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HOST PLANT RESISTANCE FOR SUSTAINABLE COCOA POD BORER MANAGEMENT (CFC/1CCO/41FT)

PROJECT COMPLETION REPORT (PCR)

Note by the Secretariat:

The attached document was submitted by the Project Executing Agency (PEA), the Malaysia Cocoa Board (MCB). The document has been reviewed by the ICCO Secretariat and is being submitted for consideration by the Executive Committee.

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I. PROJECT SUMMARY:

Project Title:	Host Plant Resistance for Sustainable Cocoa Pod Borer (CPB) Management		
Project Number:	CFC/ICCO/41FT		
Project Executing Agency (PEA):	Malaysian Cocoa Board (MCB)		
Supervisory Body:	International Cocoa Organisation (ICCO)		
Location:	Malaysian Cocoa Board (MCB), Malaysia Cocoa and Coconut Institute Limited (PNGCCIL), Papua New Guinea		
Starting Date:	October 2008		
Completion date:	April 2011		
Financing:	Total Project Cost	-	US$263,850
	CFC Grant	-	US$119,700
	Counterpart Contribution	-	US$ 144,150

II: PROJECT BACKGROUND

1. Cocoa Pod Borer (CPB) is a major insect pest that plagues cocoa production mostly in the Asia Pacific region. CPB can cause total crop loss if it is not controlled or adequately managed. The costs of controlling the pest range from 20-40% of the total cost of cocoa production. The decline in cocoa production in the ASEAN region (Indonesia, Malaysia, Philippines and more recently in Papua New Guinea) is largely due to the devastating effect of CPB.

2. Since CPB was discovered in 1980, intensive research has been carried out to identify measures to manage and control it. A number of control measures had been recommended to manage the insect pest. However, the effectiveness of the control measures varies. The current control measures for CPB have both strengths and weaknesses. Each control measure has its own limitations and no single measure has proved very successful in the control of CPB. While a combination of the control measures could improve the management of the pest, it is necessary develop a long term and more sustainable measure to control CPB.

3. The current control measures for CPB include the use of insecticides, cultural practices and biological control. Resistant planting material is an effective and sustainable method for CPB management. However, based on the present knowledge there are no clones that are completely resistant to CPB. A number of clones were reported to have traits of resistance to CPB. Lim and Phua (1984) reported that PA7, UA 30, UA 12, UA 9 and NA 34 showed the lowest dry bean loss among the clones evaluated under natural conditions. Azhar and Lim (1987) found that ICS 98, UIT 5, PA 7, UA 37 and LAFI 7 were the more tolerant clones to CPB among the clones they evaluated with LAFI 7 exhibiting the highest tolerant level. Azhar (1988) indicated that there were three components in resistance to CPB i.e. non-preference, tolerance and antibiosis. Thus, further and systematic research on Host Plant Resistance (HPR) in cocoa was required to establish resistant clones to CPB.

Rationale for the Project

4. There have been several research works on Host Plat Resistance (HPR) of cocoa to control CPB. Lim and Phua (1984) evaluated fifty-nine clones comparing the pod infestation rate and the loss of wet beans. The clones PA7, UA30, UA12, UA9 and NA34 showed the highest degree of tolerance, whereas ICS98, I594, I1206, NGK16 and WA331 were the most susceptible to cocoa pod borer infestation (Lim and Phua, 1984). However, only the tolerance component of resistance was studied. Azhar and Lim (1987) conducted an experiment on host plant resistance using the clones ICS98, UIT5, PA7, UA37, and LAF17 and found LAF17 showed the most resistance to CPB. They also indicated that not all the three components of resistance (non-preference, tolerance and antibiosis) were important in providing resistance against CPB. This indicated that no cocoa clone may have complete resistance to CPB. They noted that clones with the thickest sclerotic layer also suffered bean loss when infestation occurred before it had properly developed.

5. Day (1985) indicated that antibiosis component of resistance was the main factor that cause mortality at the first larval instar of CPB, while Azhar and Lim (1987) suggested that the hardness and thickness of the sclerotic layer contributed to tolerance. Biochemical constituents in the cocoa pod may also contribute to resistance as it matures. Therefore, further investigation on biochemical components needs to be re-examined in future.

6. There are varying results on determining the tolerance of clones to CPB. Non-preference, tolerance, antibiosis, biochemical changes are among parameters indicated as tolerance to CPB. Measuring all these parameters would lead to the identification of 'tolerant' clones. However, the procedures may be cumbersome and costly. Therefore, a quick and reliable protocol for screening of tolerant clones is needed to help cocoa breeders in the selection and identification of planting materials that are tolerant to CPB.

Project Objectives

7. The main objectives of the project are:

- To develop simple, reliable and quick screening protocols for the identification and selection of clones / hybrids tolerant to CPB; and
- To identify and select some clones tolerant to CPB.

8. The project will lead to identification of CPB tolerance cocoa varieties to enhance sustainable cocoa cultivation and increase productivity of farms and incomes of farmers.

Expected Outputs

9. The expected outcomes of the project include screening protocols for CPB tolerance clones, and clonal materials tolerant to CPB.

Component I: Morphological Characteristics of the Cocoa Pod

Physical and morphological traits of the pods of six cocoa clones at the MCB and the PNGCCIL that could contribute to resistance to CPB were measured. Pod hardness and thickness with particular emphasis on the sclerotic layer (SL) and the structure of the SL of these clones were recorded.

Outputs: Information on the physical and morphological traits of the pod of the six clones that include rogousity, thickness and hardness of the pods and the SL at each site were obtained. This information was used for selecting clones for further evaluation on the antibiosis and preference.

Activity 1.1 Determination of pod texture

The textures of the pods of the six clones at each site were recorded to determine their effect on egg-laying preference by the cocoa pod borer. In addition, the textures of the pod surface were determined by comparing the ratio of the thickness of the epicarp layer at the ridge to the secondary furrow.

Activity 1.2 Determination of SL hardness

The hardness and thickness of the sclerotic layer of the six clones were measured to monitor their effect on the survival of the larvae. The hardness and thickness of sclerotic layer were measured using a penetrometer and a pair of Vernier calipers, respectively. Measurements were taken at the point adjacent to the primary furrow at the middle portion of the pod. Thickness and hardness measurement were taken at the age of 1, 2, 3, 4, 5 and 6 months of pod ripening.

Component II: Ovipositional Preference

Ovipositional preference of CPB on the six clones was recorded both in the laboratory and in the field.

Output: Information on the ovipositional preference of CPB of six clones of each site.

Activity 2.1 Laboratory study

Choice and no choice method of assessment were adopted in this study. In the choice condition one pod from each of the six clones were arranged randomly in the netted cages. Pods of 3 – 4 months old were selected. Three to five pairs of cocoa pod borer moths collected from cocoa fields were released in the cage for 24 hours. Pods were examined for CPB eggs in the following day. For no choice condition, one pod from each clone were placed in the netted cage and 3-5 pairs of CPB moth were released for 24 hours. The number of CPB eggs laid on each pod was recorded. Both studies were repeated 3 times.

Activity 2.2 Field study

The ovipositional preference of the cocoa pod borer among the selected clones were determined by visually counting the number of eggs laid on ten randomly selected pods of 3-4 months for eggs lying at monthly intervals. Pods located at 1-2m above ground of the six clones were tagged. CPB eggs were counted at one month interval until ripening. The ripe pods were

harvested and classified into five damage categories, i.e. healthy (0), slight (1), light (2), medium (3) and heavily infested (4).

Component III: Antibiosis Study

This component determines the development, reproduction and survival of CPB of selected clones.

Output — Data on the CPB development, reproduction and survival of selected clones

Activity 3.1 — Development, reproduction and survival of cocoa pod borer on each selected clone

A pair of wild cocoa moths was caged with the individual pod of the six clones under field conditions. The number of eggs deposited were recorded and allowed to be developed until pupal stage. A dry cocoa leaf litter was placed in the cage for the pupation site. The pupa were collected and brought to the laboratory for adult emergence. A pair of new adults was then caged onto the cocoa pod of the six clones in the field for oviposition and longevity observation. Insect development was measured as a rate of increase in insect size, weight or length over time.

Component IV: Tolerance Study

The tolerance level of the six selected clones against CPB was determined.

Output — Data on the average damage severity index were determined on the six clones at each site.

Activity 4.1 — Determination of CPB infestation in the field.

About 600-1,000 fruit sets were tagged. Pods were caged when they reached 50-60mm in length. Five pods were then exposed to CPB infestation at monthly intervals, beginning 3 months until ripening.

Activity 4.2 Determination of the average severity damage of the selected clones.

Ripe pods were split and categorised according to the damaged categories to determine the average severity index.

Activity 4.3 — Data collection on entry and exit holes of CPB.

Ripe pods were harvested and shaved to determine the number of exit and entrance holes. Other parameters such as density of larvae and exit hole were recorded.

Component V: Field Assessment of Host Plant Resistance against CPB

The tolerance levels of the selected CPB tolerant clones were assessed.

Output: Tolerant clones will be identified

Activity 5.1 Propagation of clones

Six clones were propagated for the tolerance assessment in the field. Nursery grafting was used.

Activity 5.2 Field planting

The clones are planted in the box or row system. Susceptible clones were used as trapped and planted at the perimeter of the boundary or planted in rows.

Activity 5.3 CPB assessments

Once the trees bear fruit, the damage severity due to CPB infestation were monitored.

Component VI: Analysis and Interpretation of Result

Data on the pod morphological trait assessments, preference, antibiosis study, and tolerance experimentations were analysed to determine the tolerance of clonal materials against CPB prior to field planting and evaluation of the selected tolerant clones.

Output A set of criteria/parameters to identify CPB tolerant clones.

Activity 6.1 Data were analysed in both locations using standard statistical methods and packages.

Project Cost and Finance

10. The total project cost was US$ 263,550 of which US$119,700 was provided as grant by the Common Fund for Commodities (CFC). The counterpart contribution by the participating countries was US$ 143,850.

Project Management

11. The Project Executing Agency (PEA) was the Malaysian Cocoa Board (MCB) in Malaysia. The project was conducted by the MCB and Cocoa and Coconut Institute Limited in Papau New Guinea (PNGCCIL). Although similar activities were carried out at each participating institute, the clones evaluated were different with the exception of the control clones. The common clones used are those used in the International Clone Trial of the CFC/ICCO/Bioversity Project.

III. PROJECT IMPLEMENTATION AND RESULTS ACHIEVED

Resource Utilisation

12. The allocated fund of US$119,700 grant by CFC was fully utilised. The two participating institutions provided counterpart contributions that include staff costs, laboratory and field operational costs, logistics and others. Additional funds are now required to maintain the field trial on assessment of HPR established by the MCB.

Project Results

Results for Component 1 - Morphological characteristics of the cocoa pod

13. **In Malaysia**, the six clones selected for study were T85/799, EET397, ICS84, NA33, P12 and PA30. The pod colour of clones T85/799, NA33 and P12 are green, while clones EET397 and ICS84 have greenish-red and red colours respectively. NA33 and P12 clones have Amelonado pod shape. The clones with Angoleta pod shape are T85/799 and ICS84. EET397 has Amelonado-Angoleta pod shape, while the pod shape of PA30 clone is Cundeamor-Angoleta.

14. The pod length of the clones ranged between 13.72 and 19.10cm. ICS84 had the longest pod length and the shortest pod length was P12. ICS84 was recorded to have the widest pod width of 9.35cm. The clone with the smallest pod width of 7.27cm was NA33.

15. The husk thickness at primary furrow ranged from 4.75 to 8.96mm. Clone ICS84 has the highest husk thickness of 8.96mm while clone T85/799 has the lowest husk thickness of 4.75mm. On the pod surface smoothness ratio, clones ICS84, NA33, and PA30 had smooth pod surfaces. The clones that had rough pod surfaces were T85/799, EET397 and P12. The ratio of primary furrow to ridge ranged from 0.59 (T85/799) to 0.93 (ICS84). A ratio closer to 1.0 indicates the smoothness of the cocoa pod husk surface.

16. The thickness of the sclerotic layers (SL) of the pods at four months age varies among the six clones. The study noted that clone T85/799 had the highest thickness value of SL, while the smallest thickness value was recorded for clone P12. The hardness of the sclerotic layer (SL) of the six clones was measured using Penetrometer (model SP02). The hardness of SL ranged from 3.10kgf to 5.78kgf with clone ICS84 recording the highest hardness of 5.78 kgf and clone P12 registering the lowest hardness of 3.10 kgf.

17. **In Papua New Guinea**, the six clones selected for the study were PA120, ICS1, Man-15/2, k4, k9, Kakunu. Pod colours were recorded. Red and green pod colours were equally distributed among the selected clones where three clones (K9, Kakunu and ICS1) had red colour and the other three clones (MAN-15/2, PA120, and K4) hade green coloured. As the two colours were equally distributed among the selected clones, this suggests that the genes controlling colours is independent of tolerance. Therefore, the colour of the pod cannot be used in the screening protocol.

18. The shape of the cocoa pods of the clones were characterized into four categories; Calabacillo, Amelonado, Angoleta and Cundeamor. The Amelonodo and Cundeamor pod shapes were equally represented with two clones each, while Callabacilo and Amelonado had one each. K4 clone has an amelonado shape and has a relatively smooth surface as indicated by its ratio of 1.1. The closer the ratio is to 1.0, the smoother the pod surface. K9 (1.3), Kakunu (1.3) and MAN-15/2 (1.2) were the next clones with moderately smooth surface pods. ICS1 (1.4) and PA120 (1.4) had relatively rough and deeply furrowed primary furrows. Based on this information, pods with ratio of more than 1.3 can be categorized as rough, 1.2-1.3 as moderately smooth and 1.2-1.0 as smooth.

19. The average pod length of the clones studied in PNG ranged from 114mm at 2.5 months to 187mm at 5.0 months. Kakunu (144mm) was the longest and K9 (127mm) was the shortest at 2.5 months. The pod width also varied between the clones. K4 (63mm) had the highest pod width at 2.5 months and Kakunu (52mm) had the lowest. For the mature pods at 5 months, PA120 (87mm) had the highest width and both MAN-15/2 and K9 were the lowest at 77mm each.

20. Generally, for all the clones, the average SL hardness increased proportionally with age up to 4 months. The hardness then declined for some clones (MAN15/2, ICS1, K4 and Kakunu). The clones PA120 and K9 continued to show increase in pod hardness until 4.5 months but decreased thereafter. Most of the clones seem to have reached their highest pod hardness levels by 4.0-4.5 months of age. The highest hardness scores were 4.8 (MAN-15/2), 5.4 (K9), 6.2 (PA120), 4.9 (ICS1), 5.8 (K4) and 5.6 (Kakunu). Only ICS1 (6.2) reached its highest hardness late in the 5 months old pods. SL hardness at age 2.5 to 3.5 months is a very important factor, as this would be a very susceptible age for CPB infestation. Highly significant ($P<0.001$) differences were found between the clones, age groups and their interaction for the pod hardness (Table 3). K4 (4.0) had the highest hardness followed by K9 (3.5), MAN15/2 (3.0) and PA120 (3.0) at 2.5 months pod age category. Kakunu and ICS1 had the lowest hardness scores with their respective scores of 2.7 each at secondary furrow (SF). The hardness of the primary furrow (PF), secondary furrow (SF) and ridge (R) were positive and highly correlated with each other with their correlation coefficient (r) values ranging from 0.86 to 0.93. This indicated that any one of the 3 regions could be used instead of all the three when screening for pod hardness. An ANOVA performed, for pod age and pod regions (PF, SF, R) as the main effects on SL hardness, also failed to show any significant differences ($P<0.105$) among the hardness scores at PF, FS and R. This further confirmed that only one region can be used when screening for SL hardness. On the other hand, hardness in all the regions was negatively correlated with ADSI, implying that as hardness increases, starting from 2.5 months old pods, there was a decrease in bean damage.

21. The SL thickness at ridge (R) is usually the thickest followed by the secondary furrow (SF) and Primary furrow (PF). However, the clones varied in their SL thickness at all the three regions depending on the length, width, pod surface, and pod age. Starting from pod age of 2.5 to 4 months, K4 had the highest SL thickness followed by K9 and MAN-15/2. ICS 1 also showed an increased in SL thickness specifically the R, which reached the highest peaks beginning in 3.5 months to 5.0 months pod age. This high SL thickness may have been responsible for its highest hardness score for the same region and period for the given pod age category. Similarly, the clones K4 and K9 had their highest SL thickness and hardness in the early pod ages of 2.5-3.5 months. The PF thickness was positively correlated with SF thickness and R thickness with their scores; (0.87) and (0.77) respectively. This again suggested that, assessment for SL thickness from any one region (SF in this case) would be adequate because of their high correlation coefficient compared to the other two (PF and R) regions. There was also a positive correlation between the SL thickness and pod hardness. The highest correlation co-efficient was detected between PF hardness and SF thickness ($r=0.695$), followed by PF hardness and R thickness (0.685), SF hardness and SF thickness (0.666 and R hardness and SF thickness (0.629). Furthermore, the SL thickness at all the regions (PF, SF, R) seemed to be positively correlated with width of the pods. Interestingly, there was a low (weak) negative correlation (-0.318) between SF thickness and ADSI.

Results for Component 2 - Ovipositional Preference Study

22. **In Malaysia**, the ovipositional preference study conducted at the laboratory study showed that there was no specific preference of female CPB for laying eggs under choice conditions. Two eggs were laid on clone EET 397, but did not hatch. Under no choice conditions, the lowest number of CPB eggs laid on clone ICS84 was 12 and the highest number laid on clone PA 30 was 154. In contrast, the study of ovipositional preference in field indicated the existence of a relationship between the number of eggs and the Average Damage Severity Index (ADSI) values, suggesting that the number of eggs laid by female CPB influenced the ADSI values. The lowest ADSI value was recorded on clone T85/799 followed by NA33, ICS84, EET397 and PA30. But, ADSI value of 2.67 was recorded for clone P12 which had the highest number of egg laid by female CPB. This may

indicate that P12 was tolerant to CPB infestation in term of ADSI value. This means that, although the high number of eggs laid on P12 hatched and the larvae bored into the cocoa pod, the damage caused by the larvae inside the cocoa pod was minimal.

23. In **Papua New Guinea**, the study of ovipositional preference carried out in the laboratory and in the field showed that, under choice condition, the mean number of eggs laid on ICS 1(7.3) was the highest followed by PA120 (6.2). The lowest were Kakunu and K4 followed by K 9 and MA15/2. Anova showed highly significant differences ($P \leq .008$) among the clones for eggs laid. Under no choice condition, there were no significant differences among the clones for the number of eggs laid. However the clones Kakunu (6.7), ICS (6.7) and MAN15/2 seemed to have relatively lower number of eggs laid on them as compared to the rest of the clones. The clone Kakunu in particular maintained its relative ranking with low ovipositional preference on both tests. This result suggests that the CPB will ovi post on any clone under no choice condition but the eggs laid may vary depending on the substrate (pod of different clone).

24. Ovipositional preference in host plant resistance, concerns the ability of the female adult CPB to find the suitable pod to lay her egg. The choice or preference made would most probably be guided by chemical attractant or repellent stimulus in the pod. This study has clearly demonstrated this case in the choice condition test. The clones K9, K4 and Kakunu were the clones less preferred as indicated by the number of eggs laid on the pod. ICS 1, PA120, and MAN15/2 were more preferred for oviposition.

Results for Component 3 - Antibiosis study

25. **In Malaysia**, this study was carried out to determine the development, reproduction and survival of CPB on the selected clones. The results showed that, CPB development of both sexes was affected by clone factor. The male pupa that emerged from NA33 were the shortest followed by P12, EET397 and PA30. The male pupa that emerged from ICS84 and T85/799 were observed to be longest and there were no significant difference between them compared to the male pupa of the Wild population. For the female sex, there were no significant differences on the pupa that emerged from EET397, P12, PA30, T85/799 compared to the Wild population pupa. Interestingly, female pupa emerged from ICS84 and NA33 were longer compared those emerged from EET397, P12 and PA30.

26. Observation on pupa weight showed that male pupa emerged from EET397, NA33, P12 and PA30 were among the lightest and significantly different compared to male pupa from Wild population. However, male pupa that emerged from ICS84 and T85/799 were insignificantly different compared to male pupa from Wild population.

27. The size of the insect as it emerged into adult was also measured. The shortest male was found to emerge from NA33 and was significantly different compared to the rest. The male that emerged from ICS84 was the longest and significantly different compared to the Wild population. The length of the adult CPB female did not show any significant difference among the population. Significant difference was observed only on female that emerged from ICS84. The male CPB that emerged from T85/799 was the heavier and differs significantly compared to male from wild population. However, it was not significantly different when compared to male that emerged from ICS84 and NA33. Male emerged that from P12 was observed to be the lighter compared to the others. The female CPB that emerged from T85/799 was observed to be the heaviest and significantly different compared to the female that emerged from P12.

28. The fertility of female CPB is best reflected by observing their fecundity. Females that emerged from P12 were observed to have low fecundity but not significantly different compared to females that emerged from EET397, ICS84 and PA30. In contrast, females that emerged from NA33 showed higher fecundity but not significantly different compared to EET397, ICS84, PA30 and Wild population.

29. Through this study, it became clear that CPB development and reproduction were affected by cocoa clones. CPB development on NA33, P12 and PA30 seem to be affected the most. Weight of CPB that emerged from these clones was among the lowerst especially the weight of the pupa. On reproduction, the female that emerged from P12 recorded low fecandity. This was expected because the weight of the female that emerged from this clone was the lowerst compared to other clones. Insummary, antibiosis as a resistance mechanism could exist at least on NA33, P12 and PA30. Further studies are needed to develop a more clear picture on antibiosis activity in cocoa towards CPB.

30. In Papua New Guinea, The mean pupa length varied between the clones. PA120 and ICS1 had the highest of 0.72 and 0.73 cm respectively, while the lowest were K4 (0.63cm) and K9 (0.66cm). Kakunu and MAN15/2 were moderate for the pupa length. Analysis of variance for pupa length showed significant differences ($P \leq 0.006$) between the clones for pupae length (Table 6). The length of pupa on the clones K9 and K4 were significantly shorter than the rest of the clones (Kakunu, PA120, MAN15/2 and ICS1) which were not significantly different from each other.

31. The clones K9 (0.0051g) and K4 (0.0052g) had the lowest Pupa weight while three clones; PA120, MAN15/2 and ICS 1 had the highest. Analysis of variance showed significant differences ($P \leq 0.05$) on the effect of tolerant clones on the pupae weights. This is another indicator of antibiotic reaction of tolerant clones in the field situation where the insect life cycle (reproductive cycle) or normal growth is affected. The mean adult weights varied accordingly to the effects of tolerant clone used for oviposition. The trends for weight differences were almost similar to the pupae weights. Adults raised from the clones K4 (0.0034g) and K9 (0.0032g) had the lowest weight. The highest were from PA120, MAN15/2, and ICS1 with their respective score of 0.0044g, 0.0042g and 0.0041g, while Kakunu (0.0040g) was only moderate. All adult weights showed a reduction of 15-16% from the mean pupae weights. This reduction may be due to the loss of fluids from pupation to adults which is a normal biological condition. This proportional reduction did not have any effect on the trend or ranking of the adult weights. The weights of adults emerging from the tolerant clones also showed highly significantly differences ($P \leq 0.01$) similar to their pupae weight. K4 and K9 weights were significantly shorter than PA120, ICS1, MAN15/2 and Kakunu.

32. A shorter length may be a good predictor when evaluating antibiotic reaction of the pods of the more tolerant or resistant clones. The antibiosis mechanism may be due to physical hardness of the pod similar to that of rice stems with more silica which renders more resistance to borers (Day thesis), or there may be a secondary chemical involved that is in greater or lower concentration in the more tolerant clones (Kakunu, K4 and K9). Recent study on Nutritional status of cocoa in Papua New Guinea pointed out a strong relationship of increased resistance to *Pythophthora* infections with increased Zn levels (Nelson, et.al, 2011). Organic Sulfur and Manganese compounds are also known to have toxic effects on fungi growth (Agrios, 1997). Although it is not in the scope of this study, it is worth highlighting the need for future investigation into the nutrient status and chemical analysis of the cocoa pod in relation to resistance to CPB and fungal diseases such as *Pythophthora.* There may be a close relationship or similarity in the antibiosis reaction between the two. In fact, there were some observations made during the study that needs further investigation. Such observations on deformed pupae, and fungal growth on pupae upon pupal emergence are inline with this claim.

33. The pupae length and weight seem to provide good information on antibiosis mechanism of host plant resistance. As shown in the study, these variables clearly discriminated the most tolerant clones (K4 and K9) from the other clones. These clones were identified in early study as having the lowest ADSI, entry and exit holes. In this study, it would have been better to monitor the next generation of CPB population derived from the adults having shorter length or lower weight to asses or monitor their longevity. This activity will be done along side with others to further our understanding and knowledge on host plant resistance. However, previous study (Day thesis) revealed that adult moths obtained from resistant clones died earlier than those that emerged from pod of the susceptible clones. It is highly likely that a similar pattern will be observed in the future.

34. The fact that K4 and K9 have thicker or harder endocarp supports the suggestion that CPB resistance in cocoa is due to an antibiotic component and the sclerotic layer is the principle cause of this effect. Greater mortality occurred in pods with either thicker or harder stony endocarp layers in the pod wall. Larval survival was as much as 10 times greater in soft/thin-walled cultivars (Day, 1985). Day, (1985) documented clearly the various forms of larval mortalities, i.e., in the pre-scelerotic and scelerotic layer, and mortality due to parasitic predation). Entomopathogens are also readily available which account for a certain proportion (%) of mortality. Six genera of fungi are reported (Keane et.al, 1992) to infect larvae and pupae of CPB. The most effective is *Beauveria bassiana*, causing 100% death during pupation if larvae are exposed to the fungus on emerging from the pod. During the study on antibiosis, we observed several of these fungal infections. To date, we have not identified the fungus as yet and how useful it will be as a bio-control agent for CPB. More research is needed to verify the effectiveness of this fungus.

Results for Component 4 - Tolerance Study

35. In Malaysia, clones NA33 and T85/799 were infested by CPB only at the age 3 to 4 months. However on the other clones, CPB infestation seemed to occur on the pod at 5 month old. Exit holes represent the survival of CPB larvae in the pod. It was clear that survival of CPB larvae increased when infestation occurs at the right age stage of the pod. Exit holes were observed to be higher on 4 month old of infestation for NA33, T85/799, EET397 and ICS84, perhaps this was due to more egg laid by CPB. In contrast, exit hole was observed to be higher at 3 month old infestation on clone P12 and PA30.

36. Tolerance of cocoa clone towards CPB infestation was expressed by ADSI. ADSI index at different stage of pod age of the clone was undertaken. It was found that ADSI index is higher when CPB infestation occurred at 3 month old pod. While most of the clone recorded ADSI index above 3.00, NA33 recorded less then 3. Overall, NA33 shows less damaged by the CPB infestation. Correlation analysis has been done to investigate the relationship between ADSI index, entry hole and exit hole. It showed that there was significant correlation between the exit holes and ADSI ($r = 0.67$) compared to the entry holes and ADSI ($r = 0.36$) or the entry holes and exit holes ($r = 0.34$). This was because the exit holes indicated that the larva have successfully exited from the pod which received the feedings from inside pod and have caused the severity damages on the beans as scaled by the ADSI values. Meanwhile, the entry holes didn't give the direct impact on the beans infestation as some of the larva might be dead before reaching the placenta.

37. The study found that clone NA33 and T85/799 providednarrower window of time for the CPB to infest. If given the choice, CPB tend to prefer 4 month old pod for these clones. As tolerance implies the ability of the plant to recover from pest infestation, quick pod development which shortens the opportunity window for the CPB infestation was observed in NA33 and T85/799.

38. In **Papua New Guinea**, counting of entry and exit holes (EH)was done after shaving the mesocarp of the pod. Tolerance study in phase 1 showed that K9 and K4 on average had low number of entry holes per pod (7.3, and 9.8 respectively) while highest number of entry holes was recorded in MAN15/2 (36.1). Similar pattern of results were noted for the number of exit holes. Again, considerable variability within the clones for entry and exit holes was recorded. Generally, the number of exit holes per pod correlated with ADSI values where more exit holes had higher ADSI values. The ADSI varied between clones. Three clones; K9, Kakunu, and K4 had ADSI values of 0.9, 1.3, and 1.8 respectively, indicating less CPB damage. PA120 (2.1) and MAN15/2 (2.6) were moderate while ICS1 was heavily damaged by CPB. The variability within each clone is shown by the range of ADSI values. Considerable variability in CPB damage between the trees or pods of a tree for each clone was recorded. The reasons for the observation need to be substantiated with more detail study.

39. The general trend for entry holes (EH) was shown to be declining steadily starting from pod age and time of exposure at 2.5 months (which was the highest EH) to 3.5 months. There was a sharp decline of EH at pod age and exposure time of 4.0-4.5 months. The lowest was at 5.0 months of pod age and exposure. The clones ICS 1 had the highest number (54.0) of EH, while K4, MAN-15/2 and Kakunu were moderate (38-40) and the lowest were K9 and PA120 (21-25) at 2.5 months (Table 10). The same trend was observed again in 3.0 months pod age, however, there was a decline in EH for the clone K4. The lowest EH were recorded was for K4 and K9 with 27.5 EH each. A good fit of the model which is a second degree polynomial ($-0.868x^2 + 0.856x + 32.40$) seem to approximate the data on EH very well as explained by the high coefficient of determination (R^2) of 0.92 for the average EH of the different aged pods/clone. The association of EH with ADSI was positive ($r=0.483$) and highly significant. Regression analysis (Table9) also revealed a significant ($P <0.03$) relationship of EH and ADSI. This further means that an increase in the number of EH will also increase the damage of bean (ADSI value) or vice versa.

40. High EH for the pod at age of 2.5, 3.0 and 3.5 months was possible or expected because the sclerotic layer for this age group will be soft which permits high penetration by the CPB larvae. However the clones K9, K4 and PA120 had low EH for the same age as compared to the other clones. This finding seemed to conform well with findings from the study on morphological traits, where these same three clones were found to have high pod hardness and SL thickness. This suggests that the variable EH can be useful if used in the screening protocol for the identification of tolerant clones.

41. The exit holes (EXH) on average showed a similar pattern of decline but with a low magnitude; declining from its highest average of 4.9 (2.5 months pod age) to 3.5 at pod age of 4.0 months. It (EXH) did not continue to fall or stabilized at that point, but increased slightly at 4.5 months and finally dropping to its lowest level at 5.0 months. On average K9 (3.0) and PA120 (2.6) had the lowest EXH while Kakunu (5.7) and ICS1 (4.9) relatively had the highest. K4 and MAN-15/2 were only moderate with their respective scores of 4.6 and 4.1. Again, the fitted Polynomial equation seemed to display a moderately high coefficient of determination ($R^2=0.60$), suggesting that future predictions may be made confidently following the given model ($Y=0.049x^2-61x + 5.52$). The EXH and ADSI were highly significantly correlated ($r=0.613$). This relationship was also highly significant ($P<0.001$) between these traits A similar trend as with EH was displayed, where, the clone K9 and PA120 had the lowest EXH. Again the EXH was shown to be an important variable which may be used in the screening protocol in the future for identification of CPB tolerant clones. EXH had a highly significant correlation (0.613) with the damage severity (ADSI). Results showed that the

most susceptible clones can be discriminated from the tolerant ones after the assessment/ or screening process.

42. The Average Damage Severity Index (ADSI) also varied with pod age (exposure time) for the selected clones. Higher ADSI values were obtained for the 2.5 to 3.0 months age categories (Fig.9). MAN-15/2 (2.5), ICS1 (2.4) and Kakunu (2.4) had the highest and K9 (1.1), K4 (1.3) and PA120 (1.0) were the lowest for the 2.5-3.0 months old pods. Considerable decline in ADSI was observed for the 3.5, 4.0 and 4.5 pod age categories. The Average ADSI for pod age at 2.5 and 3.0 months were the same before declining at 3.5 months pod age and exposure. Moderately high mean ADSI was recorded for the clones MAN-15/2 (2.5), ICS1 (2.4) and Kakunu (2.4) and the lowest were for the clones K9 (1.1), PA120 (1.0) and K4 (1.3). An analysis for goodness of fit of the mean ADSI showed that a second degree polynomial function; $Y=0.080x^2-0.753x + 2.598$, provides a good approximation to the data. This is revealed by the high coefficient of determination value of $R^2=0.746$.

43. These results showed that under natural conditions, tolerant clones will have ADSI below 2.5 while the most tolerant varieties will have theirs ranging from 0.7-1.5. A minor increase in ADSI of the 5.0 months old age group was observed. Despite the increase, most clones were reasonably below the ADSI value of 1.5 except K4 and Kakunu with their respective ADSI above 1.5 but were still lower than the critical level of 2.5, which is the y-intercept of the polynomial function. (Model for ADSI). The late surge in the ADSI may have occurred due to the partial break down of pod hardness at the corresponding period. The hardness of all the clones except ICS1, reached their highest hardness levels for the 4.0 to 4.5 months old pods before slightly declining at 5.0 months. The reasons for this are not well established as yet, but it may be due to degradation and or softening of pod tissues before ripening. It may also be due to insect feeding behaviour and population dynamics. There fore some additional factors may be required to maintain the ADSI as low as possible with no insecticide application. Management and good farming practices such as pruning, shade reduction, pod burial and other cultural practices will help suppress the minor increases in ADSI and maintained it to its lowest level.

Results for Component 5 - Data Analysis and Interpretation of Results

44. In **Malaysia**, the stepwise multiple regression analysis was conducted to screen the significant parameters which could identify CPB tolerant clones. The response variable was the ADSI values of each tree and the independent variables or parameters used in identify the CPB tolerant clones were the five morphological characteristics (pod length, pod width, pod surface smoothness ratio (Primary Furrow/Ridge), SCL thickness and SCL hardness) and also two CPB infestation assessment (entry holes and exit holes) . The PROC REG in the SAS statistical package Version 9.1 (SAS Institute, Cary, NC), with the SELECTION=STEPWISE option was used for analysis. This method can conduct both forward addition and backward elimination, using the significance criterion ($P<0.05$). Prior to the analysis, all monthly morphological data and the CPB infestation measurement data for each tree were pooled, yielding one measurement for each pod per tree of the five trees.

45. Only exit holes per pod significantly correlated with the ADSI value. Other parameters that positively correlated with the ADSI were pod length, pod diameter and entry holes per pod while pod surface smoothness ratio, sclerotic layer of thickness and hardness have negative correlation with ADSI. However, those parameters which were not significant correlated with ADSI due to weak correlation could be used as indicator in identified the tolerant clones against CPB as SCL thickness and SCL hardness have the direct impact on CPB's larva from penetrating the pod wall into the beans.

46. The results of the stepwise regression model designed to explore the relationship between the seven independent variables (which consisted of five morphological characteristics and two CPB infestation assessments) and the ADSI value. The stepwise regression model identified exit holes per pod as factor contributed the most to the regression. It accounted for 48.11% of the output variability in the regression. Therefore, only exit holes per pod could be used as criteria to identify CPB tolerance clones.

The suggested prediction linear model of the ADSI based on the number of exit holes, *X* in determine the tolerant clones as given below. The ADSI function will be tested in the field for verification later.

$$\text{ADSI} = \begin{cases} 1.815 + 0.366X & 0 \leq X \leq 6 \\ 4.00 & X > 6 \end{cases}$$

47. In Papua New Guinea, mmorphological traits which had high positive or negative relationship to damage severity or crop losses should be considered in screening for tolerant materials. However, where two or more traits are significantly correlated to each other, only one would be considered as selection or improvement on one should also simultaneously select for the other traits.

48. The ADSI seem to be the most practically, applicable assessment variable (trait). With the information available so far from Component IV and Component I, it can be summarized that a screening method combining all the important variables and others, would be formulated to screen or identify clones for CPB tolerance, e.g

CPB Tolerant clone = Σ(≥3.8 kg/mm^2 SF_H) + (≥8.5 mm SF_T) + (≤2.1 ADSI) + Others at ≤ 3 months old pod age.

Other Activities

49. Local meetings were conducted at both sites to discuss the project's activities, work plan, progress and among others. Half yearly meetings were conducted by the project' team at the MCB. A regional meeting was held on October 20 – 24, 2008 at MCB Cocoa Research and Development Centre, Tawau between MCB and PNGCCIL. The meeting discussed the working procedures, work plan and financial allocation of the project between the participating institutions.

IV. CONSTRAINTS ENCOUNTERED

50. In Malaysia, most of the project's activities were conducted at MCB's Research and Development Centre at Madai which is 70 km from Tawau. As such frequent travels had to be made for data collection and project monitoring. Logistics issues resulted in higher expenditure incurred in the project.

51. In **Papua New Guinea**, funding for project year 1 was not received on time for implementation of the project activities. Two clones (ICS1 and ICS95) did not have the minimum required number of ten trees. Unless sufficient data is collected from these clones, they will be replaced with other international clones that are common to Malaysia and PNG. Difficulties in communications with the country resulted in delays in project implementation.

V. CONCLUSIONS AND PERSPECTIVES

52. CPB is a major concern to the cocoa industry not only in Malaysia and PNG, but also in the Asia Pacific region. Its presence has suppressed both the production and productivity of the cocoa areas, as well as the income of the growers. CPB can be managed by pod sleeving, use of insecticide, bio-control approaches, among others. However, effective management of CPB could be expensive. Tolerant plant materials would be the more ideal solution to control CPB. However, there are no clones that can claim to confer absolute tolerant to CPB. This project provided an opportunity to seek a simple screening methodology for tolerant clones to CPB. The study undertaken at the MCB suggested that the exit holes of the CPB from the pods were the parameter that can be used by the plant breeder to select tolerant clones in combination with ADSI. Pod smoothness, ratio of entry/exit holes of CPB, among others, are the other parameters that could be measured in the screening for tolerant clones.

53. The Project was the first of its kind to be undertaken at Cocoa and Coconut Institute Limited in Papua New Guinea. Prior to this project, no detailed study on host plant resistance had been conducted, meaning that with limited knowledge and technical skill, Papua New Guinea was relying on information generated by scientists from Indonesia and Malaysia, as CPB was well established in these countries and a lot of studies had been done over the past 20-30 years. New valuable information, findings, knowledge and technical skills gained from this study has given PNG greater hope to search and develop new cocoa varieties for the small holder farmers in the country PNG. PNG is now placed on a much better footing to conduct more cutting edge research into this devastating insect pest which threatens the cocoa industry in PNG. The project had revealed and confirmed that: 1) Under choice conditions, CPB female will make preference for oviposition on less tolerant (susceptible) clones by laying more eggs on them and conversely less eggs on the more tolerant clones; 2) Under no choice condition insect ovipost on any of the clones available but with variation in the egg number laid; 3) There is antibiotic properties in some cocoa varieties and that Pupa length (short) and weight (low) are good predictors of antibiosis and selection of CPB tolerant varieties/clones.

54. Because of to time and budgetary constraints, the models derived from the project are yet to be tested. The field trial to asses HPR has started and data collection is expected to commence at the end of 2012. The result obtained at the MCB will be further validated when the data from the trials are obtained.

55. It is being suggested that this project should be continued to further validate the screening models protocols obtained. Financial support is required to continue the project.

56. MCB and PNGCCIL would like to extend their gratitude to CFC for funding this project and ICCO for providing the support, guidance as well as assistance in the implementation of the project. The implementation and findings of the project were made possible with the hard work of the researchers, technicians, field staff and workers in both research institutions.