UNIVERSITY OF CAPE COAST

EVALUATION OF THE EFFICACY OF RESISTANCE SCREENING METHODS USED IN THE BREEDING OF BLACK POD DISEASE RESISTANT VARIETIES IN CACAO.

BY

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DEDICATION

To the glory of God and to my family.

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ABSTRACT

The resistance of cacao (*Theobroma cacao* L.) leaves and pods to *Phytophthora palmivora* (Butler) Butler was investigated in 25 international genotypes of cacao in five laboratory experiments and a field trial at Cocoa Research Institute of Ghana at Tafo. Tissue–paper–mount and punch-inoculation methods were used to distinguish between resistance at the penetration and post-penetration levels based on lesion frequency and size, respectively. A significant clonal difference for leaf and pod resistance at the two stages of infection was observed. Correlation between resistance of leaves and pods was positive and significant at both penetration and post-penetration stages of infection. The occurrence of such a characteristic of cacao leaf suggests the possibility of the use of leaves of cocoa seedlings for the prediction of pod resistance to the black pod disease. The linear correlation between the detached pod tests and leaf disc test was significant. A highly significant difference was observed among levels of infection of distal, proximal and mid regions of pods inoculated with *Phytophthora palmivora.*

The reliability of the tests was evaluated by correlating results of the inoculation tests with the level of field infection. These correlations were generally positive and significant, both for detached pod and leaf tests. The high positive correlation between detached leaves and pods and natural *Phytophthora* pod rot infection in the field showed that detached organs can be used for prediction of resistance in the field. SCA 6, T85/799, LAF1, ICS 1 and GU 225V were noticed to be promising clones for breeding against black

pod disease. The susceptible clones were MO 20, T79/501, VENC 4-4, PA 120 and MOCORONGO.

The results suggest that routine application of the detached leaf and pod tests if carried out under uniform conditions and in standardized manner should both be sufficient in selection of new varieties with high levels of field resistance to black pod.

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INTRODUCTION

Cocao (*Theobroma cacao* L.) of the family Sterculiaceae is a major economic crop in Ghana. The crop is an understorey tree species found in the tropical forests of Central and South America (Wood and Lass, 1985).

The cocoa bean, used in the manufacture of chocolates, cocoa powder and cocoa butter, has become a commodity of economic importance in many countries which derive a major part of their foreign exchange earnings from it. In 2002, foreign revenue derived from sale of cocoa in Ghana was 463 million US dollars, representing 22.4% of foreign revenue from agricultural and non-agricultural sources (Anon, 2003).

Pests and diseases are among the factors which limit cacao production in Ghana. Of the diseases, black pod is the most serious constraint to cacao production. The disease affects the pods, beans, flower cushions, leaves, stems and roots. Adomako (2007) reported that yield loss in cacao was largely due to black pod representing 64.1% of total yield loss. In 1985 worldwide losses due to black pod were estimated at £1540 million (Evans and Prior, 1987). On pods, the predominant symptom is a brownish or black lesion on the husk, leading to blackening and rotting of the pod. On stems, the symptoms appear as cankers (Opoku *et al.*, 2007). Even though damage due to the leaf and root infections by *Phytophthora* species are difficult to estimate, their effect on the health and productivity of cacao trees are known to be significant.

Phytophthora species is a serious pathogen in West Africa. Pod rot and stem canker caused cacao pod losses of up to 63% and the death of up to 10% of trees annually on Kar Kar Island, Papua New Guinea (Guest *et al.*, 1994). Black pod is a serious disease in Ghana and can cause between 60 -100% crop losses (Dakwa, 1988). Pod rot disease is economically important in Indonesia, the Philippines, India, the Pacific Islands and Jamaica and often causes severe losses due to loss of entire trees.

Dakwa (1987), Luterbacker and Akrofi (1993) and Opoku *et al.* (1999) have reported two species of *Phytophthora viz. Phytophthora palmivora* and *Phytophthora megakarya* as the causal agents of black-pod disease in Ghana. *Phytophthora megakarya* is of more recent occurrence in Ghana and the more destructive of the two pathogens. In areas invaded by *Phytophthora magakarya*, the pod losses have increased from an average of 15% due to *Phytophthora palmivora* to 30- 35% due to *Phytophthora magakarya* (Kebe, 1999).

The control of black pod disease is therefore a major challenge for world cocoa cultivation and selection of resistant materials is an effort being made in many producing countries to control *Phytophthora.* According to Tan and Tan (1990), several methods have been adopted by farmers to control diseases caused by *Phytophthora* species in cacao of which the most common is the use of copper–based fungicides. Although this is reasonably effective, the high cost of chemical control poses a serious challenge to peasant farmers who produce over 50% of the world cocoa. Moreover, these chemicals are toxic to animals including man and therefore pose a great danger to peasant farmers most of whom are illiterates (Anon, 1995). These

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problems have led to a greater appreciation of the need for an alternative method of control such as the use of resistant cultivars. Breeding for resistance to black-pod disease has hence been long regarded as the most economical, environmentally friendly and effective control method (Iwaro *et al.*, 2000b). Genetic studies have shown that resistance to black pod attack is polygenically inherited and could be improved by recurrent selection (Iwaro *et al.*, 1999).

Field observations of cacao cultivars have also shown consistent differences in levels of infection and although no selection has shown complete immunity, there is sufficient variability for incorporation into breeding programs. Adomako (2006) reported significant differences between cacao genotypes in the levels of black pod disease in two field trials.

In breeding for resistance to black-pod, there is the need to screen the progenies in order to select the most resistant plants. However, progress in breeding has been hampered by lack of quick screening methods to identify genotypes with superior abilities to be incorporated into breeding programmes. Selection of resistant material in the field and all the known tests for resistance based on pod inoculation necessitate waiting until a tree is bearing before its level of resistance to black pod infection can be determined (Dakwa, 1988). These methods therefore do not offer a quick assessment of the results of breeding for resistance. The pod tests for resistance and those based on pod extracts are useful for identifying resistant parents, but they require too much time for progeny testing. Consequently, tests at the seedling stage that provide information about pod resistance are earnestly sought to save time and cost. However, it has not been firmly established that there is a correlation between

the reactions of detached leaves and pod tests to *Phytophthora palmivora* infection in the field.

Wheeler (1992) emphasized the need to understand the relationship between pod resistance and leaf resistance to *Phytophthora palmivora* and the relationship between detached leaves and pod tests to *Phytophthora palmivora* infection in the field to expedite the screening of germplasm.

The main objective of the study was to evaluate the efficacy of resistance screening methods used in breeding black pod disease resistance in cacao.

The specific objectives were to determine:

1. Clonal differences in leaf and pod resistance at the penetration stage of infection.
2. Clonal differences in leaf and pod resistance at the post-penetration stage of infection.
3. The relationship between resistance of leaf and pod at the penetration stage of infection.
4. The relationship between resistance of leaf and pod at the post-penetration stage of infection.
5. The relationship between leaf disc scores and penetration and post-penetration resistance of pod, respectively.
6. The relationship between laboratory screening methods and natural field infection of *Phytophthora palmivora.*

CHAPTER TWO

LITERATURE REVIEW

The Cacao Plant

Cacao (*Theobroma cacao* L.) - which literally means "food of the gods" is one of 22 species that constitute the genus *Theobroma,* a member of the family Sterculiaceae and the principal commercially important member of the genus. Botanically, the term 'cacao' refers to the tree and its fruits whilst 'cocoa' describes the bulk commercial dried fermented beans, as well as the powder produced from the beans. Cacao in its natural environment occurs as an understorey plant.

The centre of greatest diversity and origin of the tree is the dense tropical forests of the upper Amazon and Orinoco River basins of South America, where it occurs as a sub-canopy plant. *Theobroma cacao* is assumed to have spread naturally westward and northward to Guyana and Mexico and Central America at the time of European contact in the fifteenth century. Currently, extensive production occurs in the tropics of the old world, owing to the high disease prevalence in the native American tropics of the cacao tree. Cacao production systems vary greatly within and between countries and planting materials are still largely made up of unselected seedling populations. There is also little information on the possible interaction between cacao genotypes and production systems (Eskes, 2004).

Cacao Types

The species *Theobroma cacao* comprises a large number of highly morphologically variable populations. Three main populations of cacao have been identified, namely, the Criollo, the Forastero and the Trinitario (Cheeseman, 1944). The Forasteros constitute the largest cacao population and even though they have less flavour than the Criollo, they constitute the bulk of the cocoa produced in West Africa as well as that of the world. The Trinitarios are thought to have been hybridized from Criollo and Forastero initially from Trinidad (Coe and Coe, 1996). The Criollos are the best flavoursome cocoa but are difficult to establish and prone to attacks by diseases and pests.

The Black-pod Disease

Of all cacao diseases world-wide, Black pod or *Phytophthora* pod rot causes the largest loss of cocoa production.

Phytophthora species is typically found in tropical or warm, temperate countries with high rainfall. It is believed to have arisen in South-East Asia where much genetic diversity of the *Phytophthora* species occurs (Blaha *et al.*, 1994; Mchau and Coffey, 1994). The record of *Phytophthora* species for Equitorial Guinea is from the Island of Bioko (Prior, 1985).

Taxonomy and Morphology of *Phytophthora* species

The family Pythiaceae comprises the genera *Phytophthora, Pythiogeton* and *Pythium* (Blackwell, 1949). Butler originally described *Phytophthora species* as *Pythium palmivorum*, later transferring it to *Phytophthora* because zoospore cleavage occurs inside the sporangium, not in an external vesicle.

Until 1979, it was considered that the causal pathogen of Phytophthora pod rot of black-pod was *Phytophthora palmivora*, a ubiquitous and extremely diverse species encompassing a wide range of morphological and pathogenic variants (Brasier *et al.*, 1981). A worldwide study of isolates of *Phytophthora palmivora* from diseased cacao showed that *Phytophthora palmivora* comprised a complex of organisms which included a new species, *Phytophthora megakarya* and also 'MF4' later assigned to *Phytophthora capsici* (Brasier and Griffin, 1979).

Laboratory research has demonstrated that the *Phytophthora* isolates originally designated by E.J.Butler and others as *Phytophthora palmivora* were really a complex of several species, now designated as *Phytophthora palmivora, Phytophthora megakarya, Phytophthora capsici, Phytophthora citrophthora* and possibly *Phytophthora megasperma.* These species may be distinguished by the deciduous or persistent nature of the sporangia. The deciduous species (*Phytophthora palmivora, Phytophthora megasperma and Phytophthora capsici*) can be distinguished by the length of the sporangial pedicel (Idosu and Zentmyer, 1978).

There are other differences in the morphology of sporangia, chlamydospore production, and in some of the species, in chromosome number and size. *Phytophthora palmivora* has short, thick sporangial stalks less than 5µm long. This is the most common species pathogenic to cacao worldwide.

Phytophthora megakarya has thin sporangial stalks ranging usually from 5 to 15µm in length (Brasier and Griffin, 1979). This species has limited distribution, but it is the primary black pod pathogen in Nigeria.

Sansome *et al.* (1975) and Brasier and Griffin (1979) reported that *Phytophthora palmivora* and *Phytophthora megakarya* differ in size and number of chromosomes; *Phytophthora palmivora* has 9-10 small chromosomes and *Phytophthora megakarya* has five large chromosomes.

Phytophthora capsici has long sporangial stalks, up to 250µm long and sporangia with length to breadth ratios often over 2.0. This species is found in Central and South America and in Cameroon in West Africa. It is the principal black pod pathogen in Brazil. This strange distribution, quite widespread in Latin America but in only one country in West Africa, indicates that this species must have been carried from Latin America, probably from Brazil, to Cameroon in cacao material , possibly early in the development of the cocoa industry in West Africa (Blaha *et al.*, 1994). Recently, a fourth species, *Phytophthora citrophthora,* was found to be a cause of black pod and canker of cacao in Brazil (Kellam and Zentmyer, 1981a). This species has persistent sporangia and also forms chlamydospores.

Gregory and Madison (1981) noted that differences in the epidemiology of black pod disease that have been observed in different parts of the world may be due to the presence or absence of these different species of *Phytophthora.* It is most likely that *Phytophthora arecae* was a morphological variant of *Phytophthora palmivora* described before the wider variation within the species had been determined and was considered a synonym of *Phytophthora palmivora* by Blaha *et al.* (1994).

In Ghana, the black pod disease is known to be caused by two species, *Phytophthora palmivora and Phytophthora megakarya* (Dakwa, 1987; Luterbacker and Akrofi, 1993; Opoku *et al.*, 1999).

Many differences exist in the type of symptoms produced by *Phytophthora palmivora* and *Phytophthora megakarya* on the pod and on the stem (Dakwa, 1987; Opoku *et al.*, 1996). Generally, losses due to *Phytophthora megakarya* range from 60-80% in newly affected farms to about 100% in old affected farms in the black pod season. Losses for *Phytophthora palmivora* are estimated as 4.9-19% (Blencowe and Wharton, 1961; Dakwa, 1984). In studies conducted in twenty one farms, *Phytophthora megakarya* canker area tended to be located at the base or near the base of the tree trunk (up to 40cm above ground level) whilst *Phytophthora palmivora* cankers are usually concentrated on the tree trunk at heights between 41 and 100cm from the ground level (Opoku *et al.*, 1996). Unlike *Phytophthora palmivora* which may have large lesions without any visible spores, sporulation of *Phytophthora megakarya* is generally evident even on small lesions (Opoku *et al.*, 2004).

Host Range of *Phytophthora* species

Phytophthora species infect more than 200 species of economic, ornamental, shade and hedge plants. In cool temperate countries, it is occasionally recorded on material imported from the tropics. All palms are potentially affected; *Cocos nucifera*(Coconut) and *Areca catechu*(Betelnut palm) are most commonly infected (CPC- Global module, 2000).

The primary hosts of *Phytophthora* species are *Theobroma cacao* (Cocoa), *Cocos nucifera*, *Hevea brasiliensis* (Rubber), *Carica papaya* (Pawpaw), *Areca catechu.*

The secondary hosts are *Piper nigrum* (Black pepper), *Ananas comosus* (Pineapple), *Elaeis guineensis* (Oil palm), *Areca*, *Annona*,

Artocarpus altilis (Breadnut), *Manilkara zapota* (Sapodilla), *Anacardium occidentale* (Cashew nut), Citrus, *Durio zibethinus* (Durian), plants of the palm family and (Grapefruit) (CPC- Global module, 2000).

Esenam (1971) reported that since banana plants (*Musa* spp.) are commonly interspersed in West African cacao farms, infection of banana flowers and fruits by *Phytophthora palmivora* may initiate outbreaks of black pod disease .

The Disease Cycle of *Phytophthora* species

In every infectious disease, a series of distinct events occurs in succession and leads to the development and perpetuation of the disease and the pathogen. This chain of events is called a disease cycle. The primary events in a disease cycle are inoculation, penetration, establishment of infection, colonization (invasion) , growth and reproduction of the pathogen, dissemination of the pathogen and survival of the pathogen in the absence of the host (Agrios, 1997).

Inoculation

Inoculation is the coming in contact of a pathogen with a plant. The inoculum lands on or is otherwise brought into contact with the plant to cause infection. In *Phytophthora* species, the inoculum may be spores or fragments of mycelium.

Oospores, chlamydospores and even parts of hyphae probably survive longer under harsh conditions than do the sporangia and zoospores of *Phytophthora* species; these more persistent structures can be important forms of primary inoculum (Gerlack *et al.*, 1976; Zentmyer, 1980).

There are two types of inoculum of *Phytophthora* species. Primary inoculum, which survives the dormant period and causes the original infection (Agrios, 1997). If the initial infection by primary inoculum can develop extensively enough to incite disease, then the disease is in effect a single cycle disease and the behaviour of primary inoculum is of utmost importance (Cother and Griffin, 1974). The second inoculum is produced from primary infections and it causes secondary infections. The highly simulatory effects of prolonged or repeated periods of dew, rain or saturation of soil on Phytophthora *species* do not spread rapidly enough in established hosts to incite discernible disease and that several infection foci or secondary infections are usually required according to McKenzie (1981). He further observed that, most *Phytophthora* diseases are multiple–cycle, compound interest diseases, and the behaviour of secondary inoculum, which usually takes the form of sporangia and zoospores has more impact on the rate of disease development than does the behaviour of primary inoculum, given that minimum amounts of primary inoculum are present and functioning.

The sources of inoculum of *Phytophthora* may be plant debris and the soil. In the Solomon islands, Jackson and Newhook (1978) observed that mature cocoa leaves play an important role in retaining inoculum intercepted from sporulating lesions on pods and that plant debris in leaf axils and jarquettes also contributes to available inoculum .

In Nigeria, *Phytophthora palmivora* and *Phytophthora megakarya* have been regularly isolated from the soil of cacao plantations of both young and mature cacao trees. Gregory (1981) observed that, these pathogens were not found in soils with no history of cocoa cultivation. In Cameroon, Abego

Onamena *et al.* (1985) reported that the soil is the most important source of infection and trapping of spores at the start of the rainy season (March-May) on immature pods placed on a soil suspension, and observed a population of *Phytophthora megakarya* and *Phytophthora palmivora* in the soil. Tarjot (1967) studied an old Amelonado plantation during the dry season and found *Phytophthora palmivora* in quantity on debris on the ground, in the soil, in the flower cushions from ground level to a height of over 2m in the bark of shade trees. In the state of Bahia, Brazil, de Figueirede *et al.* (1982) observed that spores of *Phytophthora* species can remain viable on heaps of pod husks for three months.

Germination of Propagules of *Phytophthora* species and Penetration of Host Plant.

Germination of sporangia of *Phytophthora* species can be divided into two types, namely, induction and formation of the germ tube and growth of the germ tube with or without formation of a new sporangium . Ho and Hickman (1967) found that germination of zoospores that had formed on plant roots began within 30min and that all zoospores had produced germ tubes within 30 hours. Agrios (1997) observed that after spores germinate, the resulting germ tube must grow or the motile secondary spore (zoospore) must move toward a site on the plant surface at which successful penetration can take place.

Phytophthora species may either penetrate plant surfaces through natural openings or through wounds. Penetration directly through the periclinal wall of an epidermal cell is most common. Hohl and Suter (1976) reported penetration via stomata as the usual mode of entry into the leaf in at

least one instance. Few quantitative data are available, but one study found that epidermal cells next to guard cells were penetrated preferentially. Agrios (1997) observed that direct penetration through intact plant surfaces is probably the most common type of penetration in fungi. *Phytophthora* species that penetrate their host plants directly do so through a fine hypha produced directly by the spore or mycelium or through a penetration peg by an appressorium.

Hohl and Stossel (1976) reported that, the extent to which haustoria are encased by cell wall appositions is believed to constitute an actual resistance mechanism. It is equally evident that susceptible host-parasite interactions involving other *Phytophthora* species are not characterized by any suppression of the formation of appositional material either on the most cell walls adjoining hyphae or around haustoria (Hohl and Stossel, 1976).

Wetness and temperature are without doubt, the main physical factors governing germination behaviour of sporangia. Duniway (1979) reported that, sporangia must be in contact with liquid water to germinate either directly by the growth of germ tubes or indirectly by the release of zoospores.

Hohl and Suter (1976) reported that penetration of the host cell most likely involves enzymatic dissolution of the wall, because no evidence of mechanical deformation of the wall microfibrils has been found. Shimony and Friend (1975) observed that, the fungus forms an infection vesicle once in the host cell from which one or more intercellular hyphae are produced.

Infection of Host by *Phytophthora* species

Agrios (1997) reported that infection is the process by which pathogens establish contact with the susceptible cells or tissues of the host plant and procure nutrients from them. Successful infections result in the appearance of symptoms, that is, discolored lesions, malformed or necrotic areas on the host plant. Between about 24 and 48 hours after infection, the host cells become necrotic. The cells often appear plasmolysed, and the structure of cell organelles becomes disorganized (Shimony and Friend, 1975). Hohl and Suter (1976) also reported rupture of the host tonoplast and degeneration of the cytoplasm as symptoms of successful infection.

Pectinolytic enzymes secreted by the fungus dissolve the pectin that holds the cells together, resulting in maceration of the tissues. The fungus grows between and through the cells. Protolytic enzymes breakdown the protoplasts of invaded cells and in some cases, cellulolytic enzymes cause complete collapse and disintegration of the cell walls. As a result, older trees show sparse foliage, shorter, cupped and yellow leaves, and dieback of twigs and branches in some diseases, such as the collar rot of apple trees, foot rot of citrus trees and black pod of cacao trees. Doke and Tomiyama (1980) observed that current physiologic knowledge of the susceptible *Phytophthora infestans*- potato interaction favours the production of soluble glucans by the fungus that can suppress the resistance response of the host. These soluble glucans are specific suppressors, characteristic of particular races of the pathogen. Kuc *et al.* (1976) reported that the insoluble glucans present in fungal cells elicit nonspecific reactions in host tissues. Cell necrosis

however, fails to restrict further development of the fungus, which continues to grow and eventually sporulates.

Kitazawa and Tomiyama (1970) observed that most striking feature of early stages of infection by *Phytophthora infestans* is the lack of host cell necrosis. The cytoplasm of infected host cells shows no detectable changes in the number or structure of organelles nor any difference in the activity of the endoplasmic reticulum. However, the cytoplasm does appear more dense around some haustoria of intercellular hyphae. The overall impression is that in initial stages of invasion of a host cell, disturbance of the cytoplasm is minimal.

Hohl and Suter (1976) observed that the length of the infection period of *Phytophthora* varies with the particular pathogen–host combination, with the stage of development of the host and with the temperature of the environment. For a successful infection to occur it is not sufficient that a pathogen to come in contact with its host; rather, several other conditions must also be satisfied. First of all, the plant variety must be susceptible to the particular race of the pathogen in which case the race of the pathogen is said to be virulent on the variety of the host plant. Also, the temperature and moisture conditions in the environment of the plant must favour the growth and multiplication of the pathogen. Agrios (1997) observed when these conditions occur at an optimum, the pathogen can invade the host plant and as a consequence, an infection occurs. In many interactions, good evidence exists that individual host cells react in apparently incompatible manner; characteristic of this cell response is the formation of deposits of papilla-like material (Aist, 1976) on host walls around the haustoria. These wall deposits

have been seen in such diverse disease situations as susceptible tobacco roots infected with *Phytophthora parasitica* var. *nicotianae* (Hanchey and Wheeler, 1971) and Soybean hypocotyls infected with *Phytophthora megasperma* f. sp *glycinea* (Stossel *et al.* 1981).

Wilson and Coffey (1980) also observed similar deposits in susceptible Irish Potato tuber and leaf tissues infected with *Phytophthora infestans*. Umaerus (1970) reported reduced penetration of the host, restricted growth of the fungus in the host and reduced sporulation capacity as incompatibility signs of host cell.

Spread of *Phytophthora* species

The pathogen exists in a number of phases during its life cycle. Wood and Lass (1985) reported that *Phytophthora* species may exist as mycelium, Chlamydospores, sporangia, oospores and zoospores. The mycelium is found in varying amounts in rotting cacao pods. Chlamydospores have especially thick walls and are present in old infected pods which remain attached to the tree. These chlamydospores will then spread rapidly through newly formed pod tissues. In cacao, infected rootlets produce sporangia, which are released into the soil as motile zoospores when there is enough moisture. Gregory and Madison (1981) and Gregory *et al.* (1984) reported that rain splash disperses this zoospore inoculum from the soil by 'ballistic droplets' at least 0.5m into the canopy, initiating primary infection foci. Rain splash on the soil and on diseased pods and leaves also creates 'aerosol droplets' that move the inoculum upwards by convection. Taylor and Griffin (1981) observed that these droplets of water, with the movement of insect vectors, especially ants, distribute the

inoculum within the canopy and to healthy pods. Infected pods can also produce stem and branch cankers which destroy the flower cushions and infect pods developing from other flowers. Cankers are also produced following the infection of wounds, caused by insects or man. Because of the absolute requirement of available water for dispersal and pathogenesis, diseases of aerial parts are worse in areas of high rainfall and high humidity, poor drainage, and where shade trees or dense plantings slow the drying of plant surfaces. Waterlogged crops are particularly susceptible to disease. In coconut, previously infected canopy tissue, nuts, soil and roots are likely sources of infection. Thévenin (1994) demonstrated that rain splash transmitted the pathogen between bunches and nuts within the canopy, but organic material in the leaf axils was not linked with disease symptoms.

Butler (1981) observed that germ tubes from zoospores or from the sporangia can penetrate the pod surface at any point on a pod of any age to give a mycelium provided there is free water on the pod surface. This mycelium will then spread rapidly through the pod tissues. On the other hand, studies of relative humidity in West Africa (Wood, 1974) have shown that long periods during which the atmospheric humidity is at saturation point are necessary for the rapid spread of the disease. Tarjot (1971) earlier reported similar findings from Ivory Coast. Recent work in Nigeria identified rainfall as the most important meteorological variation probably because it both disperses inoculum by splash and provides suitable conditions for infection.

Ward (1981) observed that in areas with heavier or more frequent rainfall, relative humidity and duration of pod surface wetness are important in determining the level of infection. The state of Bahia, Brazil, is one such area

and work there has shown that the duration of pod surface wetness is a critical factor in the rate of disease spread. The microclimatic factors of relative humidity, wind speed, air and pod temperatures were measured. The air temperature usually rose rapidly in the morning but the pod temperature lagged behind and dew therefore tended to form on each pod, giving a thin layer of free water. The probability of infection was high if the pod remained wet for more than 2-3 hours in the presence of zoospores. Butler (1980, 1981) observed that in a saturated atmosphere which often occurs during June, July and August, the pod surface remained wet for as long as 4 - 5 hours giving sufficient time for zoospore germination and pod penetration.

In Nigeria penetration by *Phytophthora* zoospores was observed within one hour of inoculation. Zentmyer (1980) observed that the duration of pod surface wetness required to initiate the infection process is very short and this is in agreement with recent work on avocado where zoospores of *Phytophthora cinnamomi* penetrated the roots within one hour of inoculation.

Gregory and Madison (1981) reported that sources of infection of a substantial percentage of infected pods are not known. These infections possibly arose from spore deposition by living vectors, rain wash or splash from sporulating cankers or splash from infected flower cushions.

Robinson (1976) observed that *Phytophthora infestans* and *Phytophthora palmivora* shed sporangia over a range of conditions varying from dry air to water. The flight range for *Phytophthora infestans* is greater than 10km, and the population can spread hundreds of kilometers in the course of one season.

Sources of Infection of *Phytophthora* species

In Nigeria, *Phytophthora palmivora* and *Phytophthora megakarya* have been regularly isolated from the soil of cacao plantations and from the superficial feeding roots of both young and mature cacao trees. Ward and Griffin (1981) reported that these pathogens were not found in soils with no history of cacao cultivation. Abogo Onamena *et al.* (1985) also reported that attempts to isolate any *Phytophthora* species capable of infecting cacao pods was not possible from soils not planted to cacao.

Gregory (1981) suggested three ways in which *Phytophthora* infecting roots could be harmful to cocoa plantations. It could provide a reservoir of inoculum for pod infections throughout the season; it could make individual trees less productive, explaining perhaps, why neighbouring trees sometimes differ so dramatically in production, or the pathogen could infect the roots of young plants on replanting, thereby stunting their growth. Kellam and Zentmyer (1982) have shown that the various species have different survival rates in artificially infected soil.

In Cameroon, the soil is a most important source of infection and trapping of spores at the start of the rainy season (March - May) on immature pods placed on a soil suspension, showed a population of *Phytophthora megakarya*, *Phytophthora palmivora*, *Phytophthora capsici* and occasionally *Phytophthora parasitica*.

Phytophthora megakarya was the most common species and was found at all the depths studied namely 0 - 2.5cm, 2.5 - 15cm , 15 - 20 cm and 20 - 30 cm. *Phytophthora palmivora* was rare at the time of year on

the surface debris but appeared in significant numbers at depths from 2.5 - 30cm.

Evans (1973) has shown that insects are involved in the spread of *Phytophthora* in Ghana. In particular the small black ant *Crematogaster striatula* uses dead plant tissues; including pieces of old infected pods, to construct tents around the peduncle of developing pods. Babacuah (1982) confirmed the role of ant or scale insect colonies on the vertical and horizontal spread of *Phytophthora* on cacao in Ivory Coast. Infection is aided and accelerated by wounds on pods caused by harvesting knives as well as rodent or insect injuries.

Kliejunas and Ko (1976) and Podger (1972) reported that mycelium , chlamydospores and oospores may be dispersed passively in transplanted infected plants; by the movement of soil, gravel and dead plant material by various agricultural, horticultural or forestry practices; or by adhering to equipment and machinery, off-road vehicles, shoes, tyres or animals.

Survival of *Phytophthora* species

Agrios (1997) reported that the chances of continued existence of a pathogen are increased if the pathogen has the capacity not only to parasitise the host but also to survive in its absence. Many successful pathogens survive as dormant or resting propagules and are also saprophytic, at first in dead host tissue and later in soil or water. Different species of *Phytophthora* vary in their capacity to persist either as dormant propagules or as saprophytic colonizers of dead organic material.

Survival in Host Tissue

Phytophthora infestans and *Phytophthora erythroseptica* survive in stored potato tubers, which provide enough inoculum to start epidemics in susceptible host populations if environmental conditions are favourable. Survival lasts at least from one season to the next, usually 6 - 8 months.

The inoculum is in the form of mycelium, which is protected and kept moist within host tissue and survives at temperatures below zero. Oospores survive for longer periods. *Phytophthora palmivora* survives 2-4 weeks on infected cocoa pods. Shea *et al.* (1980) recovered *Phytophthora cinnamomi* from dead *Theobroma cacao* roots two years after death of the plant. They concluded that the stump, lower stem and root system of *Theobroma cacao* provided a large, protected reservoir of inoculum from which large populations of zoospores invaded cacao trees. Gray (1976) reported that *Phytophthora palmivora* survives for years in dead host tissue if moisture is sufficient.

Gregory (1981) suggested that survival, germination and seasonal changes in *Phytophthora* soil populations could be associated with root growth and infection of superficial feeding roots by the pathogen.

Survival in the Absence of a Host

A pathogen must be able to survive between active parasitic phases, for example, on a crop, or it may become extinct. The surviving inoculum forms the springboard for epidemics. Okaisabor (1974) reported that most epidemics initiated by *Phytophthora* species originate from soilborne inoculum; for example, *Phytophthora palmivora*, cause of black pod disease, and *Phytophthora nicotianae* var *parasitica*, cause of fruit rot of guava.

Phytophthora may survive in soil in the form of mycelium, cysts, chlamydospores and oospores. Drying, cold and antagonistic or competitive microorganisms are limiting factors. Shea *et al.* (1980) demonstrated that *Phytophthora palmivora* survived on larger organic particles in irrigated soils. Garrett (1970) suggested that *Phytophthora* survives in the form of dormant resting bodies which have been produced in plant tissue colonized either saprophytically or parasitically.

Physical Factors Affecting Development of *Phytophthora* species

The development of fungi is governed by different kinds of biological, physical and chemical factors. Ayers and Zentmyer (1971) reported that, the reason for the stimulation of sporangium production of *Phytophthora cinnamomi* in soil seems to be the presence of exudate by certain soil bacteria, which is a chemical factor. Gisi *et al.* (1980) reported that sporangium production is governed by soil matric potential, which is a physical factor.

Temperature

Temperature is an important parameter for mycelial growth, sporulation and spore germination in *Phytophthora* species. Temperatures at and above the optimum for mycelial growth favour germ tube development by sporangia. Crosier (1974) reported that the optimal temperature for germ tube development of *Phytophthora infestans* sporangia is 24°C, whereas zoospore differentiation occurs at 12°C.

Englander and Roth (1980) reported that sporangia are formed within a wide range of temperatures and suggested that the optimal temperature range is usually very narrow. McDonald and Duniway (1978) reported that

sporangia differentiate and release zoospores in the presence of free water and at temperatures below those favouring optimal growth of the fungus.

Agrios (1997) reported that plants as well as pathogens require certain minimum temperatures to grow and carry out their activities. Pathogens differ in their preference for higher or lower temperatures. Dakwa (1988) reported that, in Ghana, throughout a wide range of controlled temperatures (10 - 34°C), sporangia in water germinated indirectly by zoospores within six hours.

Moisture

Agrios (1997) observed that the moisture level has an important influence on the growth and reproduction of *Phytophthora* species. The high moisture requirement is satisfied by high relative humidity and free water on the plant surface for species such as *Phytophthora infestans* and *Phytophthora palmivora* that are pathogens of leaves or pods.

Okaisabor (1974) observed that a film of water on the leaf for 6 - 8 hours allows infection, penetration, sporangial formation and dispersal of *Phytophthora infestans* and *Phytophthora palmivora.* These depend on the temperature and high relative humidities. Soil-borne *Phytophthora* species require high soil moisture and many species require free water for production and dispersal of zoospores. As an illustration of the difference in soil moisture characteristics, Reeves (1975) concluded that a shower of rain may suffice for sporangial formation and zoospore release on sandy but not on clay soil.

Species of *Phytophthora* vary in water requirements; for example, maximum zoospore formation and release occur on flooded soils for

Phytophthora megasperma (Pfender *et al.*, 1977) but under slightly drier conditions for *Phytophthora cinnamomi.*

Symptoms of Infected Cacao plants

In cacao, the whole plant is attacked causing black pod, bark or stem and cushion canker, cherelle wilt and chupon blight. On pods, the disease begins with a circular brown lesion that enlarges to cover the whole pod, which eventually becomes black and mummified, and sometimes covered in a white mass of sporangia. Inoculation of detached pods with various isolates of *Phytophthora palmivora* showed differences in colour, outline and rate of growth of the lesion, either discrete or confluent masses of sporangia , and varying amounts of aerial mycelium Turner (1960a).

Pod infection may also develop from the stalk *via* an infected flower cushion. Stem cankers are characterized by oval to round, rusty-brown discoloration of the external bark. The symptoms of collar infections are dark brown, irregular, water-soaked lesions with reddish-brown exudate; these lesions are not noticeable unless accompanied by a gummy exudate. Attack of young shoots results in die-back (Zadoks, 1997).

Control of Black pod disease

Breeding for resistance

It is generally believed that replacement of susceptible cultivars by ones showing durable resistance to the pathogen is one of the most effective and sustainable control methods of the disease (Iwaro *et al.*, 2000). Simmonds (1994) observed that resistance to black pod disease is horizontal.

Few cacao accessions have significant resistance to *Phytophthora palmivora*, although some resistance to stem canker has been found in some South American stock (Evans and Prior, 1987). Resistance to stem canker may not confer resistance to black pod and the majority of the world's cacao is still moderately susceptible to infection.

Leaf bioassay (Nyasse *et al.*, 1995), seedling stage screening (Pinto *et al.*, 1989) and fruit inoculation techniques (Phillips-Mora and Galindo, 1989) are now available to be used in screening for cacao varieties resistant to black pod disease. Some resistance has been found in clones TSH 1076 (Okey *et al.*, 1996), in F1 hybrids from crosses between Trinitario clones (DR, DRC, ICS) and Scavina clones (Sca6, Sca12) (Iwaro *et al.*, 1992), and in clones BE 5, EEG 8 and 9 and MA 15 (Pinto *et al.*, 1989).

Adomako (2006) also reported PA 150, Alph.B36 and Pound 7 as the most promising parents that can be used to produce progenies with enhanced resistance to black pod disease in Ghana.

Rocha and Medeiros (1968) have observed two types of resistance in cacao pods, one to the penetration of the pod by the pathogen; and the other to the growth of the pathogen after penetration.

Atanda *et al.* (1973) reported that a promising approach to control black pod disease is to breed for disease escape, so that the tree produces the bulk of its crop when climatic conditions are least conducive to the spread of the disease. He further suggested that breeders could select for short cropping cycle or for profuse flowering early or late in the season, depending on the climate of the area in question.

Methods of screening for black pod disease resistance

Field trial

Kebe *et al.* (1999) reported that field trials are the most reliable test but are expensive and can only be employed for a pre-selected number of genotypes. Paulin *et al.* (1994) observed significant genetic variation for *Phytophthora* pod rot on mature pods in cacao breeding trials. Iwaro and Butler (2001) reported significant variation in the reactions of 742 accessions which were assessed in two field trials for resistance to *Phytophthora* pod rot. Significant variation was observed among accessions and a higher level of susceptibility was observed in the third year of field observations (65%) than in the first and second years when the levels were 15 and 25%, respectively. Adomako (2007) reported significant differences of black pod infection among 20 progenies in a field trial at Tafo-Akim in Ghana. Cilas *et al.* (1998) reported that percentage pod rot observed on progenies in the same trial over nine years according to monthly harvesting data was significantly different. Iwaro *et al.* (2005) observed significant differences among 40 genotypes assessed for resistance to *Phytophthora* pod rot. Thirteen of the 40 accessions were found to be resistant (<10% pod rot), while 16 accessions were moderately resistant (10 - 25% pod rot). Eleven accessions were classified as susceptible (> 25% pod rot).

Iwaro *et al.* (2005) also reported significant differences among the years of field observations. There was a significant interaction between clones and years.

Blaha and Latode (1997) reported that a major difficulty in selecting resistant varieties was the lack of knowledge on the environmental versus

genetic factors determining field resistance. Soria (1974) reported that assessment of black pod infection in the field, under natural infection conditions can be biased by the micro-environmental conditions in which the epidemic develops.

Detached Pod tests

Iwaro and Umahaaran (1998) and Iwaro (1996) have reported that inoculation of detached pods creates an opportunity for rapid assessment of pod resistance to *Phytophthora* infection. Nyasse *et al.* (2002) reported a significant difference in detached pod inoculations which were carried out on four clones, with well-known levels of field resistance, used as parents for a diallel crossing design. They confirmed a significant positive relationship between inoculation of attached wounded pods and detached pod tests. With the detached pod test, the replicate means were positively correlated to the overall means.

Blaha *et al.* (2000) observed that detached pod tests for evaluation of *Phytophthora* pod rot can be both repeatable and reliable. The level of repeatability of the test results influences the number of replicates that has to be carried out in order to obtain reliable results. Latode (1977) reported highly significant differences in resistance of cacao clones (hybrid progenies, seedlings within progenies and clones) to *Phytophthora megakarya* when detached pods were inoculated.

Efombagn *et al.* (2004) has observed that detached pod tests, if applied under standard conditions can be of great value to speed up selection for *Phytophthora* pod rot-resistant varieties. Kebe (1999) reported that the

repeatability for the detached pod test was very significant with coefficient of rank correlation varying from 0.77 to 0.94.

Iwaro (2000) reported that if the recommended age of the pods chosen for the test needs to be inferred from the size of the pods, the reactions to infection may be more variable. In that case, two replicates of five pods each may be required. Tahi (2003) reported that the results of detached pod and leaf disc tests were well correlated.

Iwaro *et al.* (1997) established a relationship between detached pod and leaf resistance at the post-penetration stage of infection, but noted that the mechanism of resistance was different in the two organs at the penetration stage of infection.

Detached leaf tests

The detached leaf tests available are entire leaf test at penetration stage of infection, entire leaf test at post-penetration stage of infection and leaf disc test.

Nyasse *et al.* (1995, 1997) reported significant differences among cacao clones assessed for resistance to *Phytophthora* pod rot by the leaf disc test.

Blaha *et al.* (2000) observed that in the leaf disc test two replicates are considered enough to estimate the average susceptibility of clones or seedling progenies with inoculation of 40 - 60 discs from three to five clonal plants.

Kebe *et al.* (1999) reported highly significant correlations between inoculation series using the leaf disc test. They further suggested that, the high coefficients of rank correlation (r = 0.55-0.90) between average disease severity values of inoculation series can be obtained with different types of

test materials. Tahi *et al.* (2000) observed a significant correlation between leaf disc test and the average scores in the three series of pod inoculations on the same clones.

Tahi (2003) reported that, for the leaf disc test special attention needs to be paid to the availability of leaves at the right development stage for all treatments and also to the uniformity of the exposure of the leaves to light, as these factors may significantly modify the results. Tarjot (1972), Tondje *et al.* (1988), Nyasse *et al.* (1995) and Thevenin *et al.* (2004) have all reported that older leaves tend to become more resistant than younger leaves and that disease severity scores on leaf discs increase with time after inoculation.

Greathouse *et al.* (1971) recommended interflush two and three leaf stages of development for leaf disc screening tests. Tahi *et al.* (2000) reported that when field or nursery leaves of nine clones were inoculated at the hardening stage results correlated with natural field infection of pods with *Phytophthora palmivora.* Nyasse *et al.* (2002) demonstrated the same for inoculation of leaf discs collected from progeny trees from a diallel trial, when compared to general combining ability (GCA) values of the same clones for field infection levels of *Phytophthora megakarya.* Nyasse *et al.* (1995) earlier showed that the ranking of leaf disc test results obtained with a *Phytophthora palmivora* isolate was similar to that obtained with a *Phytophthora megakarya* isolate. Iwaro *et al.* (1997a) reported that leaf disc inoculations can be correlated with pod inoculations.

Eskes *et al.* (2000) recommended the leaf disc inoculation method for large-scale screening of cacao for resistance to *Phytophthora* in an

international project including evaluation of the same cacao clones in 10 different cocoa producing countries.

Iwaro *et al.* (1997b) demonstrated that resistance to *Phytophthora palmivora* existed at two levels, the penetration level, which determines lesion number and the post-penetration level, which determines lesion size.

Iwaro *et al.* (1993, 1997b), Nyasse *et al.* (1995) reported that with regards to post-penetration inoculation of leaf correlated with detached pod inoculation.

CHAPTER THREE

MATERIALS AND METHODS

Cacao Genotypes

A genetically diverse set of 25 cacao clones were used for this study. The accessions represent 25 international clones being evaluated at the Cocoa Research Institute of Ghana (CRIG). The clones were planted in the K5 extension plot (Plate 1) at CRIG using randomized complete block design with five replications.

The morphological and agronomic characteristics of the 25 clones are presented in the Table 1.

Magnification: ×2

Plate 1. A label showing the K-5 extension plot at CRIG.

Table 1: Morphological and agronomic characteristics of the 25 Cacao Genotypes

			Characteristics				
Genotype	**Origin**	**Group**	**Reaction to Black pod**	**Dry bean weight**	**Pod shape**	**% Fat**	**Yield**
PA 120	Peru	F	MR	Small	Amelonado	55.8	fair
MOCOROGO	Brazil	F	PR	Fair	Intermediate	52.6	high
LECTEEN 37i	Ecuador	C	PR	Large	Intermediate	52.3	fair
ICS 43	Trinidad	T	S	Large	Cundeamor	57.3	fair
SPEC 54i	Colombia	F	PR	Small	Amelonado	48.8	high
EQX 78	Ecuador	F	PR	Large	Angoleta	58.3	fair
EET 59H	Ecuador	F	PR	Large	Amelonado	54.1	high
PA 107	Peru	F	PR	Large	Cundeamor	56.8	high
LECTEEN 37f	Ecuador	C	PR	Large	Intermediate	53.4	high
SCA 6	Peru	F	PR	Small	Cundeamor	48.8	high
LAF 1	Peru	F	PR	Fair	Amelonado	50.9	high
T85/799	Trinidad	T	PR	Fair	Intermediate	54.2	high
AMAZ 15-15	Peru	F	PR	Large	Intermediate	48.0	low
EQX 3360-3	Ecuador	F	PR	Fair	Intermediate	53.9	high
IFC 5	Peru	F	PR	Large	Amelonado	51.5	high
IMC 47	Peru	F	PR	Large	Angoleta	53.5	high
PA 150	Peru	F	MR	Large	Elongate	56.0	high
UF 676	Costa Rica	T	PR	Large	Angoleta	56.3	high
VENC 4-4	Peru	F	MS	Fair	Amelonado	54.7	high

Table 1 Continued.

Genotype	Origin	Group	Characteristics				
			Reaction to Black pod	Dry bean weight	Pod shape	% Fat	Yield
BE 10	Brazil	F	PR	Fair	Amelonado	52.5	high
MO 20	Peru	F	MS	Large	Intermediate	48.8	high
MAN 15-2	Brazil	F	PR	Fair	Intermediate	54.9	fair
GU 225V	French	F	PR	Large	Intermediate	49.7	high
T79/ 501	Trinidad	T	MS	Fair	Amelonado	51.5	high

F= Forastero, T= Trinitario, C =Criollo

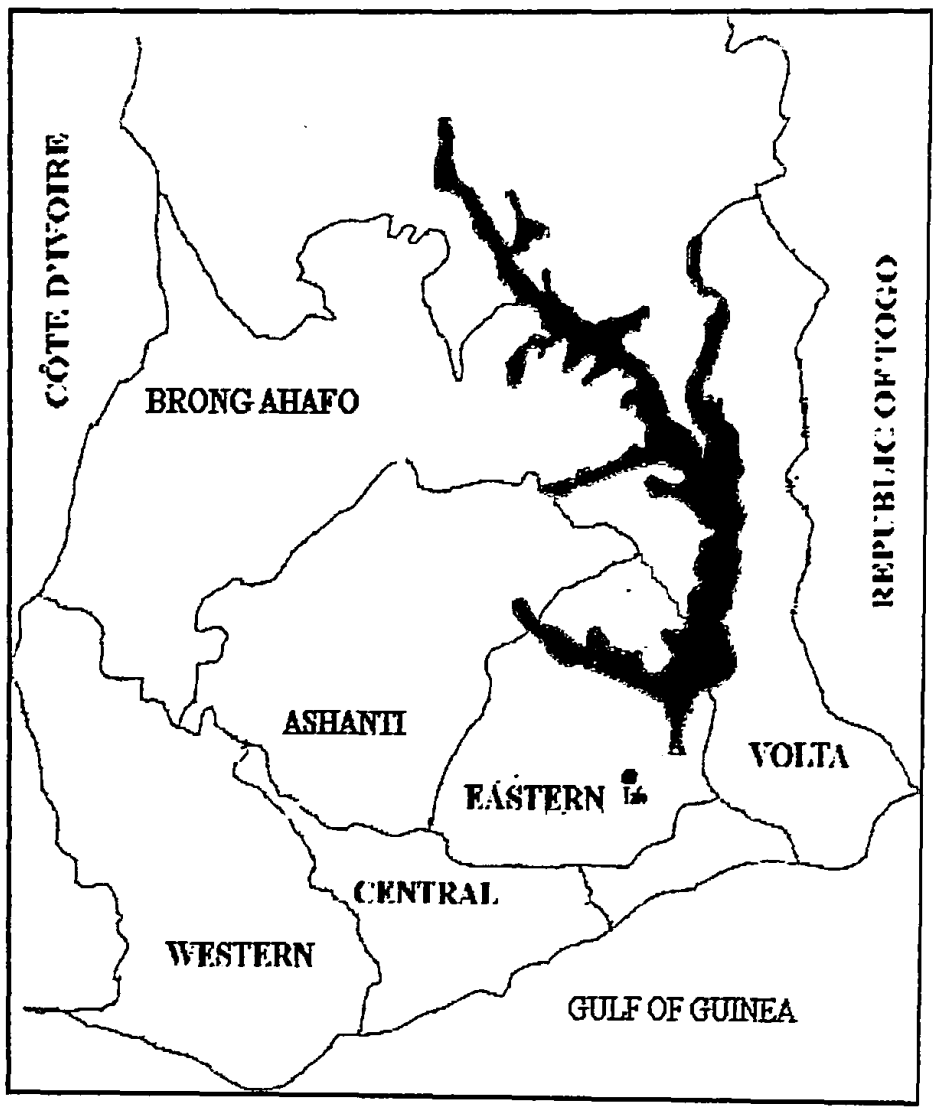
PR = Potentially resistant, S= Susceptible, MR = Moderately resistant, MS = Moderately susceptible, R= Resistant

Location and Climate

The study was conducted at Tafo in the Eastern Region of Ghana (Fig.1). The region is in the forest zone .The soil at the experimental site belongs to the forest ochrosol (Adu and Mensah-Ansah, 1969). The region has a bimodal rainfall pattern with an average range between 1200mm and 1930mm. The major rainy season is between April and July with short spells in August. The minor rainy season on the other hand, begins in September and ends in November followed by a dry very hot period from December to March, Ahenkorah *et al.* (198). Relative humidity values of 99 to 100% are generally recorded at night and early morning. Relative humidity values drop to about 70% by mid-day especially on sunny days (CRIG Meteorological

Station, 1989). Temperatures are almost uniformly high throughout the year with mean annual minimum of about 25°C Asomaning (1971).

Figure 1. Map of Ghana showing location of Eastern Region



Isolation of *Phytophthora* species from Black pod Infected Pods from the Field.

Isolation of *Phytophthora* species was done from naturally infected pods collected from the K-5 extension plot on which the 25 genotypes were planted. The diseased pods were washed and blotted dry. The pods were then surface sterilized with 70% ethanol. The infected parts of the pods were cut into 5 mm cubes and plated separately on water agar medium in Petri dishes. All inoculated plates were incubated for three days on the laboratory bench at room temperature of 25^0C. Plates were observed daily for fungal growth from tissue segments.

Fungal growths were transferred onto Carrot Agar Medium that was prepared to obtain pure culture of the isolated fungi. Emergent colonies were examined under light microscope and identification of the *Phytophthora* species was made. Based on the characteristic 'seaweed' odour of the infected pod, growth of isolate on carrot agar medium, sporangial shape and size and pedicel length, the organism was identified as *Phytophthora palmivora* (Lawrence, 1978).

The isolate was grown on a Carrot Agar Medium and from a 10-day old culture, a zoospore suspension was obtained by inundating each culture plate (9cm diameter) with 10ml sterile distilled water.

The zoospore suspension was then refrigerated for 25 minutes (5°C). The pathogenicity of the isolate was maintained by regular inoculation in the laboratory of green mature cacao pods followed by re-isolation on Carrot Agar Medium.

Sampling of leaves from the Field

The new flushes from bud break of the clones were tagged (Plate 2) so that their exact ages could be determined at the time of harvest. For each of the inoculation series, leaves were collected from all of the 25 clones in each replication. In the field, 15 leaves were harvested from each clone in each replication. The average ages of the leaves for each treatment were established by following the growth of young flushes from bud break in the field. After collecting the matured leaves (Plate 3), they were placed in labelled polyethylene bags into which a few drops of distilled water were sprayed before hand. The bags were then kept in the dark till the next morning. This was done to minimize any effect of leaf sampling time that may occur with large time lapses between harvesting of leaves (Tahi, 2003). The leaves were washed thoroughly with tap water, blotted dry with Whatman Number 3 paper and were then surface sterilized with 70% ethanol.

Magnification: ×3

Plate 2. Tagged branch end with new leaf flushes following bud break

Magnification: ×2

Plate 3. Matured leaves ready to be harvested for leaf disc test.

Leaf Inoculation Tests

Assessment of Leaf Resistance to *Phytophthora palmivora* by the Leaf Disc Test

Leaf disc inoculation as described by Nyasse *et al.* (1995) was carried out. Sixteen leaf discs of 1.5cm in diameter from each clone in a replication were cut with a cork borer, totaling 80 (5×16) discs per clone. Leaf discs were placed with their abaxial surface upwards on moist plastic foam in five trays 70 cm long, 60 cm wide and 15 cm deep. Discs belonging to the same replication were randomly arranged in groups of 25 within each tray, giving 16×25 =400 discs per tray. Inoculation was carried out the same day, after preparation of all leaf discs. After the concentration of zoospores had been determined with a hemacytometer and adjusted to 200,000 per ml, droplets of 10μL were placed on each disc (Plate 4). The trays with discs were incubated

at room temperature of 25°C and each covered with another plastic tray in the laboratory avoiding direct sunlight until observations were carried out (Plate 5). On the sixth day of incubation, disease severity symptom (DS) was recorded using a 0 - 5 assessment scale developed by Nyasse *et al.* (1995).

0= absence of symptoms,

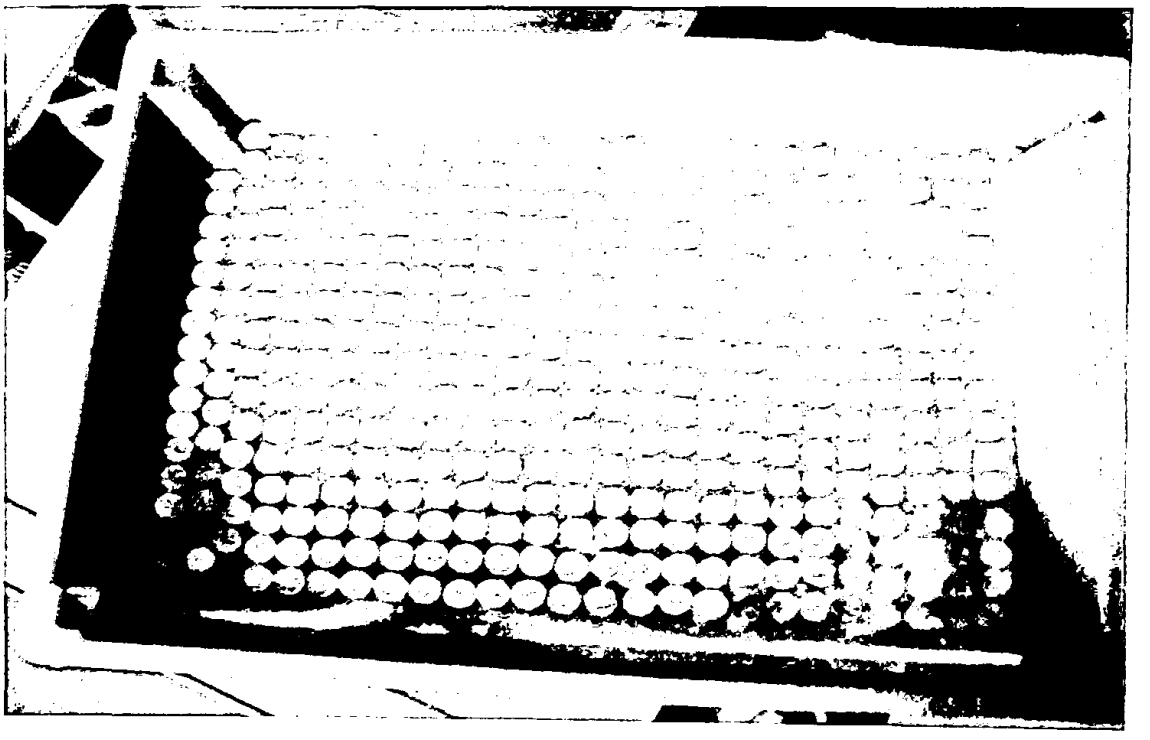
1= very small necrotic spots,

2= larger number and size of necrotic spots,

3=coalescence of brown spots into medium-sized lesions,

4= large uniform brown lesions and

5= very large brown lesions, often expanding outside the area covered by the inoculum droplet.



Magnification: ×2

Plate 4. A tray containing leaf discs inoculated with *Phytophthora palmivora* zoospore suspension.

Magnification: ×2

Plate 5. Covered trays containing inoculated leaf discs incubated at room temperature.

Assessment of Leaf Resistance to *Phytophthora palmivora* at the Penetration Level of Infection.

Leaf resistance at the penetration stage of infection was assessed with the tissue-paper mount method of inoculation as described by Iwaro *et al.* (1997b).

Mature leaves at the interflush-2 stages as described by Greathouse *et al.* (1991) were surface sterilized with 70% alcohol and rinsed in sterile distilled water. The surface - sterilized leaves were then placed in plastic trays lined with moist plastic foam, with the abaxial surface facing upwards.

From each genotype in a replication, five leaves were placed in one tray. Tissue paper-mount inoculation of the leaf samples was done by applying a 30 µL drop of zoospore suspension on the leaf surface on which a piece of tissue paper (area: 1 cm^2, thickness: 0.23 mm) was placed to allow a uniform spread of the zoospore suspension within the area covered by the tissue paper (Plate 6). A leaf from each clone was inoculated with sterile distilled water as a control in each experiment. The 25 genotypes were arranged in a randomized complete block design with five replications and incubated at room temperature in plastic trays lined with moist plastic foam and covered with another tray. After 6 days of incubation, the tissue paper mounts were carefully removed and the penetration resistance was determined by counting the number of lesions. The experiment was repeated to confirm the consistency of results obtained.

Magnification: ×2

Plate 6. Photograph showing the tissue paper mount inoculation of leaf samples in a tray.

Assessment of Leaf Resistance to *Phytophthora palmivora* at the Post-penetration Level of Infection.

Assessment of leaf resistance at the post-penetration stage of infection was based on punch inoculation method (Iwaro *et al.*, 1997b). The area of lesion formed was used as an indication of post-penetration resistance. A 4mm diameter hole was punched in the leaf lamina and the hole was covered at the adaxial surface with a spot plaster. A 4mm diameter filter paper disc previously immersed in a 200,000 zoospores per ml suspension was inserted in the hole from the abaxial side (Plate 7). A leaf from each clone was inoculated with sterile distilled water in place of zoospore suspension as a control. Inoculated leaves were arranged in a randomized complete block design with five replications and incubated at room temperature of 25°C in trays lined with moist plastic foam and covered with another tray. After incubation for six days, the lesion areas on leaves were cut and measured with a leaf area meter (MK2, Delta T Services, Burnwell, and Cambridge, England). This experiment was repeated to confirm the consistency of results obtained.

Magnification: ×2

Plate 7. Photograph showing the holes punched in the lamina of leaf samples inoculated at the abaxial surface with filter paper discs previously immersed in a 200,000 zoospore per ml suspension of *Phytophthora palmivora*.

Sampling of Pods from the Field

Mature unripe pods of sizes similar to those of ripe ones at approximately four months old were used as test samples. Pods were harvested with care and kept in labelled plastic bags. The pods were covered with cotton wool (Plate 8) in order to avoid surface damage which may occur when many pods were kept together in close contact with each other. The harvested pods were washed thoroughly with tap water and blotted dry with Whatman Number 3 paper. They were then surface sterilized with 70% ethanol.

Magnification: ×2

Plate 8. A black polyethene bag containing sampled pods covered with cotton wool.

Pod Inoculation Tests

Assessment of Pod Resistance to *Phytophthora palmivora* at the Penetration Level of Infection.

Resistance of pod at the penetration level was assessed using the multiple-point inoculation (Iwaro *et al.*, 1997b). The multiple point inoculation was performed on the pod surface, in which 10µL drops of inoculum were placed at three points along the ridges with a micropipette. A distance of about 3cm was maintained between inoculated points to avoid merging of adjacent lesions. A zoospore concentration of 200,000 per ml was used. A pod from each clone was inoculated with sterile distilled water in

place of zoospore suspension as a control. The 25 clones were replicated five times with appropriate controls and arranged in a randomized complete block design. The pods were incubated at 25°C in a 40cm×60cm transparent polyethene bags. A beaker of water to maintain a humid atmosphere was kept in the bag with the mouth of the bag closed. After 6 days, pods were assessed for the number of established lesions (Plate 9). The number of established lesions on pods was used as an indication of penetration resistance. The experiment was repeated.

Magnification: ×2

Plate 9. An experimenter counting the number of established lesions on cacao pods.

Assessment of Pod Resistance to *Phytophthora palmivora* at the Post-penetration Level of Infection.

Assessment of pod resistance at the post-penetration stage of infection was based on stab inoculation method (Iwaro *et al.,* 1997b). The area of lesion formed was used as an indication of post-penetration resistance. For the stab inoculation, a standard wound 4mm in diameter was created on the pod surface with a cork borer. The wounded spot was inoculated with a piece of cotton wool (Plate 10) previously immersed in a 200,000 zoospores per ml suspension and covered with a spot plaster. This concentration of inoculum was reported as optimum in similar inoculations conducted by Screenivasan (1995), Sitapai (1989), and Okey (1996). Inoculated pods were arranged in a randomized complete block design with five replications and incubated at room temperature in a 40cm×60cm transparent polyethylene bags (Plate11). A beaker of water to maintain a humid atmosphere was kept in the bag with the mouth of the bag closed. After incubation for six days, the size of the established lesion was traced on a transparent paper (Plate 12). The lesion size was determined from brown paper cutouts trimmed to the size of each lesion and was measured with a leaf area meter.

Magnification: ×3

Plate 10. Photograph showing punched holes on the pods inoculated with a piece of cotton wool previously immersed in 200,000 zoospores per ml suspension.

Magnification: ×3

Plate 11. Transparent polyethene bags with inoculated pods incubated at room temperature.

Magnification: ×3

Plate 12. An experimenter tracing the margin of an established lesion on the pod surface onto a transparent paper.

Distal, Proximal and Lateral Inoculation of Pods with *Phytophthora palmivora* at the Post-penetration Level of Infection.

Mature unripe pods, approximately four months old, and of sizes similar to those of ripe ones were harvested from 15 clones among the 25 clones located on the K5 extension plot. Nine pods were collected from each clone and were stabbed at distal, proximal and mid regions with a cork borer. The wounded spots were inoculated with a piece of cotton wool previously immersed in a 200,000 zoospores per ml suspension and covered with a spot plaster. Inoculated pods were arranged in a randomized complete block design with three replications and incubated at room temperature of 25°C in a

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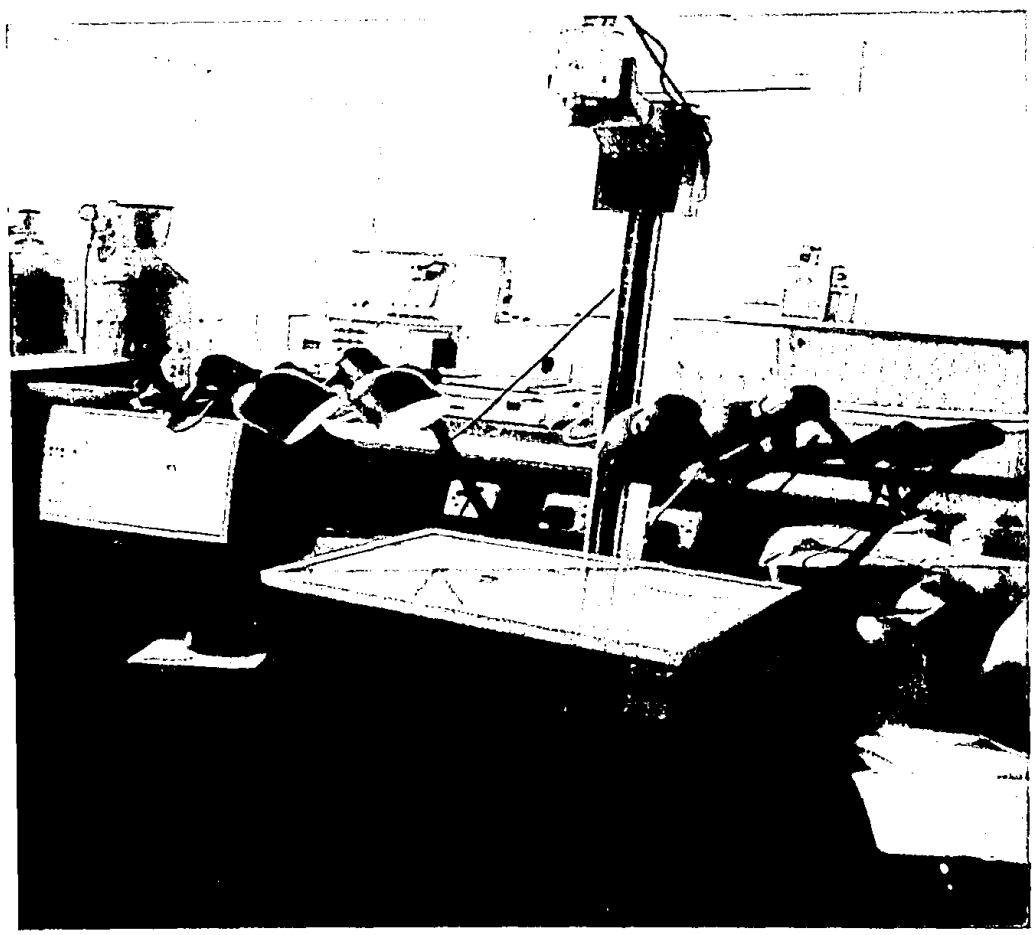
40cm×60cm transparent polyethylene bags. A beaker of water to maintain humid atmosphere was kept in the bag with the mouth of the bag closed. After incubation for six days, the size of the established lesions at the distal, proximal and mid regions of the pods was determined from brown paper cutouts trimmed to the size of each lesion and were measured with a leaf area meter (Plate 13). Data obtained from the inoculated pods were subjected to Analyses of Variance to determine the significance of differences among the 15 clones and the regions of the pod inoculated.



Magnification: ×3

Plate 13. Leaf area meter used for measuring lesion area.

Field Observation for Resistance to Black pod Disease

Data on the field observations of *Phytophthora palmivora* infection in the field were collected from all the 25 clones in each replication monthly from July to December, 2007. Each clone has eight trees per plot in the five replications. Each tree of a clone was observed monthly from July to December and the following variables were recorded:

-Number of healthy pods (that is, not showing any symptoms of *Phytophthora* pod rot).

-Number of pods with *Phytophthora* pod rot symptoms (Plate 14).

The percentage of pods affected by *Phytophthora* pod rot was expressed as:

$$\frac{\text{Numbers of pods with } \textit{Phytophthora} \text{ pod rot symptoms}}{\text{Total number of pods produced}}$$

Magnification: ×2

Plate 14. A cacao tree showing pods with *Phytophthora* pod rot symptoms in the field.

Data Analysis

The data on leaf disc scores, number of lesions, lesion sizes and field observations of black pod infection were analysed using the MINITAB version 13 statistical software to perform Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) and to determine correlation coefficients.

Multivariate analysis (cluster analysis) was carried out using the Genstart version 5 statistical package. In addition, the relationship among leaf disc scores, field infection of *Phytophthora palmivora*, penetration and post-penetration reaction to *Phytophthora palmivora* infection in leaf and pod was tested by regression analysis.

CHAPTER FOUR

RESULTS

Clonal differences in leaf and pod resistance at the penetration stage of infection.

Clonal differences in leaf and pod resistance at the penetration stage of infection are presented in Table 2a.

Analysis of variance of number of lesions obtained from the tissue-paper-mount test indicated that there were highly significant differences ($P<0.001$) among genotypes in their reactions to *Phytophthora palmivora* at the penetration stage, based on the number of lesions observed per square centimeter on leaves and pods (Table 2a). It can be seen that the number of lesions on leaves observed among the genotypes varied from 6.68 for SCA 6 to 44.96 for MO 20. They were significantly high on leaves of MO 20, PA 120, T79/ 501, EQX 78 and VENC4-4. Fewer lesions per square centimeter were recorded on leaf surfaces of SCA 6, T85/ 799, ICS1, LAF 1 and GU 225V.

There were also highly significant differences ($P< 0.001$) among genotypes for the number of lesions on inoculated pods (Table 2a). The table shows that the mean number of lesions produced on pods of T79/501 and MO 20 were significantly large, 11.08 and 13.40, respectively. Significantly fewer lesions were produced on pods of SCA 6, T85/ 799, LAF 1, and ICS 1.

The results showed that the 25 genotypes had varying levels of resistance to *Phytophthora palmivora.*

At the penetration level of resistance on leaf, 10 genotypes were observed to be resistant, six were moderately resistant and nine genotypes were susceptible (Figure 2).

The genotypes that were resistant included LECTEEN 37i, ICS 43, EET 59H, PA 107, SCA 6, LAF1, T85/799, IFC 5, ICS1 and GU 225V. The moderately resistant genotypes were LECTEEN 37f, EQX 3360-3, PA150, UF 676, BE 10 and MAN 15-2. The susceptible genotypes were PA 150, MOCORONGO, SPEC 54i, EQX 78, AMAZ 15-15, IMC 47, VENC 4-4, MO 20 and T79/501 (Table 2b)

At the penetration stage of resistance on pod, 6 genotypes were resistant, 15 genotypes were moderately resistant and 4 genotypes were susceptible (Figure 2). The resistance genotypes were EET 59H, SCA 6, LAF 1, T85/799, ICS1 and GU 225V. The moderately resistant genotypes included MOCORONGO, LECTEEN 37i, ICS 43, SPEC 54i, EQX 78, PA 107, LECTEEN 37f, AMAZ15-15, EQX 3360-3, IFC 5, IMC 47, PA 150, UF 676, BE 10, and MAN 15-2.The susceptible genotypes were PA 120, VENC 4-4, MO 20 and T79/501 (Table 2b).

Table 2a. Lesion number on leaves and pods of 25 Cacao Genotypes after inoculation with *Phytophthora palmivora*

Genotype	Mean lesion number on:			
	Leaf		Pod	
PA120	40.96	c	10.52	bc
MOCORONGO	33.72	e	8.04	efg
LECTEEN 37i	15.96	jk	7.64	fghi
ICS 43	18.80	i	7.88	efgh
SPEC 54i	30.96	f	8.20	efg
EQX 78	41.84	bc	9.24	d
EET 59H	13.28	l	5.52	jk
PA 107	13.92	kl	6.84	i
LECTEEN 37f	16.52	ij	7.16	hi
SCA 6	6.68	o	2.28	m
LAF 1	12.32	lm	5.52	jk
T85/799	10.64	mn	4.40	l
AMAZ 15-15	33.60	e	8.60	de
EQX 3360-3	16.84	ij	6.88	i
IFC 5	13.40	l	6.00	j
IMC 47	37.48	d	8.36	ef
PA 150	26.64	g	8.20	efg
UF 676	18.16	ij	8.04	efg
VENC 4-4	43.76	ab	10.00	c
ICS 1	11.44	lmn	5.16	k
BE 10	17.24	ij	7.44	ghi

Table 2a Continued.

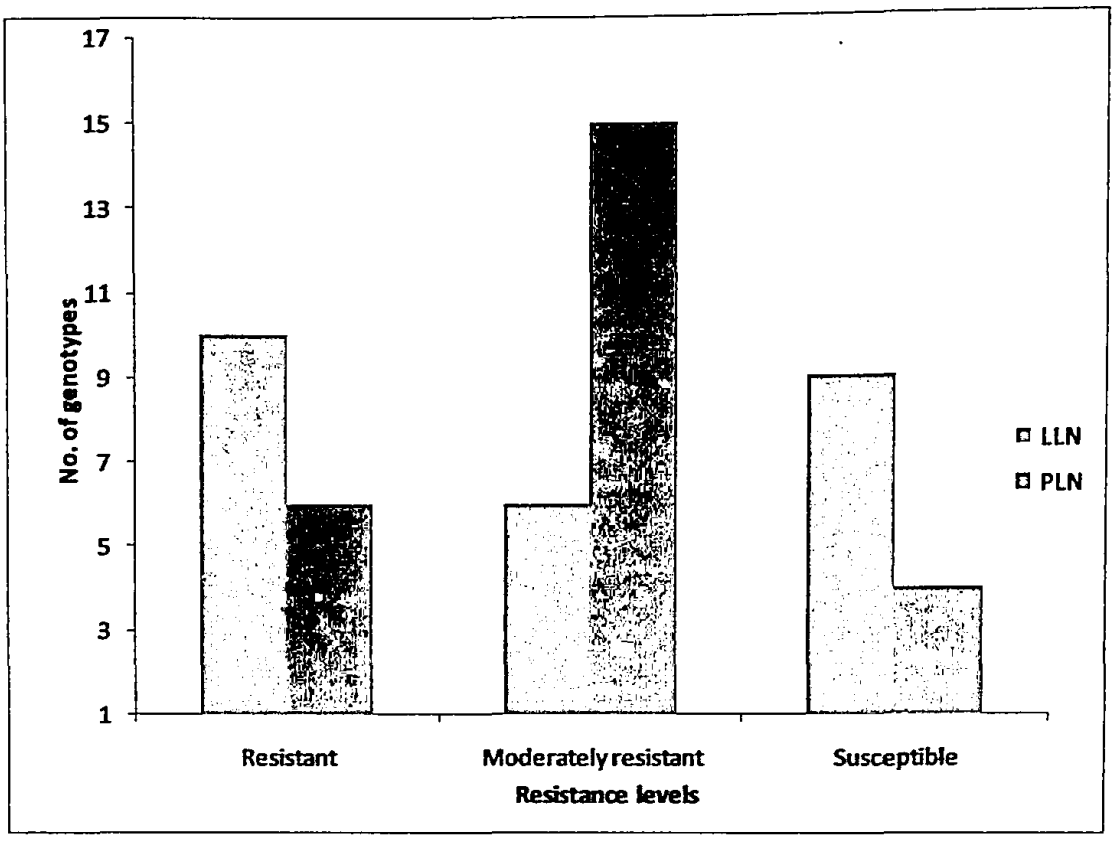
Genotype	Mean lesion number on:			
	Leaf		Pod	
MO 20	44.96	a	13.40	a
MAN 15-2	21.32	h	8.24	efg
GU 225V	9.60	n	5.68	jk
T79/501	44.28	ab	11.08	b
Lsd	2.43		0.74	
%CV	5.15		4.87	
Mean	23.8		7.61	

Table 2b. Distribution of genotypes into levels of resistance based on lesion number on leaf and pod.

Level of resistance	Leaf	Pod
Resistant genotypes	Lecteen 37i	EET 591I
	ICS 43	SCA 6
	EET 591I	LAF 1
	PA 107	T85/799
	SCA 6	ICS1
	LAF 1	GU 225
	T85/799	
	IFC 5	
	ICS1	
	GU 225V	
Moderately resistant	LECTEEN 37f	MOCORONGO
genotypes	EQX 3360-3	LECTEEN 37i
	PA 150	ICS 43
	UF 676	SPEC 54i
	BE 10	EQX 78
	MAN 15-2	PA 107
		LECTEEN 37f
		AMAZ 15-15
		EQX 3360-3
		IFC 5
		PA 150

Table 2b Continued.

Level of resistance	Leaf	Pod
		UF 676
		BE 10
		MAN 15-2
Susceptible genotypes	PA 120	PA 120
	MOCORONGO	VENC 4-4
	SPEC 54i	MO 20
	EQX 78	T79/501
	AMAZ 15-15	
	IMC 47	
	VENC 4-4	
	MO 20	
	T79/501	



LLN =Leaf lesion number, PLN = Pod lesion number

Figure 2. Distribution of resistance levels of the 25 genotypes at the penetration stage of infection of leaf and pod.

Clonal differences in leaf and pod resistance at the post-penetration stage of infection

The clonal differences on leaf and pod resistance at the post-penetration stage of infection are shown in Table 3.

Analysis of variance of lesion sizes obtained from the punch-inoculation tests of leaf indicated that lesion sizes on leaves varied highly significantly ($P < 0.001$) among the genotypes (Table 3).

SCA 6 produced the smallest lesions. Lesions were significantly large on MO 20, T79/ 501, VENC 4-4, EQX 78 and PA 120 and intermediate on PA 107, ICS43, IFC 5, BE 10, EQX 3360-3, LEECTEEN 37f and LECTEEN 37i.

A highly significant difference ($P<0.001$) was also observed among genotypes for pod resistance to *Phytophthora palmivora* invasion based on size of lesions (Table3a). Significantly smaller lesions were recorded on SCA 6. Lesions on LAF 1, T85/799, GU225V, and ICS1 were moderate, whereas significantly larger lesions were produced on the rest of the genotypes.

At the post-penetration stage of resistance on leaf, five genotypes were resistant, nine genotypes were moderately resistant and 11 genotypes were susceptible (Figure 3). The resistant genotypes included SCA 6, LAF 1, T85/799, ICS 1 and GU 225V. The moderately resistant genotypes were LECTEEN 37i, EET 59H, PA 107, LECTEEN 37f, EQX 3360-3, IFC 5, UF 676, BE 10 and MAN 15-2.

The susceptible genotypes were PA 120, MOCORONGO, ICS 43, SPEC 54i, EQX 78, AMAZ 15-15, IMC 47, PA 150, VENC 4-4, MO 20 and T79/501 (Table 3b).

At the post-penetration stage of resistance on pod, five genotypes were resistant, 13 genotypes were moderately resistant and six genotypes were susceptible (Figure 3). The resistant genotypes were SCA 6, LAF1, T85/799, ICS1 and GU225V. The moderately resistant genotypes were LECTEEN 37i, ICS 43, SPEC 54i, and EQX78, EET 59H, PA 107, LECTEEN 37f, EQX 3360-3, IFC 5, PA 150, UF 676, BE 10 and MAN 15-2.

PA 120, MOCORONGO, AMAZ 15-15, IMC 47, VENC4-4, MO 20 and T79/501 were susceptible (Table 3b).

Two distinct patterns of spread were observed among the genotypes (Plate 15). In genotypes with relatively small lesions, such as SCA 6, necrosis was restricted predominantly within the mesophyll. On the contrary, rapid spread of lesions through both mesophyll and veins was observed in MO 20, and T79/501 resulting in very large lesions (Plate 15D). Plate 15 shows how lesion sizes spread in leaves of resistant, moderately resistant and susceptible genotypes.

Plate 16 shows lesions on pods of resistant, moderately resistant and susceptible genotypes. SCA 6 and T85/799 had small lesions whilst MO 20 and T79/501 had large lesions. Lesion size on pod of MAN 15-2 was intermediate between that of SCA 6 and MO 20. The uninoculated pod of PA 120 which is a control developed no lesions.

Table 3a. Lesion size on leaves and pods of 25 cacao genotypes inoculated with *Phytophthora palmivora*

Genotype	Mean lesion size (cm^2) on:			
	Leaf		Pod	
PA120	47.46	c	140.88	b
MOCORONGO	41.58	e	84.60	d
LECTEEN 37i	32.52	jk	44.84	k
ICS 43	39.20	i	52.70	ij
SPEC 54i	40.50	f	74.12	e
EQX 78	47.72	bc	69.50	f
EET 59H	23.92	l	35.41	m
PA 107	23.08	kl	34.32	m
LECTEEN 37f	30.62	ij	64.38	g
SCA 6	4.50	o	13.08	p
LAF 1	12.24	lm	21.29	o
T85/799	10.89	mn	21.12	o
AMAZ 15-15	42.06	e	82.64	d
EQX 3360-3	30.50	ij	42.15	l
IFC 5	22.90	l	50.74	j
IMC 47	46.66	d	83.12	d
PA 150	43.40	g	63.14	g
UF 676	35.88	ij	53.06	i
VENC 4-4	47.14	ab	95.5	c
ICS 1	10.86	lmn	23.08	o

Table 3a Continued.

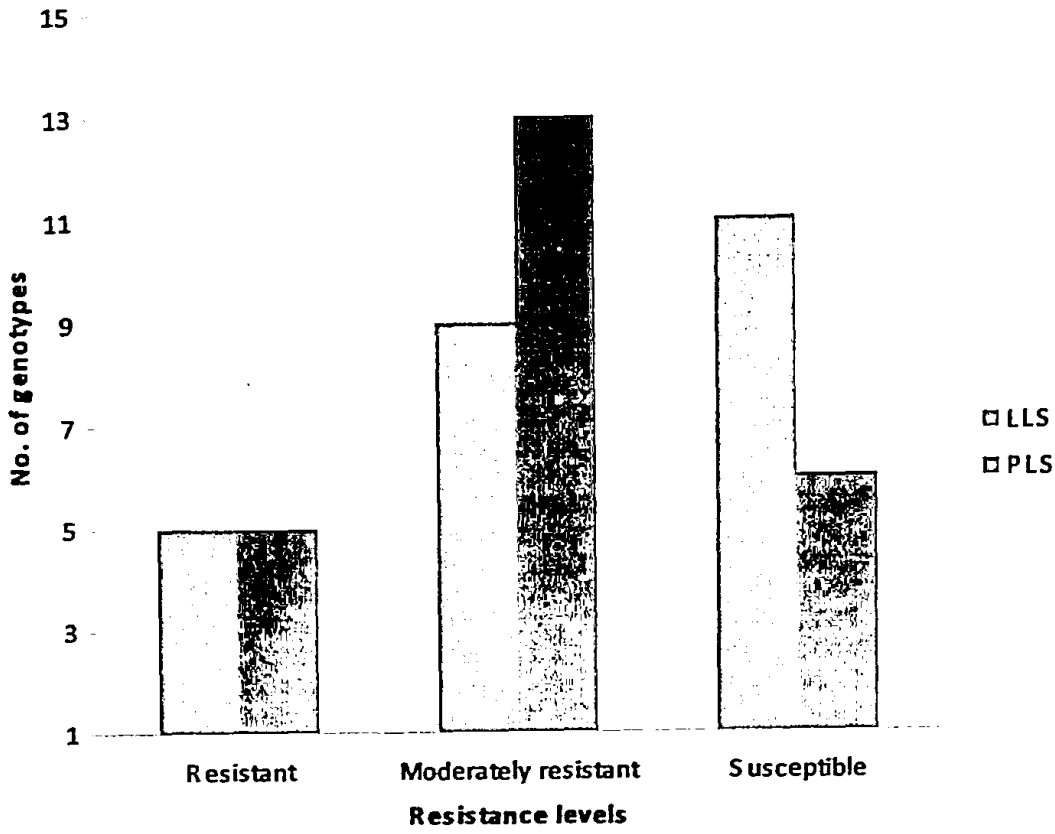
Genotype	Mean lesion size (cm^2) on:			
	Leaf		Pod	
BE 10	30.32	ij	64.54	g
MO 20	67.14	a	162.66	a
MAN 15-2	37.24	h	59.06	h
GU 225V	16.39	n	26.98	n
T79/501	59.55	ab	161.09	a
Lsd	**1.17**		**2.10**	
%CV	**1.75**		**1.63**	
Mean	**33.8**		**64.96**	

Table 3b. Distribution of the 25 into levels of resistance based on lesion size on leaf and pod

Level of resistance	Grouping of genotypes according to lesion size	
	Leaf	Pod
Resistant Genotype	SCA 6	SCA 6
	LAF 1	LAF 1
	T85/799	T85/799
	ICS 1	ICS1
	GU 225V	GU 225
Moderately Resistant	LECTEEN 37i	LECTEEN 37i
Genotype	EET 59H	ICS 43
	PA 107	SPEC 54i
	LECTEEN 37f	EQX 78
	EQX 3360-3	EET 59H
	IFC 5	PA 107
	UF 676	LECTEEN 37f
	BE 10	EQX 3360-3
	MAN 15-2	IFC 5
		PA 150
		UF 676
		BE 10
		MAN 15-2
Susceptible Genotype	PA 120	PA 120

Table 3b. Continued.

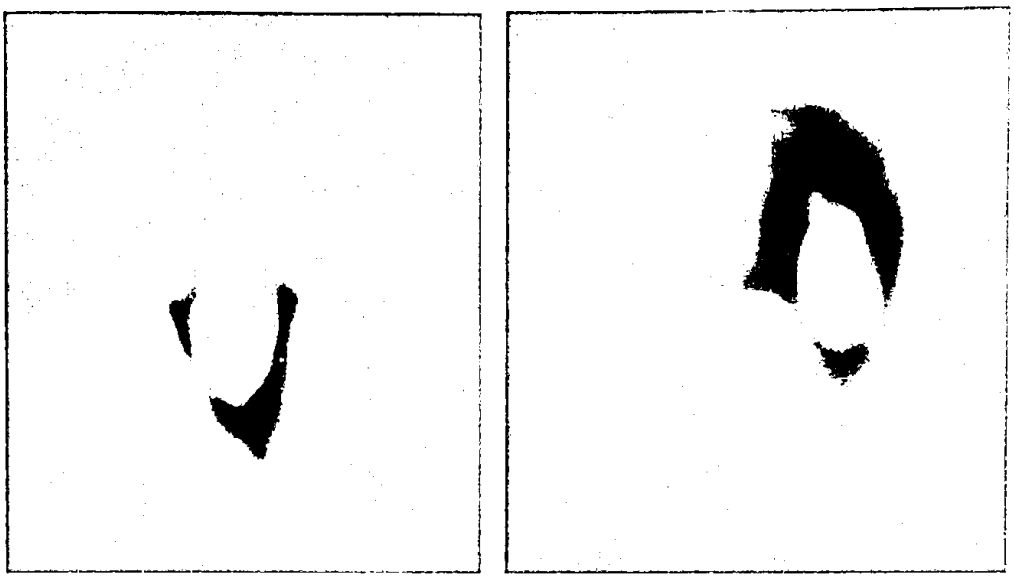
Level of Resistance	Grouping of Genotypes according to lesion size on:	
	Leaf	Pod
	MOCORONGO	VENC 4-4
	ICS 43	MO 20
	SPEC 54i	T79/501
	EQX 78AMAZ 15-15	MOCORONGO
	IMC 47	IMC 47
	PA 150	AMAZ 15-15
	VENC 4-4	
	MO 20	
	T79/501	



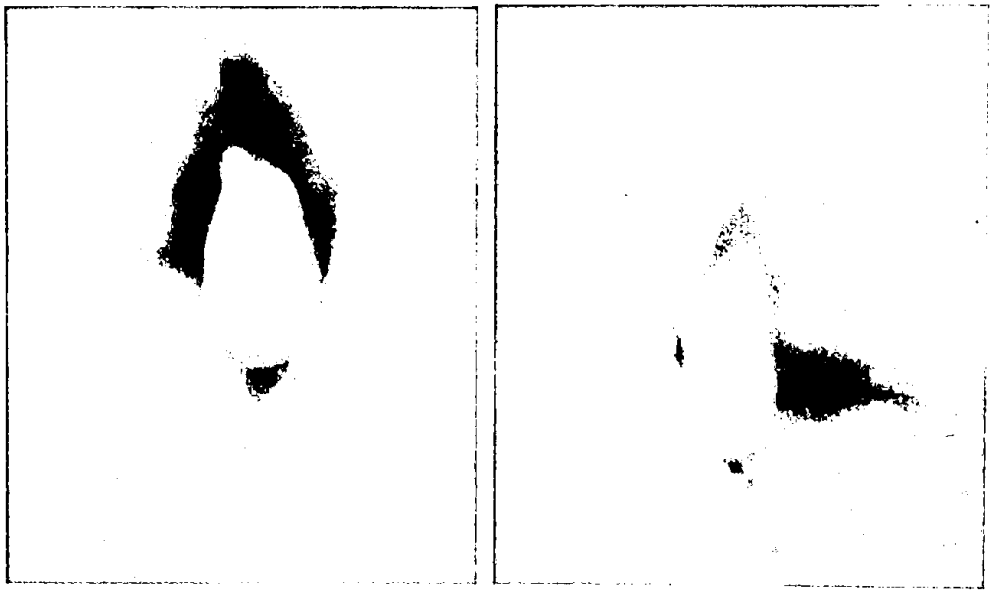
LLS = Leaf lesion size, PLS = Pod lesion size

Figure 3. Distribution of resistance levels of the 25 genotypes at the post-penetration stage of infection of leaves and pods.

(A) SCA6 (B) BE 10



(C) EET 59H (D) MO 20



Magnification: ×3

Plate 15. The pattern of spread of lesions in leaves after inoculation with *Phytophthora palmivora* at the post-penetration stage of infection. (A) SCA 6 (resistant), (B) BE 10 (moderately resistant), (C) EET 59H (moderately resistant) and (D) MO 20 (susceptible)

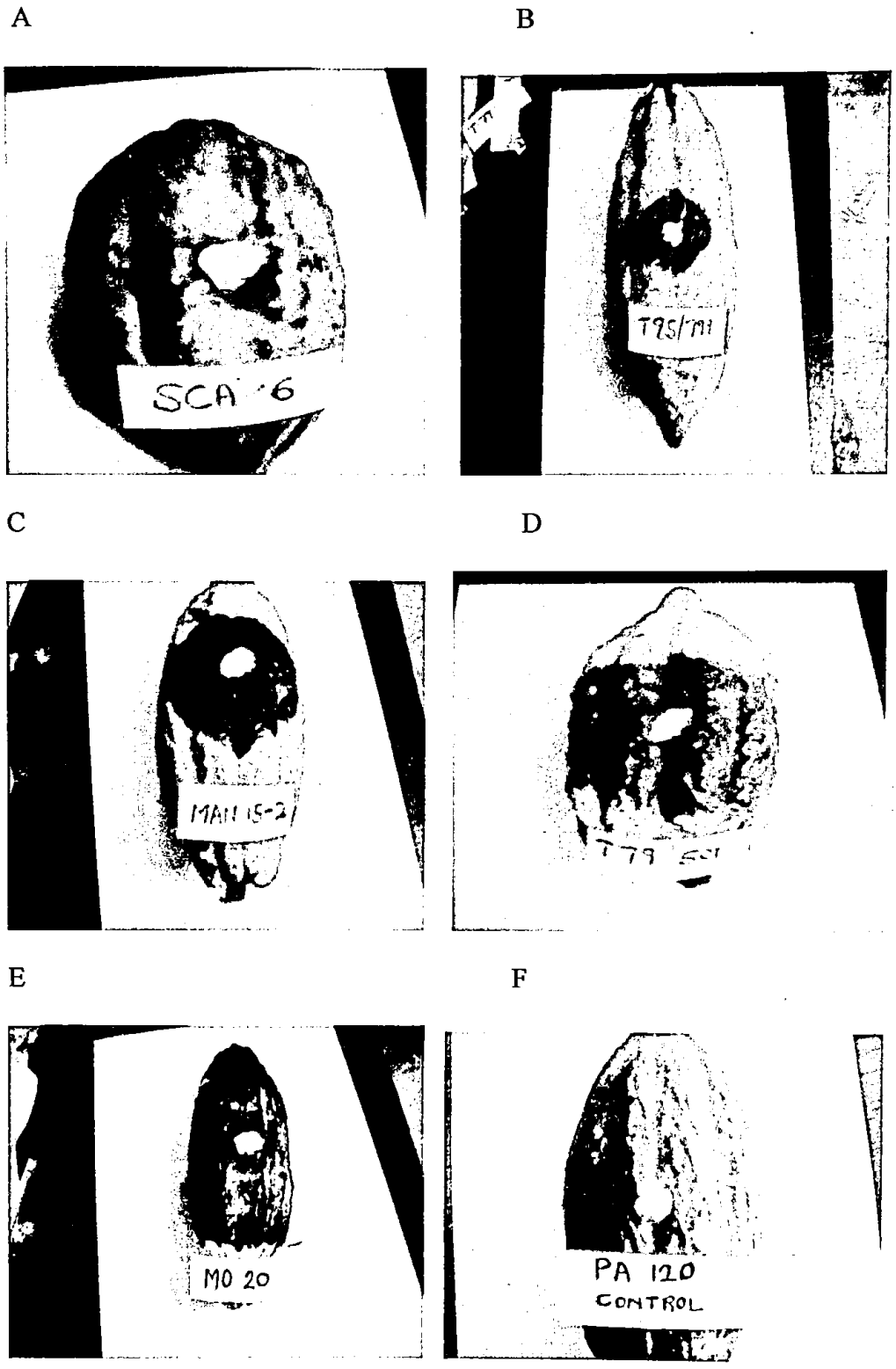


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Plate 16. The pattern of spread of lesions in pods after inoculation with *Phytophthora palmivora* at the post-penetration stage of infection.

A –SCA 6 (resistant), B-T85/799 (resistant), C-MAN 15-2 (moderately resistant), D –T79/501 (susceptible), E- MO 20 (susceptible), F – PA 120 (control).

Relationship between penetration resistance of leaves and pods.

Figure 4 is a graphical representation of the relationship between penetration resistance of leaves and pods.

A correlation analysis between number of lesions on leaves and pods showed significant correlation ($r = 0.881$ and $P < 0.001$) at the penetration level of infection (Figure 4).

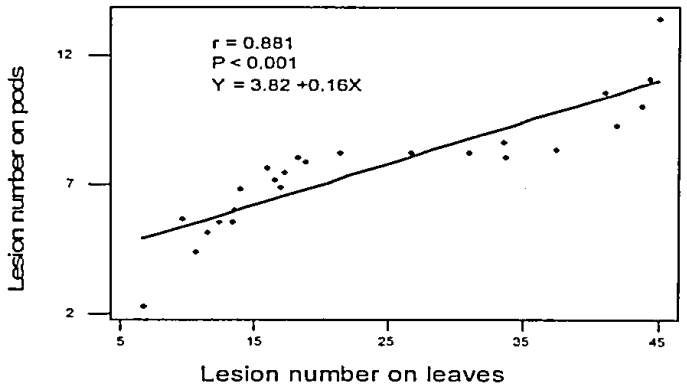


Figure 4. Relationship between number of lesions on leaves and pods of 25 Cacao genotypes inoculated with *Phytophthora palmi*vora.

Relationship between post-penetration resistance in leaves and pods.

Figure 5 is a graphical representation of the relationship between post-penetration resistance in leaf and pod. A correlation analysis between leaf lesion size and pod lesion size indicated significant correlation ($r = 0.898$ and $P < 0.001$) at the post-penetration levels of infection (Figure 5).

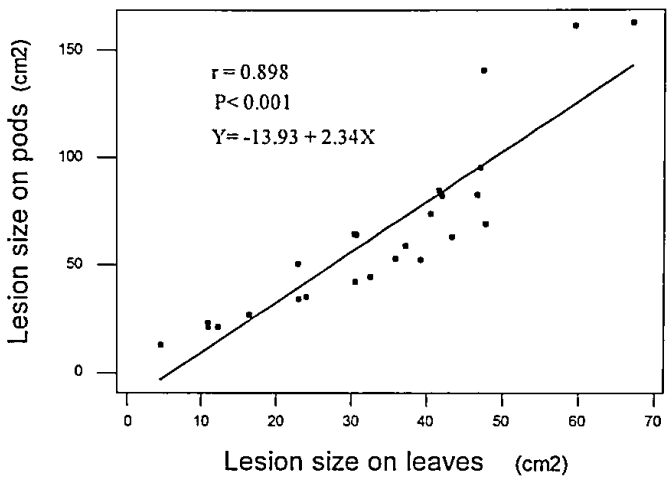


Figure 5. Relationship between size of lesions on leaves and pods of 25 cacao genotypes inoculated with *Phytophthora palmivora*.

Clonal differences in resistance scores of leaf disc tests

Table 4 shows clonal differences in resistance scores of leaf disc tests. Analysis of variance of disease severity scores on leaf discs of the 25 cacao genotypes inoculated with *Phytophthora palmivora* indicated that there were highly significant differences ($P< 0.001$) among some genotypes in their reactions to *Phytophthora palmivora*. From the table, it can be seen that the disease severity scores varied from 0.96 for SCA 6 to 4.28 for MO 20.

In Figure 6 are shown the distribution of disease severity scores of resistance to *Phytophthora palmivora* among the 25 genotypes in Trial-1, Trial-2 and Trial-3. The figure shows that disease severity varied among the 25 genotypes. None of the genotypes was highly resistant or totally immune to the *Phytophthora* disease. Also, none of the genotypes was observed to be highly susceptible to *Phytophthora palmivora* in all the trials.

Table 4 shows that in Trial-1, SCA 6 and T85/799 were resistant. The moderately resistant genotypes were EET 59H, PA 107, LAF1, IFC 5, ICS1 and GU 225V. The moderately susceptible genotypes were LECTEEN 37i, ICS 43, LECTEEN 37f, EQX 3360-3, UF 676, BE 10 and MAN 15-2. The susceptible genotypes were PA 120, MOCORONGO, SPEC54i, EQX 78, AMAZ 15-15, IMC 47, PA 150, VENC 4-4, MO 20 and T79/501.

In Trial-2, SCA 6 and ICS 1 can be seen to be resistant. The moderately resistant genotypes were EET 59H, PA 107, LAF 1, T85/799, IFC5 and GU225V. The moderately susceptible genotypes were MOCORONGO, LECTEEN 37i, ICS 43, SPEC 54i, LECTEEN 37f, AMAZ 15-15, EQX 3360-3, IMC 47, PA 150, UF 676, BE 10 and MAN 15-2. The susceptible genotypes were PA 120, EQX 78, VENC4-4, MO 20 and T79/501.

In Trial-3, SCA 6 can be seen to be resistant. The moderately resistant genotypes were ELT 59H, LECTEEN 37f, LAF 1, T85/799, IFC 5, ICS1, and GU 225V. The moderately susceptible genotypes were PA 120, MOCORONGO, LECTEEN 37i, ICS 43, SPEC 54i, EQX 78, PA 107, AMAZ 15-15, EQX 3360-3, IMC 47, UF 676, BE 10 and MAN 15-2. The susceptible genotypes were PA 150, VENC4-4 and T79/501.

Plate 17 shows how lesions developed on the surface of the leaf discs of resistant, moderately resistant and susceptible genotypes. It is clear from the Plate 17 that SCA 6 (J), LAF 1 (K), T85/799 (L), ICS1 (T) and GU 225V (X) which were the resistant genotypes remained green after inoculation with *Phytophthora palmivora*. The leaf discs of MO 20 (V), T79/501(Y), MOCORONGO (B), PA 120(A) and EQX 3360-3(N) which were the susceptible genotypes were deep brown in colour. The leaf discs of moderately susceptible genotypes were light brown in colour.

Table 4. Reaction of 25 cacao genotypes to inoculation with *Phytophthora palmivora* using the leaf disc inoculation.

Genotype	Mean leaf disc score(1-5 point scale)					
	Trial -1		Trial-2		Trial-3	
PA120	4.06	b	3.82	ab	3.36	b
MOCORONGO	3.64	c	3.44	cde	2.94	cd
LECTEEN 37i	3.10	de	3.04	gh	2.88	cde
ICS 43	3.18	d	3.08	fgh	2.54	de
SPEC 54i	3.54	c	3.28	defg	3.12	bc
EQX 78	3.62	c	3.54	cd	2.90	cd
EET 59H	2.00	g	1.96	k	1.86	fg
PA 107	2.10	g	2.32	j	2.48	e
LECTEEN 37f	3.18	d	3.02	h	2.70	cde
SCA 6	0.96	j	1.08	m	1.20	h
LAF 1	1.58	h	1.58	l	1.68	fg
T85/799	1.32	i	1.54	l	1.52	gh
AMAZ 15-15	3.60	c	3.44	cde	2.74	cde
EQX 3360-3	2.70	f	2.70	i	2.84	cde
IFC 5	2.20	g	2.16	jk	1.96	f
IMC 47	3.62	c	3.32	def	2.98	bc
PA 150	3.48	c	3.42	de	3.88	a
UF 676	3.14	de	3.20	efgh	2.98	bc
VENC 4-4	3.90	b	3.68	bc	3.92	a
ICS 1	1.52	hi	1.42	l	1.54	gh
BE 10	2.92	e	3.12	fgh	3.00	bc

Table 4 continued.

Genotype	Mean leaf disc score (1-5 point scale)					
	Trial-1		Trial-2		Trial-3	
MO 20	4.28	a	4.02	a	4.12	a
MAN 15-2	3.40	c	3.30	def	2.88	cde
GU 225V	1.48	hi	1.60	l	1.52	gh
T79/501	4.06	b	3.80	ab	3.98	a
LSD	0.21		0.23		0.36	
%CV	3.58		4.08		6.64	
S.E	0.10		0.12		0.18	

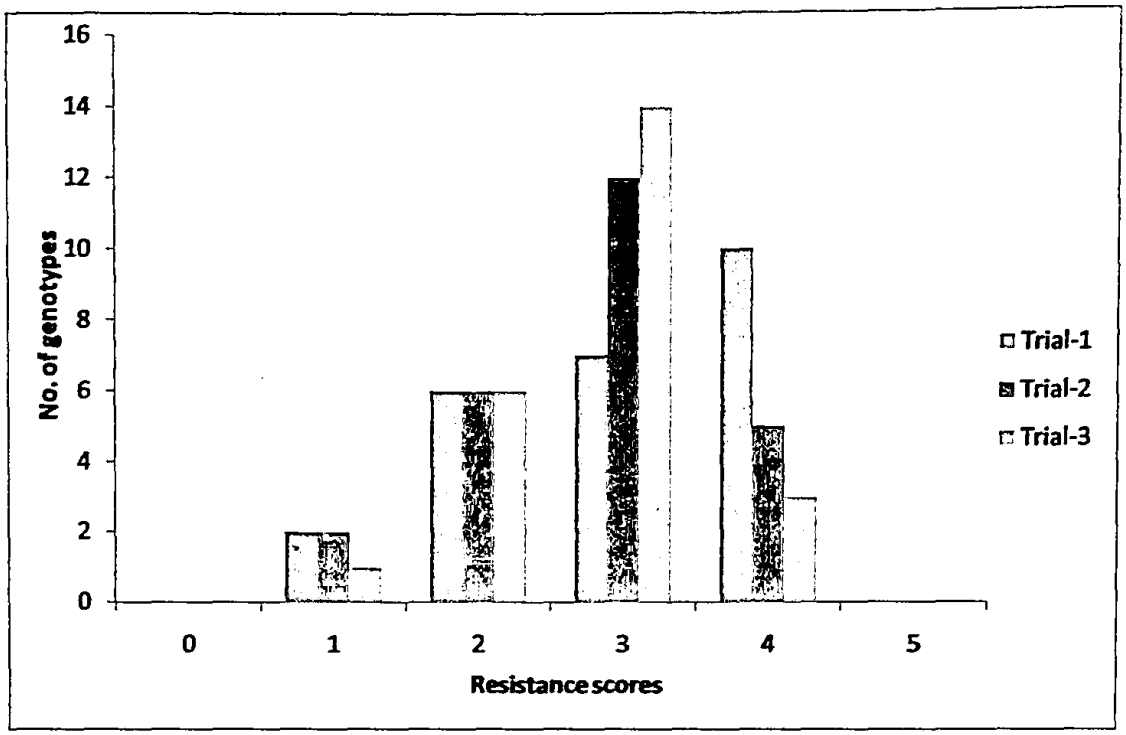
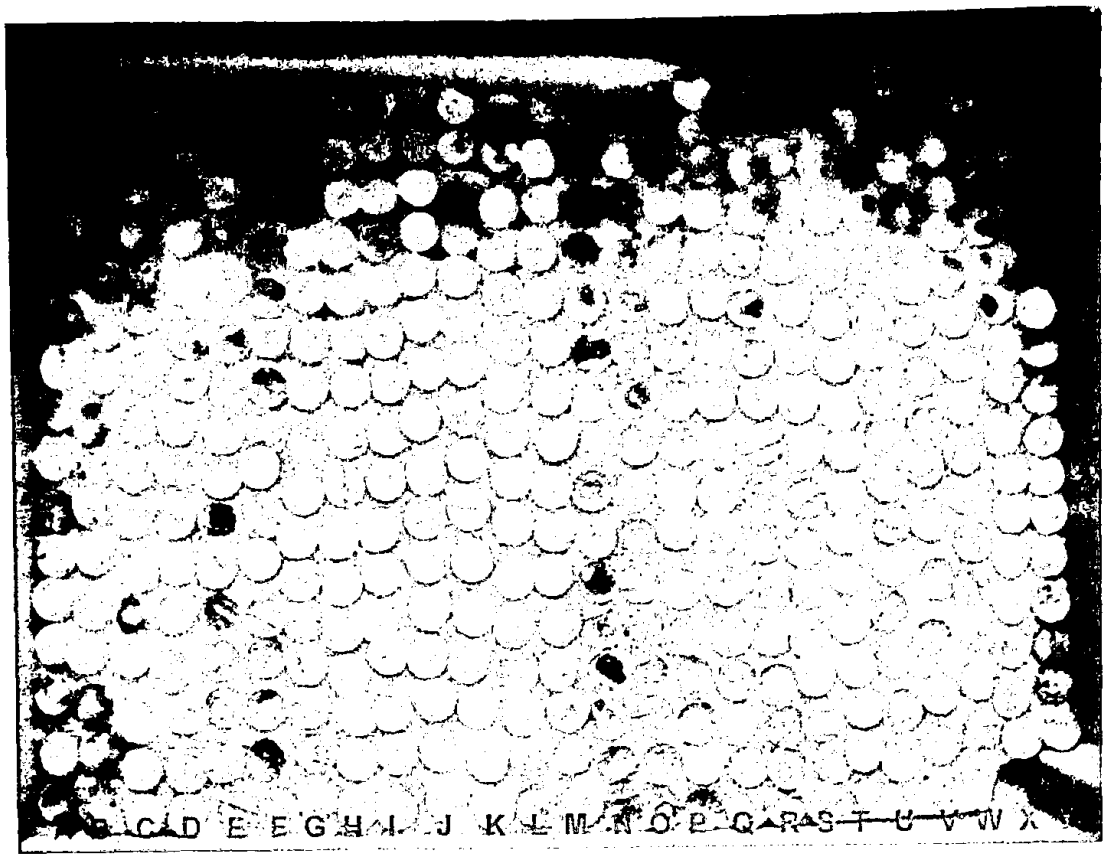


Figure 6. Distribution of scores for resistance to *Phytophthora palmivora* among 25 genotypes assessed by the leaf disc test in Trial-1, Trial-2 and Trial-3.

0= highly resistant or immune; 1 = resistant; 2= moderately resistant;

3= moderately susceptible; 4= susceptible; 5= highly susceptible.



Magnification: ×3

Plate 17. The spread of lesions on leaf discs of after inoculation with *Phytophthora palmivora.*

The alphabets in Plate 17 represent the 25 cacao clones as follows:

A = PA 120	I = LECT 37f	Q = PA 150	Y =T79/501
B = MOCORONGO	J = SCA 6	R = UF 676	
C = LECT. 37f	K = LAF 1	S = VENC 4-4	
D= ICS 43	L = T85/799	T= ISC 1	
E =SPEC 54i	M = AMAZ 15-15	U =BE 10	
F =EQX 78	N = EQX3360-3	V= MO 20	
G= EET 59H	O= IFC 5	W =MAN 15-2	
H= PA 107	P = IMC 47	X= GU 225V	

Repeatability of leaf disc test results

The repeatability of leaf disc test results is shown in Table 5. The table shows that there was a high correlation coefficient ($r = 0.993$, $P < 0.001$) between the results of Trial-1 and Trial-2. Similarly, a high correlation coefficient ($r = 0.928$, $P<0.001$) was observed between Trial -1 and Trial-3 and Trial-2 and Trial-3 ($r =0.938$, $P< 0.001$) respectively.

Table 5. Coefficients of correlation between mean disease severity values observed for genotypes in leaf disc inoculation trials in routine *Phytophthora palmivora* resistance screening experiments.

Coefficient of correlation between mean disease severity scores in inoculation trials			
Series	Trial 1	Trial 2	Trial 3
Trial 1		0.993**	0.928**
Trial 2			0.938**
Trial 3			

All coefficient of correlation were significant at 0.1% probability.

Correlation between disease severity scores in the leaf-disc test and penetration resistance in pod.

The relationship between leaf disc scores and lesion number on pods is graphically represented in Figure 7. It is clear from the figure that correlation between leaf disc score and penetration reaction of pod was significant ($r = 0.924$, $P < 0.001$).

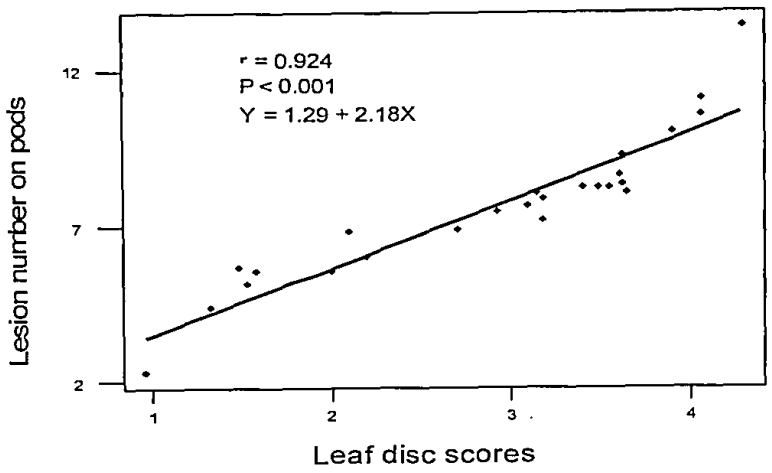


Figure 7. Relationship between number of lesions on pods and leaf disc scores in 25 cacao genotypes inoculated with *Phytophthora palmivora.*

Correlation between disease severity scores in the leaf-disc test and post-penetration resistance in pod.

The relationship between leaf disc scores and size of lesions on pods is graphically represented in Figure 8. The figure shows a significant correlation ($r = 0.846$ and $P < 0.001$) between leaf disc scores and the reaction of pods at the post-penetration stage of infection.

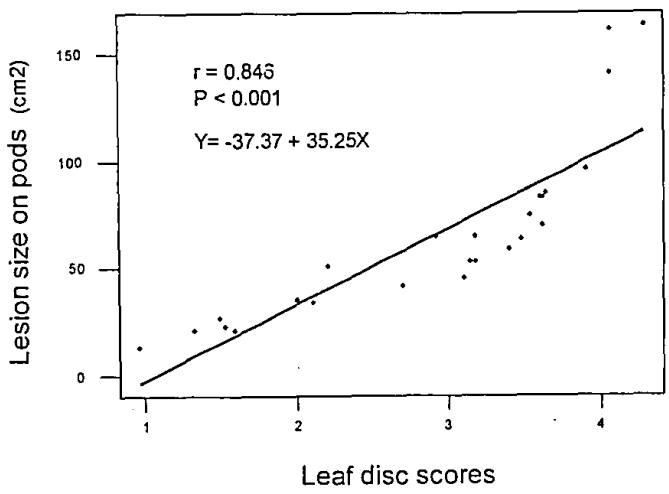


Figure 8. Relationship between lesion size on pods and leaf disc scores in the 25 cacao genotypes inoculated with *Phytophthora palmivora*.

Field Infection of *Phytophthora palmivora* of the 25 Cacao genotypes.

Table 6 contains the field infection of *Phytophthora* of the 25 cacao genotypes studied. The field resistant levels of the 25 genotypes to *Phytophthora palmivora* were significantly different ($P< 0.001$) from each other.

Figure 9 illustrates clearly the infection rates of the genotypes to *Phytophthora* infection within each of the months that the field study was carried out. In July (Figure 9a), IFC5 was least infected. The moderately infected genotypes were EET 59H, PA107, SCA6, T85/799 and GU225V whilst PA 120, MOCORONGO, LECTEEN 37i, LECTEEN 37f, ICS 43, SPEC54i, EQX78, LAF1, AMAZ15-15, EQX3360-3, IMC47, PA150, UF676, VENC4-4, ICS1, BE10, MO20, MAN 15-2 and T79/501 were highly infected.

In August (Figure 9b), it can be seen that EET59H, PA 107 and SCA 6 were least infected. The moderately infected genotypes were T85/799, IFC5 and GU225V and the highly infected genotypes included PA 120, MOCORONGO, LECTEEN 37i, ICS 43, SPEC 54i, EQX78, and LECTEEN 37f, LAF1, AMAZ15-15, EQX3360-3, IMC47, PA150, UF676, VENC4-4, ICS1, BE 10, MO 20, MAN 15-2 and T79/501.

In September (Figure 9c), MOCORONGO, LECTEEN 37i, SCA 6 and T85/799 were highly infected. The moderately infected genotypes were EQX 3360-3, IFC5 and GU225V whilst the highly infected genotypes were PA 120, ICS 43, SPEC 54i, and EQX 78, EET 59H, PA 107, LECTEEN 37f, LAF 1, AMAZ 15-15, IMC 47, UF676, VENC4-4, ICS1, BE 10, MO 20, MAN 15-2 and T79/501.

In October (Figure 9d), PA120, LECTEEN 37i, ICS 43, SCA6, T85/799, AMAZ 15-15, EQX 3360-3,IFC 5,ICS 1, MAN 15-2 and GU 225V were least infected and the moderately infected genotypes were MOCORONGO, SPEC 54i, EQX 78, EET 59H, PA 107 and LECTEEN 37f. The genotypes which were highly infected included LAF 1, IMC 47, PA 150, UF 676, VENC4-4, MO 20 and T79/501.

In November (Figure 9e), EET 59H, PA 107, SCA 6, T85/799, IFC5, IMC 47, UF676 and GU225V were least infected. The moderately infected genotypes were PA120, MOCORONGO, SPEC 54i, EQX78, LECTEEN 37f, AMAZ 15-15, PA 150, MAN 15-2 and T79/501. The highly infected genotypes were LECTEEN 37i, ICS 43, LAF 1, EQX3360-3, VENC4-4, ICS1 and MO20.

In December (Figure 9f) , PA 120, MOCORONGO, ICS43, SPEC 54i, EQX 78, EET 59H, PA 107, LECTEEN 37f, SCA6, LAF1, T85/799, AMAZ 15-15, EQX 3360-3, IFC 5, IMC 47, VENC4-4,ICS1, BE 10, MO 20, MAN 15-2 and GU 225 V were least infected. The moderately infected genotypes were LECTEEN 37i, UF 676 and T79/501 whilst the highly infected genotype was PA 150.

Table 6. Percentage of field *Phytophthora palmivora* infection observed over six (6) months.

Genotype	Months of observation (2007)					
	July	August	Sept.	Oct.	Nov.	Dec
PA120	60.4	60.4	85.0	7.4	17.4	0.0
MOCORONGO	75.0	78.9	0.0	10.8	20.0	8.7
LECTEEN 37i	87.5	92.3	0.0	0.0	33.3	13.3
ICS 43	53.7	100.0	84.6	3.0	36.4	3.3
SPEC 54i	54.3	44.8	30.8	21.7	15.8	4.8
EQX 78	48.1	54.8	41.7	19.2	17.6	5.9
EET 59H	23.8	5.9	66.7	20.6	0.0	1.1
PA 107	24.2	7.4	36.2	13.7	0.0	5.2
LECTEEN 37f	41.2	31.0	58.9	25.5	23.1	4.3
SCA 6	12.5	6.7	0.0	0.0	0.0	0.0
LAF 1	50.0	71.4	100.0	57.9	33.3	3.1
T85/799	13.5	11.1	0.0	2.3	3.0	0.0
AMAZ 15-15	51.4	65.3	37.5	8.2	22.7	0.0
EQX 3360-3	83.3	89.7	16.7	6.3	72.7	0.0
IFC 5	4.0	22.6	17.2	1.7	7.7	3.8
IMC 47	30.2	36.2	73.3	65.4	10.2	10.2
PA 150	67.2	72.1	100.0	68.8	24.0	50.2
UF 676	46.9	57.5	90.0	66.1	5.6	14.4
VENC 4-4	75.0	84.6	57.9	53.6	50.0	0.0
ICS 1	25.7	75.0	50.0	9.8	33.3	0.0

Table 6 Continued.

Genotype	Months of Observation (2007)					
	July	August	Sept.	Oct.	Nov.	Dec.
BE 10	75.0	87.8	33.3	22.7	41.7	3.8
MO 20	62.5	77.3	72.2	35.8	40.0	1.6
MAN 15-2	34.8	49.2	50.0	9.5	25.0	3.8
GU 225V	25.0	20.0	17.5	8.0	0.0	1.8
T79/501	49.4	54.6	97.8	61.9	21.4	11.5
Lsd	3.15	3.24	6.83	7.93	1.71	1.39
S.e	1.59	1.63	3.44	3.99	0.86	0.70

<10%= resistant, 10- 25%= moderately resistant, > 25% = susceptible.

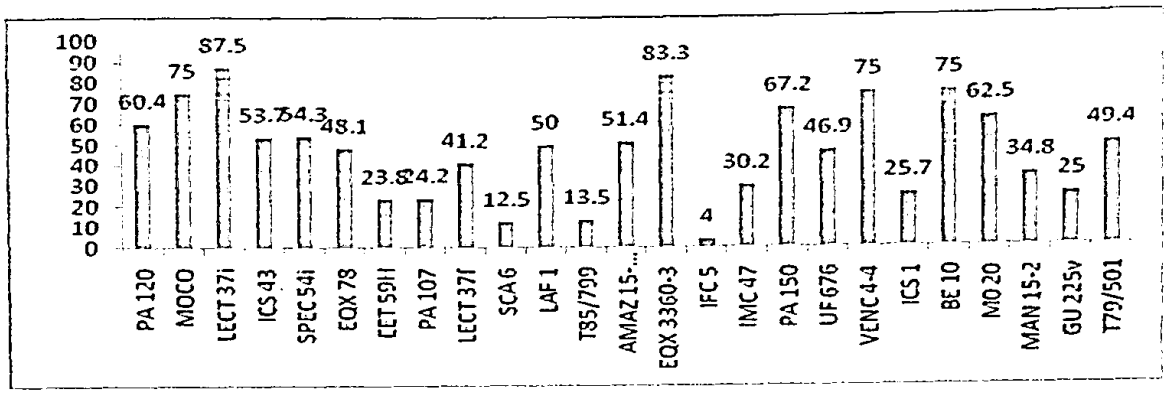


Figure 9a. Percentage black pod infection of the 25 genotypes in July.

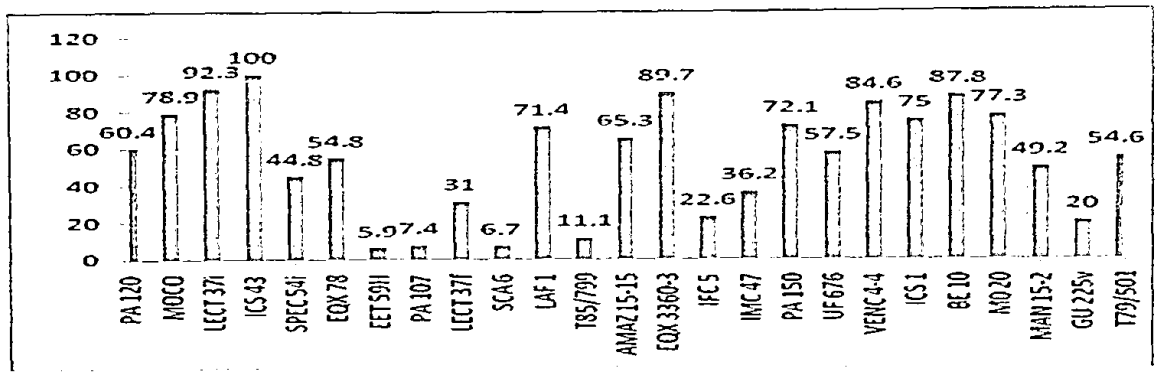


Figure 9b. Percentage black pod infection of the 25 genotypes in August.

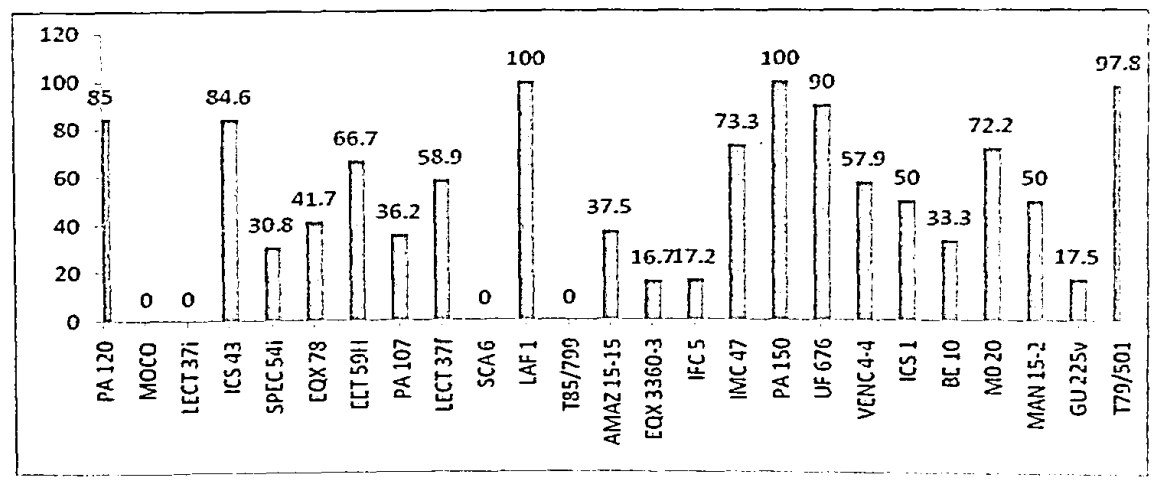


Figure 9c. Percentage black pod infection of the 25 genotypes in September.

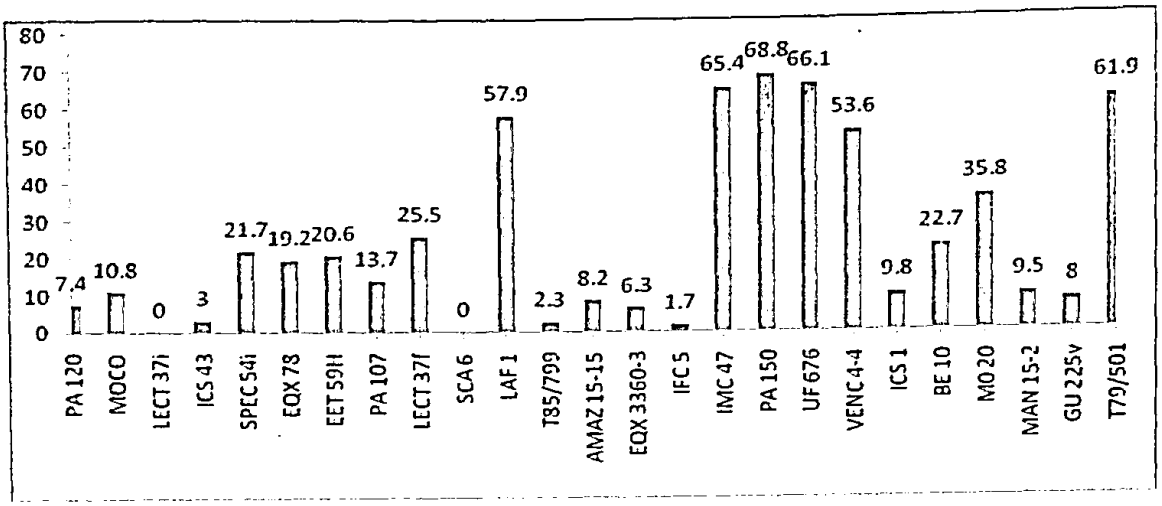


Figure 9d. Percentage black pod infection of the 25 genotypes in October.

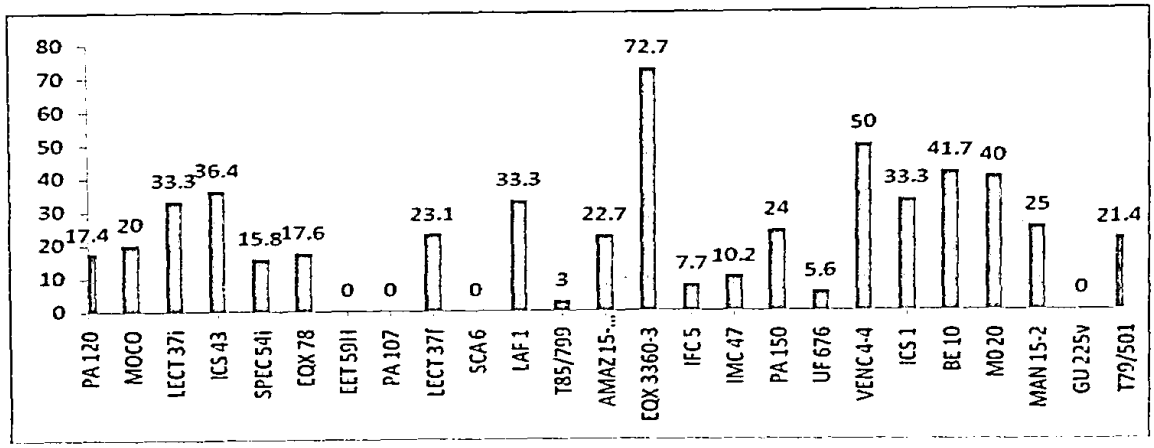


Figure 9e. Percentage black pod infection of the 25 genotypes in November.

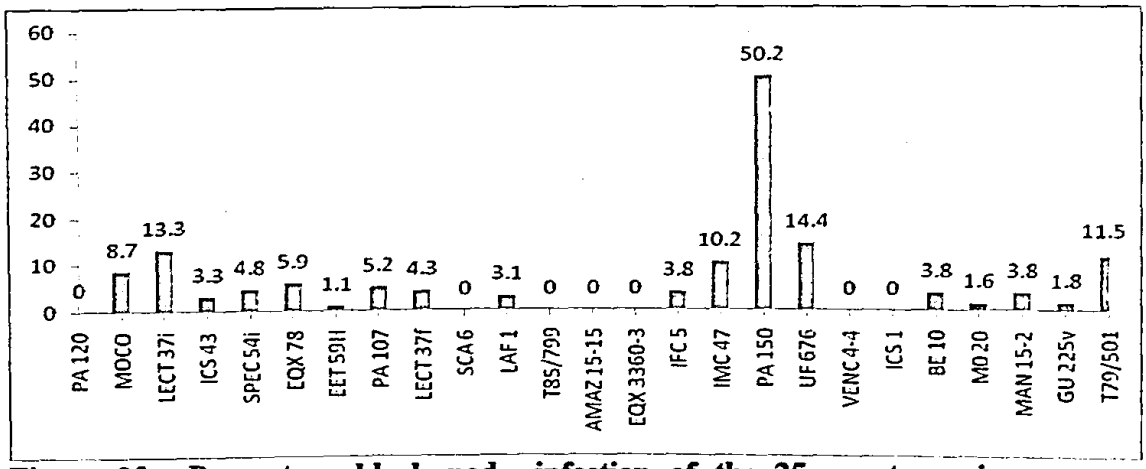


Figure 9f. Percentage black pod infection of the 25 genotypes in December.

In Table 7 are presented the meteorological data during the period when data was collected on black pod infection in the field. The table shows that, amount of rainfall, number of wet days and relative humidity were high in the months of July, August and September. Maximum temperature was however lower in the months July, August and September as compared to October, November and December.

Table7. Meteorological data for July-December 2007. CRIG meteorology centre.

Month	Rainfall (mm)	Wet days	Humidity (%)	Temp. (°C)
July	268.8	14	86.6	29.1
August	392.2	17	89.5	28.8
September	180.7	13	85.8	29.1
October	140.2	9	82.2	32.0
November	53.7	7	81.4	31.7
December	53.5	4	75.3	32.2

Variations in black-pod infection in the period of field study.

Significant differences ($P < 0.001$) were observed among the months of field observations in terms of black-pod infection. Figure 10 is a graphical presentation of the variations in black-pod infection of the genotypes in the field during the period of study.

There was a higher level of cacao pod infection of 47.0% in the month of July, 54.3% in August and 48.7% in September than 24.0% in October, 22.2% in November and 6.03% in December as shown in Figure 10.

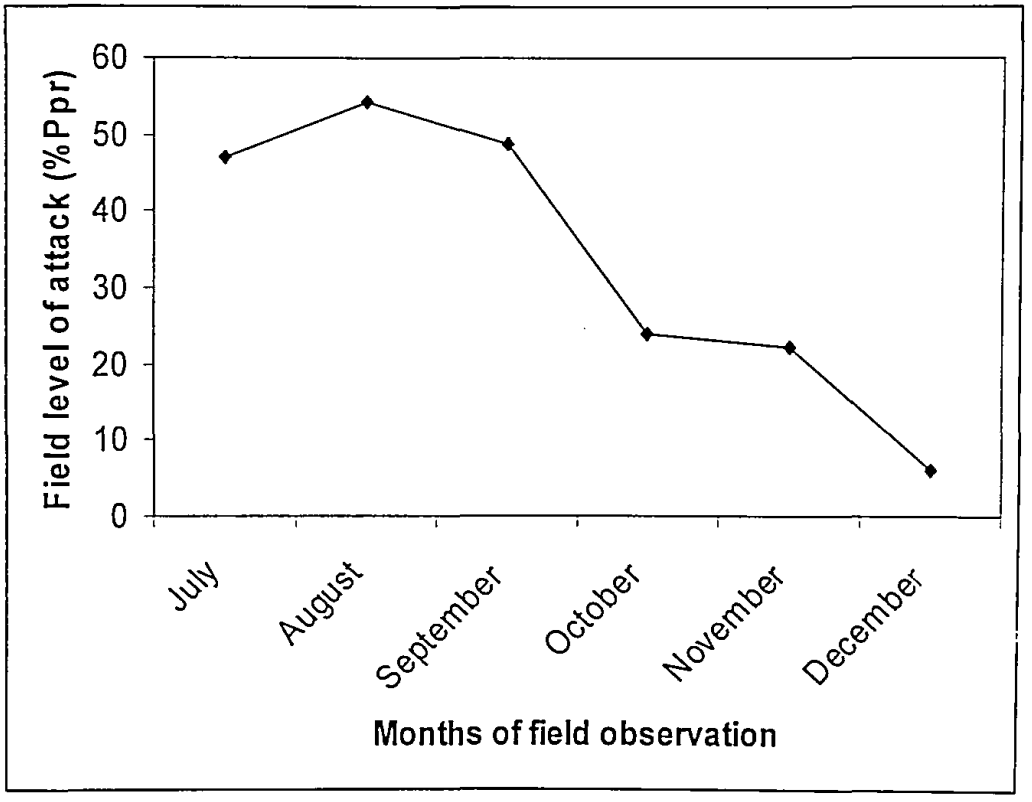


Figure 10. Percentage of *Phytophthora palmivora* infection in the field observed over six months

Average of field infection of cacao pods of the different genotypes by *Phytophthora palmivora* observed over six months are presented in Table 8.

The table shows that in the field, the genotypes studied varied in resistance to black pod infection. The resistant genotypes were SCA 6, T85/799 and IFC 5. The moderately resistant genotypes were EET59H, PA 107 and GU 225V. The susceptible genotypes were PA 120, MOCORONGO, LECTEEN 37f, ICS 43, SPEC 54i, EQX 78, LECTEEN 37f, LAF 1, AMAZ 15-15, EQX 3360-3, IMC 47, PA 150, UF 676, VENC4-4, ICS 1, BE 10, MO 20, MAN 15-2 and T79/501.

Table 8. *Phytophthrora* pod rot infection over six months, July to December, 2007.

Genotype	Mean monthly percentage infection	
PA 120	38.4	abcd
MOCORONGO	32.2	bcde
LECTEEN 37i	37.7	abcd
ICS 43	46.8	abc
SPEC 54i	28.7	bcdefg
EQX 78	31.2	bcdef
EET 59H	19.7	cdefg
PA 107	14.5	defg
LECTEEN 37f	30.7	bcdef
SCA 6	3.2	g
LAF 1	52.6	ab
T85 / 799	5.0	fg
AMAZ 15-15	30.9	bcdef
EQX 3360-3	44.8	abc
IFC 5	9.5	efg
IMC 47	37.6	abcd
PA 150	63.7	a
UF 676	46.8	abc
VENC 4-4	53.5	ab
ICS 1	32.3	bcde
BE 10	44.1	abc

Table 8 Continued.

Clone	Mean monthly percentage infection	
MO 20	48.2	ab
MAN 15-2	28.7	bcdefg
GU 225V	12.1	defg
T79/ 501	49.4	ab
L.S.D	22.72	
%CV	59	
MEAN	33.7	

<10%= resistant. 10- 25%= moderately resistant. > 25% = susceptible.

Relationship between field infection and the reaction of detached pods to *Phytophthora palmivora* at the penetration stage of infection

The relationship between average of field infection and lesion number on pods is graphically represented in Figure 11.

The results of the reaction of pods at the penetration level show a higher correlation (r = 0.622 and P =0.001) with the average of field infection in the months of July to December, 2007.

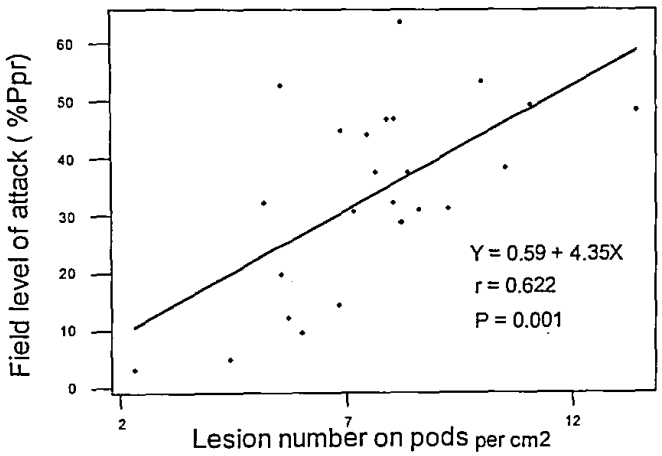


Figure 11. Relationship between lesion number on pods and average monthly *Phytophthora palmivora* pod rot infection observed over six months.

Relationship between field infection and the reaction of detached pods to *Phytophthora palmivora* at the post- penetration stage of infection

The relationship between average of field observations and size of lesions on pods is graphically represented in Figure 12.

A correlation coefficient of 0.472 (P = 0.017) was obtained between the reaction of pods at the post-penetration level of infection and the average of field infections in the months of July to December 2007, (Figure 12).

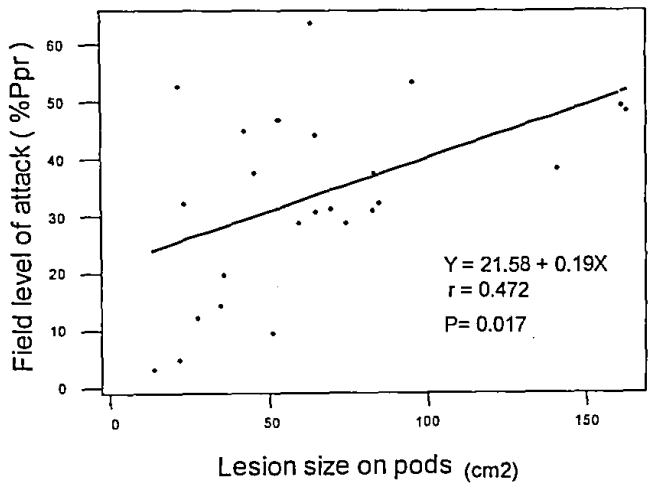


Figure 12. Relationship between size of lesions on pods and average of six months of field infection of *Phytophthora palmivora* pod rot.

Relationship between field infection and the reaction of detached leaves to *Phytophthora palmivora* at the penetration stage of infection

The relationship between average of field infection and lesion number on leaves is graphically represented in Figure 13.

The results of the reaction of leaves at the penetration level however show a significant correlation (r = 0.498, P = 0.025) with the average of field infection in the months of July to December, 2007.

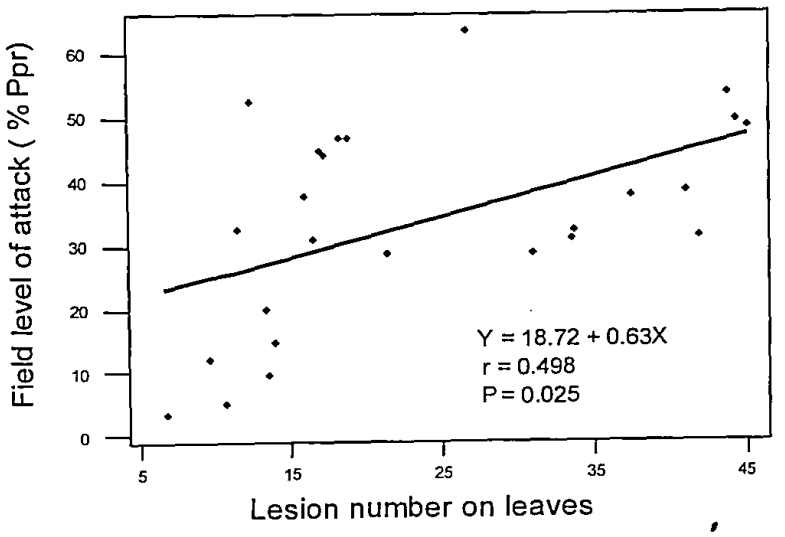


Figure 13. Relationship between lesion number on leaves and average of *Phytophthora palmivora* pod rot infection in the field over six months.

Relationship between field infection and the reaction of detached leaves to *Phytophthora palmivora* at the post-penetration stage of infection

The relationship between average of field infection and size of lesions on the leaves is graphically represented in Figure 14.

A correlation coefficient of 0.602 (P = 0.001) was obtained between the reaction of leaves at the post –penetration level of infection and the average of field infections in the months of July to December, 2007.

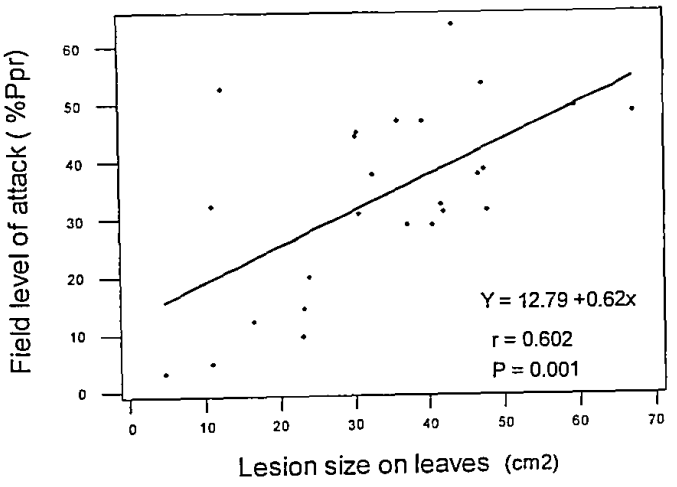


Figure 14. Relationship between lesion size on leaves and average of *Phytophthora palmivora* pod rot infection in the field over six months.

Relationship between field infection and the results of the leaf disc test

The relationship between average of field infection and leaf disc scores is graphically represented in Figure 15.

A correlation coefficient of 0.625 (P = 0.001) was obtained between leaf disc tests and the average of field infection in the months of July to December, (Figure 15) 2007.

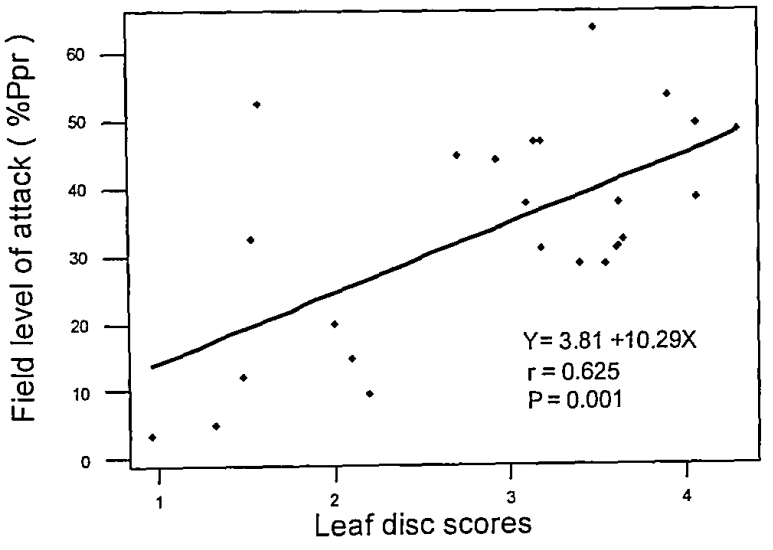


Figure 15. Relationship between leaf disc scores and average of *Phytophthora palmivora* pod rot infection in the field over six months.

Lesion sizes on distal, proximal and mid region of pods

In Table 9 are shown the sizes of lesions at the distal, proximal and mid regions of the pod, after inoculation at the respective regions with *Phytophthora palmivora*.

Analysis of Variance of lesion sizes obtained from the punch inoculation tests of parts of pods indicated that lesion sizes on pods varied highly significantly ($P < 0.001$) among the clones whether inoculated at the distal region or proximal region or the mid region.

Table 9. Lesion sizes on pods inoculated at the distal and proximal ends and at mid-region with *Phytophthora palmivora.*

Genotype	Lesion size at inoculated regions		
	Distal End	Mid region	Proximal End
ICS 43	44.1 de	51.3 de	55.7 de
SPEC 54i	62.9 c	71.3 c	76.0 c
EQX 78	62.1 c	68.9 c	74.0 cd
EET 59H	31.3 ef	36.6 ef	41.6 ef
PA 107	32.0 ef	36.6 ef	44.7 ef
LECTEEN 37f	56.0 cd	63.3 cd	68.1 cd
LAF 1	20.3 f	25.0 f	29.7 f
T85/ 799	19.4 f	22.3 f	27.1 f
PA 150	60.7 cd	66.2 cd	71.2 cd
UF 676	46.8 cde	52.2 de	57.3 de
VENC4-4	80.9 b	92.3 b	97.1 b
ICS 1	23.7 f	24.8 f	29.3 f
MO 20	108.6 a	120.1 a	124.9 a
MAN 15-2	51.9 cd	59.8 cd	64.6 cd
T79/ 501	118.6 a	131.8 a	136.8 a
Lsd	15.05	15.99	16.61
%CV	13.46	12.69	12.19
MEAN	32.8	36.9	39.9

The differences among sizes of lesions at the distal, proximal and mid regions of pods are presented in Table 10.

There were highly significant differences ($P<0.001$) among lesion sizes at the distal, proximal and mid regions of pods inoculated with *Phytophthora palmivora.* The lesions at the proximal end were larger than those at the mid region and distal end of the pods.

Table 10. Differences in lesions at different regions of pods inoculated with *Phytophthora palmivora.*

Region of pod inoculated	Lesion size
Proximal end	66.54 a
Mid-region	61.50 b
Distal end	54.63 c
Lsd	3.225
%CV	3.17

Variation of clones

A cluster analysis of the 25 genotypes based on the pooled data of the various experiments carried out in this study has been presented as a dendrogram in Figure 16. From the figure it is quite clear that the 25 genotypes were grouped into two distinct populations, one being considerably larger than the other. The larger population was made up of clusters 'I' to 'IV'. It can be seen that the two distinct populations were made up of different genetic groups.

The smaller population was made up of only cluster 'V'. Cluster 'V' as can be seen from the table consisted of only Parinari genotypes namely PA 150, PA 120 and PA 107. The three Parinari genotypes were collected from Peru and they belong to the Forastero population of cacao. The dendrogram shows that the genetic divergence between the larger population and the smaller population was wide with a similarity distance of 25.

It is clear from the dendrogram that the five clusters were not mere groupings of the genotypes with similar morphological characters, but also as distinct genetic groups. Each of the five clusters consisted of a number of genotypes.

Cluster IV had the largest intra-cluster variance followed by those of Clusters I and II. The least intra-cluster variance occurred in Cluster III and V.

It is interesting to note that all the genotypes in Cluster I originated from the lower Amazon region and they were LAF 1, LECTEEN 37f, LECTEEN 37i, EQX 78, EQX 3360-3 and BE 10. The names, origin and population of genotypes in each cluster are given in Table 11. In Cluster II were grouped some genotypes which were grouped Forasteros and they were IMC 47,

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AMAZ 15-15, SPEC54i, MAN 15-2, MOCORONGO, VENC4-4, IFC 5 and MO 20. From the cluster analysis, it can also be seen that some Trinitario genotypes; T85/799, T79/501 and UF 676 were grouped with some Forastero genotypes; SCA 6, EET 59H, and GU 225V in cluster IV. Also, Imperial College Selection (ICS) genotypes; ICS1 and ICS 43 originated from Trinidad were grouped in Cluster III.

Table 11: Distribution of the 25 genotypes into clusters

Cluster	Genotype		Origin	Population
I	LAF1	9.0	Peru	Forastero
	LECT 37f	52.6	Ecuador	Criollo
	LECT 37i	30.7	"	"
	EQX 78	37.7	"	Forastero
	EQX 3360-3	44.8	"	"
	BE 10	44.1	Brazil	"
II	IMC 47	37.6	Peru	"
	AMAZ15-15	30.9	"	"
	SPEC 54i	28.7	Colombia	"
	MAN 15-2	28.7	Brazil	"
	MOCO	32.2	"	"
	VENC 4-4	53.5	Peru	"
	IFC 5	9.5	"	"
	MO 20	48.2	"	
III	ICS 1	32.3	Trinidad	Trinitario
	ICS 43	28.7	"	"
	T85/799	5.0	"	Trinitario

Table 11 Cont.

Cluster	Genotype		Origin	Population
IV	T79/501	49.4	Trinidad	Trinitario
	UF 676	46.8	Costa Rica	"
	SCA 6	3.2	Peru	Forastero
	EET 59H	14.5	Ecuador	"
	GU225V	12.1	French	"
	PA 150	63.7	Peru	Forastero
V	PA 120	38.4	"	"
	PA 107	14.5	"	"

Lower Amazon region = Brazil, Peru and Ecuador

Upper Amazon = Costa Rica, Mexico, Trinidad, Colombia and French

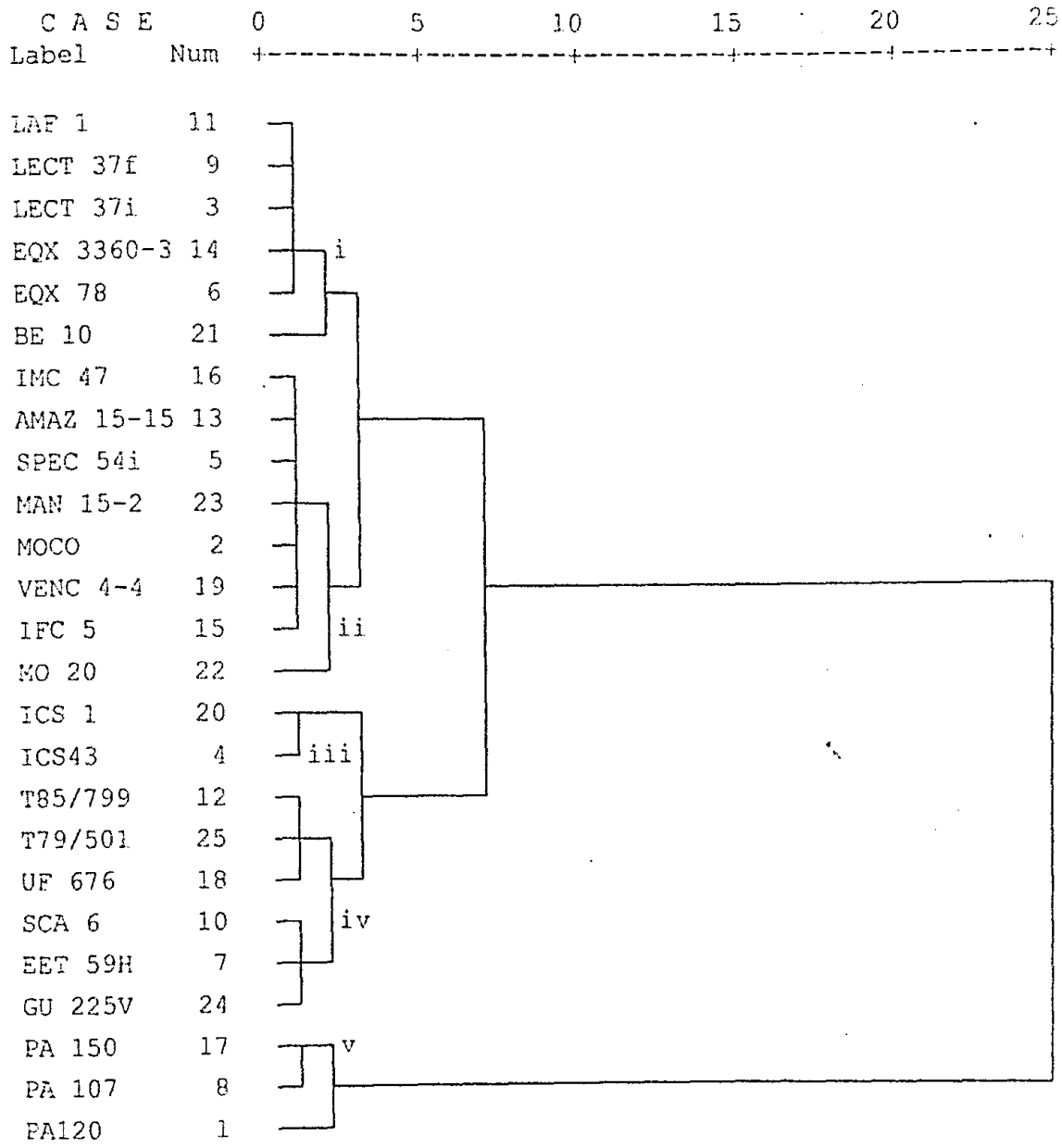

Figure 16. Variation among the 25 genotypes.

Dendrogram derived from pooled data from the various experiments following cluster analysis

CHAPTER FIVE

DISCUSSION

Generally the genotypes were observed to vary significantly in their reactions at the penetration and post-penetration stages of *Phytophthora* infection. With all the genotypes planted under the same environmental conditions the observed variations could probably be genetic. Tan and Tan (1990) and Simmonds (1994) have observed similar variations in their studies on the resistance of cacao progenies to *Phytophthora palmivora.* The significant variations among clones at the penetration and post-penetration stages of the infection processes in both leaves and pods seem to suggest further the genetic basis of the variation observed with the two different parts of the cacao tree reacting apparently similarly to the *Phytophthora palmivora* infection. Such large genetic variations have been noted by Blaha and Latode (1997), Nyasse *et al.* (1994) and Iwaro *et al.* (2005) who reported that there were significant differences among cacao genotypes after inoculation of detached pods with *Phytophthora palmivora.*

The distribution of genotypes into the various levels of resistance at the penetration and post-penetration stages of infection suggests that penetration and post-penetration inoculation of leaves and pods can effectively discriminate between the levels of resistance of genotypes.

The significant correlation between number of lesions on leaves and that on pods (see Figure 4.) suggests that the resistance mechanisms operative at the penetration stage of infection in both leaf and pod may be alike.

The observed similarity between the resistant levels to *Phytophthora* of cacao genotypes inoculated either by detached pod or detached leaf method suggest that either screening method could be used for the screening of resistance against black pod disease of cacao. This eliminates the need to use two different inoculation methods to effectively assess resistance at the penetration level of leaf and pod. Resistance of leaves at the penetration stage of infection can therefore be used to predict pod resistance. This finding is contrary to that of Iwaro *et al.* (1997) who observed poor correlation between penetration resistance in leaf and pod. They suggested that since *Phytophthora* infects both leaf and pod, resistance in leaf and pod at the penetration level of infection could complement each other.

The high positive correlation obtained between pod and leaf resistance at the post-penetration stage of infection probably suggests that the mechanism conferring post-penetration resistance within leaf and pod could be inherent.

The occurrence of such a characteristic of cacao leaf suggests the possibility of the use of leaves of cacao seedlings for the prediction of pod resistance to the black pod disease. This observation is in conformity with findings of Iwaro *et al.* (1997) who observed high positive correlation between leaf and pod at the post-penetration stage of infection.

The differences among genotypes at the post-penetration level as reflected in sizes and patterns of spread of lesions (see Plates 15 and 16) could be due to presence of some biochemical factors. Spence (1961) indicated the involvement of oxidizable phenols and polyphenol oxidase activity in determining lesion size in cacao.

The significant differences among the genotypes of cacao in their reactions to *Phytophthora palmivora* and the distribution of scores for resistance to *Phytophthora palmivora* from serial trials indicate that leaf disc test effectively discriminated the various levels of resistance in the 25 cacao accessions assessed in this study. Nyasse *et al.* (1995), Tondge *et al.* (1998) and Tahi *et al.* (2006) who studied the reaction of some cacao genotypes to *Phytophthora palmivora* infection made a similar observation.

The high correlation coefficients observed among the results of serial trials of leaf disc confirmed the repeatability of leaf disc method of screening cocoa genotypes for resistance to black pod disease of cacao.

The positive correlation between leaf disc scores and lesion number and size on pods (see Figures 7 and 8) suggests that the forms of resistance assessed by leaf disc test and detached pod test at the penetration and post-penetration levels may be inherent. Such a characteristic of cacao leaf discs suggests the possibility of the use of leaf discs of cacao seedlings for the screening of pod resistance to the black pod disease of cacao.

The observation of both significant and insignificant correlation coefficients among the monthly data collected on *Phytophthora* infection in the field suggests that field data on *Phytophthora* was not repeatable. The inconsistency in the field data (see Table 6 and Figure 9) could be due to variations in environmental factors occurring probably each month.

The higher level of infection observed in the months of July, August and September and the lower infection level in the months of October, November and December seems to suggest that probably the factors pre-disposing cocoa to *Phytophthora* infection in the first half of the field study

period were different from those in the second half of the study. The high level of rainfall, number of wet days, relative humidity and low maximum temperatures in the first three months (see Table 7) might probably be the favourable conditions for high incidence of the black pod disease. The low maximum temperature in the months of July, August and September might have probably prevented evaporation of moisture from the pod surfaces and thus led to high infection of pods.

Butler (1980, 1981) observed that in a saturated atmosphere, the pod surface remained wet for as long as 4 - 5 hours giving sufficient time for zoospore germination and penetration.

The observed high infection in the months of July, August and September and low infection in the months of October, November and December provides a promising approach to breed for disease escape. This pattern of field incidence of black pod disease is in conformity with the findings of Opoku *et al.* (1999, 2000) who observed that in Ghana, the primary infections usually occur around June, but the peak of the *Phytophthora megakarya* black pod disease generally occurs between August and October. Atanda (1973) reported that the percentage of infected mature pods is determined not only by the genetic resistance of the pods to infection but also by escape phenomena such as fruiting cycle. Kebe *et al.* (1999) reported that the fruiting cycle alone may explain 43% of the variation in field infection level.

The significant correlation between detached pod test at the penetration and post-penetration stages of infection and the average of field infection in July to December (see Figures 11 and 12) suggests that it is possible that a

stronger association may exist between the results of detached pod test at penetration and post-penetration stages of infection. The occurrence of such a characteristic of pods suggests the possibility of using pods to predict field infection of *Phytophthora.* Iwaro *et al.* (2004) and Efombagn *et al.* (2004) reported a similar observation in their studies on the relationship between detached pods and field infection of *Phytophthora.*

Also, the significant correlation between detached leaf test at the penetration and post-penetration stages of infection and the average of field infection in July to December (see Figures 13 and 14) suggests that field resistance to *Phytophthora* infection through monthly recording of infection on mature fruits could be reliably evaluated by intrinsic resistance of cacao leaf tissue to *Phytophthora palmivora* infection at the penetration and post-penetration levels of infection. Iwaro *et al.* (1997) noted a similar association between the reaction of detached leaves and attached pods to *Phytophthora palmivora* infection.

The high correlation of leaf disc test with the average of field infections for months from July to December (see Figure 15) suggests that prediction of pod resistance to *Phytophthora* infection in the field could be made from laboratory inoculation of leaf discs. A similar observation has been reported by Nyasse *et al.* (2002) in Cameroon and by Tahi *et al.* (2006) in Cote d'Ivoire, who found in their studies that when field or nursery leaves of clones were inoculated at the hardening stage, the results correlated with natural field infection of pods with *Phytophthora palmivora.*

The highly significant differences obtained among clones inoculated at distal, proximal and mid region of pods (see Table 9) further confirms that

significant differences occur among genotypes at the post-penetration level of infection of pods. The observation of significant differences among the cacao genotypes further suggests that probably any of the three regions of the pods can be inoculated to screen for resistance to *Phytophthora* infection.

The highly significant differences among lesion sizes in the interaction of distal, proximal and mid regions of pods inoculated indicated that the region of the pod inoculated influenced the rate of development of disease and therefore uniformity must be maintained during pod inoculation tests by inoculating the same region of the pods for clonal selection.

The grouping of the 25 genotypes (see Figure 16) into two distinct populations is suggestive of genetic limitations of the cacao genotypes studied. Even though the smaller population of the two which was mainly made up of Parinari genotypes which are Forasteros, seems homogenous. The larger population was more heterogenous consisting of genotypes of Criollos, Trinitario and Forasteros. Singh and Bains (1968) using cluster analysis to measure genetic diversity in upland cotton varieties obtained from different geographical regions could establish the genetic variability in the cotton varieties they studied.

It is likely that the observed variations in the field infection of the Parinaris making the distinct small population could be genetic. Even though Parinaris are Forasteros and appear to be homogenous, the variations in their monthly infections by *Phytophthora palmivora* are suggestive of the environmental factors influencing genetic base. It was clear from the results the Parinaris were heavily infected by *Phytophthora* from July to September.

However, from October to December the infection was considerably low except for PA 150. This is indicative of the possibility of using the Parinaris as a basis for breeding for cacao genotypes capable of escaping from *Phytophthora* infections. The genotypes PA150, PA 107, and PA 120 grouped in Cluster V might belong to the same class and their relationship is augmented by common features, such as their greater vigour, which sets them apart from the other genotypes. Yamada *et al.* (1982, 1996) reported genetical relation between PA 81 and PA 150. The Parinari clones belong to the Forastero population and were collected from Peru in an area bounded by the upper Amazon region.

The grouping of Criollos, Trinitarios and Forasterios in the larger distinct population made of four clusters is indicative of wider genetic diversity available in the international collection for breeding for resistance against the cacao black pod disease. Such a genetic diversity seems to be indicated by the genotypes in II and IV which contained the largest clonal variations.

In Cluster II, even though all the genotypes are Forasteros, they originated from various origins. It was clear from the results that the Forasteros were not uniformly infected by *Phytophthora* from July to December even though from July to September infections were considerably higher than from October to December.

Cluster IV had the largest intra-cluster variance. The large variance of this cluster indicates that genotypes in it are less homogeneous probably because they differ in some of their morphological characters and populations to which they belong. This was probably confirmed by the mixture of

Trinitario and Forastero populations in this cluster. The Trinitarios were more susceptible to field infection than the Forasteros except T85/799. This characteristic of T85/799 is suggestive that it could be used in breeding for disease escape.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

SUMMARY

The findings of the study are summarized as follows:

1. Clonal differences existed in leaf and pod at the penetration stage of infection.
2. Resistance levels of the 25 genotypes were different at the post-penetration stage of infection in both leaf and pod.
3. Correlation between resistance of detached leaves and pods at the penetration stage of infection was positive and significant.
4. A strong positive correlation was obtained between detached pod and leaf at the post-penetration stage of infection.
5. There was a strong positive correlation between results of detached pod and leaf discs tests.
6. Leaf discs test trials were repeatable.
7. Correlation between penetration stage infection of pods and natural *Phytophthora* infection in the field was positive and significant.
8. There was a strong positive correlation between post-penetration stage infection of pods and field infection by *Phytophthora palmivora.*
9. The coefficient of correlation between leaf disc test and natural *Phytophthora* infection in the field was positive and significant.

10. SCA 6, T85/799, LAF 1, and GU 225V and ICS 1 were the promising resistant cacao genotypes among the international collection studied.
11. The rates of the post-penetration stage of infection in pods inoculated at the distal, proximal and mid regions were significantly different.
12. The levels of black pod infection during the field study varied significantly.
13. *Phytophthora* pod rot infections in July, August and September were higher than in October, November and December.
14. The cluster analysis of the 25 international cacao collections revealed the considerable genetic diversity in the collection.

Conclusion

The results of the study revealed that significant differences existed among the 25 international cacao genotypes infected with *Phytophthora palmivora*. Detached leaf and pod inoculations were both effective in indicating the resistance levels of the 25 international genotypes at the penetration and post-penetration stages of infection. The high positive correlation between detached leaf and pod inoculation at both penetration and post-penetration stages of infection suggest that resistance levels of cacao genotypes to black pod could be assessed at the seedling level using leaf inoculation. The higher *Phytophthora* infection in July, August and September than in October, November and December in the field clearly shows the influence of environment in *Phytophthora* pod rot infection and necessitates the need to breed for black pod disease escape. A vast variation was observed among the 25 international genotypes. SCA 6, T85/799, LAF 1,

ICS1 and GU 225V were found to be resistant whereas MO 20, T79/501, VENC 4-4 and MOCORONGO were found to be susceptible. These variations in the resistance levels of the promising varieties could be utilized in the development of new cacao varieties with predictable level of *Phytophthora* resistance.

Recommendations

On the basis of the findings of this study, it is recommended that:

1. The screening of the 25 genotypes in terms of black pod resistance should be repeated with other local *Phytophthora palmivora* isolates.
2. Crosses between SCA 6, T85/799, LAF 1, ICS1 and GU 225V could be done to obtain black pod resistant cacao hybrids for they appeared to be promising resistant varieties.
3. Leaf inoculation at penetration and post-penetration stages of infection and leaf disc inoculation should be used to select cacao varieties resistant to *Phytophthora* early in the nursery before planting them in the field.
4. Detached pod inoculation at the penetration and post-penetration stages of infection could be used to select resistant varieties of cacao in the field.
5. Clones that bear majority of their pods in October, November and December should be selected for the breeding of cacao varieties with the inherent ability to escape black pod disease incidence.

6. Uniformity should be maintained during pod inoculation by inoculating the same region of the pods since the position of infection on the pod influences the rate of disease development.

REFERENCES

Abogo Onamena, A., Bakala,J., Partiot, M., Despreux, D. and Nyemb, E. (1985). Structure et evolution saisonnie're de la population de *Phytophthora* spp. Dans les sols des cacaoyeres de Nkolbisson. *Proc. 9th Int. Cocoa Res. Conf.* Lome, Togo 1994, 309-12.

Adomako, B. (2006). Combining ability analysis of black pod disease incidence in cocoa genotypes in Ghana. *Tropical Science* 2006, 46 (4), 201-204.

Adomako, B. (2007). Causes and extent of yield losses in cocoa progenies. *Tropical Science*, 47(1), 22-25.

Adu, S.V. and Mensah-Ansah (1969). Classification of Ghanaian soils for cocoa rehabilitation *Proc. 3rd Intl. Cocoa Research Conference*. Accra. 56-64.

Agrios, G.N. (1997). *Plant Pathology*. 3rd ed. Academic press, San Diego, California.

Ahenkorah, Y. and Halm, B.J. (1981). Foliar analysis as a diagnostic technique in cocoa nutrition: III Standard curves and observations. *Proceedings of the 7th International Cocoa Research Conference*, 1979, Douala, Cameroon, 177-182.

Aist, J.R. (1976). Papillae and related wound plugs of plant cells. *Annu. Rev Phytopathol.* 14, 145-163.

Anon (1995). Pest and disease. In: *Report on recent decline in cocoa production in Ghana and measures to revamp the industry*, 43-44.

Anon (2003). The state of the Ghanaian economy in 2002. Institute of Statistical Social and Economic Research (ISSER), University of

Ghana, Legon, 164.*Anon (980) La SODECAO a 6 ans. Bull. So. De. Cao* 1. SODECAO, Yaounde.

Asomaning, E.J.A. (1971). Cocoa research in Ghana. *Cocoa Growers Bull* 19, 13–18.

Atanda, O.A., Sanwo, J.O. and Jacob, V.J. (1973).Evaluation of inter-Nanay and inter-Parinari cacao progenies in Nigeria (*Phytophthora palmivora*). *Ghana J. Sci.* 15 (1),75-84.

Ayers, W.A. and Zentmyer, G. A. (1971). Effect of soil solution and two soil pseudomonads on sporangium production by *Phytophthora cinnamomi*. *Phytopathology* 61, 1188-1193.

Babacauh, K.D. (1982). Role of insect communities and water in the dissemination of *Phytophthora palmivora* (Butl.) Butl.emend Bras. and Griff in cacao tree plantations in the Ivory Coast (*Theobroma cacao*). *Café cacao The 26*(1), 31-36.

Blackwell, E. (1949). Terminology in *Phytophthora*.With definitions and descriptions. *Mycol. Pap.* 30, 1-24.

Blaha, G., Eskes, A. B., Kebe, B.I., Tahi, G.M. and Nyasse S. (2000). Early screening of resistance to *Phytophthora* spp. by means of leaf disc inoculation. Pp. 103-107 in working procedures for cocoa germplasm evaluation and selection. *Proceedings of the CFC/ICCO/IPGRI Project Workshop*, 1-6 February 1998, Montpellier. International Plant Genetic Resources Institute, Rome, Italy.

Blaha, G. and Latode, R. (1997). Contribution a la connaissance des modalites de transmission hereditaire de la resistance de cacaoyer a la pourriture brune des fruits an Cameroon. *Café Cacao, The 21*, 179-196.

Blaha, G. Hall, G.S., Warokka, J.S., Concibido E., Ortiz-Garcia C. (1994). *Phytophthora* isolates from coconut plantations in Indonesia and Ivory Coast: characterization and identification by morphology and isozyme analysis. *Mycological Research*, 98, 1379-1389.

Blaha, G and Lotode, R. (1977). Contribution to the knowledge of the means of hereditary transmission of the cocoa tree to the black pod disease (*Phytophthora palmivora)* in Cameroon. *Café Cacao The 31*(3). 179-196.

Blaha,G., Eskes A.B., Kebe, B.I., Tahi, G.M., and Nyasse S. (2001). Early screening of resistance to *Phytophthora* spp. by means of leaf disc inoculation. Pp. 103-107 in working procedures for cocoa germplasm evaluation and selection. *Proceedings of the CFC/ICCO/IPGRI project workshop*, 1-6 February 1998, Montpellier. International Plant Genetic Resources Institute, Rome, Italy.

Blencowe, J.W. and Wharton, A.L. (1961). Black-pod disease in Ghana: Incidence of disease in relation to levels of productivity. *Report on the 6th Commonwealth Mycological Conference*, London, 139-147.

Brahmana, J. Sipayung, A. Purba, P. (1993). Content of phenolic compounds as an index of resistance in coconut fruit to premature nutfall disease. *Buletin Pusat Penelitian Kelapa Sawit*, 1(1), 81-87, 5; 10 ref.

Brazier, C.M. and Griffin, M.J. (1979). Taxonomy of *Phytophthorapalmivora* fungi on cocoa. *Trans. Br. Mycol. Soc.* 72(1), 111-143.

Brazier, C.M., Griffin, M.T. and Maddison , A.C. (1981) . The cocoa black pod caused by *Phytophthora palmivora*. *Phytopathol. Pap.* Kew, England (25), 18-30.

Butler, D. (1980). Dew and thermal lag: measurements and an estimate of wetness duration on cocoa pods. *Quart. J.R. Met. Soc.* 106, 539-50. Cameroon.

Butler, D. (1981). Microclimatic factors associated with the incidence of black pod disease of cocoa. *Proc. 6th Int. Cocoa Res. Conf.* Caracas. Venezuela 1977, 171-80.

Cheeseman, E.E. (1944). Notes on the nomenclature, classification and possible relationships of cacao populations. *Tropical Agriculture*, Trinidad, 21, 144-159.

Cheeseman, E.E. (1944). Fertilization and embryony in *Theobroma cacao* L. *Ann. Bot.* 41, 107-26.

Cilas, C., Lanaud, C., Paulin, D., Nyasse, S., N'Goran, J.A.K., Kebe, B.I., Ducamp, M., Flament, M.H., Despreaux, D. (1998). La resistance a la pourriture brune des cabosses due a Phytophthora spp. Recherche de composantes de la resistance. *Plantations Researche Development 5*, 441-6.

Coe, S.D. and Coe, M.D. (1996). *The True History of chocolate*. Thames and Hudson, New York.

Cother E.J. and Griffin, D.M. (1974). Chlamydospore germination in *Phytophthora drechsleri*. *Trans. Br. Mycol. Soc* 63, 273-279.

CPC-Global module. (2000). *Phytophthora palmivora* (E.J. Butler) E.J. Butler. *CPC-Global module, 2nd Edition* (C) CAB International Wallingford, UK, 2000, 7-10.

CRIG meteorological station. (1989). Cocoa Research Institute of Ghana. *Annual report 1989*, 32-35.

Crosier, W. (1974). Studies in the biology of *Phytophthora infestans* (Mont) de Bary. *Cornell. Univ. Agric. Exp. Stn. Mem.* 155, 40.

Dakwa, J. T. (1976). Nationwide black-pod survey Joint CRIG/ Cocoa production Division Project. *Ann. Rep. Cocoa Research Institute of Ghana.* 1975/1976, 177-182.

Dakwa, J.T. (1984). National black pod survey. Joint CRIG/ Cocoa Production Division Project. *Ann. Rep. Cocoa Research Institute of Ghana,* (1976/77-1978/79), 263.

Dakwa, J.T. (1987). A serious outbreak of the black pod disease in a marginal area of Ghana. *Proc. 10th Int. Cocoa Research Conference*, Santo Domingo, 447-451.

Dakwa, J.T. (1988). A serious outbreak of the black-pod disease in a marginal area of Ghana. *Proceedings of the 10th International Cocoa Research Conference*, 1987, Santo Domingo,. Dominican Republic, 447-451. Cocoa Producers' Alliance, Lagos, Nigeria.

Doke, N., and Tomiyama , K. (1980). Suppression of the hypersensitive response of potato tuber protoplasts to hyphal wall components by water soluble glucans isolated from *Phytophthora infestans. Physiol. Plant Pathology* 16, 177-186.

Duniway, J.M. (1979). Water relations of water molds. *Annu. Rev. Phytopathol.* 17, 431-460.

Efombagn, M.I.B., Marelli J.P., Ducamp, M., Cilas, C., Nyasse S.and Vefonge, D. (2004). Effect of fruiting traits on the field resistance of cocoa (*Theobroma cacao L.*) clones to *Phytophthora megakarya. Journal of Phytopathology* 152(10), 557-562.

Englander, K. and Roth, S. (1980). Temperature regimes of sporangia formation. *Ann. Rev. Phytopathol.* 332-341.

Esenam, E.U. (1971). Banana as an important host for the black pod disease pathogen. *L.W. Afr. Sci. Ass.* 16, 13-16.

Eskes, A.B. (2004). Synthesis and conclusions of the INGENIC Workshop on Cocoa Breeding for Improved Production System *INGENIC Newsletter* 9, 2-7.

Eskes, A.B., Engels, S.M.M., Lass, R.A. (2000). Working procedures for cocoa germplasm evaluation and selection. In: *proceedings of the CFC/ICCO/ IPGRI project workshop*, 1-6 February, 1998, Montpellier, France. Rome, Italy: IPGRI.

Evans, H.C. (1973). New developments in black-pod epidemiology. *J. Agric. Soc.* Trinidad Tobago 73 (4), 434-411.FAO/INGENIC.

Evans, H.C. and Prior, C. (1987). Cocoa pod diseases: causal agents and control. *Outlook on Agriculture*, 16, 35-41.

Figueiredo, J.M. de, Campelo, A.M.F.L. and Lellis W.T. (1982) Potential infectivo de *Phytophthora* spp. em cacaneiros nas plantacoes de cacao. *Informe Tecnico-1981. CEPEC*: Itabuna, Brazil, 46-51.

Garrett, S.D. (1970). Pathogenic Root –Infecting Fungi. *Cambridge Uni. Press, London. 294 pp.Genet 80*, 258–264.

Gerlack, W.W.P., Hoitink, H.A.J., and Schmitthenner, A. F. (1976). Survival and host range of *Phytophthora citrophthora* in Ohio nurseries. *Phytopathology* 66, 309-311.

Gisi, U., Zentmyer , G.A., and Klure, L.J. (1980). Production of sporangia by *Phytophthora cinnamomi* and *Phytophthora palmivora* in soils at different matric potentials. *Phytopathology* 70, 301-306.

Gray, T.R.G. (1976). The survival of vegetative microbes. *Sec. Gen. microbial. Symp.* 26. T.R.G. Gray and J.R. Postgate, eds. Cambridge Univ. Press. Cambridge. 432pp.

Greathouse, D. C., Laetsch, W. M., and Phinney, B. O. (1991). The shoot-growth rhythm of a tropical tree (*Theobroma cacao* L.). *Am. J. Bot. 58(4)*, 281-286.

Gregory, P.H., Griffin, M.J., Madison, A.C. and Ward, M.R. (1984). Cocoa black pod: a reinterpretation. *Cocoa Growers' Bulletin*, No. 35, 5-22.

Gregory, P.H. (1981). Speculations on *Phytophthora* as a cocoa root pathogen. Pp. 267-269 in *proceedings of the 7th International Cocoa Research Conference*, Douala, Cameroun, 4-12 Nov. 1979. Cocoa Producers' Alliance, Lagos, Nigeria.

Gregory, P.H. and Madison, A.C. (1981). Epidemiology of *Phytophthora* on cocoa in Nigeria. *Phytopathological paper No. 25.* Commonwealth Mycological Institute: Kew, England.

Guest, D.I., Anderson, R.D., Foard, H.J., Phillips, D., Worboys, S. and Middleton, R.M. (1994). Long-term control of *Phytophthora* diseases of cocoa using trunk-injected phosphonate. *Plant Pathology*, 43(3). 479-492; 13 ref.

Hanchey, P. and Wheeler, H. (1971). Pathological changes in ultrastructure: Tobacco roots infected with *Phytophthora parasitica* var. *nicotianae*. *Phytopathology 61*, 33-39.

Hemmes, D.E. and Bartnicki- Garcia, S. (1975). Electron microscopy of gametangial interaction and oospore development in *Phytophthora capsici*. *Arch. Microbial.* 103, 91-112.

Hemmes, D.E. and Wong, L.D.S. (1975). Ultrastructure of Chlamydospores of *Phytophthora cinnamomi* during development and germination. *Can. J. Bot. 53*, 2945-2957.

Ho, H.H. and Hickman, C.J. (1967). Factors governing zoospore responses of *Phytophthora megasperma* var. *sojae* to plant roots. *Can. J. Bot 45*, 1983-1994.

Hohl H.R. and Suter, E. (1976). Host parasite interfaces in a resistant and a Susceptible cultivar of *Solanum tuberosum* inoculated with *Phytophthora infestans*: leaf tissue. *Can. J. Bot. 54*, 1956-1970.

Hohl, H. R. and Stossel, P. (1976). Host–parasite interfaces in a resistant and a susceptible cultivar of *Solanum tuberosum* inoculated with *Phytophthora infestans*: Tuber tissue. *Can. J. Bot. 54*, 900-912.

Idosu, G.O. and Zentmyer, G.A. (1978) *Phytophthora palmivora*: a comparative study of "typical" and "atypical" isolates from cacao (*Theobroma cacao*). *Mycologia 70*(5), 1101-1112.

Iwaro, A.D. (1996). Strategies of variety improvement in cocoa with emphasis on durable disease resistance. An external review prepared for INGENIC. pp. 23-32 in *proceedings of the International Workshop on the contribution of Disease Resistance to cocoa variety Improvement*, 24-26 November 1996, Salvador , Bahia , Brazil. INGENIC, UK.

Iwaro, A.D. (2000). Rapid screening for *Phytophthora* pod rot resistance by means of detached pod inoculation. pp. 111-113 in working procedures for cocoa germplasm evaluation and selection. *Proceedings of the CFC/ICCO/IPGRI project workshop*, 1-6 February 1998, Montpellier. International Plant Genetic Resources Institute, Rome, Italy.

Iwaro, A. D. and Butler D.R. (2001). Germplasm enhancement for resistance to black-pod and witches broom diseases. Pp 3-10 in *Proceedings of the 13th International Cocoa Research Conference:* Towards the effective and optimum promotion of cocoa through research and development, 9-14 October 2001, Kota Kinabalu, Sabah, Malaysia. Cocoa Producers Alliance, Lagos, Nigeria.

Iwaro, A.D., Screenivasan T.N. and Umaharan P. (1992). Relationship between leaf and pod resistance in cocoa to *Phytophthora palmivora* infection. *Ann. Rep. Cocoa Res. Unit , University of the West Indies*, Trinidad and Tobago, 33-39.

Iwaro, A.D., Screenivasan T.N. and Umaharan P. (1993). Relationship between leaf and pod resistance in cocoa to *Phytophthora palmivora* infection. *Ann. Rep. Cocoa Research Unit. University of the West Indies*, Trinidad and Tobago, 33-39.

Iwaro, A.D., Screenivasan T.N. and Umaharan P. (1997). Foliar resistance to *Phytophthora palmivora* as an indicator of pod resistance in *Theobroma cacao*. *Plant Disease 81*(6), 619-624.

Iwaro, A.D., Screenivasan T.N. and Umahaaran P. (1998). Cacao resistance to *Phytophthora :* Effect of Pathogen species , inoculation depths and pod maturity. *European Journal of Plant Pathology* 104, 11-15.

Iwaro, A.D, Screenivasan T.N., Butler D. R. and Umaharan P. (2000). Rapid screening for *Phytophthora* pod rot resistance by means of detached pod inoculation. Pp 109-113 in working procedures for cocoa germplasm evaluation and selection. *Proceedings of the CPC/ ICCO/ IPGRI Project Workshop,* 1-6 February 1998, Montpellier (A.B. Eskes, J,M,M. Engels and R. A. Lass, eds). International Plant Genetic Resources Institute, Rome, Italy.

Iwaro, A.D. and Singh, V. (2004). Progress report on the germplasm enhancement programme for resistance to black pod disease. pp. 43-45 in *Annual Report for 2003. Cocoa Research Unit. The University of the West Indies,* St. Augustyine, Trinidad.

Iwaro, A.D., Thevenin, J.M., Butler, D.R. and Eskes, A.B. (2005). Usefulness of the detached pod test for assessment of cacao resistance to *Phytophthora* pod rot. *European Journal of Plant Pathology* 113,173-182.

Iwaro, A.D., Umaharan P. and Screenivasan T.N. (1997b). Inheritance of foliar resistance to *Phytophthora palmivora* (Butler) Butler in cacao (*Theobroma cacao* L.). *Euphytica 96*, 377–383.

Iwaro, A.D., Umaharan P., Screenivasan, T.N. (1999). Inheritance of foliar resistance to *Phytophthora palmivora* (Butler) Butler in cacao (*Theobroma cacao L.). Euphytica 96*, 377-83.

Kebe, B.I., N'Goran, J.A.K., Tahi, G.M., Paulin, D., Clement. D., Eskes, A.B. (1999). Pathology and breeding for resistance to black pod in Cote d'Ivoire. In: *Proceedings of the International Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement,*

Salvador, Bahia, Brazil, 24-26 November 1996. International Group for Genetic Improvement of Cocoa (INGENIC), 135-40.

Kellam, M.K. and Zentmyer, G.A. (1981) Comparative survival of three *Phytophthora* species pathogenic to cocoa in inoculated cocoa pod pieces. pp. 505-509 in *proceedings of the 8th International Cocoa Research Conference* , Cartagena, Colombia , 18-23 Oct., 1981. Cocoa Producers' Alliance, Lagos, Nigeria.

Kellam, M.K. and Zentmyer, G.A. (1982). Comparative survival of three *Phytophthora* species pathogenic to cocoa in inoculated cocoa pod species. pp. 505-509 in *Proceedings of the 8th International cocoa Research conference*, Cartagena, Colombia, 18-23 Oct., 1981. Cocoa Producers Alliace, Lagos, Nigeria.

Kitazawa, K. and Tomiyama, K. (1970). Microscopic observations of infection of potato cells by compatible and incompatible races of *Phytophthora infestans*. *Phytopathol. Z. 66*, 317-324.

Kliejunas, J.T. and Ko, W.A. (1976). Dispersal of *Phytophthora cinnamomi* on the island of Hawaii. *Phytopathology 66*, 457-460.

Kuc, J., Currier, W. W. and Shih, M.J. (1976). Terpenoid phytoalexins. Pages 225-237 in: *Biochemical Aspects of Plant –parasite relationship*. J. Fried and D.R. Threlfall, eds. Academic press. London.

Latode, J.S. (1977). Evaluation methods for assessing resistance of cocoa (*Theobroma cacao L.*) resistance to *Phytophthora* black - pod disease . *Crop protection*, 14(8), 657-663.

Luterbacher, M.C. and Akrofi, A.Y. (1993). The current status and distribution of *Phytophthora megakarya* in Ghana. *Proceedings of the 11th International Cocoa Research Conference*, Yamoussoukro, La Cote d'Ivoire, 29-35.

Lawrence, J. S. (1978). Evaluation of methods for assessing resistance of Cacao (Theobroma cacao L.) cultivars and hybrids to *Phytophthora palmivora* (Butler). *Bol. Tec. 62*, 46-49.

McDonald, J.D. and Duniway, J.M. (1978). Influence of the matric and osmotic components of water potential on zoospore discharge in *Phytophthora*. *Phytopathology 68*, 751-757.

Mchau, G.R.A. and Coffey, M.D. (1994). Isozyme diversity in *Phytophthora palmivora*: evidence for a southeast Asian Centre of origin. *Mycological Research, 98(9)*, 1035-1043.

Mckenzie, D.R. (1981). Scheduling fungicide applications for potato late blight with BLITECAST. *Plant. Dis. 65*, 394-399.

Newhook, F.J. and Jackson, G.V.H. (1978). *Phytophthora palmivora* in cocoa plantation soils in the Solomon Islands. *Transactions of the British Mycological Society*. 69(1), 31-38.

Nyasse, S. (1997). Etude de la diversite de *Phytophthora megakarya* caracterisation de la resistance du cacaoyer (*Theobroma cacao* L.) a cet agent pathogene. *Ph D. Thesis, Institute. National Polytechnique*, Toulouse, France, 133-137.

Nyasse, S., Blaha G. and Cilas, C. (1994). Pathogenie de *Phytophthora megakarya* et son implication pour la mise an point d'un test precose de sensibilite a la pourriture brune sur cacaoyer. pp. 83-90 in

proceedings of the 11th International Cocoa Research Conference, 18-24 July 1993, Yamoussoukro Cote d'Ivoire. Cocoa Producers Alliance. Lagos, Nigeria.

Nyasse, S., Cilas C., Herail, C. and Blaha, G. (1995). Leaf inoculationas an early screening test for cocoa (*Theobroma cacao* L.) resitance to *Phytophthora* black pod disease. *Crop Protection 14*, 657-663.

Nyasse, S., Despreau, D. and Cilas, C. (2002). Validity of a leaf inoculation test to assess the resistance to *Phytophthora megakarya* in cocoa (*Theobroma cacao L.*) diallel mating design. *Euphytica 123*, 395-399.

Okaisabor, E.K. (1974). *Phytophthora* pod rot infections from the soil. Pages 161-168 in: *Phytophthora Disease of Cocoa*. P.H. Gregory, ed. Longman, London.

Okey, E.N., Duncan, E.J., Sirju-Charran, G. and Sreenivasan, T.N. (1996). Factors affecting the susceptibility of six cocoa clones to *Phytophthora palmivora* (Butl.) Butler bark canker in Trinidad. *Plant Pathology*, *45*, 84-91.

Okey, E.N.(1996). Histopathological and biochemical studies on cacao (*Theobromacacao*L.) canker caused by *Phytophthora palmivora* (Butler) Butler. *Ph.D. thesis. The University of the West Indies*, Trinidad and Tobago.

Opoku, I.Y., Akrofi, A.Y. and Appiah, A.A. (1996). Survey of *Phytophthora* species on cocoa in Ghana. *Rep. Cocoa Res. Inst. Ghana*, 1995/96, 105-110.

Opoku I.Y., Akrofi A.Y., Appiah A.A. (1999).The spread of *Phytophthora megakarya* on cocoa in Ghana. *J. Ghana Sci. Assoc. (Special Edition). 2(3)*, 110-116.

Opoku, I.Y., Appiah, A.A., Akrofi, A.Y. and Owusu G.K. (2000). *Phytophthora megakarya*: a potential threat to the cocoa industry in Ghana? *Ghana J. Agric. Sci. 33*, 237-248.

Opoku I.Y., Ofori-Frimpong K. and Appiah, M.R. (2004). The use of fertilizer and fungicide to sustain cocoa production in severe black pod areas. *Trop. Sci. 44*, 95-99.

Opoku, I.Y., Assuah, M.K. and Aneani, F. (2007). Management of black-pod disease of cocoa with reduced number of fungicide application and crop sanitation *African Journal of Agrocultural Research Vol. 2* (11), 601-604, November 2007.

Paulin, D., Mossu, G., Lachenaud, P. and Eskes, A.B. (1994). Genetic analysis of a factorial crossing scheme with cacao hybrids tested in four locations in Ivory Coast. In: *Proceedings of the Malaysia International Cocoa Conference*: Challenges in the 90's, 1991. Kuala Lumpur, Malaysia: Malaysia Cocoa Board, 73-83.

Pfender, W.F., Hine, R.B. and Stanghellini, M.E. (1977). Production of sporangia and release of zoospores by *Phytophthora megasperma* in soil. *Phytopathology 67*, 657-663.

Phillips-Mora, W. and Galindo, J.J. (1989). Method of inoculation and evaluation of resistance to *Phytophthora palmivora* in cocoa fruit (*Theobroma cacao*). *Turrialba. 39(4)*, 488-496.

Pinto, L.R.M., Silva, S.D.V.M. and Yamada, M.M. (1989). Screening of cacao hybrids progenitors resistant to *Phytophthora* spp. *Agrotropica, 1(2).* 104-107.

Podger, R. (1972). Herencia de la reaction del cacao a la pudricion de la mazorca causada por *Phytophthora palmivora* (Butl) Butl. In: *Proc. Workshop of the International Phytophthora Group.* Ilhaus, Bahia , Brazil, 35-40.

Prior, C. (1985). Approaches to the control of diseases of cocoa in Papua New Guinea. Journal of plant protection in the Tropics 1, 39-46. *Proc. 10th Int. Cocoa Res. Conf.* Santo Domingo, Dominican Republic, 17–23 May, 1987, 391–395.

Reeves, R.J. (1975). Behaviour of *Phytophthora cinnamomi* Rands in different soils and water regimes. *Soil Biol. Biochem.7*, 19-24.

Ribeiro, O.K., Zentmyer, G.A. and Erwin, D.C. (1976). The influence of qualitative and quantitative radiation on reproduction and spore germination of four *Phytophthora species. Mycologia 68.* 1162-1173.

Robinson, R.A. (1976). *Plant Pathosystems.* Springer-Verlag, Berlin, 184-189.

Rocha, H.M. and Medeiros, A.G. (1968). Basis for the selection of cocoa cultivars resistant to *Phytophthora palmivora* (Butl.) Butl. *International Cacao Research Conf. Mem.*, 150-156.

Sansome, E., Brasier, C.M. and Griffin, M.J. (1975). Chromosome size differences in *Phytophthora palmivora*, a pathogen of cocoa. *Nature, 255* (5511), 704-705.

Screenivasan, T. N. (1995). A rapid method of inoculation of *Phytophthora palmivora* (Butler) Butler on attached pods. 293-302 in: *Proc. Int. Cocoa Res. Conf., 9th.*

Shea S. R., Gillen, K.J. and Leppard, W.I. (1980). Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely drained *Eucalyptus marginata* Sm sites in the northern jarrah forests of South-Western Australia. *Prot. Ecol. 2,* 135-156.

Shimony, C., and Friend, J. (1975). Ultrastructure of the interaction between *Phytophthora infestans* and leaves of two cultivars of potato (*Solanum tuberosum* L.) Orion and Majestic. *New Phytol 74*, 59-65.

Simmonds, N.W. (1994). Horizontal resistance to cocoa diseases. *Cocoa Growers Bull.* 47, 42-53.

Singh, R.B. and S.S. Bains (1968). Variability and correlation studies on ginning out-turn and its components in certain varieties of upland cotton (*Gossypium hirsultum* L.) *Indian J. Agric . Sci. 3*, 227- 230.

Sitapai, E. C. (1989). Studies on the resistance in cocoa (*Theobroma cacao* L.) to *Phytophthora palmivora* (Butler) Butler. *M. Phil. Thesis. The University of the West Indies*. Trinidad and Tobago.

Soria, V.J. (1974). Sources of resistance to *Phytophthora palmivora.* In: P.H. Gregory (Ed.), *Phytophthora Disease of Cocoa*, 197–202. Longman, London.

Spence, J.A. (1961). Black pod disease of cocoa II: A study in host-parasite relations. *Ann. Appl. Biol. 49*, 723-734.

Stossel, P., Lazarovits, G. and Ward, E.W.B. (1981). Electron microscope study of race-specific and age-related resistant and susceptible reactions of soybeans to *Phytophthora megasperma* var. *sojae*. *Phytopathology 71*, 617-623.

Tahi, G.M. (2003). Evaluation sur Feuilles de Cacaoyer (Theobroma cacao L.) de la Resistance a *Phytophthora palmivora* (Butler) Butler, Agent de la Pourriture Brune des Cabosses: Influence de Certains Facteus et Etude de l'Heritabilite du caractere. Abidjan, Cote d'Ivoire: *Universite de Cocody, Ph.D Thesis.*

Tahi, M., Kebe, I., Eskes, A.B., Ouattara, S., Sangare, A. and Mondeil, F. (2000). Rapid screening of cacao genotypes for field resistance to *Phytophthora palmivora* using leaves, twigs and roots. *European Journal of Plant Pathology* 106(1), 87-94.

Tahi, G.M., Kebe, B.I., N'Goran, J.A.K., Sangare, A., Mondeil, F., Cilas, C. And Eskes, A.B. (2006). Expected selection efficiency for resistance to cacao pod rot (*Phytophthora palmivora)* comparing leaf disc inoculations with field observations. *Euphytica 149*, 35-44.

Tan, A.M. (1990). New recommendations for the control of black stripe. *Planters' Bulletin Rubber Research Institute of Malaysia*, 202, 14-17.

Tan, G.Y. and Tan, W.K. (1990). Additive inheritance of resistance to pod rot caused by *Phytophthora palmivora* in cocoa. *Theor Appl*, 32-46.

Tarjot, M. (1967). Etude de la résistance des cacaoyersà la pourriture des cabosses due au *Phytophthora palmiwora* (Butl.) en Côte d'ivoire.Deuxième partie. *Revue Café, Cacao, Thé, 11*,(l), 3-13.

Tarjot, M. (1971). Some details concerning the role of the environment on the behaviour of cocoa pods towards *Phytophthora palmivora* (Butl) Butl in the Ivory Coast . *Café Cacao The 15*(3), 235-240.

Tarjot, M. (1972). Contribution a l'etude du comportement des feuilles de cacaoyer envers le *Phytophthora palmivora* .*Cafe, Cacao, The 16*(4), 333-338.

Taylor, B. and Griffin, M.J. (1981). The role and relative importance of different ant species in the dissemination of black pod disease of cocoa. In: Gregory P.H., Madison, A.C. eds. *Epidemiology of Phytophthora on Cocoa in Nigeria*. Wallingford, UK: CAB International, 132-144.

Thévenin JM, (1994). Coconut *Phytophthora* diseases in Indonesia etiological aspects. In: CIRAD-CP, eds. *Coconut Phytophthora, Workshop Proceedings*. Montpellier, France: CIRAD-CP, 27-31.

Thevenin, J.M., Ducamp, M., Kebe, B.I., Tahi, M., Nyasse, S. and Eskes, A. (2004). Planting material screening by controlled inoculation. In: Cilas C, Despreaux D, eds. *Improvement of Cocoa Tree Resistance to Phytophthora Diseases*. Montpellier , France: CIRAD, 119-30.

Tondje, P.R., Bakala, J., Partiot, M. and Mouen Bedimo, J.A. (1988). Essai de mise au point d'un test de vis de *Phytophthora* sp. pp. 413-419 in *proceedings of the 10th International Cocoa Research Conference*, Santo Domingo, Dominican Republic, 17-23 May 1987.Cocoa Producers' Alliance , Lagos , Nigeria.

Turner, P. D. (1960a). Resistance and Tolerance: Laboratory Studies. *Rep. W. Afr. Cocoa Res.Inst.*, 1959-60, 29-32.

Turner, P.D. (1960b). Comparative sporulation as an indication of resistance by cacao to *Phytophthora* pod rot. *Tropical Agriculture* Trinidad 42. 305-309.

Umaerus, V. (1970). Studies of field resistance to *Phytophthora infestans*. 5. Mechanisms of resistance and applications to potato breeding .*Z. Pflazenzuecht. 63*, 1-23.

Ward, M.R. (1981). Analysis of an epidemic on a plot at Gambari in 1977. In P.H. Gregory and A.C. Maddison (eds.) Epidemiology of *Phytophthora* on cocoa in Nigeria. *Phytopathological paper No. 25. Commonwealth Mycological Institute:* Kew, England, 96- 114.

Ward, M.R. and Griffin, M.J. (1981). Soil phase of *Phytophthora*. In P.H. Gregory and A.C. Maddison (eds.) Epidemiology of *Phytophthora* on cocoa in Nigeria. *Phytopathological paper No. 25. Commonwealth Mycological Institute,* Kew,England, 50-62.

Wheeler, B.E. J. (1992). Assessment of resistance to major cocoa diseases. Pages 139-145 in: *Proc. Int. Workshop on Conservation, Characterization and Cocoa Genetic Resources in the 21st Century.* Cocoa Res. Unit, University of the West Indies, Trinidad and Tobago.

Wilson, V.E. and Coffey, M.D. (1980). Cytological evaluation of general resistance to *Phytophthora infestans* in potato foliage. *Ann. Bot. (London) 45*, 81-90.

Wood, G.A.R. and Lass, R.A. (1985). *Cocoa.* 4th ed. Longman Singapore Publishers (Ptc) Ltd. 11, 267–282

Wood, G.A.R. (1974). Black pod: meteorological factors (*Phytophthora palmivora*, fungus disease of cacao), 153-159. In *Phytophthora Disease of cocoa*, (P.H. Gregory, ed.).

Yamada, M.M., Bartley, B.G.D., Lopes, U.V. and Pinto, L.R.M. (1996). do fator compatibilidade em *Theobroma cacao* L. Relacoes fenotipicas em genotipos adicionais do grupo Parinari (PA). *Agrotropica 8(2)*, 51-52.

Yamada, M.M., Bartley, B. G. D., Castro, G.C.T. and Melo, G.R.P. (1982). Heranca do fator compatibilidade em *Theobroma cacao* L. Relacoes fenotipicas na familia PA (Parinari), *Revista Theobroma (Brazil) 12(3)*, 163-167.

Yoshikawa, M. and Masago, H. 1977. Effect of cyclic AMP on catabolite-repressed zoosporangial formation in *Phytophthora capsici*. *Can. J. Bot. 55*, 840-843.

Zadoks, J. C. (1997). Disease Resistance Testing in Cocoa. *Plant Disease, 75(5)*, 532-535.

Zentmyer, G.A. (1980a) *Phytophthora cinnamomi* and the diseases it causes. Monograph No. 10. *American Phytopathological Society*. St. Paul, Minnesota, U.S.A., 113-115.

Zentmyer, G.A. (1980b). *Phytophthora cinnamomi* and the diseases it causes. Monograph No. 10. *American Phytopathological Society*. St. Paul, Minnesota, U.S.A., 96-100.

Zentmyer, G. A., Mircetich, S. M., and Mitchell, D. M. (1968). Tests for resistance of cacao to *Phytophthora palmivora*. *Plant. Dis. Rep. 52*, 790-791.

LIST OF APPENDICES

APPENDIX 1

Analysis of variance table for leaf disc scores

Sources of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Replication	4	0.44672	0.11168	4.13	
Treatment	24	114.11872	4.75495	176.02	<.001
Error	96	2.59328	0.02701		
Total	124	117.15872			

P= 0.001

APPENDIX 2

Analysis of variance table for lesion size on pods

Sources of Variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Replication	4	58.745	14.686	5.25	
Treatment	24	198021.842	8250.910	2949.88	< .001
Error	96	268.515	2.797		
Total	124	198349.102			

P= 0.001

APPENDIX 3

Analysis of variance table for lesion number on pods.

Sources of Variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Replication	4	2.9779	0.7445	2.17	
Treatment	24	633.0355	26.3765	76.72	<.001
Error	96	33.0061	0.3438		
Total	124	669.0195			

P= 0.001

APPENDIX 4

Analysis of variance table for lesion size on leaves

Sources of Variance	d.f.	s.s.	m.s.	v.r.	F.pr.
Replication	4	198.5721	49.6430	57.19	
Treatment	24	29265.5766	1219.39	1404.72	< .001
Error	96	83.3349	0.8681		
Total	124	29547.4837			

P= 0.001

APPENDIX 5

Analysis of variance for table for lesion number on leaves

Sources of Variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Replication	4	59.650	14.912	3.99	
Treatment	24	19321.052	805.044	215.27	<.001
Error	96	359.006	3.740		
Total	124	19739.708			

P= 0.001

APPENDIX 6

Method used to prepare the carrot agar medium.

The carrot was peeled and cut into pieces. 200g of the pieces of carrot was measured and boiled on a hot plate for 20 minutes. The boiled carrot was blended with 400ml of sterile distilled water. The blended carrot was filtered with a piece of white cloth which was autoclaved. 20g of agar was added to the filtrate and then autoclaved. The media was poured into petri dishes and allowed to solidify. The petri dishes were covered and packed into a fridge.

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