Phytophthora megakarya*, *P. palmivora*, *P. capsici* and *P. citrophthora* (Brasier & Griffin, 1979). Losses due to *P. megakarya*, the most important species in Africa, amount to 60–80% when no control measures are taken. In Côte d'Ivoire, the major cacao producing country in the world, *P. palmivora* is still the prevailing species, causing 10–20% yield losses. However, *P. megakarya* has already been reported in the country near to the border with Ghana and is advancing towards the major cacao producing areas in the centre and in the west of Côte d'Ivoire (BIK, unpublished data).

In searching for genetic resistance to the disease, in addition to field observations, different types of artificial inoculation tests have been used. Inoculation of cacao leaves or leaf discs with zoospores of *Phytophthora spp.* has recently become a routine method for screening for resistance to PPR. Several researchers have participated in developing this test, studying inoculum concentration, temperature of incubation, light intensity during incubation and the duration of incubation (e.g. Tondjé *et al.*, 1988; Iwaro, 1995; Nyassé *et al.*, 1995; Thévenin & Motilal, 2000; Ducamp & Cilas, 2000; Tahi, 2003; Thévenin *et al.*, 2004). By inoculating leaves in the hardening stage (50 to 60 days old), either when collected in the field or in the nursery, Tahi *et al.* (2000) showed that results may be correlated with natural infection of pods with *P. palmivora* in the field. The heritability estimates for leaf resistance obtained in factorial and diallel crossing designs (Iwaro *et al.*, 1997a; Tahi *et al.*, 2006a) suggest that selection can be considerably accelerated by using leaf or leaf disc inoculations. Recently, the effect of leaf age on the expression of resistance and on the correlation with field levels of attack was reported (Tahi *et al.*, 2006b).

The objective of the present research was to evaluate the possible effects of light intensity and timing of leaf

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Accepted 7 August 2006

collection in the field on the expression of resistance in leaf discs of nine cacao clones. The interactions of the treatments with clone effects and the correlations of the results of the leaf disc test with natural infection in the field, as well as with artificial inoculation of pod, were analysed.

Materials and methods

Plant materials

Leaves and pods from 12 different clones were used, which were chosen because of their variable resistance to PPR in field trials (Table 1). The percentage of PPR was estimated by field observations of nine clones over 6 years in the trial BL7 planted in 1986 in Bingerville, Côte d'Ivoire (Anon, 1998), and by general combining ability estimates for the percentage of pod infection in the field of three genotypes based on observations over 10 years in the hybrid trial B8 planted in 1979, also in Bingerville (Cilas *et al*., 1998; Tahi *et al*., 2000). The IFC5 clone, growing in the Bingerville collection, was used as a susceptible control clone. All clones were grown without any overhead shade.

Leaf disc tests

The inoculation technique used in all leaf disc experiments and the scoring of disease severity (DS) was done 7 days after inoculation, using a 0 to 5 point scale according to Nyassé *et al*. (1995). The *P. palmivora* isolate used was obtained in 1995 from a naturally-infected pod from the Bingerville station in Côte d'Ivoire. The pathogenicity of the isolate was maintained by regular inoculation in the laboratory of green mature cacao pods and by re-isolation afterwards on pea agar medium in tubes placed at 26°C in the dark. This isolate has shown to have an average level of aggressiveness in leaf disc tests, with DS scores varying between 3 and 4 for susceptible cacao genotypes (Tahi *et al*., 2000; Tahi *et al*. 2006a; Tahi *et al*., 2006b).

Initially, the effects of time of day of leaf collection and of light intensity were studied separately in three trials (Trials 1, 2 and 3). The combined effect of both variables was then studied in Trial 4. In all trials, semi-hardened leaves of about 2 months old were used, which is considered the optimum age for leaf disc tests (Tahi *et al*., 2006a).

Effect of time of leaf collection, with leaf disc preparation and inoculations carried out on the same day (Trial 1)

Leaves were collected from four clones (332, 1020, 1633 and IFC5) at four different times of the day. The time of collection, temperature and relative humidity (RH) variations observed in the field during the period of leaf collection were i) between 06·30 and 07·00 hours, 27 to 29°C and 82 to 92% RH; ii) between 10·00 and 10·30 hours, 32 to 34°C and 61 to 67% RH; iii) between 13·30 and 14·00 hours, 35 to 37°C and 43 to 57% RH; and iv) between 17·00 and 17·30 hours, 29 to 31°C; 53 to 66% RH.

Three leaves from each of four trees were collected per clone. Ninety-six leaf discs 15 mm in diameter were cut with a cork borer immediately after collection. Discs were randomly distributed in groups of 24 discs per clone in each of four trays of 70 × 60 × 10 cm on a wetted plastic foam layer 1 cm thick. Inoculation was carried out in the evening of the same day, at about 20·00 hours.

Effect of time of leaf collection and overnight storage of leaves and leaf discs (Trial 2)

The second trial investigated if conservation of the leaves or leaf discs overnight at high relative humidity would have any effect on the results of Trial 1. Leaves were

Table 1 Genetic origin of clones studied (UA = Upper Amazon origin) and their field level of resistance to phytophthora pod rot (PPR), based on 6 and 10-year field observations in trial BL7 and B8, respectively

Field trial	Clone number	Genetic origin	Field PPR (%)
Bingerville (clone trial BL7)	332	P7 × IFC5 (UA × Amelonado)	5·6 a[a]
	735	PA150 × IFC1 (UA × Amelonado)	10·0 ab
	1041	SCA6 × IFC5 (UA × Amelonado)	11·5 abc
	1020	T85/799 × IFC15 (UA × Amelonado)	13·7 bc
	1514	T60/887 × IFC5 (UA × Amelonado)	16·3 de
	330	IMC67 × IFC1 (UA × Amelonado)	16·5 de
	1202	NA79 × IFC1 (UA × Amelonado)	17·2 e
	202	T60/887 × IFC1 (UA × Amelonado)	23·4 f
	1633	T63/967 × IFC5 (UA × Amelonado)	25·7 f
Bingerville (hybrid trial B8)	PA150	UA	**11·1**[b] a
	T60/887	PA7 × NA32 (UA × UA)	**19·3** bc
	NA79	UA	**26·7** cd
Control clone	IFC5	Amelonado	_

[a]Different letters indicate significant differences within columns according to the Newman and Keuls test at 5% probability (Anon, 1998).
[b]General combining ability values (in bold) for field resistance in trial B8 (Cilas *et al*., 1998).

collected from a moderately resistant clone (T60/887) at 07·00 and 10·00 hours and at 13·00, 16·00 and 17·00 hours. Ten leaves were harvested at each collection time and placed in slightly humidified plastic bags. Five leaves (group 1) were used to prepare leaf discs the same evening (after 17·00 hours), whereas the other five leaves (group 2) were left at 100% relative humidity overnight and discs were prepared from these leaves the following morning between 18·00 and 20·00 hours. Thirty discs were prepared for each time of leaf collection/inoculation treatment and distributed over three trays (replicates). Inoculation of 50% of the leaf discs from group 1 was carried out the same evening of the day of leaf collection (time of inoculation treatment no. 1) and the other 50% of the leaf discs the next morning around 09·00 hours (time of inoculation treatment no. 2). Leaf discs from group 2 leaves were inoculated the next morning, also around 09·00 hours (time of inoculation treatment no. 3).

Effect of light intensity in the canopy prior to collection (Trial 3)

Three clones with different levels of field resistance (PA150, T60/887 and NA79) were used in this trial to study the effect of using leaves exposed to three levels of light intensity. The average light intensity at the different canopy sites of leaf collection was measured with a LAI-2000 light meter (Welles, 1990) on a sunny day for the three treatments applied. The three categories are as follows: i) 'Unshaded' leaves taken from the outside of the canopy (generally small, thin and pale green leaves): exposure to maximum solar radiation; ii) 'Medium shaded' leaves taken more inside the canopy (green and relatively thick leaves): average exposure 8·38% of maximum solar radiation; and iii) 'Highly shaded' leaves taken from the middle lower part of the canopy (dark green large leaves, but not as thick as in treatment 2): average exposure 0·78% of maximum solar radiation. There was no clonal effect on the amount of radiation received in the different treatments ($P = 0{\cdot}57$) and also no interaction effect between clones and radiation levels ($P = 0{\cdot}52$).

Semi-hardened leaves of similar age were obtained during the morning in the canopy of three trees of each of the three clones, and at the three levels of shade indicated above. One hundred and twenty leaf discs were prepared of each clone, distributed over four trays, and inoculated the same day.

Combination of time of leaf collection, light intensity and clonal effects (Trial 4)

In this factorial trial, all possible 60 combinations of three light intensity levels, two times of leaf collection, and ten clones were tested. Shade levels were as indicated under Trial 3, the timing of leaf collection was between 06·30 and 09·00 hours and between 13·00 and 15·30 hours. The nine clones tested (332, 735, 1041, 1020, 1514, 330, 1202, 202 and 1633) were known for their level of field resistance (Table 1). A susceptible control clone was also used (IFC5). For each of the treatments, three leaves were collected from each of six trees per clone. The leaves were conserved at 100% humidity overnight and leaf discs were prepared the next day. For each of the 60 treatments, 48 leaf discs were prepared and distributed over four trays (replicates). Inoculation was done 24 hours after leaf collection for all treatments.

Pod inoculation tests (Trial 5)

Approximately 4-month-old mature green pods of the same nine clones used in Trial 4 were inoculated in the field and in the laboratory during a relatively dry period in the rainy season (September 1997). The isolate of *P. palmivora* was the same as in the leaf disc tests. For the laboratory test, three or four pods from five different trees per clone were used (a total of 15–20 pods per clone). The pods from each clone were distributed over five trays of $145 \times 115 \times 40$ cm, with all pods from one tree being placed in the same tray. The pods were placed on top of wooden laths to avoid direct contact with the wetted plastic foam layer that was placed on the bottom of each tray. Before inoculation, a metal point of 2 mm diameter was used to create wounds of 8 mm depth at two opposite points in the middle of the pod. Within each of the wounds, 30 μL of a suspension of 5×10^5 zoospores mL^{-1} was applied with a pipette. The field inoculations were carried out one day after the laboratory test. Four to eight pods of each of five to seven trees were inoculated per clone (totalling 20–40 pods per clone), using the same method as applied in the laboratory. After inoculation, the pods were covered with a plastic bag containing a wetted piece of cotton wool in order to maintain 100% relative humidity.

Measurements of the largest diameter (D) and the smallest diameter (d) of the necrotic lesions were carried out on the fifth day after inoculation in the laboratory and in the field. The surface of the lesions on the fifth day after inoculation (SurfD5) was calculated by the following formula: $(D \times d \times \pi)/4$.

Statistical analyses

The effects of the main factors studied (cacao genotype, time of day leaves were harvested and light intensity), as well as of their interactions, were estimated with the SAS statistical package (SAS, 1989), using a linear model for the analysis of variance. The leaf disc and pod inoculation trial was analysed according to a randomized block design, with the trays being considered as blocks. The Newman and Keuls test was used to identify differences between the mean values of the treatments. Laboratory (disease scores in the leaf disc test) and field (percentage of natural pod infection) inoculations were compared using Spearman's rank correlation (Spearman, 1904). Because of the environmental correlation reported between the number of pods on the tree and the percentage of infection (Anon, 1998), the average percentages of pod infection in

the field for the clones were adjusted by using the total number of pods per tree as co-variable. The surface of the lesions obtained with the pod inoculations were analysed after carrying out square root transformations.

Results

Effect of time of day of leaf collection (Trials 1 and 2)

In Trial 1, highly significant effects of time of leaf collection and of clones ($P < 0{\cdot}001$) were observed. Lower DS scores were obtained for leaves collected in the morning than for leaves collected in the afternoon and maximum disease scores were obtained early in the afternoon (Table 2). The average DS scores of the clones in the leaf disc tests were correlated to the level of field resistance. Ranking of the clones did not change substantially, although the interaction effect between clones and time of leaf collection had a P value of 0·06.

Trial 2 verified whether incubation of leaves and leaf discs overnight before inoculation in the morning might stabilize the physiological conditions of the leaf tissue, and hence decrease the effect of timing of leaf collection. The results (Table 3) show again a highly significant effect of time of leaf collection for leaf discs inoculated in the evening of the day of leaf collection, with highest disease scores for the leaves collected at 16·00 hours, confirming the results of Trial 1. However, the effect of time of leaf collection is less pronounced when leaf discs are prepared and inoculation is done the following morning.

Effect of exposure of leaves from three clones to different light intensities (Trial 3)

Higher average DS scores were observed for leaves exposed to direct sunlight (DS = 3·65) compared to leaves exposed to low or medium light intensity in the canopy (2·99 and 3·04, respectively). The effect of the clones was also highly significant, as was the interaction between clones and light intensity. At high light intensity, leaves of the moderately resistant clone T60/887 appeared to be as susceptible as that of the most susceptible clone NA79 (Table 4).

Combined effect of time of collection and exposure to light intensity of leaves from nine clones (Trial 4)

In Trial 4, leaves were collected in the morning and afternoon from nine cacao clones at three levels of sunlight exposure in the canopies. Leaf disc preparation and

Table 2 Susceptibility to *Phytophthora palmivora* of leaf discs obtained from leaves collected at different times of the day and inoculated the same day (Trial 1) compared with field level of phytophthora pod rot (PPR) of the same clones

Clone	Field PPR(%)	Time of leaf collection 06·30–07·00 h	10·00–10·30 h	13·30 to 14·00 h	17·00–17·30 h
332	5·6[a]	0·73 a[b]	0·80 a	1·48 a	1·29 a
1020	13·7	1·23 b	1·44 b	1·97 a	1·80 b
1633	25·7	1·63 c	2·23 c	2·94 b	2·95 d
IFC5 (Control)	_	1·86 c	2·36 c	2·93 b	2·57 c
Mean	15	1·36 A	1·71 B	2·33 D	2·15 C
CV (%)[c]	_	12	11	14	11

[a]Percentage of rotten pods observed in a clone trial from 1989 to 1994 (Anon, 1998) see Table 1.
[b]Different letters indicate significant differences within columns (lower case) or rows (upper case) according to the Newman and Keuls test at 5% probability.
[c]Coefficient of variation.

Table 3 Susceptibility of leaf discs to *Phytophthora palmivora* from leaves of a moderately resistant clone (T60/887) collected at different times of the day and inoculated the same day or the next day (Trial 2)

Time of leaf collection	Leaf discs prepared on 20/01/01 between 18·00 and 19·00 h: Inoculation on 20/01/01 at 20·00 h	Inoculation on 21/01/01 at 09·00 h	Leaf discs prepared on 21/01/01 between 07·00 and 08·00 h: Inoculation on 21/01/01 at 09·00 h
07·00 h	1·70 a[a]	2·03 a	2·20 a
10·00 h	2·03 b	2·03 a	2·23 a
13·00 h	2·57 c	2·17 a	2·37 a
16·00 h	3·07 d	2·77 b	2·80 a
17·00 h	2·23 b	1·97 a	2·50 a
Mean	2·32 AB	2·19 A	2·42 B
CV (%)[b]	6	14	11

[a]Different letters indicate significant differences within columns (lower case) or rows (upper case) according to the Newman and Keuls test at 5% probability.
[b]Coefficient of variation.

Table 4 Susceptibility of leaf discs to *Phytophthora palmivora* from leaves collected at three levels of light intensity in the canopy of three cacao clones (Trial 3) compared with field level of phytophthora pod rot (PPR) of the same clones

		Light intensity in the canopy		
Clone	Field PPR (%)	Low	Medium	High (no shade)
PA150	11·1[a]	1·89 a[b]	2·18 a	2·39 a
T60/887	19·3	3·03 b	2·79 b	4·22 b
NA79	26·7	4·06 c	4·14 c	4·33 b
Mean	19·03	2·99 A	3·04 A	3·65 B
CV (%)[c]	_	17	17	13

[a]Percentage of rotten pods observed in a clone trial from 1989 to 1994 (Anon, 1998) (see Table 1).
[b]Different letters indicate significant differences within columns (lower case) or rows (upper case) according to the Newman and Keuls test at 5% probability.
[c]Coefficient of variation.

Table 5 Analysis of variance for susceptibility of leaves to *Phytophthora palmivora* collected in the morning and evening at three levels of light intensity in the canopy of ten cacao clones (Trial 4)

Source of variance	Df	MS	*F*	*P*
Time of leaf collection (T)	1	5·95	76·76	< 0·001
Light intensity (LI)	2	7·20	92·92	< 0·001
Clone (C)	9	3·69	47·57	< 0·001
C × T	9	0·28	3·60	< 0·001
C × LI	18	0·28	3·67	< 0·001
T × LI	2	0·38	4·92	< 0·008
C × T × LI	18	0·28	3·7	< 0·001
Error		180	0·08	

inoculation was carried out early on the following day. The effect of all three main factors was highly significant (Tables 5 and 6). The results confirm, as in Trials 1, 2 and 3, the effects of time of leaf collection and light intensity exposure on resistance of cacao leaf tissue to *P. palmivora* (Table 6). The variances due to interactions were also significant, although with much lower *F*-values than observed for the main effects. The ranking of the clones sometimes changed significantly as a result of the combination of factors studied. Such was the case for clone 1020, a moderately resistant clone for field resistance. Its leaves showed high resistance when collected in the morning from shaded parts of the canopy (disease score of 1·4), but high susceptibility when collected in the afternoon and exposed to high light intensity in the canopy (disease score of 3·3).

Attached and detached pod inoculations (Trial 5)

Attached and detached pods of the same clones as tested in Trial 4 were inoculated after wounding. The necrotic area that developed on the pod wall after 5 days was significantly larger for detached pods than for attached pods (Table 7). However, the interaction between inoculation methods (attached or detached pods) and clones was not significant ($P = 0{\cdot}26$), suggesting that both methods may produce similar results. This was confirmed by a significant coefficient of rank correlation ($r = 0{\cdot}77$), obtained between the two pod inoculation methods.

Table 6 Susceptibility of leaf discs to *Phytophthora palmivora* from leaves collected in the morning (06·30–09·30 h) and afternoon (13·00–16·00 h) at three levels of light intensity in the canopy of ten cacao clones (Trial 4) compared with field level of phytophthora pod rot (PPR) of the same clones. Leaf discs were prepared and inoculated one day after leaf collection.

		Leaves collected between 06·30 and 09·30 h Light intensity in the canopy				Leaves collected between 13·00 and 16·00 h Light intensity in the canopy			
Clone	Field PPR (%)	Low	Medium	High (no shade)	Mean	Low	Medium	High (no shade)	Mean
332	5·6[a]	1·5 a[b]	1·8 a	1·6 a	1·63 a	1·7 a	1·7 a	2·0 a	1·81 a
735	10	1·6 ab	2·2 abc	2·4 b	2·06 bcd	2·2 bc	2·7 def	3·1 cd	2·67 de
1041	11·5	2·0 bcd	2·0 ab	2·6 bc	2·17 bcde	2·4 c	2·1 abc	2·6 b	2·35 c
1020	13·7	1·4 a	2·2 abc	2·4 b	2·01 bc	2·3 c	2·4 bcde	3·3 d	2·68 de
1514	16·3	1·8 abc	2·3 abc	3·0 cd	2·35 cde	2·6 c	2·8 ef	3·1 cd	2·87 e
330	16·5	1·8 abc	2·0 ab	1·6 a	1·82 ab	1·7 ab	1·9 ab	2·3 ab	1·99 a
1202	17·2	2·1 cd	2·4 bcd	2·7 bcd	2·4 def	2·4 c	2·5 cde	2·6 b	2·51 cd
202	23·4	2·0 bcd	2·4 bcd	3·0 cd	2·46 ef	2·6 c	2·3 bcd	3·2 d	2·68 de
1633	25·7	2·3 d	2·7 d	3·1 d	2·71 fg	2·7 c	2·6 def	3·1 d	2·81 e
IFC5 (Control)	_	3·0 e	2·6 cd	3·0 cd	2·87 g	2·5 c	3·1 f	4·2 e	3·26 f
Mean	15·5	1·95 A	2·2 B	2·5 C	2·25	2·31 A	2·41 A	2·95 B	2·56
CV (%)[c]	_	13	13	12	14·7	12	12	10	8·2

[a]Percentage of rotten pods observed in a clone trial from 1989 to 1994 (Anon, 1998) (see Table 1).
[b]Different letters indicate significant differences within columns (lower case) or rows (upper case) according to the Newman and Keuls test at 5% probability.
[c]Coefficient of variation.

Table 7 Lesion area (mm^2) 5 days after inoculation of wounded attached and detached pods of nine cacao clones inoculated with *Phytophthora palmivora*

		Type of inoculation	
Clone	Field PPR (%)	Attached pods (field)	Detached pod inoculations (laboratory)
332	5·6[a]	76·1 b[b]	91·9 a
735	10	85·8 cd	98·1 ab
1041	11·5	66·9 a	94·5 ab
1020	13·7	80·5 bc	103·1 abc
1514	16·3	76·1 b	103·4 abc
330	16·5	80·5 bc	100·2 ab
1202	17·2	89·9 de	114·3 bc
202	23·4	90·7 de	106·1 abc
1633	25·7	92·1 e	120·2 c
Mean	15·5	82·1 A	103·5 B
CV (%)[c]	_	9·6	18·9

[a]Ppr (%): percentage of rotten pods observed in a clone trial from 1989 to 1994 (Anon, 1998) see Table 1.
[b]Different letters indicate significant differences within columns (lower case) or rows (upper case) according to the Newman and Keuls test at 5% probability applied to square root transformed data.
[c]Coefficient of variation.

Resistance of nine clones as measured by leaf disc and pod inoculation, compared to natural infection in the field

The results of both pod inoculation tests were significantly correlated with natural infection in the field, but the coefficient of correlation was higher for the detached pod test (r = 0·97) than for the attached pod test (r = 0·70). For the leaf disc test (Table 8), the most significant correlation with natural infection in the field was obtained for leaves growing in medium shade collected in the morning (r = 0·83). The correlation of field resistance with average DS scores of the leaves collected in the morning was significant (r = 0·78), but not so for leaves collected in the afternoon (r = 0·50). A similar pattern was observed when comparing the leaf disc results with that of the detached pod inoculation tests, with highest coefficients of correlation being observed for leaves collected in the morning (r = 0·64 to 0·97, Table 8).

Discussion

The results confirm the value of the leaf disc inoculation tests to predict the level of field resistance of cacao clones to phytophthora pod rot. When carried out under standardized conditions, the leaf disc test may explain 60 to 90% of the variation in field resistance to *P. palmivora* (Table 8; Tahi *et al*., 2000; Tahi *et al*., 2006b). However, the predictive value of the test varies with the test conditions. Previously, it was demonstrated that leaf age is important (Tahi *et al*., 2006b), with more reliable results being obtained for leaves that start hardening-off (approximately 50–60 days old). This is the type of leaf used in the current studies. It has now been demonstrated that the time of leaf collection and the degree of exposure of the leaves to light in the canopy also significantly affect the level of resistance, as well as the reliability, of the test. The interactions of these factors with cacao clones were significant, although the *F*-values for the interactions were much lower than those for the clone effects (Table 5). However, this would explain why the coefficients of correlation between the DS scores observed in the leaf disc test and natural infection in the field varied considerably according to the treatments applied. The time of leaf collection seems particularly important; leaves collected in the afternoon provide much less reliable results than leaves collected in the morning (Table 8). Furthermore, leaves collected under medium shade conditions (about 10% of total radiation) seem to provide more reliable results than leaves collected from more shaded parts of the canopy or from parts exposed to direct sunlight.

The underlying mechanisms of the increased susceptibility of leaves collected in the afternoon and of leaves exposed to higher light conditions is not well understood. Similar effects have been observed in other pathosystems,

Table 8 Coefficients of rank correlation between disease severity of leaf discs of nine cacao clones, from leaves collected in the morning and afternoon from different light intensities in the canopy, and on attached and detached pods inoculated with *Phytophthora palmivora* compared to phytophthora pod rot (PPR) from natural infection

		Light intensity of leaves collected in the canopy						
Time of leaf collection	Pod test	Low	Medium	High	Low + Medium	Low + High	Medium + High	Low + Medium + High
Leaves collected between 06·30 and 09·30 h	Field PPR (%)[a]	0·76*[b]	0·83*	0·76*	0·84**	0·77*	0·78*	0·78*
	Attached pods	0·53 ns	0·70*	0·42 ns	0·58 ns	0·54 ns	0·56 ns	0·58 ns
	Detached pods	0·64 ns	0·97***	0·83**	0·84**	0·82**	0·86**	0·83**
Leaves collected between 13·00 and 16·00 h	Field PPR (%)[a]	0·73*	0·25 ns	0·40 ns	0·54 ns	0·61 ns	0·17 ns	0·50 ns
	Attached pods	0·36 ns	0·23 ns	0·28 ns	0·39 ns	0·48 ns	0·16 ns	0·28 ns
	Detached pods	0·82*	0·53 ns	0·57 ns	0·74*	0·70*	0·43 ns	0·68*

[a]Percentage of rotten pods observed in a clone trial from 1989 to 1994 (Anon, 1998).
[b]Significance of coefficients of correlation: ns = non significant; *significant at 5% ; **1% ; ***0·1% probability.

e.g. for resistance to coffee leaf rust, also evaluated by leaf disc inoculations (Eskes, 1989). The effects of time of leaf collection and light intensity might be related, as leaves collected in the afternoon have been exposed to more light, at least during the day of leaf collection. Possibly, exposure of leaves to higher light intensity or to longer periods of light during the day, increases the amount of assimilates inside the tissue, turning it into a more suitable substrate for growth of the pathogen. The results further suggest that more favourable conditions for pathogen growth would decrease the reliability with which genetic differences in resistance are estimated between clones. The same was the case when comparing resistance of very young leaves, which are more susceptible, with older more resistant leaves (Tahi *et al*., 2006b). There may also be a parallel with results reported by Blaha & Lotodé (1977), who indicated that genetic differences in PPR resistance were much smaller at a site that is more favourable for the development of the pathogen in Cameroon (Nkolbisson) than at a site that is less favourable for the pathogen (Nkoemvone). Therefore, conditions highly conducive to pathogen growth appear to reduce the ability to discriminate between susceptible and resistant clones in resistance tests.

Inoculation of detached pods resulted in higher susceptibility than inoculation of attached pods. This confirms earlier results with pod inoculations (e.g. Tarjot, 1969; Iwaro *et al*., 1997b). Although the rank correlation of the results between these tests was significant, the detached pod inoculations provided a better correlation with field results than attached pod inoculations. This might be due to the more uniform environment to which detached pods are exposed compared to attached pods. Correlations between results obtained with leaf disc and pod inoculations varied according to the test conditions. The highest correlations were observed for leaf discs collected in the morning with the detached pod test (Table 8). This was expected, as these are the same conditions that provide best correlations with field results for both tests. Iwaro *et al*. (2005) have recently demonstrated the predictive value of a detached pod test using spray inoculation. The latter test has the advantage that apparently fewer pods are required than for wound inoculations in order to obtain significant differences between clones.

Both leaf and pod inoculation tests are presently being used as routine methods to screen for resistance to phytophthora pod rot. The current results show that standardization of the conditions under which these tests are carried out is essential to obtain more reliable results. With regard to the leaf disc test, besides the reported effect of leaf age (see Tahi *et al*, 2006b), the degree of exposure to sunlight and the time of day the leaves are harvested and the leaf discs are prepared are important factors to be considered. As a standard method, it is recommended that 50–60-day-old leaves growing under medium shade conditions are collected in the early morning (07·00 to 09·00 hours) and that leaf discs are prepared the same day for inoculation at the end of the afternoon. However, when large experiments need to be undertaken, with a more extended time needed for leaf collecting, the leaves should be kept overnight in the dark at 100% humidity with leaf discs being prepared and inoculated the following day. When these conditions cannot be fulfilled (for example, no leaves of the best age are available for all clones), the use of more replications in time will be needed to obtain reliable results (Tahi *et al*., 2006b).

Acknowledgements

This research was partially financed by CAOBISCO (Brussels-based Association of the Chocolate, Biscuit and Confectionery Industries of the European Union).

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