

Screening of cocoa seedlings for resistance to *Phytophthora palmivora*

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SUMMARY

A new method for screening cocoa seedlings for resistance to black pod caused by the fungus *Phytophthora palmivora* (Butl.) Butl. is described in which pregerminated seeds are inoculated with a culture of the fungus. Seedling mortality at 8 weeks appears to be related to the susceptibility of the parents to black pod in the field. Unlike previous methods for testing resistance, the new method may make possible the screening of large quantities of seedling progenies from clones with known field resistance, leaving relatively resistant seedlings for field planting. The method offers prospects for studying the genetics of black pod resistance.

INTRODUCTION

Differential susceptibility of cocoa varieties to black pod has been reported in almost all the cocoa growing areas of the world (TURNER, 1961; ASOMANING and WHARTON, 1962; ASOMANING, 1964; ROCHA, 1965; WESTSTELJN, 1966). That these differences in susceptibility are heritable has also been demonstrated (SPENCE and BARTLEY, 1966; TARJOT, 1967).

The most popular method of control of black pod has been by chemical protectants. This method has proved difficult because of the rather heavy rains in cocoa growing areas and the high cost of the chemicals (DADE, 1927; THOROLD, 1959; BLENCOWE and WHARTON, 1961). Breeding for resistance appears to be the most practicable approach to the solution of the problem of black pod control. While no trees immune to the disease have yet been found, many clones have been reported in all cocoa-growing countries to possess some measure of field resistance to the disease. These have been summarized by ROCHA (1965).

Tests of first generation progenies of some resistant clones selected at Tafo and planted in a field most favourable for black pod infection (WHARTON, 1960) showed that no immune lines segregated, but varying levels of susceptibility ranging from very high to low were present (AMPONSAH and DARWA, 1967). Wide variations in pod susceptibility within crosses have also been noted at Tafo from accumulated individual tree yield records (AMPONSAH, 1969). The planting of segregants with very low susceptibility in pure stands

could reduce disease incidence to negligible proportions. In the circumstances, it would appear that the most rapid means of obtaining adequately resistant material from existing sources lies in devising a quick method for screening large quantities of seedling progenies to eliminate susceptible ones at the seedling stage, leaving only the resistant ones to go to the field.

This line of approach has been studied for some time (AMPONSAH and ASARE-NYAKO, 1972), and the present paper reports a new method evolved for screening seedlings for resistance to *Phytophthora palmivora*.

MATERIALS AND METHODS

Seedlings of the following parentage : K5 × Y44, K5 × Sca6, K5 × Lafi 7, Na32 × A12, U6 × T85/799, K5 × Pa7 and K5 × T79/501 were used in this work. The features of the clones involved in these crosses are summarized below :

Clone	Origin	Field reaction to black pod
K5	Local selection from the Kumasi area	? Resistant
Y44	Local selection from the Anyinam area	Resistant
Sca6	Pound's collection from the Upper Amazon	Resistant
Lafi 7	Selection from Samoa	? Resistant
Na32	Pound's collection from the Upper Amazon	Susceptible
A12	Local selection from the Aburi area	Susceptible
U6	Local collection from the Suhum area	? Resistant
T85/799	Trinidad Introduction. A progeny from IMC60 × Na34 all of Pound's collection from the Upper Amazon	? Resistant
T79/501	Trinidad Introduction. A progeny of Na32 × Pa7 both from Pound's collection from Upper Amazon	Resistant
Pa7	Trinidad Introduction. Pound's collection from the Upper Amazon	Resistant

The clones described as resistant are those that consistently give low black pod incidence in the field.

Cocoa beans with their testa removed were pregerminated between two sheets of wet blotting paper lining plastic troughs for four days. The troughs were covered with wooden lids and left at room temperature (25 to 30 °C) in the laboratory. At the end of four days, the cotyledons had expanded and the radicles were about 3 cm long, but no plumule had emerged.

Meanwhile, six-day old cultures of *P. palmivora* on oatmeal agar in 9 cm Petri dishes has been raised at room temperature in the dark.

Ten plates of *P. palmivora* cultures thus prepared were comminuted in a Waring blender with 150 ml of distilled water for a minute or two till a slurry was obtained. The slurry was made up with more distilled water to 1/1 to obtain the stock solution. To determine the appropriate concentration for subsequent studies, the following concentrations were tested :

Concentration	How made up
A	1/2 stock solution
B	1/4 "
C	1/16 "
D	1/32 "
E	1/64 "
0	No inoculum (control)

After tests with these dilutions of the stock solution, a concentration of 1/16 the stock solution was selected for this work. Concentrations of 1/4 and 1/8 were found to be too potent while 1/64 was too mild in effect.

Pregerminated seeds were submerged for periods of 1, 3 and 6 minutes to determine the effective periods of exposure to the inoculum prepared as detailed above. There was no significant difference in seedlings emergence when submersion in the inoculum was between 1 and 6 minutes but there were indications of varietal differences. It was thus decided to submerge seedlings for 3 minutes in all subsequent tests.

Twenty pregerminated seeds of each hybrid were submerged for 3 minutes in 120 ml of the inoculum in a beaker. The inoculum was decanted and all 20 seeds planted immediately in a seed box, 60 cm × 30 cm × 10 cm, filled with heat sterilized soil. Watering was done before planting and thereafter once daily. There were a minimum of 75 inoculated seeds (four seed boxes) and 20 uninoculated seeds (one seed box) for each variety tested at any one time.

Twelve days after inoculation, emerged seedlings were counted. Eight weeks later, the surviving seedlings were individually examined and finally graded 'healthy' (fit to be raised for field planting) or 'reject' (unfit for field planting). The 'healthy' seedlings were transplanted to polythene potting bags singly and kept in the nursery till the planting season. Surviving

seedlings that had severe root and stem infections were discarded. Such plants were usually stunted in growth.

In some few cases, seedlings that had underground infection, but suffered no apparent set-back in growth or general physiological condition escaped rejection at eight weeks but died after transplantation into potting bags.

RESULTS

Emergence (that stage when elongation of the hypocotyl had lifted the cotyledon clear of the soil) started about five days after planting and was complete by the twelfth day in the case of the uninoculated seeds. Of the inoculated seeds, some did not emerge (pre-emergence death); others emerged but subsequently wilted and collapsed (early and late post-emergence deaths). Emergence of some inoculated seeds was delayed for about two to three weeks. Both pre and post-emergence deaths occurred in progeny of 'resistant' and 'susceptible' varieties, but were always less numerous among the former than the latter.

Table I gives a summary of results obtained from three tests. The rankings of the crosses in all three tests are consistent. K5 × Sca6 had the highest percentage surviving seedlings followed by K5 × Y44, K5 × Lafi 7 and Na32 × A12. The parent clones (Sca6 and Y44) of the two best crosses are known to be highly resistant to pod infections in the field. Na32 and A12 are two clones known to be highly susceptible to the disease at Tafo (WHARTON, 1960 ; GLENDINNING, 1966). The results of these seedling tests are therefore consistent with known field reactions of the parent clones.

About 12 days after inoculation, the emergence rates were higher for crosses between resistant parents than those between susceptible parents. K5 × Y44 had a consistently slightly higher rate of emergence than K5 × Sca6, but the latter cross tended to have a higher percentage of healthy plants at eight weeks.

Table II is a summary of four separate tests of three crosses : U6 × T85/799, K5 × Pa7 and K5 × T79/501. U6 × T85/799 was consistently the most susceptible of the three crosses. The rankings of the more resistant crosses — K5 × Pa7 and K5 × T79/501 — were consistent in three of the four tests. In the totals of the four tests, K5 × Pa7 and K5 × T79/501 were not significantly (5%) different in resistance but both were significantly more resistant (1%) than U6 × T85/799.

DISCUSSION

All the known tests for resistance based on pod inoculation (TURNER, 1961, 1963 ; MADEIROS, 1965 ; PRENDERCAST, 1965 ; SPENCE and BARTLEY, 1966 ; TARJOT, 1967 ; DAKWA, 1968) necessitate waiting until a tree is in bearing before its susceptibility to block pod infection can be determined. This limitation also applies to tests with pod extracts (ORELLANNA,

TABLE I
EMERGENCE AND SURVIVAL RATES OF SEEDLINGS OF FOUR COCOA VARIETIES
INOCULATED WITH *PHYTOPHTHORA PALMIVORA* (BUTL.) BUTL.

Crosses	Known field reaction	Treatment	No. of seedlings tested	% Emergence after 12 days	Surviving seedlings 8 weeks after inoculation	
					No. rejected	% Healthy
K5 × Y44	?R × R	Control	20	100.0	0	10.00
		Inoc.	201	64.2	10	54.7
K5 × Sca6	?R × R	Control	15	100.0	0	100.0
		Inoc.	160	61.9	17	55.6
K5 × Laf7	?R × ?R	Control	20	100.0	0	100.0
		Inoc.	178	59.0	23	48.9
Na32 × A12	S × S	Control	20	100.0	0	100.0
		Inoc.	274	25.5	6	21.9
K5 × Y44	?R × R	Control	20	100.0	0	100.0
		Inoc.	100	76.0	8	56.0
K5 × Sca6	?R × R	Control	20	100.0	0	100.0
		Inoc.	75	65.3	10	56.0
K5 × Laf7	?R × ?R	Control	20	100.0	0	100.0
		Inoc.	96	52.1	3	34.4
Na32 × A12	S × S	Control	20	100.0	0	100.0
		Inoc.	107	41.1	6	26.2
K5 × Y44	?R × R	Control	20	100.0	0	100.0
		Inoc.	180	71.1	12	65.6
K5 × Sca6	?R × R	Control	20	100.0	0	100.0
		Inoc.	130	70.0	15	79.2
K5 × Laf7	?R × R	Control	20	100.0	0	100.0
		Inoc.	170	67.1	5	60.6
Na32 × A12	S × S	Control	20	100.0	0	100.0
		Inoc.	180	50.0	7	34.4

1964; TURNER, 1965). These methods therefore do not offer a quick means of breeding for resistance. The pod tests for resistance and those based on pod extracts are useful for identifying resistant parents, but they require too much time to be widely used in progeny testing.

Inoculated roots of clones were shown by ASOMANING (1964) to exhibit differential susceptibility which conformed with the levels of pod infection in the field. This method was later modified by ZENTMYER (1968). The extent of canker development on stems of seedlings has been used as a measure of resistance (ZENTMYER *et al.*, 1968; ZENTMYER, 1971). The results obtained also agreed with known pod susceptibility in the field. Tests based on stem and on root inoculations are useful in identifying resistant varieties, but involve destruction of the individual seedlings. In Ghana, where large scale propagation by cuttings has not been found practicable, the resistant parents can only be used for further breeding. Individual tree yield records, including yields of progenies from resistant and susceptible clones at Tafo, show wide variations in levels of black pod infection within the seedling progenies (AMPONSAH, 1969), even among these from the same pod. It is apparent, therefore, that in breeding for resistance to black pod, there is the need to screen

the progenies in order to remove the most susceptible plants at the seedling stage. This need is fulfilled by the new method.

Unlike the other tests for resistance, the end result of this test is the planting material which goes to the field. Thus, not only are superior parents and varieties identified but resistant plants are directly obtained for field planting and for further breeding.

This work has eliminated any doubts of some cocoa seedlings surviving *P. palmivora* inoculation at an early stage of germination in Ghana. The fact that clones known to show high field resistance consistently gave higher proportions of resistant FI progeny than highly susceptible clones suggests that the field resistance or susceptibility of those clones are heritable. The Tafo Series V trials (AMPONSAH, 1970) showed that the percentages of black pod incidence for the first seven years of bearing in U6 × T85/799, K5 × Pa7 and K5 × T79/501 were 30%, 18% and 19% respectively. The higher susceptibility of U6 × T85/799 compared with K5 × Pa7 and K5 × T79/501 thus recorded in the field was confirmed in four separate tests using the new laboratory method (Table II). The new laboratory method therefore could provide a reliable and quick means of assessing the progeny of crosses for field resistance to black pod infection.

TABLE II
SURVIVAL RATES OF SEEDLINGS OF THREE COCOA VARIETIES
INOCULATED WITH *PHYTOPHTHORA PALMIVORA*

Date of inoculation	Crosses	Known field reaction	Treatment	No. of seedlings tested	Surviving seedlings 8 weeks after inoculation		
					% Rejected	% Healthy	Ranking
5-11-70	U6 × T85/799	?R × ?R	Control	40	0	100.0	
			Inoc.	290	7.2	33.4	
	K5 × Pa7	?R × R	Control	40	0	100.0	1
			Inoc.	180	6.1	59.4	
19-11-70	U6 × T85/799	?R × ?R	Control	40	0.0	100.0	
			Inoc.	180	9.4	51.7	
	K5 × Pa7	?R × R	Control	40	0.0	100.0	3
			Inoc.	180	7.8	36.7	
14-12-70	U6 × T85/799	?R × ?R	Control	40	0.0	100.0	
			Inoc.	180	7.2	55.0	
	K5 × Pa7	?R × R	Control	40	0.0	100.0	3
			Inoc.	170	4.7	50.5	
19-11-72	U6 × T85/799	?R × ?R	Control	40	0	100.0	
			Inoc.	180	1.9	64.8	
	K5 × Pa7	?R × R	Control	20	0	100.0	3
			Inoc.	100	5.0	37.0	
Grand total	U6 × T85/799	?R × ?R	Control	20	0	100.0	
			Inoc.	100	6.0	56.0	
	K5 × Pa7	?R × R	Control	20	0.0	100.0	2
			Inoc.	100	8.0	65.0	
Grand total	U6 × T85/799	?R × ?R	Control	140	0.0	100.0	
			Inoc.	750	7.7	34.1	
	K5 × Pa7	?R × R	Control	140	0.0	100.0	2
			Inoc.	630	6.0	53.2	
Grand total	K5 × T79/501	?R × R	Control	140	0.0	100.0	
			Inoc.	640	7.0	57.6	

The fact that higher concentrations of inoculum considerably reduced the number of surviving seedlings, and also that higher dilutions increased the survival rate, showed that surviving seedlings at a given concentration are not immune to infection. Seedlings surviving at higher concentrations are probably, on the average, more resistant than those surviving at lower concentrations. The death of some seedlings before emergence and others early or late after emergence as well as the occurrence of surviving seedlings which include both severely infected and little or non-infected plants suggest varying levels of susceptibility. Crosses with high proportions of surviving seedlings correspond to those with high field resistance while crosses with low proportions of surviving seedlings are susceptible in the field. The inference is made that the surviving seedlings are the more resistant of the progenies tested at that concentration. The most susceptible seedlings were those that died before emergence while the most resistant were those that gave no apparent indication of infection. Between the

two extremes were various levels of susceptibility as indicated by the varying periods of death or expressions of symptoms of infection. The existence of varying levels of resistance among seedling progenies from clones with high field resistance has also been observed in the field (AMPONSAH, 1971). This is further evidence of the similarity of the seedlings reaction to artificial inoculation and observed field reaction of older trees.

It would appear that seedlings dying before emergence are more susceptible than those dying after emergence. A higher rate of emergence, however, does not necessarily imply a higher rate of survival. An indication of the degree of resistance can be observed at the emergence stage, but ranking within susceptible or resistant groups at this stage could be misleading. The relative resistances of given crosses are better estimated on the surviving plants eight weeks after inoculation.

It is evident that no variety or clone used in these tests was homozygous for black pod resistance. All the crosses at the inoculum concentrations studied

contained resistant plants (Tables I and II). This was confirmed by AMPONSAH (1971) when he reported that beans in the same pod were heterogeneous for black pod resistance. This observation was true for both resistant and susceptible clones or crosses.

The mean surviving percentage of 26.7 in Table I for the susceptible cross Na32 × A12 was not due to escapes; a fact which is confirmed by the presence of varying levels of infection in 'healthy' plants at the eight week stage and also by the presence of *P. palmivora* inoculum in healthy plants 10 months after inoculation. It must be emphasised that no immune plants have been found and that by increasing the inoculum concentration all plants can be killed.

HOLLIDAY (1955) used a similar method in testing susceptibility of cocoa to witches broom. *Marasmius perniciosus* is obligately parasitic on cocoa. HOLLIDAY thus used basidiospores obtained from *M. perniciosus* grown on the host plant. This method is also similar to that used for testing wheat against stem rust. *Puccinia graminis tritici* (STAKMAN, 1954). In the screening method described, however, comminuted agar cultures of the facultative pathogen — *P. palmivora* — containing three spore types, sporangia, zoospores and chlamydospores, as well as vegetative mycelia were used in the inoculations. The seeds with removed testa were pregerminated as in the case of HOLLIDAY's tests before inoculation.

The results obtained suggest the presence of major genes for resistance to *P. palmivora* in cocoa. A similar situation exists between *P. infestans* a non-obligate parasite and potato, *Solanum* spp. (BLACK *et al.*, 1953). It must be noted that both *P. palmivora* and *P. infestans* have a wide host range (MACFARLANE, 1968). On the whole the results indicate that the heredity behaviour of resistance is determined by and dependent upon a certain balance of genetic factors which appear to be governed by a number of factors whose action is in part complementary and in part cumulative.

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