EVALUATION OF METHODS FOR ASSESSING RESISTANCE OF CACAO *Theobroma cacao* L. CULTIVARS AND HYBRIDS TO *Phytophthora palmivora* (Butler) Butler

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EVALUATION OF METHODS FOR ASSESSING RESISTANCE OF CACAO (THEOBROMA CACAO L.) CULTIVARS AND HYBRIDS TO PHYTOPHTHORA PALMIVORA (BUTLER) BUTLER *

*J. S. Lawrence***

SUMMARY

This study was conducted to select or develop a rapid and easy but reliable standardized inoculation method for preliminary screening of cacao for resistance to *P. palmivora*. Previously published methods and various modifications of some of them were examined using the same cacao cultivars and the same *P. palmivora* isolate throughout. Results from these methods were compared with each other and with data of natural infection in the field.

Screening methods examined were: 1) Inoculation of attached pods using zoospore suspension without wounding or mixed mycelial-sporangial suspension with and without wounding, 2) Inoculation of detached pods using zoospore suspension with and without wounding, 3) Inoculation of blocks of pod pericarp tissue with zoospore suspension, 4) Inoculation with mycelium-agar discs of seedling-stems and branches on the tree, 5) Inoculation of attached leaves with mycelium-agar discs or zoospore suspension, 6) Inoculation of seedling roots with zoospore suspension, 7) Inoculation of germinated seeds with zoospore suspension or mycelial-sporangial suspension.

Methods varied in the degree with which they differentiated between resistant and susceptible cultivar reactions, and in consistency over replicated tests. However, the most resistant cultivars (Pound 7, Scavina 6, Scavina 12 and Catongo) were graded as resistant in most methods. The most reliable screening method for use with fruiting trees was point-inoculation of unwounded attached pods using zoospore suspension and recording percentage infection and lesion diameters. Inoculation of pre-germinated peeled seeds with a drop of zoospore suspension and, following planting, recording percentage emergence of healthy seedlings was the most satisfactory method for screening hybrid progenies.

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INTRODUCTION

On a world-wide basis *Phytophthora* pod rot (or black pod disease), caused by *Phytophthora palmivora* (Butler) Butler, is the most important disease of cacao (*Theobroma cacao* L.). Although there are some cacao diseases which locally may be more severe and cause greater losses, *Phytophthora* pod rot is responsible for more total crop losses than any other disease. It occurs in all cacao-growing countries (Krug & Quartey-Papafio, 1964; Thorold, 1975) and annual losses sustained by the disease have been estimated to represent 20-30% of the world production. However, the extent of the losses varies considerably from one country to another and pod losses of up to 80% or more can be experienced some years in certain areas (Opeke & Gorenz, 1974).

Losses can be reduced by cultural methods, particularly phytosanitation, frequent harvesting, reduction of shade, maintenance of soil fertility and adequate drainage, and by the use of fungicides. However, these practices, especially fungicide applications, are often costly in relation to the quantity of cacao saved and many growers regard them as economically unfeasible, particularly when cacao prices are low. In the long term, the use of resistant cultivars (especially those with stable, race non-specific resistance) is often the most effective and economic method of controlling plant diseases, and in the last 20 yr or so more attention has been focussed upon the selection and development of cacao types with resistance to *P. palmivora*.

Amongst those cacao cultivars in the world whose reaction to *P. palmivora* is known, most are more or less susceptible to *Phytophthora* pod rod and so far no immune cultivars have been found, although a number in different areas have been reported to show good resistance (Rocha, 1965; Soria, 1974). Consequently, there is a continuing need to search for resistant material for use in breeding and improvement programmes and to develop adequate methods for evaluating resistance to aid in this search.

A wide variety of test methods, most of which have been reviewed by Rocha (1965), Spence & Bartley (1966), Thorold (1967), Rocha & Medeiros (1968), Weststeijn (1969) and Blaha (1974), have been used by many workers in different countries to evaluate resistance to *P. palmivora* (Table 1). Thus, tests currently carried out are very diverse with respect to methods and cacao material used, often leading to considerable discrepancies in results, so that it is difficult to make direct comparisons of levels and types of resistance between cultivars from different areas. Therefore, as has frequently been pointed out, the need arises to standardize test methods used by different investigators so that results obtained in one country may have some significance for another (Rocha, 1965; Spence & Bartley, 1966; Medeiros, 1967; Rocha & Medeiros, 1968; Gregory, 1969; Weststeijn, 1969; Blaha, 1974).

The final test of performance of a cultivar or hybrid has to be in the field, but field evaluation is slow and requires large plots and large quantities of seedling progenies. Furthermore, it has been pointed out (Blenclowe, 1962) that absolute reliance should not be placed upon field evaluation as it only distinguishes *useful* resistance for a given area

Table 1. References to types of method used to assess resistance of cacao to *P. palmivora*.

Natural infection in the field (selected references)	Amponsah & Dakwa (1969), Besse (1969) Dakwa (1968), Gunawardena (1966), Soria & Esquivel (1966, 1970,a,b), Thorold (1956), Thrower (1960), Weststeijn (1966a, 1967, 1968), Wharton (1957, 1960, 1961, 1962).
Inoculation of attached pods	1. With zoospore or zoospore-sporangial suspension a) without wounding: Adebayo (1971), Blaha (1967, 1971, 1972), Blaha & Lotodé (1976, 1977), Dakwa (1968), Gorenz (1971), Lellis & Peixoto (1960), Medeiros (1967), Medeiros & Melo (1966), Medeiros & Rocha (1964, 1965), Partiot (1975), Rocha (1966), Rocha & Mariano (1969), Rocha & Medeiros (1968), Rocha & Vello (1971), Sreenivasan (1975), Tarjot (1969a,b), Toxopeus & Gorenz (1970), Wharton (1959, 1960). b) With wounding: Medeiros (1967), Medeiros & Rocha (1965), Partiot (1975). 2. With mycelium a) no wounding: Rocha & Vello (1971). b) wounding: Adebayo (1975), Akinrefon (1971), Medeiros & Machado (1967), Rocha & Machado (1972), Rocha & Vello (1971), Turner (1962).
Inoculation of detached pods	1. Zoospore, sporangial or zoospore-sporangial suspension a) no wounding: Blaha (1967), Gorenz (1971), Hislop & Park (1962), Holliday (1954), Leather (1966), Lellis & Peixoto (1960), Medeiros (1967), Medeiros & Melo (1966), Medeiros & Rocha (1964, 1965), Orellana (1953, 1954a), Prendergast (1965), Prendergast & Spence (1967), Sreenivasan (1975), Tarjot (1967b, 1969a,b), Thorold (1953, 1955), Thrower (1960). b) wounding: Lellis & Peixoto (1960), Medeiros (1967), Prendergast & Spence (1967), Turner (1963). 2. Mycelium or fragments of diseased pods a) no wounding: Medeiros (1967), Prendergast & Spence (1967), Ram & Ram (1973). b) wounding: Prendergast (1965), Prendergast & Spence (1967), Rocha & Mariano (1969), Spence (1961a), Tarjot (1965, 1967a), Turner (1961, 1962, 1963, 1965a).

Table 1. Cont.

Inoculation of pod husk pieces with zoospore suspension	Prendergast (1965), Rocha & Mariano (1969), Rocha & Vello (1971), Spence & Bartley (1966).
P. palmivora development in media incorporating pod-tissue extracts	1. Growth of mycelium in liquid medium containing pod-husk tissue: Orellana (1954b), Prendergast (1965). 2. Growth of mycelium in liquid or solid media with endocarp extract: Turner (1963). 3. Zoospore and sporangial germination in liquid medium with endocarp extract: Turner (1962).
Inoculation of leaves	1. Zoospore or sporangial suspension (no wounding): Adebayo (1975), Hansen (1961), Holliday (1954), Siller & McLaughlin (1950), Tarjot (1972d). 2. Mycelium (no wounding): Galindo & Salazar (1965).
Inoculation of seedling stems	1. Zoospore or zoospore-sporangial suspension: Sreenivasan (1975). 2. Mycelium: Adebayo (1975),Akinrefon (1971), Legg (1970), Mircetich (1965), Zentmyer (1969, 1972), Zentmyer, Mircetich & Mitchell (1968).
Inoculation of seedling or cutting roots	1. Zoospore or sporangial suspension: Asomaning (1964), Asomaning & Wharton (1963), Mircetich (1965), Prendergast (1965), Turner & Asomaning (1963), Weststeijn (1965, 1966b), Zentmyer, Mircetich & Mitchell (1968). 2. Mycelium: Partiot (1975), Prendergast (1965), Ravisé (1970), Zentmyer (1969).
Inoculation of pregerminated seeds with zoospore or mycelial-sporangial suspension	Amponsah & Asare-Nyako (1973), Partiot (1975), Tarjot (1977).

and may not indicate intrinsic resistance. For example, cultivars which are inherently susceptible may exhibit disease escape in a given area (which occurs when fruit of a cultivar do not mature at times of normal peak pod production and therefore escape the full severity of *P.palmivora* attack). Although basically susceptible, these cultivars would be classified as resistant by field evaluation. Also there may be a direct correlation between the severity of black pod and the number of fruit present; as yield increases so does percentage infection (Thorold, 1953; Wharton, 1959; Blencowe & Wharton, 1961; Muller, 1971), an effect which

may be more pronounced with more susceptible cultivars (Esquivel, 1973). In these cases a low yielding cultivar, therefore, may appear to be more resistant in field trials than it really is. Further problems with field trials are that selection of resistance is more difficult or less reliable when disease incidence is low (Blencowe, 1962), and that it is not possible to be sure that all pods on all trees in a given area are subjected to the same intensity of infection (Thorold, 1953). Thorold (1953) maintained that the degree of resistance and differences in reactions of individual trees in the field could be confirmed only with the aid of artificial inoculation. Thus, inoculation tests are useful not only for rapidly screening breeding material and hybrid progenies but also in complementing field evaluations.

The principal object of the study presented here was to develop or select from existing methods reliable and reproducible standard tests for assessing resistance of cacao to *P. palmivora* that can be easily and rapidly carried out. While the primary purpose of the selected tests was for use in identifying in the CATIE cacao collection promising resistant cultivars for production of hybrid seed and to test the hybrid progenies themselves, it is also hoped that this investigation might contribute to the development and acceptance of more reliable screening tests for use in all cacao-growing areas.

MATERIALS AND METHODS

Cacao Cultivars, Fungus Isolate and Inoculum Preparation

To facilitate comparison between the 7 basic types of test method examined the same group of cultivars was used throughout all experiments. All cultivars were clonal material raised from cuttings and under Costa Rican conditions represented a range in response to *P. palmivora* infection from very susceptible (UF 677, Pound 12, UF 29), moderately susceptible (CC 41, UF 296) and intermediate (UF 613) to tolerant (CC 42, Pound 7, Catongo, Scavina 6, Scavina 12) (Table 2). UF 29 and CC 41 exhibit disease escape in the Atlantic Zone of Costa Rica but their inherent susceptibility has been demonstrated (Esquivel, 1973). The Catongo used in these studies had been obtained as cuttings from the original accession at CEPLAC-CEPEC, Itabuna, Brazil.

The same *P. palmivora* isolate was used in all tests. Many single-sporangium isolates were made from cacao pods collected from the major cacao-growing areas of Costa Rica. All conformed to morphological form or morphotype 1 (Waterhouse, 1974; Sansome, Brasier & Griffin, 1975), other morphotypes not being collected. The test isolate selected, of compatibility type A2, showed no loss in pathogenicity or sporulation capacity *in vitro* after 3 yr of weekly transfers on V-8 juice medium, but to maintain infective potential it was passed through pod tissue every 1-2 months. Except for certain seed inoculation tests, the fungus was always cultured on 20% V-8 juice-calcium carbonate agar and inoculum was prepared from 10-day-old cultures grown in 9 cm Petri dishes incubated under normal laboratory conditions with daylight illumination. With cultures less than 10 or more than 14-days-old, zoospore production was lower.

Table 2. Percentage cacao pods naturally infected by *P. palmivora*.

Cultivar	Percentage infected pods
1) Avs for the 6 years 1965-1970 at *La Lola* cacao farm (Esquivel, 1973)	
CC 42	3.4
UF 29	7.2+
CC 41	7.6+
UF 613	11.7
UF 296	13.5
UF 677	24.1
2) Avs for the 2 years 1972-1973 at *La Lola*	
Pound 7	3.6
Scavina 6	5.8
Pound 12	16.8
3) Avs for the 2 years 1972-1973 at CATIE, Turrialba	
Catongo	4.5
Scavina 12	6.0

+ Exhibit disease escape at *La Lola*.

Zoospore suspensions were prepared by flooding plate-cultures with 20 ml of distilled water chilled to about 10°C and placing them in a refrigerator at 5°C for 20 min. After a further 20 min at 25°C in a darkned incubator they were checked for adequate zoospore sporulation and the suspension filtered through Whatmans No. 1 filter paper to remove unwanted sporangia and mycelium. Originally, zoospore concentrations were adjusted to 2 x 10^5 spores/ml by means of a haemocytometer but it was found that if the procedure described was strictly adhered to, values close to 2 x 10^5/ml were consistently obtained and the tedious practice of calibrating with a haemocytometer was obviated. This concentration was chosen arbitrarily and for convenience rather than for any special reason and because it gave satisfactory infection of the test material.

More elaborate methods for producing zoospore suspension (Prendergast, 1965; Leather, 1966; Medeiros, 1967, 1977; Weststeijn, 1969, Blaha, 1971)

were considered unnecessary as the same isolate was used throughout and suspensions adequate for the purposes of this study were obtained by the method described here. For use in the field, zoospore suspensions were prepared in the laboratory immediately prior to departure and carried to the field in a vacuum flask to prevent encystment of the zoospores before inoculation, which might be caused by prolonged exposure of the suspension to elevated temperatures.

In all screening methods, replicate tests were carried out at different times and, as far as possible, in the same locations and under the same environmental conditions.

Inoculation of Detached Pods

Fully developed but unripe 21-25-wk-old pods of the test cultivars free from visible wounds were harvested from the field (whenever possible, only 1 pod from each tree) immediately prior to use and washed in tap water. Pods were randomly placed in 90 x 35 x 20 cm wooden boxes lined and sealed with transparent polyethylene sheeting and containing a reservoir of water. Pods (usually 6 per box) were kept out of contact with the water and each other. Ten replicate pods of each cultivar were point-inoculated without wounding at 2 positions, on top of the pericarp ridges, in the middle of the peduncular and apical hemispheres of the uppermost surface, using a 0.01 ml drop of zoospore suspension. Rings or tubular devices were not used to retain the inoculum droplet. Immediately following inoculation, always carried out after 3 pm, the boxes were sealed and the pods incubated under laboratory conditions. Percentage infection was subsequently recorded and daily measurements were made of lesion diameters. Diameters were assessed by measuring the lesion in two directions at right angles to each other, parallel to the longitudinal and transverse axes of the pod, and calculating the mean of the two values. This method essentially duplicated those reported by Holliday (1954), Orellana (1954a), Prendergast (1965) and Tarjot (1967b, 1969a,b).

In one series of tests, the boxes were permanently sealed for the duration of the experiment, humidity within (as measured by a thermohygrograph) remaining at 100% r.h. throughout. In another series, boxes were opened after 24 h and thereafter opened daily at 7 am and resealed at 4:30 pm. On resealing, the boxes (but not the pods) were atomizer-sprayed with water to raise r.h. to 100% as rapidly as possible. In further tests, boxes were maintained permanently sealed but pods were point-inoculated with zoospore suspension in conjunction with a needle wound to a depth of 1 mm at the inoculation point.

Inoculation of Pod-Tissue Blocks

The pod-tissue block method of Prendergast (1965) was followed exactly. Small blocks of pericap tissue measuring 5 cm^2 were carefully cut without damaging the epidermis from the equatorial region of fully developed but unripe pods which had been surface sterilized with 5% sodium hypochlorite solution. Twenty blocks, 2 from each of 10 pods per cultivar, were inoculated by placing 0.01 ml of zoospore suspension at the centre of the epidermal surface and incubated at 100% r.h. in the same boxes and under the same conditions as described for detached pods.

Blocks were randomly placed in the boxes with 20 blocks per box. After 4 days, the number of blocks in each cultivar that showed infection was recorded.

Inoculation of Attached pods

Fully developed but unripe pods (21-25-wk-old) of the test cultivars were selected in areas uncontaminated by fungicide applications. Replicate pods, never less than 10 per cultivar and as far as possible only 1 or 2 from each tree, were point-inoculated without wounding in 2 places located laterally and opposite each other on the pod, using 0.1 ml of zoospore suspension. The inoculum drop was retained by small cups fashioned from modelling-clay affixed to the pod surface. Cups were placed on top of the pericarp ridges, not in the furrows, to prevent leakage of the inoculum drop from the base of the cup. The pods were enclosed in polyethylene bags, containing 10 ml of distilled water, which were tied with string around the pod peduncle. Punctures were made with a needle approximately 2 cm above the lower-most points of the bags to prevent them from filling up with rain water. All inoculations were conducted after 3 pm, or whenever the weather was cloudy and cool, to avoid harmful effects to the inoculum from heat or direct sunlight. The polyethylene bags were retained on the pods for the duration of the test to protect them from insects and to provide a more uniform microenvironment. The modelling-clay cups, which could be easily dislodged from the pod surface without removal of the bags, were detached 3 days after inoculation to allow daily records to be made. Percentage infection was recorded and daily measurements taken of lesion diameters, calculated in the same way as for detached pods. This method was very similar to those reported by Blaha (1967, 1971), Tarjot (1969b) and Blaha & Lotodé (1976, 1977).

Using the same basic technique, other inoculation methods were examined. In one test, mixed mycelial-sporangial suspension was prepared by scraping sporulating mycelium from *P. palmivora* cultures into 20 ml of distilled water. This suspension was employed in place of zoospore suspension to point-inoculate either without wounding or with a 1 mm-deep wound at the inoculation point. In all other respects the procedure was the same as above. In further tests, point-inoculation was replaced by spraying zoospore suspension applied by a De Vilbiss atomizer to the lower two-thirds of the pod, each pod receiving about 2 ml of suspension. Four days later, the number of lesions which had developed were recorded. This method was based on those reported by Lellis & Peixoto (1960), Medeiros & Rocha (1964, 1965), Medeiros & Melo (1966), Medeiros (1967), Rocha & Medeiros (1968) and Rocha & Mariano (1969).

Cacao is genetically very heterogeneous and one of the problems this poses in artificial inoculation tests is great variability of the cacao material employed, although variability can be reduced to some extent by the use of cloned material. In pod inoculation, therefore, it is important to minimize variability due to other, non-genetic causes, such as by using pods of the same age. However, it was not known what age of pod would be most suitable for inoculating, especially since pods of different ages show varying degrees of susceptibility to *P. palmivora* (Rocha, 1966; Medeiros & Machado, 1967; Tarjot, 1972a; Adebayo, 1975). To determine the optimum age of pods for use in inoculation tests, 10 replicate pods of 6 of the test cultivars, derived from hand pollinations, were

inoculated at precisely 2, 3, 4 and 5 months and when fully mature (slightly more than 6 months for most cultivars at Turrialba) using the point-inoculation zoospore suspension method without wounding described above. The inoculations were carried out from July to November, a period coinciding with the time of highest rainfall and highest *P. palmivora* incidence for the area.

Inoculation of Seedling Stems, Marcot Stems and Tree Branches

The method of Zentmyer *et al.* (1968) and Zentmyer (1969, 1972) was adopted to inoculate seedling stems. Seedlings were obtained from self- and cross-pollinations of the test cultivars (depending on compatibility) and raised in the greenhouse, each seedling grown individually in perforated black polyethylene bags containing methyl bromide-sterilized soil. When 4-months-old, the stems of not less than 10 replicates per cross were inoculated by making small vertical cuts with a scalpel in the cortex and inserting 3 mm-diam discs cut with a sterile cork-borer from the perifery of 10-day-old cultures of *P. palmivora*. The cuts were bound with adhesive tape and the seedlings kept in the greenhouse at ambient temperatures. After 18 days, the bark was removed and the outlines of the developed cankers traced on paper so that the lesion areas could be determined with a planimeter.

Four-month-old marcots of the test cultivars and branches growing on the trees were inoculated in exactly the same way, except that in the second branch-test, measurements were made 25 days after inoculation rather than after 18 days. As far as possible, sections of branches of much the same age were selected by choosing inoculation points where there were the same number of leaf flushes towards the tip, and by selecting branch diameters of 6-8 mm to approximate the width of 4-month-old seedling stems. Only 1 branch was used per tree.

Leaf Inoculations

Leaves attached to trees in the field were inoculated with mycelium or zoospore suspension. Leaves of the same age, approximately 6-wk-old, were selected, being those which had emerged in the flush previous to the one just emerging. Twenty replicate leaves of each cultivar were point-inoculated with 3 mm-diam discs from *P. palmivora* cultures, using 1 disc per leaf. The discs were placed between major veins in the centre of the adaxial surface of the leaf lamina and the leaf enclosed in a transparent perforated polyethylene bag containing 10 ml of distilled water. All inoculations were carried out after 3 pm to avoid the heat of the day. Percentage infection was recorded and daily measurements made of the developing lesions. Galindo & Salazar (1965) reported a similar method.

Following the inoculation methods of Siller & McLaughlin (1950) and Hansen (1961), the above procedure was repeated using zoospore suspension. Leaves were inoculated by atomizer-spraying the adaxial surface with zoospore suspension applied in a central band either side of the midrib, each leaf receiving about 0.5 ml. Numbers of infected leaves, numbers of lesions formed, and numbers of lesions per unit area of leaf were recorded.

Essentially the same procedures were carried out with 6-month-old seedlings grown in the greenhouse and raised from seed obtained from self- and cross-pollinations with the test cultivars. The adaxial surface of the third and fourth youngest leaves of 10 replicate seedlings per cross were inoculated with mycelial discs or sprayed with zoospore suspension in the same way as in the field. Zoospore-inoculated leaves were immediately enclosed in moistened perforated polyethylene bags. Following inoculations with mycelial discs, leaves were enclosed in unperforated bags of 5 replicate plants of each cross, the rest remaining uncovered. As in the field, numbers of infected leaves and numbers of lesions developing and their sizes were recorded.

Inoculation of Seedling Roots

Seedling roots were inoculated following the methods reported by Asomaning (1964) and Weststeijn (1965, 1966b). Seedlings were obtained from self- and cross-pollinations with the test cultivars and raised in the greenhouse. They were grown singly in perforated black polyethylene bags containing 3 l of methyl bromide-sterilized soil. Seedlings were selected for uniformity of size and only those which appeared vigorous were inoculated. When 12-wk-old, the roots of 10 plants from each of the self- or cross-pollinations were inoculated without uprooting by applying 10 ml of standard zoospore suspension to the soil surface at a radius of about 3-4 cm around the base of the stem. Five plants from each of the pollinations were simultaneously treated with 10 ml of sterilized distilled water as controls. Eight weeks after inoculation, the plants were harvested, taking care to extract all roots without damage, and analysed for dry weight of the entire plant. Reaction of the crosses to *P. palmivora* infection was assessed by the differences in dry weight between the inoculated plants and the controls.

Seed Inoculations

Seeds were extracted from mature pods obtained from crosses made with the test cultivars, and the mucilage and integuments removed. The peeled seeds were germinated in the laboratory for 2 days by placing them in a gauze bag and immersing them in water in glass beakers protected from direct light but not kept in the dark. Plastic tubes from the water supply were inserted into each beaker to produce a very slow but constant replacement of the water. Germinated seeds were placed in dishes lined with moistened filter paper and each seed inoculated on the uppermost cotyledon with a 0.1 ml drop of zoospore suspension. Immediately following inoculation, seeds were planted in perforated black polyethylene bags containing 3 l of methyl bromide-sterilized soil with a 4 cm superficial layer of sterile sawdust, 20 seeds being placed in each bag. A total of 100 seeds were inoculated for each cross while a further 40 untreated seeds were included as controls. Seedlings were raised under greenhouse conditions and 3 wk after inoculation, the number of healthy seedlings which had emerged were counted. After a total of 2 months, the surviving seedlings were examined and the number which appeared vigorous and healthy was recorded. Response of the crosses to *P. palmivora* infection was assessed by expressing seedling emergence as a percentage of the emergence of the untreated controls.

In preliminary tests with seed inoculations, it was discovered that the use of a 2×10^5/ml zoospore suspension produced seed and seedling mortalities which were too high to allow clear cut distinctions in responses of the crosses. Reducing the suspension concentration to 1×10^5/ml yielded much more satisfactory results and was thereafter adopted for all future tests. Initially, seeds were obtained from self- and cross-pollinations with the test cultivars, depending on compatibility, but in the last 2 tests, all cultivars were crossed with the same male parent (the highly susceptible UF 667) to minimize variation and to facilitate comparison between the crosses. With the same male parent, differences in reaction to *P. palmivora* infection could be more reliably attributed to the differences in susceptibility shown by the test cultivars (female parents).

The method of Amponsah & Asare-Nyako (1973) was included in the last 2 tests for comparison. Several major differences in technique were adopted by these authors. Seeds were germinated between sheets of moist filter paper kept in the dark for 4 days. Inoculum was prepared as follows: the contents of 10 9 cm Petri dish cultures of *P. palmivora* grown on oatmeal agar for 6 days at room temperature in the dark were mixed in a Waring blender with 150 ml of distilled water for a minute or two until a slurry was obtained. This mixture was made up to 1 l with more distilled water to form a stock solution which was then further diluted to one-sixteenth to provide the inoculum. Lots of 20 germinated seeds were inoculated by immersing them in 120 ml of the inoculum solution for 3 min and then were immediately planted in sterilized soil in the greenhouse. Seedling emergence was counted after 12 days, and 8 wk later the surviving seedlings were examined and graded as fit or unfit for transplanting to the field. Partiot (1975) and Tarjot (1977) reported similar methods.

In the tests comparing the 2 methods, Amponsah & Asare-Nyako's method was adopted exactly except that seeds were germinated in slowly running water for 2 days and seedling emergence was recorded after 3 wk.

RESULTS AND DISCUSSION

Inoculations of Detached Pods and Pod-Tissue Blocks

In inoculations of detached pods, rings of modelling-clay (Tarjot, 1969b) petroleum jelly (Blaha, 1967; Tarjot, 1967b) or glass (Holliday, 1954) or tubular devices (Adebayo, 1971; Sreenivasan, 1975) were not employed to retain inoculum droplets since, if the incubating boxes were undisturbed, the droplet was small enough to be retained by adhesion to the pod surface. Furthermore, it was noted that under the high humidity conditions of the boxes the inoculum droplet retained by some rings or tubes was still present 6-7 days later and subsequent lesion appearance and development was greatly retarded, possibly because post-penetration development by *P. palmivora* is slowed or inhibited in saturated pericarp tissues. In support of this view, it was noticed that when attached pods were inoculated with an inoculum droplet retained by a modelling-clay cup, lesions established and developed on a level with, or slightly above, the upper meniscus of the droplet rather than on the area of pericarp submerged and covered by the droplet. Marking inoculation

points on detached pods was unnecessary as their location was known and became visible when infection occurred. The chance that contamination from field infection would occur in the vicinity of the inoculation site appeared to be remote and was never observed.

Separate analyses were performed on the 3 methods adopted with detached pods; (a) permanently sealing the boxes, (b) opening the boxes daily, and (c) wounding the pods and permanently sealing the boxes (Table 3). There was a very highly significant ($P < 0.001$) effect of cultivars on $\log_{10}$ of lesion diam for all 3 methods but for method (a) there was also a significant ($0.01 < P < 0.05$) interaction between cultivars and tests, indicating a degree of inconsistency between tests. There was a highly significant effect of cultivars on percentage infection for all 3 methods, $P < 0.001$ for methods (a) and (c) and $0.001 < P < 0.01$ for (b). For method (c), however, the large groups of replicate pods with all 10 infected led to poor differentiation between the cultivars.

Responses of the test cultivars in all experiments with detached pods (Table 3) generally did not compare well with the known reaction of these cultivars to natural infection in the field (Table 2). Whereas pods of some tolerant cultivars, such as Pound 7 and Scavina 6, maintained their low susceptibility when removed from the tree, susceptibility of Catongo greatly increased when pods were detached, a phenomenon first reported by Medeiros & Rocha (1965). To some extent the same effect occurred with UF 613, a moderately tolerant cultivar in the field. Conversely, Pound 12, normally a highly susceptible cultivar, showed slightly reduced susceptibility when detached pods were inoculated (Table 3, Fig.1).

It has been reported that the susceptibility of pods is increased when they are maintained at high humidity (Tarjot, 1972b). To investigate the possibility that the increase in susceptibility of detached pods of Catongo might be partly due to the necessity of incubating the pods at a constant high humidity, additional tests were conducted in which, after 24 h incubation, the boxes were opened daily for the rest of the test. Under these conditions, however, Catongo still showed the same increase in susceptibility (Table 3). Furthermore, with all cultivars percentage successful infections were lower, probably due to the fact that even after epidermal penetration by *P. palmivora* relatively high ambient humidities and sufficient pericarp moisture-content are necessary for establishment of infection. Lesions 5 days after symptom appearance were smaller than those obtained in sealed boxes since rates of lesion development were lower.

Wound-inoculation of detached pods presented no advantage. When compared with unwounded pods in sealed boxes very similar results were obtained, except that incubation periods were reduced. Susceptibility of Pound 7, Scavina 6 and CC 42 was not increased by a 1 mm-deep wound (Table 3).

In all detached pod tests, no clear differences were shown between cultivars when incubation periods and percentage infection were compared with susceptibility as determined by lesion size. Only Scavina 6 and Pound 7 demonstrated any degree of resistance to establishment of infection (Table 3).

In the course of the tests involving unwounded pods incubated in sealed boxes and with laboratory fungicide screening trials utilizing

Table 3. Point-inoculation of detached cacao pods with *P. palmivora* zoospore suspension.

CULTIVAR (10 replicate Pods per test)		WITHOUT WOUNDING										WITH WOUNDING			
		Incubation boxes permanently sealed						Boxes opened daily after 24 h				Boxes permanently sealed			
		Percentage successful infections			Av lesion diam (cm)+			Percentage infection		Av lesion diam+		Percentage infection		Av lesion diam+	
	Test	1	2	3	1	2	3	1	2	1	2	1	2	1	2
Pound	7	*	*	70	*	*	1.6	*	*	*	*	40	60	1.8	2.0
Scavina	6	*	*	50	*	*	2.0	*	*	*	*	20	35	1.4	2.2
Catongo		100	100	*	5.2	5.3	*	95	80	3.7	3.2	100	100	5.6	5.0
CC	42	100	75	80	1.8	1.2	2.2	75	65	0.6	0.5	95	100	1.9	2.1
UF	613	100	95	100	4.0	3.1	4.2	50	60	1.5	1.8	100	100	4.1	3.7
CC	41	100	100	100	3.8	3.6	3.0	85	75	3.1	2.6	100	100	3.5	4.2
UF	296	95	100	95	3.5	2.8	4.4	60	50	2.8	2.0	100	100	2.6	3.6
Pound	12	85	95	95	2.4	3.0	2.5	75	50	2.0	1.5	100	100	3.7	3.5
UF	677	100	85	95	5.6	5.5	5.1	55	65	3.5	3.0	100	100	6.5	4.8

+ Recorded 5 days after symptoms first appeared.

* Cultivar not included in the test.

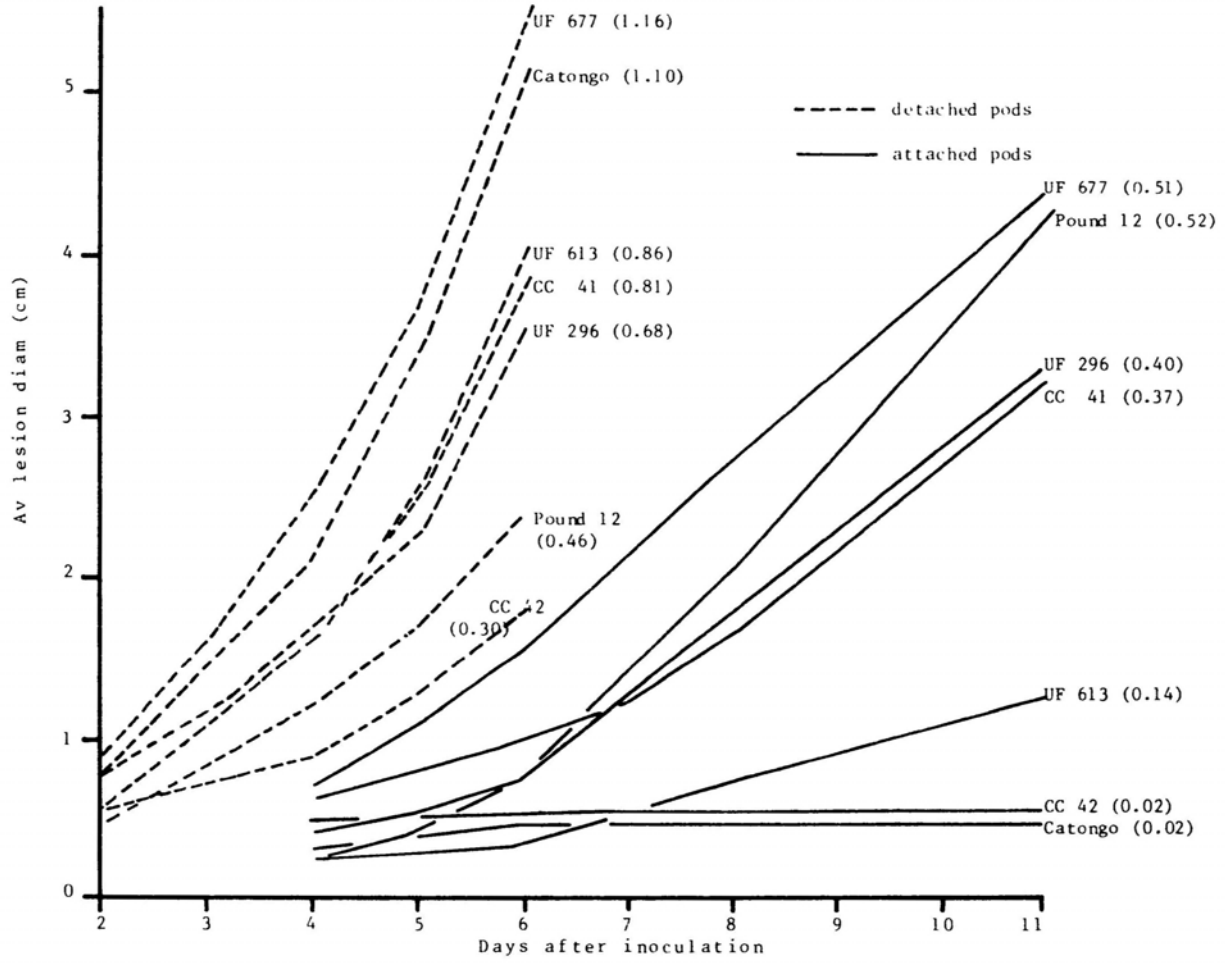

Fig. 1. Lesion development on attached and detached pods of 7 cacao cultivars following point-inoculation without wounding. Attached pods were permanently enclosed in bags, detached pods were inucubated in seal ed boxes. Values in parentheses following cultivar designations represent av daily increase in lesion diam.

the identical inoculation procedure, a constant difference in lesion size was noted according to the location of the inoculation site on the pod. A total of 105 pods of the highly susceptible UF 677 (none treated with fungicide) were point-inoculated with zoospore suspension in the centre of the proximal and distal halves of the uppermost surface of the pod. Av lesion sizes measured 5 days after appearance of first symptoms were 4.7 cm on the proximal half and 5.8 cm on the distal. Variation in pod susceptibility according to inoculation site was reported by Medeiros (1967) and choice of the inoculation site for attached pods was influenced by this feature.

The tests with pod tissue blocks (Table 4) yielded results very similar to percentage infection data for whole, detached pods. χ^2 tests of the significance of the difference between cultivars in number of blocks infected revealed that the data just failed to show evidence of a difference at the 5% level. Thus, no distinct differences were detected between cultivars, although fewer blocks of Scavina 6 and Pound 7 were infected. Using the same method, Spence & Bartley (1966) and Rocha & Mariano (1969) found Scavina 6 to be highly resistant, producing no infection. However, the use of fewer replicates and different *P. palmivora* isolates could have accounted for this difference. Post-penetration development of *P. palmivora* in pod tissues could not be adequately assessed by this meth-

Table 4. Point inoculation of cacao pod-tissue blocks with *P. palmivora* zoospore suspension without wounding.

Cultivar		Nº of blocks infected after 4 days (20 inoculated)	
		Test 1	2
Pound	7	11	14
Scavina	6	8	10
CC	42	16	17
UF	613	18	16
CC	41	20	20
UF	296	17	19
UF	29	19	20
Pound	12	18	17
UF	677	19	19

od as the tissue blocks are too small to allow measurement of lesions beyond a few days. Despite the small size of material inoculated in this test, it provided little advantage over tests with whole detached pods since the same number of pods per cultivar were harvested from the field and brought to the laboratory in either test. Only the number of incubation boxes used was reduced.

Inoculations of Attached Pods

In preliminary tests with attached pods, the polyethylene bags were removed 3 days after inoculation, similar to the procedure described by Blaha (1967, 1971) and Blaha & Lotodé (1976, 1977). It was reasoned that if maintaining pods in a saturated atmosphere increases their moisture content and consequently their susceptibility to post-penetration growth by *P. palmivora* (Dakwa, 1968; Tarjot, 1972b), a more realistic assessment of response to *P. palmivora* would be obtained by exposing pods to natural field conditions after first allowing a period for infection to become well established. However, in practice when bags were removed from the pods, they were subjected to too wide a variation in environmental conditions, especially humidity, and lesion development tended to be excessively retarded, ceasing altogether, even with susceptible cultivars, during unforeseen dry spells. Similar results were obtained when the bases of the bags were cut open after 3 days, the bags remaining tied to the peduncle to form a tent-like cover open on the underside. Since artificial inoculation experiments should provide uniform and optimum conditions for infection and development by a pathogen, it was decided to accept the possible disadvantage of maintaining pods at high humidity in favour of reducing variation in the pod micro-environment. Consequently, in all future tests pods were enclosed in bags for the duration of the test.

Analyses carried out on the $\log_{10}$ of lesion diam in all attached pods tests showed that lesion diam was very highly significantly different ($P < 0.001$) for different cultivars, both for zoospore and mycelial-sporangial suspensions. There was no significant interaction between cultivar and replicate test for zoospore suspension but with mycelial-sporangial suspension wounding did interact significantly with cultivars at $P < 0.001$, showing that the relative values for the cultivars depended on the presence or absence of wounding. When zoospore suspension was used, cultivars had a highly significant ($P < 0.001$) effect on percentage of successful infections. However, only Pound 7, Scavina 6 and Scavina 12 differed significantly from the other cultivars. There were insufficient data to test whether there was an interaction between cultivar and replicate test for percentage infection and there was insufficient replication to test the effect of cultivars on percentage infection with mycelial-sporangial suspension.

Results from tests with zoospore point-inoculations without wounding (Tables 5 & 7) corresponded well with the responses of the test cultivars to natural infection in the field (Table 2). Cultivar reaction was generally very consistent; Pound 7, Scavina 6 and CC 42 uniformly demonstrated low susceptibility while Pound 12, UF 29 and UF 677 were consistently the most susceptible. Incubation periods were very similar on both resistant and susceptible cultivars, initial symptoms appearing on all cultivars within a 24 h period, and no clear relationship existed between degree of resistance and length of incubation period.

Table 5. Point - inoculation of attached cacao pods with *P. palmivora* zoospore or mycelial-sporangial suspension.

CULTIVAR (10-20 replicate pods per test)	Test	ZOOSPORE SUSPENSION								MYCELIAL-SPORANGIAL SUSPENSION			
		No wounding								No wounding		Wounding	
		Percentage successful infections				Av lesion diam (cm)+				Percentage infection	Av lesion diam+	Percentage infection	Av lesion diam+
		1	2	3	4	1	2	3	4				
Pound	7	*	*	70	30	*	*	0.6	0.7	*	*	*	*
Catongo		95	*	*	*	0.5	*	*	*	*	*	*	*
Scavina	6	*	*	40	20	*	*	0.6	0.6	30	0.8	40	1.3
Scavina	12	*	*	*	45	*	*	*	0.7	*	*	*	*
CC	42	90	75	100	80	0.6	0.8	1.0	0.8	75	0.6	95	1.7
UF	613	80	100	95	100	1.1	1.3	1.2	1.5	85	1.3	100	6.0
CC	41	95	100	100	100	2.7	2.2	3.0	2.5	100	2.2	100	3.5
UF	296	95	65	100	100	2.8	3.0	3.6	2.3	95	3.0	100	3.8
UF	29	*	75	100	100	*	3.9	5.5	4.1	*	*	*	*
Pound	12	85	90	100	95	3.5	4.0	4.1	3.6	85	3.9	100	5.3
UF	677	95	85	85	100	3.8	4.5	4.2	3.7	100	3.9	100	5.3

+ Recorded 7 days after symptoms first appeared.
* Cultivar not included in the test.

Differences in symptoms and lesion development were noted in some cultivars. Lesions on pods of Pound 7 and Scavina 6 tended to be water-soaked with fine, dark stippling within the boundary of the affected area and rarely developed into the solid, brown spots characteristic of black pod lesions. In contrast, lesions on CC 42 were usually very dark brown or nearly black. Following establishment of infection on pods of these 3 cultivars, lesions often developed very slowly, frequently ceasing growth altogether 2-3 days after symptom appearance. With CC 42 this was particularly pronounced and appeared to be akin to the hypersensitive reaction observed with other plant diseases. Similar reactions were noted by Spence (1961b), Tarjot (1972c) and Blaha & Lotodé (1976).

The use of a mixed mycelial-sporangial suspension to point-inoculate pods without wounding produced very similar results to those obtained with zoospore suspension (Table 5). However, when mycelial-sporangial suspension was used in conjunction with wounding, percentage infection was slightly increased, incubation periods were shortened, and, consequently, lesions tended to be larger 7 days after first symptom appearance.

From tests with point-inoculated attached pods, indications were that resistance in UF 613 is mostly due to resistance to penetration since pod susceptibility was significantly increased by wounding, to the same degree as shown with detached pods of this cultivar. Conversely, Scavina 6 appeared to be relatively resistant to both penetration and post-penetration growth by *P. palmivora*, as wounding did not appreciably increase susceptibility of attached pods of this cultivar. Pound 7 and CC 42 seemed to be moderately susceptible to penetration but demonstrated resistance to growth of the fungus within pod tissues.

Spraying attached pods with zoospore suspension yielded unsatisfactory results (Table 6). When an analysis of variance based on $\log_{10}$ of lesion numbers was carried out, the difference between cultivars was highly significant ($P < 0.001$). However, a comparison of means showed that in both tests only Scavina 6 produced significantly fewer lesions. There was also a significant ($P < 0.01$) interaction between cultivars and test indicating that results were not consistent for the 2 tests. Therefore, only Scavina 6 presented a more or less distinct resistant reaction while clear-cut differences between other cultivars were not apparent. Furthermore, this method only allowed determination of percentage infection, and hence resistance to penetration, since the numerous lesions produced by this method coalesced too rapidly for lesion diameters to be measured beyond 2 or 3 days after symptom appearance. It was not found possible to evaluate cultivar reaction on the basis of lesion development up to 8 days after inoculation as reported from Brazil (Lellis & Peixoto, 1960; Medeiros & Rocha, 1965; Medeiros & Melo, 1966; Rocha & Medeiros, 1968; Rocha & Mariano, 1969). Possibly, more satisfactory results could have been obtained using a lower zoospore concentration.

Inoculations of pods of various ages indicated that with all cultivars pods were more resistant to *P. palmivora* infections at 3 months of age, thereafter susceptibility increasing progressively with age (Table 7). Very young pods of 2 months of age were more susceptible than those at 3 months, being comparable in susceptibility with 4-month-old pods.

Pods at 2 months were too small to allow continuous measurements of lesion diameters up to 7 days after first symptom appearance since lesions

Table 6. Unwounded attached cacao pods sprayed with *P. palmivora* zoospore suspension.

Cultivar (10 replicate pods)		Av nº lesions after 4 days	
		Test 1	2
Pound	7	17.7	20.2
Scavina	6	10.1	6.9
CC	42	21.3	16.8
UF	613	18.1	12.8
CC	41	29.7	34.4
UF	296	19.9	26.3
Pound	12	31.2	16.6
UF	677	20.5	29.5

covered the entire pod surface within 2-4 days after inoculation. Figures comparative with other pod ages, therefore, could not be obtained and lesion sizes of these pods were excluded from Table 7. At 3 months, with some cultivars too few infections resulted from the inoculations such that, due to a poor sample, lesion sizes at this age may be unreliable.

Analysis of the $\log_{10}$ lesion diam showed that there was a very highly significant difference ($P < 0.001$) between cultivars but no significant interaction between age and cultivars. Also, there was no significant difference ($P > 0.05$) between cultivars in the number of successful infections. Thus, different ages showed no evidence of giving inconsistent results so the optimum age for inoculation could be chosen using other criteria.

Inoculation results were supported by observations of natural infection on a number of the experimental pods, all of which were unprotected throughout the experiment (when naturally infected, these pods had to be abandoned for inoculation purposes). These natural infection data are not included here as the experiment was not designed for this purpose and, since the sample size changed constantly as pods became infected or were inoculated, too few pods remained towards maturity for the data to be statistically reliable. Neverthless, indications were that the pods attained an age of about 1 month without suffering much loss from natural *P. palmivora* attack, although some were lost from cherelle wilt due to causes other than *P. palmivora*. However, at about 1 1/2 - 2 months many of

Table 7. Point-inoculation with *P. palmivora* zoospore suspension of unwounded attached cacao pods of different ages.

Cultivar (10 replicate pods at each age)		Age of pods (months) 2	3	4	5	Mature (>6)
42	Percentage successful infections	70	10	50	65	95
	Av lesion diam (cm)+	*	1.4	0.5	0.8	1.0
613	Percentage infection	100	0	85	80	100
	Lesion diam	*	0	0.9	1.2	1.2
41	Percentage infection	85	10	70	95	100
	Lesion diam	*	0.5	1.5	3.0	3.2
296	Percentage infection	80	10	65	50	95
	Lesion diam	*	0.9	1.8	3.2	3.0
I2	Percentage infection	85	30	75	75	90
	Lesion diam	*	1.0	2.3	3.4	4.0
677	Percentage infection	85	20	65	85	90
	Lesion diam	*	2.2	2.0	3.6	4.3

+ Recorded 7 days after symptoms first appeared.
* No value obtainable.

the experimental pods were infected by *P. palmivora* before they could be artificially inoculated, indicating that pods of this age were moderately susceptible. Incidence of natural infection on the experimental pods was lower at about 3 months of age while older pods were more frequently infected. What causes these changes in pod susceptibility is not known but it may be related to changes in polyphenoloxidase production or activity within the pericarp tissues (Turner, 1965b; Rocha, 1966).

A similar effect of changing susceptibility in relation to pod age was reported by Rocha (1966) who in Costa Rica inoculated approximately 2-, 4- and 6-month-old unwounded pods of a resistant (UF 613), a moderately susceptible (UF 29) and a highly susceptible cultivar (UF 221). Rocha stated that pods were less susceptible at 2 months than at 4 or 6 months, but this discrepancy may be explained by the fact that he did not use

pods derived from controlled pollinations and, therefore, could not be sure of their exact age. Thus, his approximately 2-month-old pods may well have been closer to 3-months-old, an age when they are least susceptible. Tarjot (1972a) also reported that susceptibility increased progressively towards maturity and that cherelles showed maximum resistance. However, it appears that pods derived from hand pollinations were not used in this case, either, since ages were not ascribed to the 4 developmental stages inoculated. Cherelles were described as less than 10 cm long and, therefore, these, too, may have been correspondingly older than pods of 2 months of age in Costa Rica. When considering losses to *P. palmivora* infection sustained by young cherelles, it would be surprising if they represented the most resistant age.

An opposite age effect was reported by Medeiros & Machado (1967). Wound inoculating pods of the cultivar *Comum* at 5 developmental stages, they found that, using lesion areas as a criterion, pods became less susceptible towards maturity. Using essentially the same method, Adebayo (1975) found that when susceptibility was assessed on the basis of lesion diameters, there was no significant difference in the susceptibility of young, fully developed but unripe, and mature pods of the cultivars Na 32, N 38 and CF 62. Only when a *difference ratio* was calculated from lesion diameters measured 3, 6 and 9 days after inoculation was susceptibility of pods shown to increase with age. In both reports, pods were inoculated by means of relatively deep wounds and, therefore, it is probable that susceptibility of mesocarp and endocarp tissues, rather than the entire pericarp and epidermis, was being assessed. Different resistance mechanisms probably operate in inner pericarp tissues (possibly connected with content of simple sugars) and could account for the contrary results reported by these workers.

The determination of pod susceptibility at different ages was carried out to select the optimum age of pods for use in inoculation tests. An ideal pod age would be that which combines least resistance to *P. palmivora* with the greatest useable surface area. Mature pods satisfy both these requirements but an objection to their use is that resistance mechanisms may become inoperative or less active at maturity and, therefore, their reaction to *P. palmivora* infection may not represent the same as that of unripe pods (Prendergast & Spence, 1967). Mature pods are also subject to considerable attack by rodents and birds whereas unripe pods are not. Pods 1 month from maturity (i.e. 83-85% of time to maturity completed) are the same size as mature pods and almost as susceptible to *P. palmivora* and it was concluded that this represents the optimum age for inoculations. The only disadvantage with the use of unripe pods is that, before screening tests with pods of known age can be conducted, the av time to maturity for each of the test cultivars in a given area first has to be established.

At first, in attached pod tests, hand pollinations were carried out to obtain pods of the same age but later it was realised that this was unnecessary when screening fruiting cultivars since pod tissue is maternal in origin and the pollen source is unimportant. Pods of the required age were obtained simply by tagging very young pods produced by open pollination when they were 3-10 mm in length, that is, not more than 1-wk-old. Thus, the laborious and often unsuccessful process of hand pollination was avoided.

Inoculations of Seedling Stems, Marcot Stems and Tree Branches

The method employing seedling-stem inoculations was examined principally as a technique for screening newly produced hybrid progenies rather than for use in testing fruiting cultivars, since for the latter purpose, even using seeds from open-pollinated fruit, there was a delay of up to 4 months until seedlings were ready for inoculating. Furthermore, if this method is used to test cultivars, the necessity for using hybrid crosses with self-incompatible types introduces unwelcome variability. However, the stem-inoculation technique was investigated using marcots or tree branches as a means of directly assessing resistance of the test cultivars.

For both seedling-stem and branch inoculations there was a very highly significant ($P < 0.001$) effect of cultivars on the $\log_{10}$ of lesion area, while there was no significant interaction between cultivar and replicate test. In seedling-stem tests, consistent gradations in response between resistant and susceptible crosses were obtained and results from self-crosses revealed much the same hierarchy of resistance-susceptibility as shown by the parent cultivars in the field (Table 8). However, the range in lesion sizes between the most resistant and susceptible, being only about 3-fold, limited the facility with which distinctions between degrees of susceptibility could be made. This is in contrast with the results published by Zentmyer *et al.* (1968) and Zentmyer (1969) who reported larger lesions and a greater size range between resistant and susceptible material. However, these workers measured lesion lengths 6 wk, not 18 days, after inoculation. Zentmyer (1972) later modified his method to record lesion areas after 18 days, the technique that was followed here. This proved much more suitable under Turrialba conditions since 6 wk after inoculation lesions on susceptible material often completely girdled the seedling stems along their entire length and many seedlings were killed, thus greatly complicating measurement and interpretation of results. Even exactly following Zentmyer's method, however, much smaller lesions were obtained than reported by him (Zentmyer, 1972). Presumably, these discrepancies arose from environmental differences affecting rate of seedling growth and vigour; the use of different *P. palmivora* isolates could be largely discounted since Zentmyer included a Turrialba isolate in his tests.

Results from branch inoculations correlated well with cultivar responses to natural infection (Table 8). In comparison with seedling stems variation was less, lesion sizes amongst replicates being very uniform. Lesions were small, however, and the size range between the most resistant and most susceptible cultivars was less than 3-fold. In a repetion of this test, therefore, branches were left an additional week after inoculation. Except that lesions were larger, similar results were obtained (Table 8).

It was anticipated that inoculation of marcots probably would have duplicated results obtained with branches, but in this experiment insufficient marcots produced roots or survived transplanting to provide an adequate test sample. Rooted cuttings probably would have served equally well but it was thought that marcots would provide stem diameters suitable for inoculating quicker than would cuttings. The use of marcots finally was considered unsatisfactory as their preparation requires too much time and effort and they are wasteful of material since many fail to form roots or die after transplanting. To a lesser extent, the same objections can be levelled at the use of cuttings.

Table 8. Inoculation of cacao-seedling stems and living branches with discs from *P. palmivora* cultures.

Cacao Material[+]	Av lesion areas (cm^2) after 18 days				
	Seedlings (10-20 replicates)			Branches (10 reps)	
Test	1	2	3	1	2[++]
Pound 7 (Catongo)	*	2.9	3.2	1.0	1.7
Catongo	*	3.8	3.0	1.2	1.5
Scavina 6 (Catongo)	*	4.0	2.1	1.6	2.0
Scavina 12 (Catongo)	*	3.3	2.5	1.7	2.2
CC 42	2.6	5.4	3.8	1.9	2.4
UF 613 (UF 296)	2.9	4.6	4.5	2.0	2.4
CC 41	3.7	5.6	3.9	1.8	2.6
UF 296	3.2	5.9	4.9	1.8	2.4
UF 29	*	6.3	5.0	1.9	3.0
Pound 12 (UF 677)	4.5	6.0	5.7	2.0	3.5
UF 677	3.7	7.2	6.7	2.7	3.3

[+] Cultivars in parentheses refer to male parents used for producing seed with self-incompatible types. All other cultivars were selfed.

[++] Measurements made 25 days after inoculation.

* Cultivar not included in the test.

Leaf Inoculations

Results from leaf inoculations were very variable and inconclusive. Attached leaves were used in preference to leaves, or leaf pieces, removed from the tree to avoid any possible changes in susceptibility such as encountered with detached pods. However this precaution proved ineffectual in view of the great variability in the results obtained. With both field and greenhouse tests using mycelial inoculum, lesion development on replicate leaves of each cultivar was very erratic. In some cases, lesions developed relatively slowly, even ceasing growth altogether on certain leaves, while in others they developed very rapidly and killed the entire leaf in a few days. Assessment of lesion diameter was

not easy, either, as lesions tended to be very irregular in shape. As a result, av lesion sizes were too varied to allow comparisons between cultivars, although it was noticed that with the more resistant cultivars fewer inoculated leaves were killed. In the greenhouse tests, it was established that leaves inoculated with a mycelial disc required a constant high atmospheric humidity for adequate lesion development since, although infection occurred immediately under the agar disc, there was no further lesion development if the leaves were not enclosed in a moistened bag.

The use of a zoospore suspension spray inoculum proved to be totally unsatisfactory. Often numerous lesions were formed coalesced and they could not always be accurately counted. Great variation between replicate leaves occurred and no obvious differences between cultivars were obtained. Point-inoculation with zoospore suspension would have been preferred but a suitable means of retaining the suspension drop on attached leaves was not devised.

Root Inoculations

As with seedling-stem inoculations, the root inoculation method was investigated primarily as a technique for screening hybrid progenies. For the same reasons mentioned with seedling-stem inoculations, root inoculations were regarded as being less appropriate to test cultivars in production.

Results from seedling-root tests were somewhat ambiguous and corresponded only partially with natural infection responses of the parent cultivars (Table 9). The difference in weight between infected and control plants was significantly different at $P < 0.001$ for the different cultivars. However, there was also a very highly significant ($P < 0.001$) interaction between cultivars and tests, indicating that results were not consistent for the 2 tests. As reported elsewhere (Asomaning, 1964; Prendergast, 1965), a gain in weight over the controls was obtained with infected seedlings of the more resistant crosses. Infected seedlings of the more susceptible crosses mostly lost weight in comparison with the untreated controls, so some differentiation in response between resistant and susceptible crosses was possible. However, the variation occurring between tests with some crosses, infected seedlings losing weight in one test and gaining weight in the other, indicated that this type of test is not sufficiently reliable.

Seed Inoculations

Seed inoculation methods also were examined as a possible means of evaluating hybrid progenies rather than for screening fruiting cultivars, although for the latter purpose, if seeds from open-pollinated fruit are used, there is no delay in the preparation of test material as mature pods are immediately available in the field. However, the use of open-pollinated seeds for test cultivars introduces unwelcome variability since the male parent is unknown and could be different with each pod used.

When seeds were inoculated with zoospore suspension, numbers of healthy seedlings emerging were consistently greater with resistant crosses than with susceptible crosses and results from self-crosses compared well with

Table 9. Inoculation of cacao-seedling roots with *P. palmivora* zoospore suspension.

Cacao Material	Av whole plant dry weight (g) after 8 wk					
	Infected (10 replicates)		Untreated Control (5 reps)		Difference (Control-infected)	
Test	1	2	1	2	1	2
Catongo selfed	6.5	6.7	5.6	6.4	-0.9	-0.3
UF 613 x Catongo	7.0	7.8	7.0	6.8	0	-1.0
CC 42 selfed	5.3	4.2	4.8	5.0	-0.5	+0.8
CC 41 selfed	3.3	5.6	4.9	5.6	+1.6	0
UF 296 selfed	6.4	3.5	6.7	6.1	+0.3	+2.6
UF 29 selfed	4.0	4.1	5.7	6.0	+1.7	+1.9
UF 677 selfed	5.1	4.1	6.5	6.3	+1.4	+2.1

the responses of the parent cultivars to natural infection (Table 10). Some variation between tests was experienced but a similar gradation of reactions of the crosses was obtained in all tests, the distinction between resistance and susceptibility always being distinguishable.

Significance tests conducted with angle transformations of the data from tests using zoospore suspension showed that cultivars had a significant ($P < 0.001$) effect on percentage emergence, emergence as a percentage of the control, and percentage vigorous plants of surviving seedlings after 2 months. The first 2 of these variates gave cultivar means with the same rank order, but this was to be expected since seedling emergence of the cultivars used in this study was consistently high (95-100%). However, emergence must be expressed as a percentage of that of the control as a contingency for those hybrids which may have lower seedling emergence.

When this method was compared with that of Amponsah & Asare-Nyako (1973) both methods produced similar gradations of responses (Table 11). Both cultivar and type of suspension had significant ($P < 0.001$) effects on angle percentage emergence and emergence as a percentage of the control. There was also a significant ($0.01 < P < 0.05$) effect of the type of suspension on percentage vigorous surviving plants. There was no significant interaction between cultivar and type of suspension, signifying that there was no difference between cultivar response according to suspension. However, percentage emergence values were slightly higher when mixed mycelial-sporangial suspension was used and the range in values between resistance and susceptibility was less. This difference probably was due to the greater virulence of the zoospore inoculum. Duplications of Amponsah & Asare-Nyako's method produced higher percentage emergence values than they reported. Discrepancies probably were due to differences in environmental conditions, cacao material and *P. palmivora* isolate used.

Examining modifications of Amponsah & Asare-Nyako's method, Tarjot (1977) found that unpeeled seeds showed lower mortalities than peeled seeds at given inoculum concentrations and, therefore, recommended that seed integuments be retained. However, seed inoculation is a highly artificial method and bears little relation to natural infection processes and, provided that adequate distincitions between resistance and suscepti-

Table 10. Inoculation of pre-germinated cacao seeds with *P. palmivora* zoospore suspension.

Cacao Material (100 seeds treated 40 untreated)	Seedling emergence as % of untreated control 3 wk after inoculation			Percentage vigorous plants of surviving seedlings after 2 months		
Test	1	2	3	1	2	3
Pound 7 x Catongo	60	58	69	98	95	90
Catongo selfed	56	52	65	100	94	78
Scavina 6 x Catongo	65	61	59	95	85	83
Scavina 12 x Catongo	63	54	50	72	91	81
CC 42 selfed	30	49	41	69	81	66
UF 613 x UF 296	48	47	37	94	59	89
CC 41 selfed	24	25	28	96	52	85
UF 296 selfed	20	44	34	79	52	67
UF 29 selfed	37	17	20	92	47	89
Pound 12 x UF 677	16	21	10	81	75	50
UF 677 selfed	20	29	20	60	79	50

bility can be obtained by adjustment of the inoculum concentration, it would seem to be solely a question of convenience whether seed integuments are retained or not.

Tarjot (1977) also reported that inoculating seeds by immersion in a crushed mycelium solution, rather than in a zoospore suspension, produced seedling mortality levels more appropriate for distinguishing resistant and susceptible reactions because mycelium solutions gave percentage seedling emergence which increased proportionately to dilution whereas zoospore suspension did not. In the present study, it was found that seed inoculation by immersion in any kind of inoculum suspension could not be completely standardized since, even if the suspension was calibrated, it was not possible to ensure that all seeds received the same amount of inoculum. Depositing a zoospore-suspension droplet of known volume and concentration on each seed provided a more controlled inoculation procedure and selection of the optimum concentration for distinguishing between the most resistant and most susceptible cacao types tested was accomplished without difficulty. Peeled seeds were preferred with this technique since all of the inoculum droplet was retained within the convolu-

Table 11. Inoculation of pre-germinated cacao seeds with *P. palmivora* zoospore suspension or mycelial-sporangial suspension.

Hybrid (100 seeds treated 40 untreated)	Zoospore suspension				Mycelial-sporangial suspension			
	Seedling emergence as % of untreated control after 3 wk		% vigorous plants of surviving seedlings after 2 months		Seedling emergence as % of untreated control after 3 wk		% vigorous plants of surviving seedlings after 2 months	
Test	1	2	1	2	1	2	1	2
Pound 7 x UF 667	62	55	87	95	86	76	96	99
Scavina 6 X UF 667	64	69	92	82	92	74	94	87
Scavina 12 x UF 667	55	60	91	77	76	64	97	84
UF 613 x UF 667	64	49	83	94	40	69	90	94
Pound 12 x UF 667	14	25	85	79	45	50	84	76
UF 677 x UF 667	20	34	90	76	43	36	81	80
UF 667 x UF 667	9	26	56	81	50	48	76	60

tions of the exposed cotyledon whereas with unpeeled seeds most of the droplet ran off.

Under Turrialba conditions, recording percentage seedling emergence after 12 days, as adopted by Amponsah & Asare-Nyako (1973), proved to be too early to provide clear distinctions between resistant and susceptible reactions, and it was found that a period of 3 wk was necessary to yield satisfactory results. Under Cameroon conditions, Tarjot (1977) reported that a period of 1 month provided best results. The methods of seed germination used by Amponsah & Asare-Nyako (1973), Partiot (1975) and Tarjot (1977) were not adopted since the length of hypocotyls after 4 days on moist filter paper or sawdust is such that they are much more susceptible to injury when planted than those of seeds germinated for 2 days immersed in water. Germinating seeds by the latter method reduced this risk and appeared to be quite satisfactory since seedling emergence with the untreated controls was always at or close to 100%.

Contrary to the findings of Amponsah & Asare-Nyako (1973), it was concluded that the percentage of surviving seedlings after 2 months which were fit for transplanting to the field was a less useful criterion for assessing reaction of the cacao material tested. Although there were significant differences between crosses and a degree of agreement between cultivar rank order with this criterion and the rank order assumed to indicate inherent susceptibility (see p.24), clear, visible distinctions between resistance and susceptibility were not readily apparent (Tables 10 & 11). It seems unlikely that percentage of vigorous seedlings amongst those surviving after 2 months would bare direct relation to the effects of *P. palmivora* infection, these being manifested well before 2 months with no subsequent secondary infection. It was considered that what remained after 2 months probably merely represented a population of uninfected seedlings which exhibited the natural variability in vigour normally experienced with hybrid progenies.

Comparison of Pod Inoculation Techniques

Zoospore inoculum was preferred in all pod inoculation tests since it is regarded as the principal means of dissemination of the disease and represents the most common natural infection process of the pods. Because a zoospore suspension can be easily calibrated and standardized, it provides a much more uniform and reproducible inoculum than mycelium-agar discs or mycelial-sporangial suspension. Mycelium-agar discs are difficult to standardize completely since quantities, activity and age of mycelium differ according to location in the fungus colony and it is difficult to entirely exclude sporangia. Furthermore, mycelium probably less commonly acts as natural inoculum, such as when infected pod material is transported by insects and other invertebrates, rodents, birds and cutting implements, and might fail to take into account the inhibitory or stimulatory effects that the pod epidermis may have on spore germination and germ-tube growth and penetration. Standardization of mycelial-sporangial suspension also poses problems since relative amounts of mycelium, sporangia and zoospores in the inoculum vary considerably and cannot be controlled. Although it is possible to standardize pure sporangial suspensions (Orellana, 1954a), they were not used as it is difficult to ensure that all sporangia germinate either directly or indirectly. Cultivation of *P. palmivora* isolates was preferred on V-8 agar as sporangial

sporulation was much more abundant on this medium than on potato-dextrose, cassava-dextrose or oatmeal agar (on all of which vegetative growth was much greater) and higher concentrations of zoospore suspension were obtained.

Point-inoculation of pods with a standard quantity and concentration of zoospore suspension was preferred to spray applications of the inoculum. Point-inoculations provided a more uniform and reproducible inoculum and allowed the individual discrete lesions so produced to be measured daily up to the point where most of the pod surface was covered. Two distinct types of pod resistance to *P. palmivora* have been recognised by several workers (Lellis & Peixoto, 1960; Spence, 1961b; Medeiros, 1967; Prendergast & Spence, 1967; Rocha & Medeiros, 1968; Rocha & Mariano, 1969; Rocha & Vello, 1971; Tarjot, 1972c); a resistance to penetration or establishment of infection and a resistance to post-penetration growth by *P. palmivora* within pericarp tissues. Point-inoculation of pods allows determination of both types of resistance since it enables percentage infection and lesion development to be recorded. Zoospore spray-inoculations (Lellis & Peixoto, 1960; Medeiros & Rocha, 1965; Prendergast, 1965; Rocha & Medeiros, 1968; Rocha & Mariano, 1969) or immersing pods in zoospore suspension (Wharton, 1959; Toxopeus & Gorenz, 1970), may yield more accurate percentage infection data and are, therefore, appropriate for determining degrees of resistance to initial penetration, but the numerous lesions produced by these techniques coalesce too rapidly to allow measurements of individual lesions beyond a few days, so that post-penetration resistance is not so easily assessed. A further objection is that the quantity of inoculum used is less exact since it is difficult to control the amount of suspension each pod receives. The same objections apply to the cotton-pad (Toxopeus & Gorenz, 1970; Gorenz, 1971) and the filter-paper (Wharton, 1959) placement methods; the inoculum is not fully standardizable and the many lesions arising from the zoospore-suspension impregnated squares coalesce too rapidly to allow rate of lesion development to be followed for a sufficient period.

Retaining the zoospore suspension droplet by means of a small modelling-clay cup adhering to the pod surface (Blaha, 1967, 1971, 1972; Tarjot, 1969a,b; Blaha & Lotodé, 1976, 1977) proved satisfactory. The same end is achieved with the method reported by Adebayo (1971), in which zoospore suspension is retained in a plastic tube mounted on pods held horizontally on the tree by string, but it is more time-consuming and there is a danger of splitting the peduncle and producing what would amount to a detached pod.

The use of *P. palmivora*-infested wheat grains inserted into short lengths of drinking-straw attached to pods by means of modelling-clay (Sreenivasan, 1975) possesses the advantage that it is relatively simple and convenient and a constant area of pod surface is exposed to inoculum. A drawback to this method is that the inoculum is not completely standardized since there is no control over the concentration of zoospore suspension eventually produced when the straw is filled with water. Furthermore, it was found in the present study that retaining the inoculum in tubular structures often resulted in much longer incubation periods, especially with more resistant cultivars, compared with the use of a more open receptable. However, this method merits examination of its suitability for inoculating seedling and cutting stems.

Two inoculum points on opposite sides of the pod (Tarjot, 1969a,b)

were preferred to only 1 inoculum site (Blaha, 1967, 1971, 1972; Blaha & Lotodé, 1976, 1977) since they provided additional replication and, even when both points produced infection, measurement of lesion diameters was still possible for a sufficient period and up to an adequate lesion size before the two coalesced. Three inoculum points (Weststeijn, 1969) proved less convenient since on susceptible cultivars with smaller pods the lesions coalesced too rapidly. Inoculations of detached pods showed that lesions developing from equatorially (laterally) located inoculations were larger that those produced nearer the peduncular or apical ends of the pod. The region of greatest diameter, therefore, represented the most susceptible portion of the pod and provided the maximum surface area for expansion of the lesions before they coalesced. In all tests employing point-inoculations with zoospore suspension, a 0.1 ml drop was found to be adequate with the concentration of suspension used. Greater quantities as reported by other workers (Tarjot, 1969a,b; Weststeijn, 1969; Adebayo, 1971) were not required, although it is realised that if lower zoospore concentrations are used, larger volumes of suspension may be needed to provide adequate inoculum.

It has been reported that susceptibility of attached pods may increase when the epidermis and pericarp are wounded at the inoculation site (Medeiros & Rocha, 1965; Prendergast & Spence, 1967; Rocha & Mariano, 1969). In tests conducted with point-inoculated detached pods, lesion sizes on all cultivars were very similar on both wounded and unwounded pods (Table 3). When attached pods were wound-inoculated, however, with all cultivars, except Scavina 6, incubation periods were reduced and lesion sizes 7 days after symptom appearance were larger than those on unwounded pods (Table 5). It was concluded that wound-inoculating detached pods had very little effect but that wounding attached pods increased their susceptibility to a level comparable with that of detached pods. This effect was particularly noticeable with UF 613.

Wound-inoculation of attached pods, however, appeared to have only a slight effect upon percentage infection (Table 5), largely because percentage infection was almost as high on unwounded as on wounded pods and the effect of wounding on infection percentage could not be easily detected. According to Tarjot (1972c, 1974) *P. palmivora* always penetrates the pod epidermis of resistant and susceptible types alike but with resistant cacaos migration of the fungus into inner-epicarp and mesocarp and endocarp tissues is greatly retarded, and often completely prevented, by very slow development through a layer of small-celled parenchyma located in the superficial layers of the epicarp immediately below the epidermis. It is of interest to note that if penetration does in fact always occur, the term *resistance to penetration* should be abandoned in favour of *resistance to development within the epicarp* or *resistance to epicarp penetration*, this in turn representing a type of post-penetration resistance. Wounding of the epidermis and underlying epicarp may overcome this type of resistance and substantially shorten the incubation period of *P. palmivora* (Tarjot, 1967b, 1974). According to Tarjot's findings, therefore, depth of wounding might assume importance in overcoming resistance to development within the epicarp, a possibility lent support by results from Rocha & Vello (1971) who reported that with a 1 mm wound Scavina 6 was still very resistant but with a 4 mm wound its resistance was lower. Presumably, a 1 mm needle-wound in pods of some resistant cultivars in some way is insufficient to allow the fungus to readily overcome the obstacle presented by the superficial epicarp layers mentioned by Tarjot,

whereas a 4 mm wound enables the fungus to completely and rapidly penetrate this layer and to extend freely throughout the pericarp tissues. However, it is uncertain whether a 1 mm-deep wound would fail to overcome resistance to epicarp penetration in all cultivars since the depth of the epicarp cells implicated by Tarjot might vary from cultivar to cultivar. A much more important consideration, however, is that wounding fails to take into account the possible role of the cuticle and epidermis in resistance to initial penetration. As Tarjot reported (1974), even if slight abrasions of the epidermis of some resistant pods are made with a scalpel at the inoculation site, rapid penetration and rotting of the pod ensues. In view of the uncertainties and disadvantages associated with wound-inoculation, it seems advisable not to adopt this practice for attached pods.

In all pod tests, pod material of the same age was used, thus eliminating variability due to age differences. For this reason, it was found that as few as 10 replicate pods per cultivar allowed satisfactory evaluation of response to infection, although whenever possible 20 replicates were preferred. The 50-100 replicates employed by Blaha (1971), while obviously providing a statistically more satisfactory sample, are too many for rapid screening when more than 1 or 2 cultivars are included in the same test. Some variation was found in the reaction of pods from different trees of each cultivar, even with cloned material. Thus, there is a danger that if pods of a given cultivar are all or mostly obtained from 1 tree, the test material will be less representative of that cultivar and misleading results might be obtained. To avoid this risk, as far as possible only 1 or 2 pods were used from each tree.

With point-inoculation of whole pods, 2 criteria for assessing susceptibility were adopted, (1) percentage successful infection to detect differences in penetration resistance, whether epidermal or epicarpal, and (2) av lesion diameters to determine post-penetration resistance, a system adopted by many workers (Thrower, 1960; Medeiros & Rocha, 1964, 1965; Blaha, 1967, 1971, 1972; Medeiros, 1967; Tarjot, 1967a,b, 1969a,b; Rocha & Medeiros, 1968; Rocha & Mariano, 1969. Initially, measurement of lesion areas (Medeiros & Rocha, 1964, 1965; Rocha & Medeiros, 1968; Rocha & Mariano, 1969; Rocha & Vello, 1971) rather than diameters was contemplated. However, no improvement in evaluation of cultivar response was derived from area measurements. As lesion areas were difficult to measure with any accuracy on pods with pronounced ridging and since tracing lesion outlines on to paper and measuring them with a planimeter also was laborious and time-consuming, the simpler diameter measurements were preferred. Pod lesions were always more or less circular and their diameters were easy to assess.

Although daily measurements of lesions were made in all pod tests, it was considered that 1 measurement made a given number of days after inoculation would yield the same information concerning post-penetration resistance as daily measurements carried out for 10 days or so. Measurements made only once not only greatly facilitated screening tests involving large quantities of test material but also produced just as clear-cut distinctions between resistance and susceptibility as values for av daily increase in lesion size (Fig. 1). It was concluded, therefore, that rate of lesion development could be more conveniently and just as reliably determined by measuring lesions only once after a standard number of days (Medeiros & Rocha, 1964, 1965; Prendergast, 1965; Rocha & Medeiros, 1968; Rocha & Mariano, 1969; Rocha & Vello, 1971; Rocha & Machado, 1972).

In the experiments reported here, all lesion measurements on attached pods were made a standard 7 days after the appearance of first symptoms. However, *P. palmivora* incubation periods and rates of lesion development varied according to seasonal temperature differences. Therefore, to standardize evaluation of susceptibility and to enable stricter comparisons between each test, in future screening it is recommended that measurements be made when lesion sizes on pods of the most susceptible cultivars attain an av diam of 6 cm. In this way, only pods of the most susceptible cultivar need to be measured more than once and results between different test runs will be more comparative. An av of 6 cm is recommended since it represents the maximum size range that the 2 opposite lesions can attain before coalescing on highly susceptible cultivars with very small pods. Under Turrialba conditions, when lesions on highly susceptible cultivars reached this diameter, the dispersion of lesion sizes between the most resistant and most susceptible cultivars was sufficient to allow adequate evaluation of cultivar response.

It was difficult to combine the 2 criteria, percentage infection and lesion size, to form a single, convenient yet significant numerical evaluation of cultivar response. Frequently, there was no correlation between the two (Tables 3 & 5) neither was it always certain whether absence of infection reflected resistance to penetration or was merely due to inoculation failure. With most cultivars, infection nearly always occurred at least at 1 inoculation point on all replicate pods and high infection values of 75-100% were usually obtained. Even with the cultivars that showed significantly lower percentage infection values (Pound 7, Scavina 6 and Scavina 12) resistance was equally well demonstrated by lesion sizes. It is possible, therefore, that for many cultivars records of percentage infection are of less value and that lesion sizes or rates of development may be the sole criterion required. However, it is recommended that percentage infection data be recorded to identify those cultivars whose resistance is based solely on resistance to penetration of the epidermis or superficial epicarp layers.

Although it may be difficult to combine the 2 criteria numerically, it is not difficult to draw conclusions by considering them jointly. For example, pod inoculations of the cultivar EET 338 in the CATIE collection produced only 35% successful infections, indicating low susceptibility to epidermal or epicarp penetration. However, whenever lesions did form they developed as rapidly as on the most susceptible cultivars. In contrast, EET 59 was highly susceptible to initial penetration (100% infection) but lesions developed very slowly and often ceased growth altogether 2-3 days after symptom appearance. The conclusion which can be drawn considering the 2 criteria is that, other things being equal, under natural conditions percentage pod losses probably are greater with EET 338 than with EET 59. Although infection may be low with EET 338, whenever it does occur the pods rot quickly. With EET 59 proportionately more pods may be infected but probabaly fewer become rotted before harvest. Observations tended to support this conclusion but pod or bean loss data were not available to confirm it. If this conclusion is correct, it further demonstrates that lesion size may more accurately reflect intrinsic susceptibility than percentage infection.

While recognising that percentage infection was a suitable criterion for evaluating resistance to epidermal penetration, Blaha & Lotodé (1976, 1977) maintained that av daily increase in lesion area was an unsatisfactory criterion for evaluating post-penetration resistance since, especially in the first 4 days of lesion expansion, it was influenced by percentage infection

and, therefore, tended to reflect resistance to epidermal penetration. This may be a limitation inherent in susceptibility criteria which involve recording daily increments in lesion expansion. In the present study, no such limitation was noted since av lesion diameters or areas, measured when the lesions were well developed, not only bore little relation to percentage infection but adequately reflected resistance to post-penetration. Consequently, the evaluation of post-penetration resistance devised by Blaha & Lotodé (1976, 1977), a *lesion growth index* derived from the slope of a line obtained by plotting log-transformed av lesion areas measured between the 7th and 10th days after inoculation, may be more complicated than is necessary for a simple and rapid screening test.

Since no correlation between resistance and length of incubation period was obtained, incubation period as a criterion for assessing resistance was not adopted. For the same reason, the *susceptibility index* of Tarjot (1967 a,b, 1969b) was not used since, with relatively uniform incubation periods, this proved to be just another expression for percentage infection values.

The amount of sporulation occurring on pod-lesion surfaces recorded a given number of days after inoculation (Turner, 1963, 1965a) was not adopted as a criterion, either, as this proved difficult to determine with any accuracy or consistency and, thus, was difficult to standardize. Recording the time for appearance of sporulation on inoculated pods (Blaha & Lotodé, 1976, 1977) also was considered but proved to be of little value since not only was time for sporulation erratic on replicate pods of the same cultivar but, as pointed out by Akinrefon (1971), it seemed to be related to lesion size (lesions of less than 6 cm diam on inoculated pods were never seen to sporulate) and, thus, to rate of pod tissue colonization by the fungus. Time to sporulation, therefore, tended to duplicate information obtained more reliably from measurements of lesion diameters.

Comparison of All Test Methods

The purpose of the screening method to be selected in these investigations was 2-fold; to evaluate resistance of fruiting cultivars or hybrids and to screen newly produced hybrid progenies for resistance to *P. palmivora*. With all methods examined in these investigations it was realised that no single method could adequately satisfy both requirements and it was decided to select 2 methods, 1 for each purpose. Consequently, all methods were considered and compared in the light of these 2 purposes, whilst also bearing in mind the requirements of a good screening test. Briefly, a screening test should (1) be rapid and easy to carry out and not involve complicated techniques or require sophisticated equipment, (2) be as comparative, standardized and reproducible as possible, (3) provide good correlation with response to natural infection and accurately reflect intrinsic resistance and (4) be capable of dealing with large numbers of test plants or plant parts.

Pod, leaf and branch inoculations were more appropriate for screening established cultivars since they utilized material immediately available in the field and there was no delay in preparing test material for inoculation. Seedling-stem, seedling-root and seed inoculations were less suitable for this purpose since they required longer periods for preparation of the cacao material to be tested. These latter methods, however, were more appropriate for testing hybrid progenies since they utilized the earliest hybrid material available, namely, seeds or seedlings.

Correlation methods were used to compare those screening methods which showed significant cultivar differences and consistency over replicate tests, namely those presented in Tables 3, 5, 6, 7, 8, 10 and 11. Eighteen variates, representing measures of susceptibility used in these methods, were compared with each other by means of product-moment correlation coefficients to determine the degree of alikeness between all paired combinations of these variates. Spearman rank correlation coefficients also were calculated to determine the relative agreement between the cultivar rank order in each of the same 18 variates plus a standard measure of inherent susceptibility. Ranks assigned to cultivars to express this inherent susceptibility were based on natural infection data, modified when necessary by av performance derived from the experimental data to overcome the difficulty posed by those cultivars (CC41, CC42 and UF29) whose intrinsic susceptibility was not reliably indicated by field evaluation. The pairwise correlations were based on the ranks of those cultivars present for both susceptibility measures. The number of degrees of freedom associated with the correlation between each pair of susceptibility measures provided a guide as to the weight to be attached to the corresponding values.

A feature of the analysis using product-moment correlation was the very close association (coefficients ranging from 0.80 to 1.00) between the following variates: log lesion diam (point-inoculation with zoospore suspension of unwounded attached pods; Table 5), log lesion diam (point-inoculation with mycelial-sporangial suspension of unwounded attached pods; Table 5), log lesion diam (point-inoculation with zoospore suspension of unwounded attached pods of different ages; Table 7), log lesion area (seedling-stem inoculations; Table 8), angle transformation of % emergence and emergence as % of control (seed inoculations with zoospore suspension; Table 10), angle % emergence and emergence as % of control (seed inoculation with zoospore suspension or mycelial-sporangial suspension; Table 11).

The primary interst in the second analysis was the comparison between each of the measures of susceptibility and the supposed true-ranking of the cultivars based on the measure of inherent susceptibility. Ranks within the same group of 8 self-consistent variates mentioned above together with log lesion area (branch inoculations; Table 8) correlated best with the ranks assumed to indicate inherent susceptibility, coefficients ranging between 0.90 and 1.00.

On the basis of these results and those from significance tests, and in conjunction with other considerations, such as rapidity, convenience and reproducibility of the test, the following conclusions were drawn.

Of all the pod inoculation methods examined, point-inoculation with zoospore suspension of unwounded attached pods provided the most reliable and consistent results. Detached pods of nearly all cultivars were more susceptible than attached pods and the degrees of resistance among the cultivars were slightly less apparent. This increase in susceptibility of detached pods, noted by many workers, including Medeiros & Rocha (1964, 1965), Medeiros & Melo (1966), Blaha (1967), Rocha & Mariano (1969) and Tarjot (1969b), would not be important if it were uniform with all cultivars. Unfortunately, some cultivars (notably Catongo) exhibit a disproportionate increase in susceptibility when pods are removed from the tree, while others may show increased resistance (Medeiros, 1967). Because of these anomalies, detached pod tests may not be reliable and

cannot be recommended. The same objection applies to the use of pod tissue blocks, a further disadvantage with this method being that it merely assesses resistance to epidermal or epicarpal penetration. It is regrettable that detached pod material is less suitable for evaluation methods as this kind of test is rapid and convenient, and, since it can be conducted under controlled conditions in the laboratory, is strictly comparative and highly standardized and reproducible.

Branch inoculations produced results which for evaluating cultivars in the field were as reliable as those obtained with point-inoculated unwounded attached pods. However, this method did not discriminate so satisfactorily between the test cultivars as did point-inoculation of attached pods. It is also a destructive method since whole branches have to be removed to record results, and branch inoculations may be less convenient than pod inoculations since with taller cacao trees, few branches of the required diameter or age are low enough to be easily reached without the awkward use of a ladder.

The leaf inoculation methods examined here were totally unsuitable. To avoid the type of anomalous results experienced with detached pods, leaves still attached to the plant were inoculated, but investigation of methods utilizing detached leaves or leaf portions in the laboratory (Tarjot, 1972d; Adebayo, 1975) might be fruitful.

Seedling-root inoculations gave poor and inconsistent results and as a method for screening hybrid progenies it was not as reliable as seed or seedling-stem inoculations. Results from seed and seedling-stem inoculations were basically similar. Both gave results which were consistent between replicate tests and good correlation with the known responses of the crosses to *P. palmivora* infection were obtained. However, with the seedling-stem method, gradations in response between resistant and susceptible material were slightly less distinct. The main advantage of the seed inoculation method over seedling-stem inoculations is that it is rapid and easy. The earliest possible hybrid progeny material available is used, thus reducing delays, and recording of results is exceptionally simple. Large quantities of hybrid progenies can be tested at a time and the test material is not wasted since, after results have been gathered, the test seedlings can be transplanted to the field for further investigations in the breeding programme. In comparison, seedling-stem inoculations were less rapid, requiring a delay of up to 4 months before seedlings had attained a size suitable for inoculating. Furthermore, the method is destructive and recording of data was more laborious and time-consuming than with seed inoculations.

Of the 2 seed inoculation methods investigated, that using zoospore suspension was preferred since, even though the inoculation procedure did not simulate the natural infection process of seeds, the inoculum was more standard and slightly clearer distinctions between resistant and susceptible reactions were obtained.

For the sake of comparison between all screening methods examined, the same *P. palmivora* isolate was used throughout, selected on the basis of pathogenicity on pods. The use of a single isolate is recognised as a limitation since not only may individual isolates show varying degrees of virulence on different cultivars (Leather, 1966; Ram & Ram, 1973; Zentmyer, 1972) but some isolates, even of the same morphotype, appear to exhibit differential virulence according to the cacao organ attacked (A.G. Medeiros, personal communication). Consequently, the use of a pod

isolate may not have represented the most appropriate strain of *P. palmivora* for use in tests involving inoculation of cacao organs other than pods. However, adequate infection and lesion formation appeared to have been obtained with stem, branch, root and leaf inoculations, and cultivar responses in most screening tests were similar to responses to natural pod infection in the field. Therefore, the limitation imposed by the use of the same fungus isolate in all tests may not have been too severe. Neverthless, selection of the *P. palmivora* isolate to be used in screening tests is important (Blaha, 1974). Since *P. palmivora* isolates may show differential virulence to certain cacao cultivars, it is advisable that many isolations be made initially and tested on pods, branches, seedling-stems or seeds (depending on the screening method selected) of several known resistant and susceptible cultivars to ensure selection of the most virulent or predominant strain (race or morphotype) of the fungus present in the testing area.

Methods involving measurements of *P. palmivora* development in media containing pod tissue extracts (Orellana, 1954b; Turner, 1962, 1963) were not examined in this study since it was considered that they fail to take into account possible physical and chemical resistance mechanisms (active or passive) imparted by or present in intact epidermal and pericarp tissues.

CONCLUSIONS AND RECOMMENDATIONS

With established trees in the field bearing fruit (cultivars or hybrids) the most reliable and consistent method for screening for resistance to *P. palmivora* was point-inoculation of unwounded attached pods using zoospore suspension. Pods of known age, 1 month from maturity (that is, when they have completed 83-85% of their time to maturity), should be used. A minimum of 10 pods per cultivar should be inoculated, although 20 replicates are preferable, and if possible, each pod should be from a different tree. Each cultivar should be tested at least twice at different times. Both percentage successful infection and av lesion diameters should be recorded, the latter when lesion sizes on pods of a standard, susceptible cultivar attain an av diam of 6 cm. This standard, susceptible cultivar, whose response to natural *P. palmivora* infection should be known and whose performance in pod inoculation tests should be well established, should be included in each test to provide a basis of comparison for the cultivars being screened as well as to check the efficiency and success of the inoculation procedure. A second, resistant, control cultivar may be included to provide a standard for levels of desirable resistance. In regions with pronounced seasonal differences it would be advisable to conduct tests during the times of highest natural incidence of *P. palmivora* when more favourable environmental conditions would help to ensure greater inoculation success and more reliable results.

If for any reason young, non-bearing trees need to be evaluated for resistance to *P. palmivora*, the branch inoculation technique would provide the most reliable substitute for pod inoculations.

The most satisfactory method for screening hybrid progenies was inoculation with zoospore suspension of pre-germinated seeds. The zoospore concentration to be used will be governed by the virulence of the *P. palmivora* isolate used but care should be taken to select the concentra-

tion which will provide the clearest distinction between the most resistant and most susceptible cacao types to be tested. Following planting, percentage emergence of healthy seedlings after a given interval (to be determined according to local climatic conditions and virulence of the *P. palmivora* isolate used) should be recorded. At least 100 seeds per hybrid should be inoculated together with at least 40 untreated control seeds and each test should be repeated. A standard susceptible hybrid should be included in each test to provide a basis of comparison and to check the success of the inoculation procedure.

The Prospects for Standardized Screening Methods

It is generally recognised that world standardization of methods for screening cacao for resistance to *P. palmivora* would be valuable in providing comparative information which would be of some significance for all cacao-producing countries. As Blaha (1974) pointed out, and as this study indicates, a single universal method cannot be recommended since certain methods are appropriate for certain objectives only and no others. Therefore, more than 1 method would have to be recommended to satisfy the requirements of various, differing objectives.

To be truly effective, international acceptance and adoption of such methods must be unanimous but, because opinions concerning test procedures vary widely among cacao investigators, this ideal may not be so easily realised. Furthermore, althought these screening methods might suffice for local needs to test cacao cultivars to indigenous strains of *P. palmivora*, material identified as resistant in 1 locality might well be much more susceptible in another where different environmental conditions might prevail and where other morphotypes and races of *P. palmivora* might be present. Theoretically, this limitation can be cirumvented by distributing cultivars identified as promising in various parts of the world and testing them in each cacao-producing region against local strains of *P. palmivora* (the converse, to test local cultivars against a range of imported *P. palmivora* isolates, would entail too great a risk to local cacao industries). In practice, however, considerable hazards could be attendant upon this kind of world-wide exchange of cacao material. At present, many cacao-growing countries do not maintain rigorous enough phytosanitary or quarantine precautions for the despatch or receipt of plant material and the risk of disseminating virulent types of *P. palmivora* or other serious cacao pathogens could be unacceptably high. The ideal solution, therefore, would be to establish international testing facilities in a country where cacao will never be in production, similar to those provided by the Coffee Rust Research Centre in Portugal for work on *Hemileia vastatrix*.

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LITERATURE CITED

ADEBAYO, A.A. (1971). A technique of inoculation of fixed cocoa pods with *Phytophthora palmivora* (Butl.) Butl. Turrialba 21:280-282.

ADEBAYO, A.A. (1975). Effect of age of host tissues on susceptibility of *Theobroma cacao* L. to *Phytophthora palmivora* (Butl.) Butl. and its relation to screening for resistance. *In* III Meeting African subgroup on *Phytophthora palmivora*, Tafo, Ghana, 1975. 15 pp. (mimeograph).

AKINREFON, O.A. (1971). Studies on the varietal reaction of cacao in Nigeria to *Phytophthora palmivora* (Butl.) Butl. Phytopath. Z. 70:359-365.

AMPONSAH, J.D. & ASARE-NYAKO, A. (1973). Glasshouse method for screening cocoa (*Theobroma cacao* L.) for resistance to black pod caused by *Phytophthora palmivora* (Butl.) Butl. Trop. Agric. (Trinidad) 50:143-152.

AMPONSAH, J.D. & DAKWA, J.T. (1969). Preliminary genetic assessment of a black-pod resistance trial in Ghana. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 162-165.

ASOMANING, E.J.A. (1964). Varietal resistance of young clones and seedlings of cacao (*Theobroma cacao* L.) to root infection by *Phytophthora palmivora*. Trop. Agric. (Trinidad) 41:251-256.

ASOMANING, E.J.A. & WHARTON, A.L. (1963). Black pod disease. Root infection by *Phytophthora palmivora* Butl. Studies on varietal resistance to root infection. Ann. Rep. W. Afr. Cocoa Res. Inst., 1961-1962. pp. 27-28.

BESSE, J. (1969). Bilan des travaux de sélection et d'amélioration du cacaoyer en Côte d'Ivoire. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 57-70.

BLAHA, G. (1967). *Phytophthora palmivora* (Butl.) Butl.: Variation de la pathogénie en fonction de la source de l'inoculum. Café-Cacao-Thé 11:331-336.

BLAHA, G. (1971). Contribution à l'étude de la sensibilité du cacaoyer à *Phytophthora palmivora* au Cameroun. *In* III Intern. Cocoa Res. Conf., Accra, Ghana, 1969. Tafo, Cocoa Res. Inst. Ghana. pp. 410-421.

BLAHA, G. (1972). Recherche de cultivars résistants à *Phytophthora palmivora*. Étude comparative des réactions aux infections experimentales effectuées sur cabosses. *In* IV Intern. Cocoa Res. Conf., Trinidad & Tobago, 1972. Government of Trinidad and Tobago. pp. 435-445.

BLAHA, G. (1974). Methods of testing resistance. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 179-195.

BLAHA, G. & LOTODÉ, R. (1976). Un critère primordial de sélection du cacaoyer au Cameroun: La résistance à la pourriture brune des cabosses (*Phytophthora palmivora*). Café-Cacao-Thé 20:97-116.

BLAHA, G. & LOTODÉ, R. (1977). Contribution à la connaissance des modalités de la transmission héréditaire de la résistance du cacaoyer à la pourriture brune des cabosses (*Phytophthora palmivora*) au Cameroun. Café-Cacao-Thé 21:179-196.

BLENCOWE, J.W. (1962). Planes futuros para mejoramiento genético en Ghana. Cacao (Costa Rica) 7(1):11-12.

BLENCOWE, J.W. & WHARTON, A.L. (1961). Black pod disease in Ghana: Incidence of the disease in relation to levels of productivity. Rep. VI Commonw. Mycol. Conf., Kew, 1960. pp. 139-147.

DAKWA, J.T. (1968). Black pod disease. Ann. Rep. Cocoa Res. Inst. Ghana, 1966-1967. pp. 30-33.

ESQUIVEL, O. (1973). Estudios sobre la reacción de resistencia de algunos cultivares de cacao (*Theobroma cacao* L.) a la pudrición de frutos causada por *Phytophthora palmivora* (Butl.) Butl. en la región atlántica de Costa Rica. M.S. Thesis, IICA, Turrialba, Costa Rica.

GALINDO, J.E. & SALAZAR, A. (1965). Development of a technique to evaluate the resistance of cacao pods to *Phytophthora palmivora* at the seedling stage. Ann. Rep. Director Res. to Board Directors Amer. Cocoa Res. Inst., 1964-1965.

GORENZ, A.M. (1971) The cotton-pad placement method of inoculating cocoa pods in the field or laboratory. *In* I Réunion de sous-groupe de travail Afrique sur le *Phytophthora palmivora*, Yaoundé, Cameroun, 1971. (mimeograph).

GREGORY, P.H. (1969). Black pod disease project report. London, The Cocoa, Chocolate and Confectionery Alliance. pp. 36 & 43.

GUNAWARDENA, K.J. (1966). Yield characters of some foreign and local cocoa clones and their susceptibility to pod rot. *In* Papers II Session FAO Tech. Working Party on Cocoa Production and Protection, Rome, 1966. FAO Working Pap. Ca/66/2. 5pp.

HANSEN, A.J. (1961). Resistance and susceptibility of different cacao varieties to *Phytophthora palmivora*. Cacao (Costa Rica) 6(3):8.

HISLOP, E.C. & PARK, P.O. (1962). Studies on the chemical control of *Phytophthora palmivora* (Butl.) Butl. on *Theobroma cacao* L. in Nigeria. I. Laboratory bio-assay of fungicides on detached pods. Ann. appl. Biol. 50:57-65.

HOLLIDAY, P. (1954). Notes on the control of black pod disease and witches' broom disease in Trinidad. *In* V Réunion Comité Téc. Interamer. del Cacao, IICA, Turrialba, Costa Rica, 1954. Vol.1 Sección Fitopatología, Doc. 15. 5 pp.

KRUG, C.A. & QUARTEY-PAPAFIO, E. (1964). World cocoa survey. FAO Agric. Studies No. 63. Rome. 242 pp.

LEATHER, R.I. (1966). Studies on the reaction of some cocoa varieties to *Phytophthora palmivora* (Butl.) Butl. in Jamaica. Expl. Agric. 2:107-112.

LEGG, J.T. (1970). Black pod disease. Stem cankers. Ann. Rep. Cocoa Res. Inst. Ghana, 1968-1969. p.36.

LELLIS, W.T. & PEIXOTO, O. (1960). Comunicação preliminar sobre a resistência do cacau *Catongo* ao *Phytophthora palmivora* Butl. Inst. Cacau Bahia, Bol. Inf. No. 61. pp. 26-31.

MEDEIROS, A.G. (1967). Methodology of research to study resistance of cacao to *Phytophthora palmivora* (Butl.) Butl. *In* Conf. Intern. Recherches Agron. Cacaoyères, Abidjan, Ivory Coast, 1965. Paris. pp. 205-211.

MEDEIROS, A.G. (1977). Método standard para produção de zoosporângios e zoósporos de *Phytophthora palmivora* (Butl.) Butl. Revista Theobroma 7:107-111.

MEDEIROS, A.G. & MACHADO, A.D. (1967). Variação da resistência do tecido de fruto de cacau à *podridão parda*. Inf. Téc. CEPLAC, Centro Pesquisas Cacau, Itabuna, Brasil, 1967. pp. 47-48.

MEDEIROS, A.G. & MELO, W.J. (1966). Seleção de cacaueiros resistentes à podridão parda. Inf. Téc. CEPLAC, Centro Pesquisas Cacau, Itabuna, Brasil, 1966. pp. 45-50.

MEDEIROS, A.G. & ROCHA, H.M. (1964). Programação dos trabalhos de seleção de cacaueiros resistentes à *podridão parda* no Estado da Bahia, Brasil. Phytopathology 55:125-126 (Abstr.).

MEDEIROS, A.G. & ROCHA, H.M. (1965). Estudo da resistência do cacau Catongo à *podridão parda* (*Phytophthora palmivora* (Butl.) Butl.). Relatório Anual CEPLAC, Centro Pesquisas Cacau, Itabuna, Brasil, 1964. p. 29.

MIRCETICH, S.M. (1965). Studies on the pathogenicity of some *Phytophthora palmivora* isolates to cacao varieties. Ann. Rep. Director Res. to Board Directors Amer. Cocoa Res. Inst., 1964-1965.

MULLER, R.A. (1971). À propos de l'épidémiologie de *Phytophthora*. *In* I Réunion de sous-groupe de travail Afrique sur le *Phytophthora palmivora*, Yaoundé, Cameroun, 1971. (mimeograph).

OPEKE, L.K. & GORENZ, A.M. (1974). *Phytophthora* pod rot: symptoms and economic importance. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 117-124.

ORELLANA, R.G. (1953). Studies on *Phytophthora* pod rot of cocoa in Costa Rica. *In* Rep. Proc. Cocoa Conf., 1953. London, The Cocoa, Chocolate and Confectionery Alliance. pp. 117-120.

ORELLANA, R.G. (1954a). Estudios sobre la podredumbre de las frutas del cacao causada por *Phytophthora palmivora* en Costa Rica. Turrialba 4:35-38.

ORELLANA, R.G. (1954b). Growth of *Phytophthora palmivora* of cacao in liquid media containing cacao shell from different clones as a basis for assessment of resistance. *In* V Reunión Comité Interamer. del Cacao, IICA, Turrialba, Costa Rica, 1954. Vol. 1 Sección Fitopatología, Doc. 29. 3 pp.

PARTIOT, M. (1975). La résistance horizontale du cacaoyer au *Phytophthora* sp. Méthodes d'évaluation précoce. Café-Cacao-Thé 19:123-136.

PRENDERGAST, W.N.E. (1965). Studies in the resistance of *Theobroma cacao* L. to *Phytophthora palmivora* (Butl.) Butl. M.Sc. Thesis, Univ. W. Indies, St. Augustine, Trinidad & Tobago.

PRENDERGAST, W.N.E. & SPENCE, J.A. (1967). A contribution to the study of the resistance of *Theobroma cacao* L. to *Phytophthora palmivora* (Butl.) Butl. *In* Conf. Intern. Recherches Agron. Cacaoyères, Abidjan, Ivory Coast, 1965. Paris. pp. 212-216.

RAM, C. & RAM, A. (1973). Virulence of some selected isolates of *Phytophthora palmivora* in cacao. Revista Theobroma 3(3):12-16.

RAVISÉ, A. (1970). Modalités du parasitisme de souches de *Phytophthora* de Bary sur jeunes cacaoyers. Café-Cacao-Thé 14:295-302.

ROCHA, H.M. (1965). Cacao varieties resistant to *Phytophthora palmivora* (Butl.) Butl. A literature review. Cacao (Costa Rica) 10(1):1-10.

ROCHA, H.M. (1966). La importancia de las sustancias polifenólicas en el mecanismo de la resistencia de cacao (*Theobroma cacao* L.) a *Phytophthora palmivora* (Butl.) Butl. M.Sc. Thesis, IICA, Turrialba, Costa Rica.

ROCHA, H.M. & MACHADO, A.D. (1972). Pesquisas sobre *podridão parda* na Bahia, Brasil. *In* IV Intern. Cocoa Res. Conf., Trinidad & Tobago, 1972. Government of Trinidad and Tobago. pp. 360-378.

ROCHA, H.M. & MARIANO, A.H. (1969). Seleção de cultivares de cacau resistentes à *Phytophthora palmivora* (Butl.) Butl. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 166-169.

ROCHA, H.M. & MEDEIROS, A.G. (1968). Basis for selection of cacao cultivars resistant to *Phytophthora palmivora* (Butl.) Butl. Cacao (Costa Rica) 13(4):10-17.

ROCHA, H.M. & VELLO, F. (1971). Estudos sobre resistência do cacau (*Theobroma cacao* L.) à *Phytophthora palmivora* (Butl.) Butl. *In* III Intern. Cocoa Res. Conf., Accra, Ghana, 1969. Tafo, Cocoa Res. Inst. Ghana. pp. 430-438.

SANSOME, E., BRASIER, C.M. & GRIFFIN, M.J. (1975). Chromosome size differences in *Phytophthora palmivora*, a pathogen of cocoa. Nature (London) 255:704-705.

SILLER, L.R. & MCLAUGHLIN, J.H. (1950). A method of evaluating fungicides for the control of *Phytophthora palmivora* on *Theobroma cacao* L. Cacao (Costa Rica) 2(10):1-3.

SORIA, J. (1974). Sources of resistance to *Phytophthora palmivora*. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 197-202.

SORIA, J. & ESQUIVEL, O. (1966). Niveles de infección de *Phytophthora palmivora* sobre cultivares de cacao en condiciones de campo. Fitotecnia Latinoamericana 3:119-124.

SORIA, J.& ESQUIVEL,O. (1970a). Herencia a la resistencia a enfermedades del cacao. Resistencia a *Phytophthora palmivora*. Cacao (Costa Rica) 15(3):5-6.

SORIA,J.& ESQUIVEL, O. (1970b). Mejoramiento genético del cacao. Selección de plantas para rendimiento y enfermedades y perpetuación con propagación asexual. Cacao (Costa Rica) 15(3):7.

SPENCE, J.A. (1961a). Black pod disease of cacao. I. A comparison of isolates of *Phytophthora palmivora* (Butl.) Butl. Ann. appl. biol. 49:717-722.

SPENCE, J.A. (1961b). Probable mechanism of resistance of varieties of cacao to black pod disease caused by *Phytophthora palmivora* (Butl.) Butl. Nature (London) 192:278.

SPENCE, J.A. & BARTLEY, B.G. (1966). Testing of breeding material of *Theobroma cacao* for resistance to black pod disease (*Phytophthora palmivora*). *In* Papers II Session FAO Tech. Working Party on Cocoa Production and Protection, Rome, 1966. FAO Working Pap. Ca/66/6. 6pp.

SREENIVASAN, T.N. (1975). A new method for screening cocoa for resistance to *Phytophthora palmivora* (Butler) Butler. *In* V Intern. Cocoa Res. Conf., Ibadan, Nigeria, 1975. 12pp. (mimeograph).

TARJOT, M. (1965). Étude de la résistance des cacaoyers à la pourriture brune des cabosses dûe au *Phytophthora palmivora* (Butl.) Butl. en Côte d'Ivoire. I. Inoculations expérimentales par blessure et par fragment de culture. Café-Cacao-Thé 9:126-133.

TARJOT, M. (1967a). Étude de la résistance des cacaoyers à la pourriture brune des cabosses dûe au *Phytophthora palmivora* (Butl.) Butl. en Côte d'Ivoire. Inoculations expérimentales par blessure et fragment de culture. *In* Conf. Intern. Recherches Agron. Cacaoyères, Abidjan,

TARJOT, M. (1967b). Étude de la résistance des cacaoyers a la pourriture dûe au *Phytophthora palmivora* (Butl.) Butl. en Côte d'Ivoire. II. Inoculations expérimentales au laboratoire par dépôt d'une goutte de suspension de zoospores sur la cabosse sans blessure. Café-Cacao-Thé 11:3-13.

TARJOT, M. (1969a). Variations de sensibilité des cabosses de cacaoyer envers le *Phytophthora palmivora* suivant la période de l'année. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 213-218.

TARJOT, M. (1969b). Étude de la résistance des cacaoyers à la pourriture brune des cabosses dûe au *Phytophthora palmivora* (Butl.) Butl. en Côte d'Ivoire. III. Inoculations expérimentales sur le terrain. Café-Cacao-Thé 13:297-309.

TARJOT, M. (1972a). Relations possibles entre la variation de la teneur en éléments minéraux de la cabosse desdifférents cacaoyers et la sensibilité envers le *Phytophthora palmivora*. *In* IV Intern. Cocoa Res. Conf., Trinidad & Tobago, 1972. Government of Trinidad and Tobago. pp. 398-405.

TARJOT, M. (1972b). Étude d'un facteur de sensibilité des cabosses de cacaoyer envers le *Phytophthora palmivora*: La teneur en eau des tissus du péricarp. *In* IV Intern. Cocoa Res. Conf., Trinidad & Tobago, 1972. Government of Trinidad and Tobago. pp. 406-411.

TARJOT, M. (1972c). Étude anatomique de la cabosse de cacaoyer en relation avec l'attaque du *Phytophthora palmivora*. Café-Cacao-Thé 16:123-134.

TARJOT, M. (1972d). Contribution à l'étude du comportement des feuilles de cacaoyer envers le *Phytophthora palmivora*. Café-Cacao-Thé 16: 333-338.

TARJOT, M. (1974). Physiology of the fungus. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 103-116.

TARJOT, M. (1977). A study of black pod in the cacao-tree due to *Phytophthora palmivora*. A first adaptation to Cameroon conditions of an early sensitivity test on young seedlings. *In* VI Intern. Cocoa Res. Conf., Caracas, Venezuela, 1977. 11pp. (mimeograph).

THOROLD, C.A. (1953). The control of black pod disease of cocoa in the Western Region of Nigeria. Rep. Proc. Cocoa Conf., 1953. London, The Cocoa, Chocolate & Confectionery Alliance. pp. 108-115.

THOROLD, C.A. (1955). Observations on black pod disease (*Phytophthora palmivora*) of cocoa in Nigeria. Trans. Brit. Mycol.Soc. 38:435-452.

THOROLD, C.A. (1956). Observations on cacao production in relation to the incidence of black-pod disease, caused by *Phytophthora palmivora*. J. hortic. Sci. 31:149-155.

THOROLD, C.A. (1967). Black pod disease of *Theobroma cacao*. Rev. appl. mycol. 46:225-237.

Ivory Coast, 1965. Paris. pp. 217-225.

THOROLD, C.A. (1975). Diseases of cocoa. Oxford, Clarendon Press. pp. 197-198.

THROWER, L.B. (1960). Observations on the diseases of cacao pods in Papua-New Guinea. I. Fungi associated with mature pods. Trop. Agric. (Trinidad) 37:111-120.

TOXOPEUS, H. & GORENZ, A.M. (1970). *Phytophthora* pod rot. A routine screening technique for resistance. Ann. Rep. Cocoa Res. Inst. Nigeria, 1968-1969. pp. 109-110.

TURNER, P.D. (1961). Black pod disease. Strains of *Phytophthora palmivora*. Ann. Rep. W. Afr. Cocoa Res. Inst., 1959-1960. pp. 20-24.

TURNER, P.D. (1962). Black pod disease. Resistance and tolerance. Laboratory studies. Ann. Rep. W. Afr. Cocoa Res. Inst., 1960-1961. pp. 29-32.

TURNER, P.D. (1963). Black pod disease. Resistance and tolerance. Laboratory studies. Ann. Rep. W. Afr. Cocoa Res. Inst., 1961-1962. pp. 21-25.

TURNER, P.D. (1965a). Comparative sporulation as an indication of resistance by cacao to *Phytophthora* pod rot. Trop. Agric. (Trinidad) 42:305-309.

TURNER, P.D. (1965b). Polyphenoloxidase activity in cacao selections showing variable resistance to *Phytophthora* pod rot. Pl. Dis. Reptr. 49:319-321.

TURNER, P.D. & ASOMANING, E.J.A. (1963). Black pod disease. Root infection of cocoa by *Phytophthora palmivora* Butl. Infection of Amelonado seedlings. Ann. Rep. W. Afr. Cocoa Res. Inst., 1961-1962. pp. 25-27.

WATERHOUSE, G.M. (1974). *Phytophthora palmivora* and some related species. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 51-70.

WESTSTEIJN, G. (1965). Black-pod studies. Root infection by *Phytophthora palmivora*. Ann. Rep. W. Afr. Cocoa Res. Inst., 1963-1964. pp. 48-51.

WESTSTEIJN, G. (1966a). Susceptibility of *Theobroma cacao* L. to *Phytophthora* pod rot disease: Field observations on clonal cacao. *In* Papers II Session FAO Tech. Working Party on Cocoa Production and Protection, Rome, 1966. FAO Working Pap. Ca/66/29. 19 pp.

WESTSTEIJN, G. (1966b). Studies on black-pod disease. Ann. Rep. W. Afr. Cocoa Res. Inst., 1964-1965. pp. 65-71.

WESTSTEIJN, G. (1967). Studies on black pod disease. Search for resistance. Ann. Rep. W. Afr. Cocoa Res. Inst., 1965-1966. pp. 118-122.

WESTSTEIJN, G. (1968). Studies on *Phytophthora* pod rot disease. Search for resistance. Ann. Rep. Cocoa Res. Inst. Nigeria, 1966-1967. pp. 71-74.

WESTSTEIJN, G. (1969). Methods of screening *Theobroma cacao L.* for resistance to *Phytophthora* pod rot disease. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 157-161.

WHARTON, A.L. (1957). Black pod disease resistance and tolerance. *In* Proc. Cocoa Breeding Conf., W. Afr. Cocoa Res. Inst., Ghana, 1956. pp. 7-9.

WHARTON, A.L. (1959). Black pod disease. Ann. Rep. W. Afr. Cocoa Res. Inst., 1957-1958. pp. 25-30.

WHARTON, A.L. (1960). Black pod disease. Resistance and tolerance. Field investigations. Ann. Rep. W. Afr. Cocoa Res. Inst., 1958-1959. pp. 26-27.

WHARTON, A.L. (1961). Black pod disease. Resistance and tolerance. Ann. Rep. W. Afr. Cocoa Res. Inst., 1959-1960. pp. 24-25.

WHARTON, A.L. (1962). Black pod disease. Resistance and tolerance. Field investigations. Ann. Rep. W. Afr. Cocoa Res. Inst., 1960-1961. pp. 28-29.

ZENTMYER, G.A. (1969). Resistance of cacao to *Phytophthora palmivora*. *In* II Conf. Intern. Pesquisas Cacau, Salvador & Itabuna, Brasil, 1967. Itabuna, Centro Pesquisas Cacau. pp. 147-149.

ZENTMYER, G.A. (1972). Resistance of cacao to *Phytophthora palmivora*. *In* IV Intern. Cocoa Res. Conf., Trinidad & Tobago, 1972. Government of Trinidad and Tobago. pp. 469-470.

ZENTMYER, G.A. (1974). Variation, genetics and geographical distribution of mating types. *In* Gregory, P.H., ed. *Phytophthora* disease of cocoa. London, Longman. pp. 89-101.

ZENTMYER, G.A., MIRCETICH, S.M. & MITCHELL, D.M. (1968). Test for resistance of cacao to *Phytophthora palmivora*. Pl. Dis. Reptr. 52:790-791.

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