



International Journal of  
**Agricultural  
Research**

ISSN 1816-4897



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## **Thickness of the Cocoa Pod Husk and its Moisture Content as Resistance Factors to *Phytophthora* Pod Rot**

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### **ABSTRACT**

Pod husk thickness and percentage moisture content were evaluated for their influence on *Phytophthora palmivora* infection of resistant, moderately resistant and susceptible selections of cocoa. The aim of this work was to elucidate if thickness of the pod husk and its moisture content are resistance factors to *Phytophthora* pod rot in cocoa genotypes. Twelve cocoa genotypes were inoculated with *Phytophthora palmivora* using detached pod test at penetration and post-penetration stages of inoculation. Cocoa genotypes resistant to *Phytophthora palmivora* in the field and in the laboratory inoculation (SCA 6, GU225V, T85/799 and LAF 1) had the thickest pod husk and lowest moisture content in the current study suggesting that pod husk thickness and moisture content factors were involved. Susceptible cocoa clones had the thinnest pod husk and highest percentage moisture content of pod husk, suggesting that thinner pod husk with high moisture content favours *Phytophthora* pod rot infection. A significant negative correlation was obtained between pod husk thickness and lesion number and size on pods indicating that as thickness of the pod husk increases, lesion number and size decreases. Correlation between moisture content of pod husk and lesion number and size on pod was positive and significant suggesting that as moisture content increases, lesion number and lesion size also increases. These results suggest pod husk thickness and moisture content as resistance factors in cocoa. Nevertheless, other pod husk factors could not be excluded.

**Key words:** *Theobroma cacao*, resistance, *Phytophthora palmivora*, thickness, moisture, genotypes

### **INTRODUCTION**

Cocoa (*Theobroma cacao* L.) is an important cash crop in Ghana. The low productivity of the crop is attributed to several factors, the most important being black pod disease. Adomako (2007) observed that yield loss was largely due to black pods representing 64.1% which was equivalent to an annual loss of 62.8 kg ha<sup>-1</sup> of dry cocoa beans. The disease is responsible for instability of productivity and important yield losses in Ghana.

Two species of *Phytophthora*, *Phytophthora palmivora* and *Phytophthora megakarya* have been reported as the causal agents of the disease in Ghana (Opoku *et al.*, 1999).

In Ghana, losses ranged from 60-80% in farms in which the disease had only recently appeared to 100% in old infected farms (Dakwa, 1987).

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 or systemic fungicides. The

fungicides are prone to weathering by persistent rainfall and pod growth dilution and for a treatment to be effective, constant frequent re-applications have to be made in a season. Sustainable crop production would therefore, depend on high investment in fungicides to reduce black pod disease. This approach may however not be economically feasible for the resource-poor farmers in the developing world. This increases the overall cost of cocoa production compared to the limited income from cocoa sales. Moreover, persistent use of fungicides, as with all chemicals is harmful and damaging to the environment. There is increase awareness that heavy metals, present in soil have negative consequences on human health and on the environment (Abrahams, 2002; Selinus *et al.*, 2005). Cultural practices alone are not effective in controlling black pod disease (Akrofi *et al.*, 1997). Studies conducted by Luterbacher (1994) on removal of leaf litter around cocoa trees and the application of fungicides to the ground showed that these practices had little or no effect on black pod disease progress. This necessitates the need to shift emphasis from fungicides to more integrated approach to the disease. The breeding of high yielding and resistant material is generally agreed to be the most effective, economic and environmentally safe control method (Iwaro *et al.*, 2000). Although complete resistance has not been detected, differences in susceptibility among clones or among hybrids derived from crosses have been observed in various countries, including Ghana (Adomako, 2007). This makes it feasible to reduce yield loss due to *Phytophthora* infection through genetic improvement.

Although the majority of cocoa in Ghana are very susceptible to the disease, less susceptible individuals have been found including SCA 6, T85/799 and GU 225 V (Nyadanu, 2008).

Like many other agronomic traits, resistance to *Phytophthora* exhibits a continuum of phenotypic variations in the species *Theobroma cacao* suggesting the implication of several genes or factors in resistance to *Phytophthora* (Blaha and Latode, 1976; Cilas *et al.*, 1998). However, little is known about what factors contribute to resistance to *Phytophthora*. Iwaro (1995) obtained a strong correlation between the joint effect of stomatal frequency, size and lesion frequency on pods. He indicated that the mechanism of resistance at the penetration stage of infection could be attributed in part to these two morphological characteristics. Spence (1961) and Prendergast (1965) suggested the involvement of a polyphenol oxidase enzyme system as factors of resistance at the post-penetration stage of infection. Knowledge of the factors underlying resistance is important for breeding programs since the different resistance mechanisms have different effects in plant physiology and may affect plant performance and fitness in combination with other stresses (Prats *et al.*, 2007) or they may also influence the durability of resistance.

The goal of this study was to investigate the role cocoa pod husk thickness and moisture content play in the resistance of cocoa to *Phytophthora palmivora*.

## **MATERIALS AND METHODS**

**Experimental site:** The experiment was conducted at the Cocoa Research Institute of Ghana (CRIG), Tafo, in the Eastern region of Ghana. The plot from which the 12 cocoa genotypes used in this study were selected was established in 2001. Cocoa trees were planted in a Randomized Complete Block Design (RCBD) with 5 replications at 2.5×2.5 m spacing with shade trees and had uniform and optimal cover (approximately 50% of the solar radiation pass through the canopy). No fertilizers were applied. Regular pruning was performed on the exceeding plagiotropic and orthotropic branches, along with the removal of mistletoes and chupons. The soil at the experimental site belongs to the forest ochrosol. The region has a bimodal rainfall pattern with an average range between 1200 and 1930 mm. 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 (<http://www.crig.org/home.php>).

**Cocoa genotypes:** The cocoa clones used in this study represent resistant (T85/799, GU 225V, SCA 6, LAF1), moderately resistant (MAN 15-2, IFC 5, LECTEEN 37i, AMAZ 15-15) and susceptible (VENC4-4, PA 120, MO 20 and MOCORONGO) genotypes reported in the previous study by Nyadanu (2008). The clones represent 12 of the 25 international clone trials supported by the CFC/ICCO/Biodiversity project being evaluated in Ghana.

**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 segments. The segments were then plated on carrot agar medium. All inoculated plates were incubated for 3 days on the laboratory bench. Plates were observed daily for fungal growth from tissue segments.

Fungal growths from tissue segments were transferred onto another carrot agar medium to obtain pure culture of the isolated fungi. Emergent colonies were examined under light microscope and identification of the *Phytophthora* specie 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*.

The isolate was grown on a carrot agar medium and from a ten-day-old culture, a zoospore suspension was obtained by inundating each culture plate (9 cm diameter) with 10 mL sterile distilled water.

The zoospore suspension was then refrigerated for 25 min (5°C) and transferred into an incubator for 25 min. The pathogenicity of the isolate was maintained by regular inoculation in the laboratory of green mature cocoa pods followed by re-isolation on carrot agar medium.

**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 labeled plastic bags. The pods were covered with cotton wool in order to avoid surface damage which may occur when many pods are kept together in close contact with each other. The harvested cocoa pods were washed thoroughly with tap water and blotted dry with Whatman Number 3 paper. They were then surface sterilized with 70% ethanol.

**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.*, 1997). The multiple point inoculation was performed on the pod surface, in which 10 µL drops of inoculum were placed at 3 points along the ridges with a micropipette. A distance of about 3 cm was maintained between inoculated points to avoid merging of adjacent lesions. A zoospore concentration of 200,000 per mL was used. This concentration of inoculum was reported as optimum in similar inoculations conducted by Screenivasan (1995), Sitapai (1989) and Okey (1996). A pod from each clone was inoculated with sterile distilled water in place of zoospore suspension as a negative control. The 12 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

40×60 cm transparent polyethene bags. A beaker of water was kept in the bag with the mouth of the bag closed. After 6 days, pods were assessed for the number of established lesions. The number of established lesions on pods was used as an indication of penetration resistance. The experiment was repeated twice.

**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.*, 1997). The area of lesion formed was used as an indication of post-penetration resistance. For the stab inoculation, a standard injury 4 mm in size was created on the pod surface with a cork borer. The wounded spot was 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 five replications and incubated at room temperature in a 40×60 cm transparent polyethylene bags. A beaker of water 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. The lesion size was determined from brown paper cutouts trimmed to the size of each lesion and was measured with a leaf area meter. The experiment was repeated twice.

**Assessment of thickness of pod husk:** Pods were cut transversely at the equator to expose the thickness of the husk using a very sharp knife. The thickness of the husk was measured using a transparent ruler. Each pod was measured at seven different points on the ridge and furrow. Five pods were assessed per each genotype per 5 replication to obtain a mean measurement. The experiment was repeated twice.

**Assessment of moisture content of pod husk:** The percentage moisture content of the pod husk was determined following the method of Susheelamma *et al.* (1990). Five pods for each genotype per replication were collected from the field and tissues were obtained from the pod husk at the equator of the pod using a cork borer (18 mm diameter). The fresh weight (M1) of the tissue was determined using an electronic balance (Mettler Toledo, Model PB3001-L, made in Switzerland). The tissue was then dried in an oven at 80°C for 72 h and the dry weight (Md) recorded. The percentage Moisture content (Mc) was calculated using the formula:

$$Mc = \frac{M1 - Md}{M1} \times 100$$

**Statistical analysis:** The data on number of lesions, lesion sizes, thickness of pod husk and percentage moisture content of pod husk were subjected to Analysis of Variance (ANOVA) using the Genstart software 10.0, after which residual plots were inspected to confirm data conformed to normality. MINITAB statistical software was used to perform correlation and regression analysis. The significance of mean differences among genotypes was evaluated at  $p < 0.05$ , using Duncan's Multiple Range Test (DMRT).

## RESULTS

**Clonal differences in pod resistance at the penetration and post-penetration stages of infection:** Clonal differences in pod resistance at the penetration stage of infection are presented in Table 1. There were highly significant differences ( $p < 0.001$ ) among genotypes for the number of lesions on inoculated pods.

Table 1: Mean lesion number and lesion size on pods of 12 cocoa clones after inoculation with *Phytophthora palmivora*

Clone	Lesion No. cm <sup>-2</sup>	Lesion size (cm <sup>2</sup> )
T85/799	4.40 <sup>e</sup>	18.64 <sup>e</sup>
GU 225V	5.68 <sup>e</sup>	25.46 <sup>e</sup>
SCA 6	2.28 <sup>f</sup>	12.14 <sup>f</sup>
LAF 1	5.52 <sup>e</sup>	19.92 <sup>e</sup>
MAN 15-2	8.24 <sup>d</sup>	58.48 <sup>d</sup>
IFC 5	6.00 <sup>e</sup>	49.98 <sup>d</sup>
LECT 37i	7.64 <sup>d</sup>	45.68 <sup>d</sup>
AMAZ 15-15	8.60 <sup>d</sup>	80.52 <sup>d</sup>
VENC 4-4	10.00 <sup>b</sup>	94.20 <sup>c</sup>
PA 120	10.52 <sup>b</sup>	140.26 <sup>b</sup>
MO 20	13.40 <sup>a</sup>	159.72 <sup>a</sup>
MOCORONGO	8.04 <sup>d</sup>	84.08 <sup>d</sup>
Lsd (p<0.05)	0.58	2.63
S.e.	0.46	2.06

Values with the same letter in the same column are not significantly different (p<0.05)



Fig. 1: The spread of lesions on leaf and pod of resistant (Sca6) and Susceptible (MO 20) cocoa genotypes

It can be seen that the number of lesions on pods observed among the genotypes varied from 2.28 for SCA 6 to 10.52 for PA 120. Table 1 shows that the mean number of lesions produced on pods of PA 120, MO 20 and VENC 4-4 were significantly large. Significantly fewer lesions were produced on pods of SCA 6, T85/799, LAF 1, and GU 225V.

Analysis of variance of lesion sizes obtained from the punch-inoculation tests of pod indicated that lesion sizes on pods varied highly significantly (p<0.001) among the genotypes (Table 1). SCA 6 produced the smallest lesion size. Lesions were significantly large on MO 20, PA 120, VENC 4-4, and MOCORONGO and intermediate on MAN 15-2, IFC 5, LECTEEN 37i and AMAZ 15-15. There were no significant difference among the lesion sizes of T85/799, GU 225V, SCA 6 and LAF 1. Two distinct patterns of spread of lesions were observed among the genotypes (Fig. 1a-d). In genotypes

with relatively small lesions, such as SCA 6, necrosis was restricted predominantly within the mesophyll and the pod husk. On the contrary, rapid spread of lesions through both mesophyll and pod husk was observed in MO 20 resulting in very large lesions.

**Clonal differences in thickness and moisture content of pod husk:** There was a significant difference ( $p < 0.05$ ) among clones in thickness both at the ridge and furrow points of the cocoa pod (Fig. 2, 3). The clones SCA 6, GU 225V, T85/799 and LAF 1 had a significantly thicker pod husk compared with the other clones, while VENC 4-4, PA 120, MO 20 and MOCORONGO had the thinnest. In general, the pod husk was thicker at the ridge than at the furrow in all the clones tested. At the Furrow point however, IFC 5 and MAN 15-2 which were the selected moderately resistant clones had thicker husk than T85/799, GU 225V, SCA 6 and LAF 1 which were the selected resistant clones. At the ridge and furrow there were no significant differences among the selected susceptible clones. These clones were grouped separately from selected resistant and moderately resistant clones.

The moisture content of the pod husk varied significantly ( $p < 0.05$ ) among the clones (Fig. 4). T85/799, GU 225V, SCA 6, LAF 1 and LECTEEN 37i had the lowest moisture content while MAN

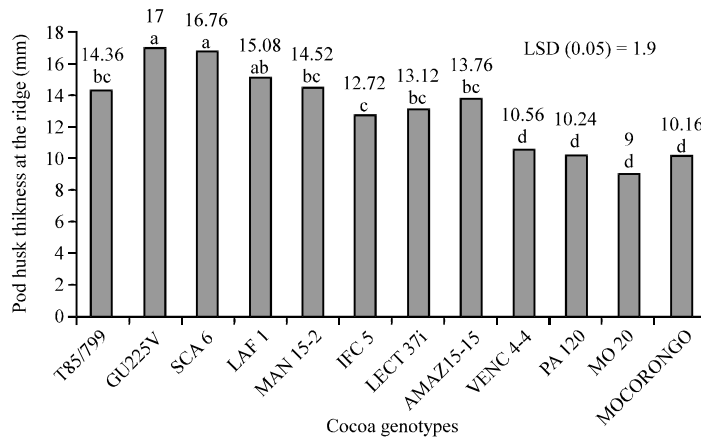


Fig. 2: Differences in pod husk thickness (ridge) of the 12 cocoa genotypes. Bars with same letters show not significant differences

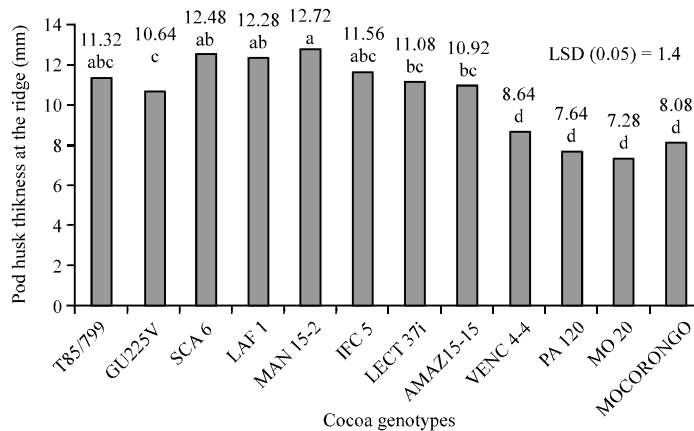


Fig. 3: Differences in pod husk thickness (furrow) of the 12 cocoa genotypes. Bars with same letters show not significant differences

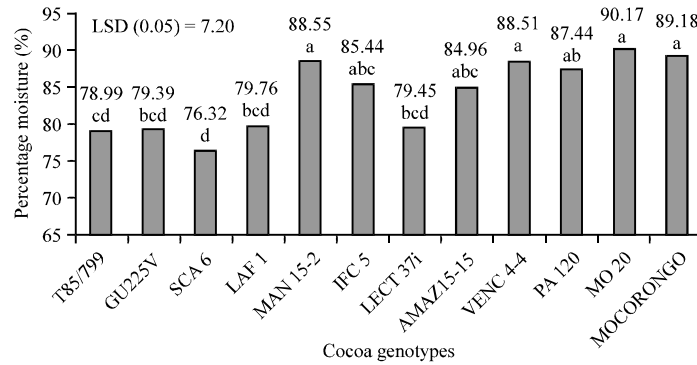


Fig. 4: Differences in percentage moisture content of pod husk of the 12 cocoa genotypes. Bars with same letters show not significant differences

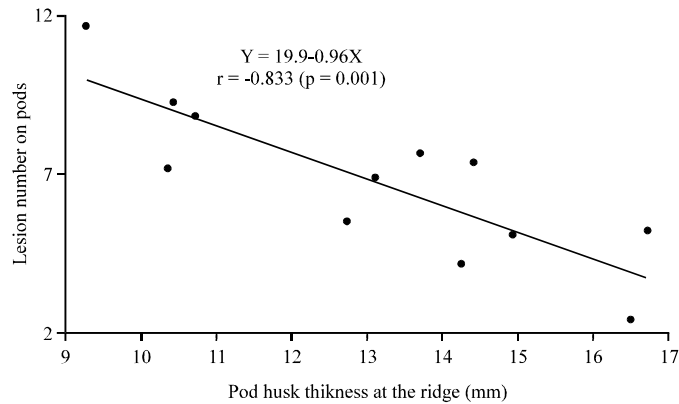


Fig. 5: Relationship between lesion number on pods and thickness of pod husk at the ridge

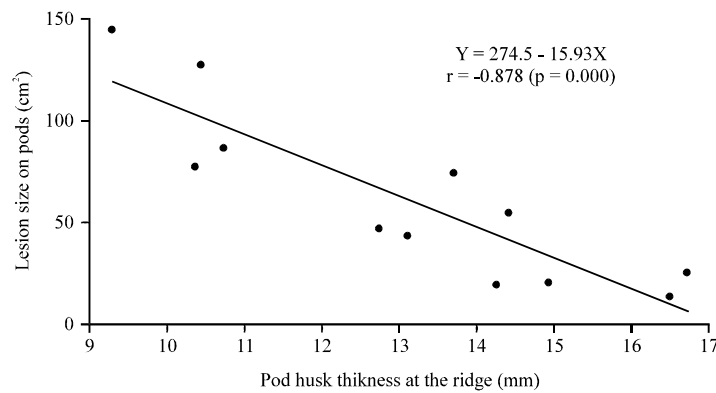


Fig. 6: Relationship between lesion size on pods and thickness of pod husk at the ridge

15-2, VENC 4-4, PA 120, MO 20 and MOCORONGO had the highest moisture content under the same conditions.

**Relationship between thickness of pod husk and pod resistance at penetration and post-penetration stages of infection:** A correlation analysis between number of lesions on pods and the thickness of pod husk at the ridge (Fig. 5) showed highly significant negative correlation



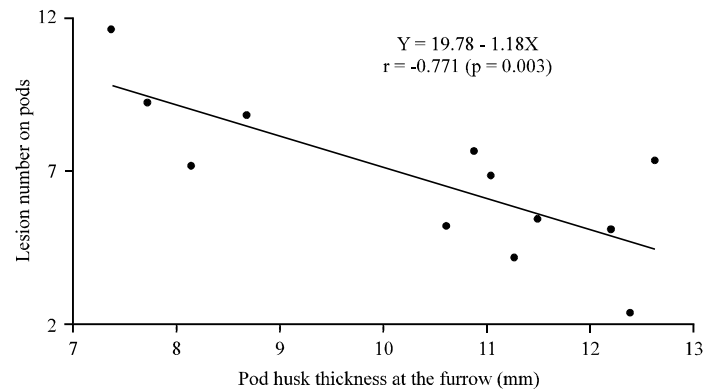


Fig. 7: Relationship between lesion number on pods and thickness of pod husk at the furrow

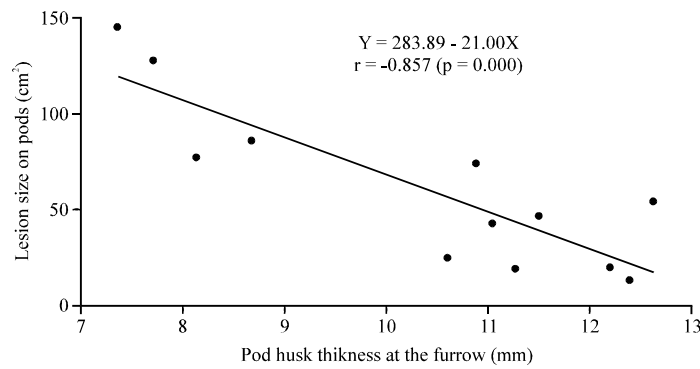


Fig. 8: Relationship between lesion size on pods and thickness of pod husk at the furrow

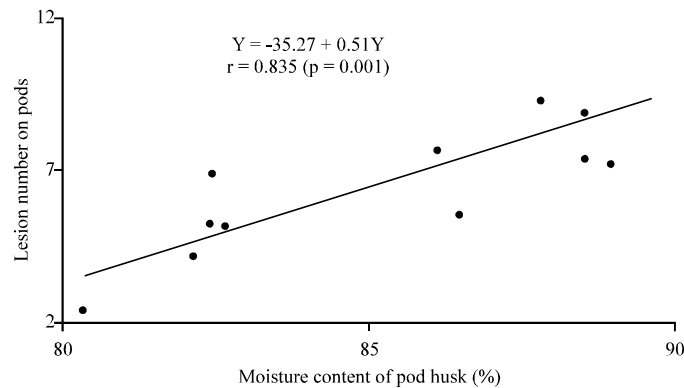


Fig. 9: Relationship between lesion number on pods and percentage moisture content of pod husk

( $r = -0.833$ ,  $p < 0.05$ ). Similarly, a highly significant negative correlation ( $r = -0.878$ ,  $p < 0.05$ ) was obtained between lesion size on pods and the thickness of pod husk at the ridge (Fig. 6). Lesion number and size on pods and thickness of pod husk at the furrow displayed similar relationship with correlation coefficients of ( $r = -0.771$ ,  $p < 0.05$ ) and ( $r = 0.857$ ,  $p < 0.05$ ), respectively (Fig. 7, 8).

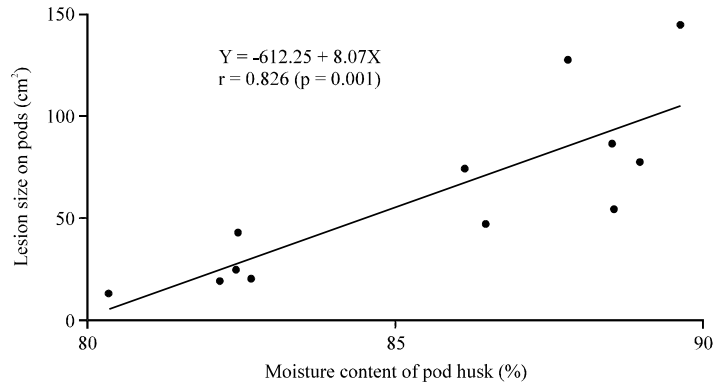


Fig. 10: Relationship between lesion size on pods and percentage moisture content of pod husk

**Relationship between moisture content of pod husk and pod resistance at penetration and post-penetration stages of infection:** A highly significant positive correlation ( $r = 0.835$ ,  $p < 0.05$ ) was obtained between percentage moisture content of pod husk and lesion number on pods (Fig. 9), suggesting that moisture content of cocoa pod husks can be used for the prediction of resistance in pods at the penetration level of infection. A similar relationship was observed between moisture content of pod husk and lesion size on pod with correlation coefficient of ( $r = 0.826$ ,  $p < 0.05$ ) (Fig. 10).

## DISCUSSION

Generally the genotypes were observed to vary significantly in their reactions at the penetration and post-penetration stages of *Phytophthora palmivora* infection. With all the genotypes planted under the same environmental conditions the observed variations could probably be genetic. This finding is in agreement with the results of Tan and Tan (1990) and Simmonds (1994) who have also observed similar variations in their studies on the resistance of cacao progenies to *Phytophthora palmivora*. Such large genetic variations have also 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 findings also agrees with the observations of Djocgoue *et al.* (2006) and Akaza *et al.* (2009) who also reported significant differences in levels of resistance between the genotypes of cacao progenies. Ahmed *et al.* (2006) also observed similar findings in reaction of chickpea to *Ascochyta* blight following detached leaf and whole-plant inoculation with an aggressive isolate of *Ascochyta rabiei*.

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.

Tosa and Shishiyama (1984) reported that attacked cells may prevent fungal penetration by forming cell wall appositions named papillae. It has also been reported by Carver and Carr (1978) that resistance mechanisms acting after haustorium formation lead to a restriction of nutrient flow to the pathogen, restricting the number of secondary, tertiary etc haustoria, colony size and number of conidiospores. Ponmurugan and Baby (2007) also observed that growth characteristics, physiological and biochemical parameters were reduced significantly in infected plants rather than healthy plants. However, the reduction was more prominent in susceptible cultivar than in tolerant

ones. The reduced lesion number and lesion size observed in SCA 6, T85/799, LAF 1 and GU 225V might be explained by these penetration and post-penetration resistance mechanisms.

The significant negative correlation between thickness of pod husk and lesion number and size indicated that genotypes of cocoa with thicker pod husk tended to be more resistant than genotypes with thinner pod husk based on infection of *Phytophthora palmivora*. Therefore, pod husk thickness is an important resistant factor that needs to be incorporated into breeding lines for resistance to *Phytophthora palmivora*. This finding supports the observations of Bong and Lee (1996) who reported thickening of cell wall of callus cells of clones resistant to vascular streak dieback in cocoa. Puttha *et al.* (2008) also reported relationship between peanut bud necrosis virus resistance and some agronomic traits in peanut. They observed that PBNV susceptibility is somewhat associated with larger seed and might hinder the progress of breeding for large-seeded peanut with resistance to PBNV. Future research will address whether increasing pod husk thickness is correlated with factors that determine the strength of the pod husk, such as lignin content, which may inhibit lesion movement. The significant positive correlation between lesion number and size on pods and percentage moisture content of pod husk indicates that genotypes of cocoa with lower percentage moisture content tended to be more resistant than genotypes with higher percentage moisture content of pod husk. The cocoa genotypes with thicker pod husks would encounter thicker endocarp than genotypes with thinner pod husks, which may explain why cocoa genotypes with thicker pod husk had lower moisture content. The susceptibility of cocoa genotypes with high percentage moisture in pod husk to *Phytophthora* indicates that the high moisture content favours the growth of the fungus. These results support those of Okey (1996), Bertrand *et al.* (1976) and Tippett and Hill (1983) which showed a positive correlation between bark moisture content and level of canker susceptibility. Clones which had high moisture content were found to be more susceptible while those with low moisture content were less susceptible to *Phytophthora palmivora* stem infection.

Of the clones investigated, only the susceptible clones (PA 120, VENC 4-4, MO 20 and MOCORONGO) had a higher percentage moisture content and thinner pod husk. The grouping of susceptible clones in this manner suggests that they might be genetically related in terms of these characteristics.

The strong relationship between lesion size and percentage moisture content of pod husk in Fig. 10 especially at the lower end of the scale, suggests that percentage moisture content may predict moderate to high levels of resistance at the post-penetration levels of infection. The inability to predict accurately the level of susceptibility, indicated by a level of scattering at the upper extreme may indicate that following initial entry other factors may be involved in the successful establishment of infection point. This agrees with observations of Agrios (1998) which stated that other modes of penetration could be attributable to chemical stimuli emanating from stomata, wounds, scars and the base of the epidermal hairs. Penetration in these areas could also be due to physical stimuli related to the structure of the stomata, epidermal hairs and the nutrient gradient present in wounds or scars.

The study identifies SCA 6, GU 225V, LAF 1 and T85/799 as useful sources of resistance at the penetration and post-penetration stages of infection. These clones had relatively lower moisture content and thicker pod husks. These clones combine both penetration and post-penetration resistance and so are valuable source of resistance for cocoa breeding programmes.

In conclusion, our results suggest that thickness and lower moisture content of pod husk are factors involved in resistance to *Phytophthora* pod rot in the cocoa genotypes assayed. However,

these cannot completely explain the resistance observed, suggesting that other cocoa pod characteristics (phenolic compounds, lignin content and wax composition) are also functioning in the resistance to *Phytophthora* pod rot caused by *Phytophthora palmivora*.

#### ACKNOWLEDGMENT

We wish to thank the Common Fund for Commodities (CFC) for financial support. Our sincere gratitude goes to the technical staff of Plant Pathology and Plant Breeding Divisions, CRIG, for their help in this study. This research article was published with the kind permission of the director of the Cocoa Research Institute of Ghana.

#### REFERENCES

- Abrahams, P.W., 2002. Soils: Their implications to human health. *Sci. Total Environ.*, 291: 1-32.
- Adomako, B., 2007. Causes and extent of yield losses in cocoa progenies. *Tropical Sci.*, 47: 22-25.
- Agrios, G.N., 1998. *Plant Pathology*. 3rd Edn., Academic Press, San Diego, California.
- Ahmed, H.U., K.F. Chang, S.F. Hwang, B.D. Gossen, R.J. Howard and T.D. Warkentin, 2006. Components of disease resistance in desi and kabuli chickpea varieties against ascochyta blight. *Plant Pathol. J.*, 5: 336-342.
- Akaza, M.J., J.A.K.N. Goran, S.P.A.N. Guetta, B.I. Kebe, G.M. Tahi and A. Sangare, 2009. Resistance to *Phytophthora palmivora* (Butler) butler assessed on leaf discs of cacao (*Theobroma cacao* L.) hybrid trees. *Asian J. Plant Pathol.*, 3: 106-118.
- Akrofi, A.Y., I.Y. Opoku and A.A. Appiah, 1997. On-farm farmer managed fungicide trials to control black pod disease caused by *Phytophthora megakarya* in Ghana. *Proceedings of the 1st International Seminar on Cocoa Pests and Diseases*, Nov. 6-10, Accra, pp: 109-118.
- Bertrand, P.F., H. English, K. Uriu and F.J. Schick, 1976. Late season water deficits and development of cytospora canker in French prune. *Phytopathology*, 66: 1318-1320.
- Blaha, G. and R. Latode, 1976. The primary criteria for selection of cacao in Cameroun: The resistance to pod rot losses: Variations in reactions and development of lesions due to ecological and physiology of fruits. *Cafe Cacao*, 20: 97-116.
- Blaha, G. and R. Latode, 1997. Contribution of and recognition of modalities of transmission of hereditary of resistance of cacao to pod rot of fruits in Cameroon. *Cafe Cacao*, 21: 179-196.
- Bong, C.L. and M.T. Lee, 1996. Resistance to vascular streak dieback: Research and applications in breeding and disease management. *Proceedings of the International Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement*, Nov. 24-26, Salvador, Bahia, Brazil, pp: 195-204.
- Carver, T.L.W. and A.J.H. Carr, 1978. Effects of host resistance on development of haustoria and colonies of Oat mildew. *Ann. Applied Biol.*, 88: 171-178.
- Cilas, C., C. Lanaud, D. Paulin, S. Nyasse and J.A.K. N'Goran *et al.*, 1998. The resistance to pod rot losses due to *Phytophthora* spp. research on components of resistance. *Plant. Res. Dev.*, 5: 441-446.
- Dakwa, J.T., 1987. A serious outbreak of the black pod disease in a marginal area of Ghana. *Proceedings of the 10th Institute Cocoa Research Conference, (ICRC'87)*, Santo Domingo, pp: 447-451.
- Djocgoue, P.F., T. Boudjeko, D.J. Nankeu, M.I.B. Efombagn, S. Nyasse and D.N. Omokolo, 2006. Comparative assessment of the resistance of cocoa (*Theobroma cacao* L.) progenies from SNK10 x SNK413; ICS84 x ICS95 to *Phytophthora megakarya* in cameroon by measuring size of necrotic lesion along the midrib. *Plant Pathol. J.*, 5: 329-335.

- Iwaro A.D., T.N. Screenivasan, D.R. Butler and P. Umaharan, 2000. Rapid Screening for Phytophthora Pod Rot Resistance by Means of Detached Pod Inoculation. In: Working Procedures for Cocoa Germplasm Evaluation and Selection, Eskes, A.B., J.M.M. Engels and R.A. Lass (Eds.). International Plant Genetic Resources Institute, Rome, Italy, pp: 109-113.
- Iwaro, A.D., 1995. Mechanisms of resistance to *Phytophthora palmivora* (Butler) Butler in cacao (*Theobroma cacao* L.) and their genetic basis. Ph.D. Thesis, The University of the West Indies, Trinidad and Tobago,
- Iwaro, A.D., J.M. Thevenin, D.R. Butler and A.B. Eskes, 2005. Usefulness of the detached pod test for assessment of cacao resistance to *Phytophthora* pod rot. *Eur. J. Plant Pathol.*, 113: 173-182.
- Iwaro, A.D., T.N. Sreenivasan and P. Umaharan, 1997. Foliar resistance to *Phytophthora palmivora* as an indicator of pod resistance in *Theobroma cacao*. *Plant Dis.*, 81: 619-624.
- Luterbacher, M.C., 1994. The identification, epidemiology and control of *Phytophthora megakarya* on cocoa in West Africa. Ph.D. Thesis, University of London.
- Nyadanu, D., 2008. Evaluation of the efficacy of resistance screening methods used in the breeding of black pod disease resistant varieties in cacao. M. Phil Thesis, University of Cape Coast, Cape Coast, Ghana.
- Nyasse, S., G. Blaha and C. Cilas, 1994. The pathogenicity of *Phytophthora megakarya* and its implication for spray and point inoculation tests to predict sensitivity of pod rot in cacao. Proceedings of the 11th International Cocoa Research Conference, July 18-24, Cocoa Producers Alliance, Lagos, Nigeria, pp: 83-90.
- Okey, E.N., 1996. Histopathological and biochemical studies on cacao (*Theobroma cacao*L.) canker caused by *Phytophthora palmivora* (Butler) Butler. Ph.D. Thesis, The University of the West Indies, Trinidad and Tobago.
- Okey, E.N., E.J. Duncan, G. Sirju-Charran and T.N. Screenivasan, 1996. Factors affecting the susceptibility of six cocoa clones to *Phytophthora palmivora* (Butl) Butler bark canker in Trinidad. *Plant Pathol.*, 45: 84-91.
- Opoku, I.Y., A.Y. Akrofi and A.A. Appiah, 1999. The spread of *Phytophthora megakarya* on cocoa in Ghana. *J. Ghana Sci. Assoc.*, 2: 110-116.
- Ponmurugan, P. and U.I. Baby, 2007. Morphological, physiological and biochemical changes in resistant and susceptible cultivars of tea in relation to phomopsis disease. *Plant Pathol. J.*, 6: 91-94.
- Prats, E., T.L.W. Gay, B.J. Thomas and L.A.J. Mur, 2007. Enemy at the gates: Interaction-specific stomatal responses to pathogenic challenge. *Plant Signal Behav.*, 2: 275-277.
- Prendergast, W.N.E., 1965. Studies in the resistance of *Theobroma cacao* L. to *P. palmivora* (Butl.) Bul. M.Sc. Thesis, University of the West Indies, Trinidad.
- Puttha, R., S. Jogloy, S. Wongkaew, J. Sanitchon, T. Kesmala and A. Patanothai, 2008. Heritability, phenotypic and genotypic correlation of peanut bud necrosis virus resistance and agronomic traits in peanut. *Asian J. Plant Sci.*, 7: 276-283.
- Screenivasan, T.N., 1995. A rapid method of inoculation of *Phytophthora palmivora* (Butler) Butler on attached pods. Proceedings of the 9th International Cocoa Research Conference, (ICRC'95), Lagos, Nigeria, pp: 293-302.
- Selinus O., B. Alloway, J.A. Centeno, R.B. Finkelman, R. Fuge, U. Lindh and P. Smedley, 2005. Essentials of Medical Geology: Impacts of the Natural Environment on Public Health. Elsevier, Amsterdam, ISBN: 0126363412, pp: 812.

- Simmonds, N.W., 1994. Horizontal resistance to cocoa diseases. *Cocoa Growers Bull.*, 47: 42-53.
- 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.
- Spence, J.A., 1961. Black pod disease of cocoa II: A study in host-parasite relations. *Ann. Applied Biol.*, 49: 723-734.
- Susheelamma, B.N., M.S. Jolly, K. Giridhar and K. Sengupta, 1990. Evaluation of germplasm genotypes for drought resistance in Mulberry. *Sericologia*, 30: 327-341.
- Tan, G.Y. and W.K. Tan, 1990. Additive inheritance of resistance to pod rot caused by *Phytophthora palmivora* in cocoa. *Theor. Applied Genet.*, 80: 258-264.
- Tippet, J.T. and T.C. Hill, 1983. The relationship between bark moisture and invasion of *Eucalyptus marginata* by *Phytophthora cinnamomi*. *Aust. Plant Pathol.*, 12: 40-41.
- Tosa, Y. and J. Shishiyama, 1984. Defense reactions of barley cultivars to an inappropriate *Forma specialis* of the powdery mildew fungus of gramineous plants. *Can. J. Bot.*, 62: 2114-2117.