European Journal of Plant Pathology **106:** 87–94, 2000.

Rapid screening of cacao genotypes for field resistance to *Phytophthora palmivora* using leaves, twigs and roots

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Accepted 12 November 1999

Key words: black pod disease, breeding, correlations, early screening, *Theobroma cacao*

Abstract

Black pod, caused by *Phytophthora* spp. is one of the most important diseases of cacao occurring worldwide. Losses due to black pod caused by *P. palmivora* are still moderate in Côte d'Ivoire but *P. megakarya* causes high losses in Ghana and other Central African countries. Variation in field attack has been observed between cacao genotypes, but evaluation of pod losses is unsuitable for obtaining rapid progress in breeding. Results of inoculation tests using young detached leaves, twigs and roots, obtained from field and nursery plants, are presented here and compared to field resistance of similar genotypes observed over a 10-year period. Nine different Upper Amazon Forastero genotypes were tested together with progenies obtained by crossing these with the susceptible check IFC5 (Amelonado genotype). Rank correlations between the early screening tests and the level of field attack were positive and mostly significant ($r = 0.58$–0.95). The coefficient of correlation was slightly higher for leaves ($r = 0.88$) and roots ($r = 0.89$) than for twigs ($r = 0.76$). Also, resistance of the different plant organs was correlated ($r = 0.6$–0.9). Resistance of the Upper Amazon parents was well correlated with the resistance of their cross progenies ($r = 0.7$–0.9), suggesting that resistance is highly heritable. Resistance of leaves and twigs from the nursery was better correlated with field resistance than resistance of leaves and twigs from the field, which might result from more uniform growing conditions in the nursery. Inoculation of leaves appears the most suitable early screening method for black pod resistance. Application of this test in breeding more resistant cacao cultivars is discussed.

Introduction

Cacao (*Theobroma cacao*) represents important export revenues for producing countries. Africa is responsible for about 65% of world production of dry cocoa (2.6 million tons in 1996), Côte d'Ivoire being the largest producer with about one million tons (ICCO, 1996).

Cacao black pod disease can be caused by five different *Phytophthora* species. It induces crop losses estimated as 30% of the world production (Wood and Lass, 1985). *P. palmivora* is present worldwide and is so far the only one identified in Côte d'Ivoire where it causes losses of 10–25% (Kebe, 1993). A second species, *P. megakarya*, is present in most other African cacao producing areas with losses estimated to be more than 50% (Blaha and Lotodé, 1976; 1977). This species is advancing in Ghana (Lutherbacher and Akrofi, 1993) and has recently reached the border with Côte d'Ivoire. Chemical control of black pod is possible, but not always efficient, and is expensive, considering the low yields (400 kg/ha).

Significant genetic variation for black pod attack of mature fruits (pods) has been observed in cacao breeding trials in different countries (Wood and Lass, 1985; Paulin et al., 1994). Pathologists have, however, argued that precise observation of field resistance should include records of infected young fruits (cherelles) as well as pods. In a few countries, weekly observations

of infected cherelles and pods during main infectious periods were carried out, but no correlations between the two measures of field resistance were reported. Inheritance of field resistance, observed by both methods, is predominantly governed by additive gene action (Despreaux et al., 1989; Paulin et al., 1994; Cilas et al., in press). A correlation between field resistance of a restricted number of parental genotypes and their offspring has been reported in Côte d'Ivoire and Cameroon (Sounigo et al., 1993; Nyassé, pers. comm.).

Evaluation of resistance of individual cacao genotypes has involved mainly inoculation of attached or detached pods (Tarjot, 1972; 1973; Blaha, 1974; Blaha and Lotodé, 1976; Partiot, 1976; Lawrence, 1978; Babacauh, 1980; Philips-Mora and Galindo, 1989). Pod inoculations are not efficient for breeders, who require early screening tests. Therefore, inoculation of leaves, twigs and roots has been tried (Asomaning, 1964; Blaha, 1974; Lawrence, 1978; Muller, 1987; Tondje et al., 1987). Although a certain relationship between different test methods has been noted (Muller, 1987), results have been inconclusive with regard to establishment of correlations between these tests and long-term field data. Recently, resistance of detached leaves or leaf discs was shown to be related to pod resistance (Nyassé et al., 1995; Iwaro et al., 1997).

Few studies on interaction between host and pathogen genotypes have been carried out. Ranking of root resistance of cacao genotypes to different *P. palmivora* isolates in Côte d'Ivoire appeared to be correlated (Partiot, 1976), suggesting that use of one isolate may be sufficient to evaluate resistance to this species. Recently, indications have been obtained that resistance to *P. palmivora* and *P. megakarya*, as observed by leaf inoculations and in the field, may be correlated (Nyassé et al., 1995; Cilas et al., in press).

The objective of the present study was to evaluate resistance on different plant organs that can be used for early screening of black pod resistance, and relate the results to available long-term field data in Côte d'Ivoire.

Materials and methods

Plant material and field resistance evaluations

The cacao genotypes were either parental genotypes (clones) or seedling progenies (crosses between clones, hereafter called 'cross progenies' or 'progenies') grown at the experimental station of the Centre National de Recherche Agronomique (CNRA) at Bingerville, Côte d'Ivoire. Resistance of nine Upper Amazon (UA) Forastero clones and one Amelonado control clone (IFC5) were compared to resistance of progenies grown in the field (crosses between the nine UA clones and four Amelonado clones) or in the nursery (crosses between the nine UA clones and IFC5). The field progenies were part of a variety trial, planted in 1979 at Bingerville, made up of a crossing scheme using 16 UA genotypes as female parents and 4 locally selected and genetically related Amelonado genotypes as male parents (IFC1, 2, 5 and 15). The trial was planted with complete randomisation of single tree plots with 15–20 trees for each cross progeny. The genetic analysis of the Bingerville experiment indicated a large effect of the female parents on percentage of rotten ripe fruits (pods), a small effect of the male parents and absence of interaction between male and female parents (Paulin et al., 1994). The data from the Bingerville field trial over a 10-year period (1982–1987 and 1990–1994) were used to estimate the general combining ability (gca) for field resistance of the female UA genotypes. For the nine UA genotypes used in this study, the gca values varied from 11% to 28% pod losses (Cilas et al., in press) and are indicated as '%Rot, 10y' in Table 1. Field resistance was also estimated in the same Bingerville trial by observing weekly incidence of infected cherelles and pods on all trees during the entire epidemic period in 1990 (Kebe, 1990). The gca values for the percentage of infected cherelles and pods varied between 10% and 25% and is indicated as '%Rot, w90' in Table 1 (no statistical analysis available).

The resistance tests were carried out in 1995. Plant organs were collected from four different trees growing in the field of each of nine cross progenies (crosses of the UA genotypes with IFC5) and of the 10 parental clones. These field trees were multiplied as rooted cuttings for the tests carried out on nursery materials. IFC5 was used as susceptible control clone in all tests. Organs obtained from the same four field trees of the genotypes were used in all experiments, except for twig inoculations carried out on nursery progenies for which 36 seedlings derived from hand pollinations (UA × IFC5) were used. Plant organs were harvested between 6.30 and 9.30 am one day before inoculation (leaf test) or on the day of inoculation (twigs).

Fungal material

One isolate (M3-7.2) of *P. palmivora*, obtained in 1995 from a naturally infected pod from the Bingerville

station, was used in all inoculation studies. This isolate was multiplied each 4–6 weeks by transfer of mycelial discs, taken from the border of fungal colonies, on pea agar in tubes placed at 26 °C in the dark (Huguenin and Boccas, 1971). Aggressiveness on cacao was ensured by inoculation with mycelial discs of detached sterilised green fruits, incubated at 100% relative humidity under laboratory conditions, between each sub-culture of the isolate on pea agar. Inoculum was obtained by sub-culturing the isolate on pea agar in Roux culture flasks of $15 \times 25 \times 7$ cm size laid down horizontally, or in Petri dishes. The type of inoculum varied according to the plant organs tested: suspensions of zoospores for leaves, mycelial discs for twigs and ground mycelia, zoosporangia and zoospores for roots. Zoospore suspensions were obtained after incubation of culture flasks for six days in darkness followed by 10 days with alternating 12 h darkness and fluorescent light. To obtain zoospore release, the cultures were flooded with 40 ml distilled water at 4 °C and incubated at least for 40 min under incandescent light of 60 W. For twig inoculations, mycelial discs of 3 mm diameter were obtained with a cork borer from 5-day-old cultures grown in Petri dishes in the dark at 26 °C. The cultures used for root inoculation were grown on liquid medium (20 g maltose in 1 l of pea juice) for 14 days in the dark at room temperature followed by exposure for seven days to continuous fluorescent light (Partiot, 1976). The liquid cultures were ground for 2 min in a mixer and diluted five times for inoculation.

Inoculation techniques

Fully expanded leaves were taken from twigs that were changing from green to brown colour, corresponding to about two-month-old tissue. These were placed upside down on a wetted plastic foam layer in 60 × 70 cm trays and incubated under laboratory conditions for 24 h in darkness at 100% relative humidity (Nyassé et al., 1995). The leaf petioles were covered with wet cotton wool to avoid desiccation. One leaf from each of the four trees per genotype (clone or cross progeny present in the field or nursery) was used and these were placed in different trays. Each tray contained 10 leaves and was covered with black plastic sheets. The following day, inoculation was carried out by placing 50 droplets of a 10 µl suspension of 3×10^5 zoospores per ml onto the lower surface of each leaf. After inoculation, the trays were covered again with the black plastic sheet and incubated under laboratory conditions at 27 ± 4 °C.

For twig inoculations, the top 18 cm of stems (nursery plants) or secondary branches (field plants) of similar diameter and physiological age were used, i.e., twigs showing a change of the green surface colour into brown. A needle was used to make a small wound at 9 cm from the apex and a mycelial disc was placed on each wound (Despréaux, 1988). For each genotype and for each test, in total 36 twigs coming from four different trees were inoculated and then distributed randomly over three culture flasks (replicates). Each flask contained 100 ml water agar with 5 mg of antibiotic (tifomycin) and 5 mg benomyl at the bottom. The twigs were planted with the cut bottom end in the water agar. Flasks were sealed and placed vertically into incubators in the dark at laboratory temperature (26–28 °C).

For each root inoculation test, 42 rooted cuttings obtained from four field plants of each cross progeny or clone were used. Cuttings of 10 cm long were rooted in the nursery in pots containing sterilised composted sawdust and inoculated six weeks later. The pots were distributed over six rooting compartments (replicates). Inoculation was done in the nursery by pouring 50 ml of inoculum suspension around the rooted cutting in the pot. The rooting compartments were closed with transparent plastic film.

Resistance evaluations on infected tissues

For the leaf test, disease severity was scored on the seventh day after inoculation on a 0–5-point scale developed by Blaha (Nyassé et al., 1995), with 0 indicating absence of symptoms and 1–5 increasing size of infected area and increasing intensity of necrosis (from 4 to 5). For the twigs, the length in mm of the necrosis below the cortex was measured nine days after inoculation. Root resistance was evaluated 16 days after inoculation and expressed as the percentage of primary roots showing infected steles (brown or translucent), observed after removal of the cortex.

Statistical analyses

The statistical design of all inoculation tests included two sources of variance: genotypes and replicates (see above). Analyses of variance were done with the SAS statistical package. Normality of the residuals and homogeneity of variances was verified before carrying out the analyses. Means were compared with the Bonferroni test at 5% probability. Coefficients of rank

correlation were calculated according to the Spearman method.

Results

Level of resistance

The general combining ability (gca) for field resistance of nine UA genotypes is shown in Table 1 as well as the results of the inoculation tests using leaves, twigs and roots of the UA clones (C), of the cross progenies (P) of these genotypes with IFC5 and of IFC5 (control clone). The results for field resistance, evaluated by two methods [infected mature pods over 10 years (%Rot, 10y) and infected cherelles and pods during 1990 (%Rot, w90)], indicated lower gca values for three parental genotypes (PA150, SCA6 and P7) than for the other genotypes tested. Pod losses of the most resistant genotypes were 60–80% lower than for the most susceptible ones.

The results of the different inoculation tests also show significant differences among parental clones and cross progenies, except for the leaf test applied to the field progenies (Pf, Table 1). The coefficients of variation (CV) tend to be lower for the twig inoculations than for the root and leaf tests. For the leaf and root tests the CV are higher for the cross progenies than for the parental clones, which could be due to the genetic variation present in the cross progenies. The Amelonado control clone appears to be as susceptible as the most susceptible UA clones.

Correlations between resistance tests

Because of the different scales used for resistance evaluations, only coefficients of rank correlation are considered here. The ranking of the genotypes for each test is given in Table 2. Average ranking scores for all tests applied to the same type of plant organ are also indicated. The results of the two methods for field resistance evaluation (Cilas et al., in press; Kebe, 1990) are significantly correlated ($r = 0.80^{*}$). The coefficients of correlation among different inoculation tests carried out on the same plant organs are all significant ($r = 0.73$–0.93). Resistance of parental genotypes is always significantly correlated with resistance of progenies tested on the same plant organ (Table 3).

The coefficients of correlation between inoculation tests using different plant organs vary from 0.46 to 0.95. Seventeen out of 21 correlations are significant at the 5% probability level (Table 3). The highest correlation is between the leaf and root tests applied to parental clones and the lowest correlation is between the twig and root tests applied to cross progenies. Cross progenies tested as leaves or twigs from the nursery (Pn) are

Table 1. Field resistance of cacao genotypes [clones (C) and cross progenies (P)] to *P. palmivora* compared with resistance observed after innoculation of leaves, twigs and roots obtained from field (f) or nursery (n) plants

Parental genotype	Field resistance[1]		Leaves (0–5 point scale)			Twigs (lesion length in cm)			Roots (% infected)	
	%Rot, 10y	%Rot, w90	Cf	Pf	Pn	Cf	Pf	Pn	Cn	Pn
PA150	11.1 a	9.7	1.7 abc	1.4 a	0.9 ab	46.0 ab	38.9 a	40.0 ab	24.8 a	25.7 abc
SCA6	11.5 a	5.5	1.3 ab	0.7 a	0.8 ab	33.7 a	33.1 a	33.3 a	29.8 a	25.0 abc
P7	13.7 a	6.8	1.1 a	1.2 a	0.7 a	41.9 a	39.0 a	37.4 a	25.0 a	11.8 a
T85/799	18.8 b	18.7	2.1 bc	2.2 a	1.1 ab	51.4 ab	42.1 a	41.1 ab	50.5 bc	41.7 bcd
T60/887	19.3 bc	10.8	1.8 abc	2.1 a	0.9 ab	45.0 ab	46.6 a	48.3 ab	32.7 ab	23.7 ab
NA32	24.5 bcd	23.0	2.2 cd	2.2 a	1.2 ab	63.2 abc	44.9 a	48.9 ab	60.2 cde	50.4 cde
IMC67	25.1 bcd	16.7	1.7 abc	2.0 a	1.1 ab	38.4 a	37.3 a	41.4 ab	52.7 cd	33.6 abcd
NA79	26.7 cd	24.5	3.2 e	2.5 a	2.3 bc	78.6 bcd	73.3 b	56.7 bc	68.7 de	52.6 de
IMC78	28.7 d	19.9	3.3 e	1.7 a	1.2 ab	93.0 cd	44.3 a	46.1 ab	68.3 cde	37.8 bcd
Control: IFC5	—	—	2.7 de	2.6 a	2.8 c	109.0 d	115.1 c	67.8 c	77.9 e	72.8 e
Coefficient of variation (%)	—	—	15	52	45	18	9	12	19	36
P[2]	—	—	***	n.s.	***	***	***	***	***	***

[1]General combining ability for percentage fruit rot evaluated at harvest over a 10-year period (%Rot, 10y), according to Cilas et al. (in press), and by weekly counts of infected cherelles and pods in 1990 (%Rot, w90), according to Kebe (1990), in crosses with four Amelonado genotypes (15–20 trees per cross).

[2]P = probability of F-test: $^{*} = 0.05$, $^{**} = 0.001$, and n.s. = non-significant.

Table 2. Ranking of field resistance of cacao genotypes to *P. palmivora* compared to resistance observed in laboratory/nursery tests. For explanation of abbreviations see Table 1

Parental Clone	Field resistance		Resistance tests										
			Leaves				Twigs				Roots		
	%Rot, 10y	%Rot, w90	Cf	Pf	Pn	$\bar{x}$	Cf	Pf	Pn	$\bar{x}$	Cn	Pn	$\bar{x}$
PA150	1	3	3	3	3	3	5	3	3	4	1	4	2
SCA6	2	1	2	1	2	2	1	1	1	1	3	3	3
P7	3	2	1	2	1	1	3	4	2	3	2	1	1
T85/799	4	6	6	7	5	6	6	5	4	5	5	7	6
T60/887	5	4	5	6	4	5	4	8	7	6	4	2	4
NA32	6	8	7	8	7	7	7	7	8	7	7	8	7
IMC67	7	5	4	5	6	4	2	2	5	2	6	5	5
NA79	8	9	8	9	9	9	8	9	9	9	9	9	9
IMC78	9	7	9	4	8	8	9	6	6	8	8	6	8

Table 3. Coefficients of rank correlation (r) between laboratory/nursery resistance tests of cacao clones (C) or cross progenies (P) using leaves, twigs or roots collected from field (f) or nursery (n) plants for inoculation with *P. palmivora*

	Leaf			Twig			Root
	Cf	Pf	Pn	Cf	Pf	Pn	Cn
Leaf							
Pf	0.73*,[1]						
Pn	0.93**	0.76*					
Twig							
Cf	0.88**	0.61 n.s.	0.76*				
Pf	0.71*	0.78*	0.61 n.s.	0.91**			
Pn	0.93**	0.85**	0.83**	0.73*	0.75*		
Root							
Cn	0.88**	0.71*	0.95**	0.66*	0.60 n.s.	0.78*	
Pn	0.80*	0.78*	0.86**	0.71*	0.46 n.s.	0.70*	0.80*

[1] p = probability of r values: * = 0.05, ** = 0.01, *** = 0.001, n.s. = non-significant.

generally better correlated with other tests than when these organs were obtained from the field (Pf).

The coefficients of correlation between the average ranking for resistance on different plant organs (see Table 2) are less significant for roots × twigs ($r = 0.78^{*}$) than for roots × leaves ($r = 0.96^{**}$) and for leaves × twigs ($r = 0.90^{**}$).

Correlation between resistance tests and field resistance

Coefficients of correlation between resistance tests and the weekly observations carried out in the field in 1990 (%Rot, w90) are all significant at the 1% probability level, with r values varying from 0.73 to 0.95 (Table 4). Correlations between individual resistance tests and the 10-year field observations (% Rot, 10y) were also positive ($r = 0.56$–0.93), but four out of eight were not significant at the 5% probability level. Correlations were generally lower for the twig inoculations compared to the leaf and root inoculations. This is also confirmed by the coefficients of correlation between the two types of field results and the average ranking scores of resistance tests carried out on different plant organs: 0.85^{**} and 0.66 n.s. for the twig test, 0.95^{**} and 0.80^{*} for the leaf test, and 0.93^{**} and 0.85^{**} for the root test, respectively. Correlations between screening tests and field results were generally higher (Table 4) with leaves and twigs taken from nursery progenies (Pn) than from field progenies (Pf).

Table 4. Coefficients of rank correlation (r) between field resistance of cacao genotypes to *P. palmivora* and resistance tests of clones (C) or cross progenies (P) inoculated by using leaves, twigs or roots obtained from field (f) or nursery (n) plants

Field resistance	Laboratory/nursery tests							
	Leaf			Twig			Root	
	Cf	Pf	Pn	Cf	Pf	Pn	Cn	Pn
%Rot, 10y	0.81*,1	0.58 n.s.	0.88**	0.60 n.s.	0.56 n.s.	0.71*	0.93**	0.58 n.s.
%Rot, w90	0.91**	0.88**	0.95**	0.83**	0.73**	0.90**	0.90**	0.90**

[1]Probability of r values: * = 0.05, ** = 0.01, *** = 0.001, n.s. = non-significant.

Discussion

Variation for resistance between cacao genotypes

Cross progenies of three UA genotypes (PA150, SCA6 and P7) showed significantly higher average long-term field resistance to *P. palmivora* than the other six UA genotypes evaluated. The same three genotypes also showed the highest average ranking for resistance in the artificial inoculation tests, which revealed further high susceptibility of the Amelonado control genotype IFC5. Resistance of PA150, SCA6 and P7 to pod inoculations with *Phytophthora* spp. has been reported earlier in other countries (Luz et al., 1989; Phillips-Mora and Galindo, 1989). Cilas et al. (in press) observed similar ranking of several cacao genotypes for field resistance to *P. palmivora* in Côte d'Ivoire and *P. megakarya* in Cameroon and Togo, suggesting that resistance to black pod in one country might be effective in other countries and to different *Phytophthora* species. However, more studies on host–pathogen interaction will be needed to verify this conclusion for a larger number of genotypes.

Resistance of the UA parental genotypes, as observed in the screening tests, was well correlated with resistance of the offspring (crosses with susceptible Amelonado clones). This suggests that resistance to black pod, if correctly evaluated, can be highly heritable and that field resistance to *P. palmivora* of hybrid progenies can be efficiently predicted from the performance of the parental genotypes in early screening tests.

Number of plants used per clone or cross progeny

In the screening tests, four plants or trees were used to estimate resistance of the cacao genotypes (clones or cross progenies). This is a relatively low number, especially for the cross progenies which may be segregating for resistance. It may explain why differences between genotypes for the leaf inoculation test were less significant for the cross progenies than for the clones (Table 1). Nevertheless, significant coefficients of correlation were obtained between parents and offspring with this screening test (Table 3) indicating that the ranking for resistance was correct. In future research, it is recommended to use at least 15 plants to assess differences in resistance between and within cross progenies.

Correlation studies

The two methods for evaluation of field resistance (Table 1, Kebe, 1990; Cilas et al., in press) were significantly correlated ($r = 0.8$), indicating that weekly observations on cherelles and pods during one infectious period can give similar results to long-term losses of mature fruits due to black pod. However, the weekly observations were generally better correlated with the early screening tests than the overall infection level of mature pods. Therefore, observations on cherelles and pods during the main infectious period might estimate the intrinsic resistance level more correctly than observations on mature pods at harvest time. The differences may be due to mechanisms of escape (genotypes producing more pods in the dry season) or to other phenomena involved in field resistance at the mature pod level, possibly maturation time of pods affecting risk of infection (Berry and Cilas, 1994).

Resistance of the different plant organs tested (leaves, twigs and roots) were largely related, which confirms earlier results (Muller, 1987). Correlations between the leaf and root inoculations were always significant (Table 3), suggesting that similar resistance mechanisms may be involved. However, resistance of twigs was less well related to that of roots

and of leaves, and also to field resistance. The difference appears to be largely due to the IMC67 genotype, which shows higher resistance of the twigs than of leaves or roots (Tables 1 and 2). This could be explained by the already known high resistance of this genotype to stem infections (Okey et al., 1993). Twig inoculations may therefore be less reliable for predicting pod losses in the field due to *Phytophthora* than leaf or root inoculations. On the other hand, twig inoculations might be better related to resistance to bark canker, also caused by *P. palmivora*, which is an important cacao disease in other countries, such as in Papua New Guinea.

Correlations with the leaf and twig tests applied to cross progenies were generally lower when the plant organs were taken from field plants than from nursery plants. This holds for comparisons among resistance tests as well as for the correlation between resistance tests and field observations (Pf and Pn in Tables 3 and 4). This may reflect more uniform growing conditions in the nursery compared to the field. The possible effect of environment on the reliability of the screening tests would deserve further studies.

Conclusions for resistance breeding

The most reliable early screening test results were obtained with the leaf and root inoculations carried out on nursery materials. The root test is destructive and more laborious, therefore the leaf test is the most suitable for early screening. This test also allows replication in time, which will be needed to reliably evaluate resistance of individual nursery plants. The use of leaf discs, as proposed by Nyassé et al. (1995), rather than entire leaves would permit evaluation of a larger number of leaves of many genotypes in one test and may give a better estimate of resistance of these genotypes.

The present results show that field resistance of cacao genotypes to black pod in Côte d'Ivoire can be predicted using early screening tests, which can accelerate the selection process. The significant relationship between resistance of parental clones and of their off-spring would allow identification of suitable parents for pre-breeding, aiming at accumulation of resistance genes in cacao populations, or for practical breeding, aiming at combining resistance with other agronomic or quality traits in new cacao cultivars.

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