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Variation of the response of clonal cocoa to attack by cocoa pod borer *Conopomorpha cramerella* (Lepidoptera: Gracillariidae) in Sabah

Chong-Lay Teh[a,*], Joe Thau-Yin Pang[a], Cheng-Tuck Ho[b]

[a]Golden Hope Research Sdn Bhd, P.O. Box 135, 91007 Tawau, Sabah, Malaysia
[b]Golden Hope Research Sdn Bhd, P.O. Box 207, 42700 Banting, Selangor, Malaysia

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Abstract

Eight cocoa clones were examined for variation in resistance to cocoa pod borer (*Conopormorpha cramerella* Sn) in Sabah, Malaysia. Over a 13 month period up to 1200 ripe pods of each were shaved back to the sclerotic layer and the number of larval entry and exit holes were counted. There were statistically significant differences in the proportion of larvae that were able to exit, with PBC123, a popular commercial clone in Sabah, and IMC23 is more resistant than BAL244 and KKM22. The six more resistant clones were characterized for pod hardness, using a penetrometer. The correlation with the entry/exit ratio suggested that the measurements could be used for screening clones for resistance. The data indicated that at relatively low rates of infestation, the proportion of larvae that can exit from the pods is negatively correlated with the number that enter, which would amplify the effect of partial resistance. The partial resistance of PBC123 may be sufficient to greatly reduce moth populations in a large enough pure stand of the clone. Pod borer resistance should be given a high weighting in an economic index for cocoa clone selection, and should be combined with tolerance of infestation in the form of low damage to the cocoa beans despite infestation.

Keywords: Cocoa pod borer; Clone; Resistance; Pod hardness

1. Introduction

Cocoa is the principal host of the cocoa pod borer (CPB, *Conopomorpha cramerella* Sn., Lepidoptera: Gracillariidae) in Sabah. It is one of the more recalcitrant cocoa pests, capable of causing great financial loss, estimated at 31% of the value of the crop on a plantation in 1994. Although damage can be contained through use of insecticides (Teh and Yeow Kok Cheng, 1995) there is great concern that they are losing effectiveness over time (Lee Chin Tui, 1996), as well as risks to consumers and environment alike.

The number of eggs laid on the surface of cocoa pods by individual female moths is thought to be between 60 (Azhar and Long, 1996) and 150 (average assumed by Day (1985), for a simulation model). Moths have a preference for the primary and secondary furrows of rough-surfaced pods (Azhar, 1990). When the eggs are laid, the pods need to have reached at least 10 cm in length (ten weeks after fertilization, Humphries, 1943) for the larvae to be able to reach the full-grown stage (Wessel, 1983). Egg predation was taken as 15% by Day (1985) for his simulation model. Egg parasitism ranged from 5 to 50% through the crop cycle (Day, 1985). It fluctuated with the egg population, which is in phase with the cropping cycle, so overall egg mortality is highly variable. Lim (1983) reported that the mean percentage of parasitism of cocoa pod borer eggs per pod was 23.6%, with a range of 0–100%.

The proportion of first instar larvae penetrating to the core of the pod rises sharply to about 80% once pods are 10 weeks old. On average, there is about 15% larval mortality in the pre-sclerotic layer of the pod (Day, 1985). Larval mortality is a function of larval numbers in the pod, increasing as numbers rise, and of the development of the sclerotic layer. This layer develops towards the end of pod maturation, with genotypic variation in its thickness rather than density (Adomako and Fordham, 1985). Whether there is genotypic variation in the timing of its development

*Corresponding author.
E-mail address: clteh@goldenhope.com (C.-L. Teh).

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is unknown. Larval entry and survival declined as the pods matured. Day reported that some 75% of larvae survived in pods aged about 13 weeks, and very few in pods that were ripening. Typically, pods of the widely cultivated Forastero varieties take about 24 weeks from fertilization to ripening, but some clones of Trinitario type take up to 30 weeks (Williams, 1977). Azhar and Long (1996) estimated that only 6% of larvae emerged from the pod. The larvae spin down to the ground, pupating in the leaf litter. Some 40% of pupae are preyed upon. The life cycle is approximately 22–33 days, shorter the more severe the infestation (Chong-Lay Teh, unpublished observations).

Once inside the pod, the larvae feed within the placenta, mucilage and sometimes the testa of the developing seeds (beans). This causes the beans to clump and to stop developing. In severe infestations, the pod stops developing, and ripens prematurely. A low level of infestation does little economic damage but once it exceeds about 60% of pods, an increasing proportion of the beans become unextractable, and those beans that are obtained are of inferior quality (Day, 1985). The incidence of cocoa pod borer is highly variable within fields, varying widely over quite short distances (Chong-Lay Teh, 1994 unpublished observations).

Early Indonesian work on genetic variation in losses from CPB was summarized by Wessel (1983). There was variation in the incidence of damage by CPB, smooth-surfaced pods attacked less at the beginning of the harvest period, but not later. This was thought to be because rough surfaces provided better sites for egg- laying, and protection against eggs being washed off by rain. Forastero types generally were thought to be less susceptible than "Criollos", but the hardness of the pods (i.e. thickness of the sclerotic layer) was not thought to be a factor.

The variety West African Amelonado, with hard pods, and the clones PA7 (hard pods) and SCA12 (soft pods) were examined in detail by Day (1985). Day observed that there was no difference between the genotypes in larval survival at either the pre-sclerotic layer or inside the pods. Overall survival was much higher in SCA12, with soft pods, than in the other two genotypes, and was correlated with estimates of pod hardness obtained with a penetrometer. In a simulation model, the crop loss with PA7 was half that of SCA12.

Azhar and Lim (1987) confirmed and extended Day's (1985) findings. In a study of five clones, including PA7, they showed that differential inward and outward penetration of the sclerotic layer was correlated with its hardness. In a more extensive study, Azhar et al. (1995) classified 59 clones for low ovipositional preference, large husk thickness ratio, harder and thicker sclerotic layer and low average damage index. PA7 was classified as moderately resistant, whilst other Upper Amazon clones especially from Pound's (1938) Nanay population were classified as resistant. Several clones with affinities to the Nicaraguan "Criollos" were classified as susceptible.

Resistance to CPB will be most usefully deployed as part of an integrated pest management strategy. The most valuable contribution would be genotypes that reduce the moth's breeding success. In the early stages of selection, with high pest pressure, such genotypes may well show a high incidence of infestation and of damage from the pest, because a large pest population will be maintained by the numerous susceptible trees. Once the proportion of more resistant trees rises, the pest population and the damage caused by it should fall. For this reason, we focused on a search for traits that were expected to reduce the breeding success of the moth, and for a rapid method of screening large numbers of genotypes in a field-based selection programme.

2. Materials and methods

Eight clones (Table 1) were chosen for study because they varied in the roughness, the pod surface and the hardness of the pod wall. PBC123 and BR25 were two of the of most popular clones for commercial planting in Sabah in the mid-nineties, while others were of interest as parents in the breeding programme at Golden Hope Research Centre, Sabah (formerly Scientific Department, BAL Plantations Sdn Bhd).

All the clones were growing in unreplicated plots in a single field. It was not practicable to take pods from a replicated trial as no established trial included the required range of pod types. The field was managed as part of the commercial plantation, with standardized control of CPB as described by Teh and Yeow (1995). This entailed regular

Table 1
Origin and characteristics of the eight clones

Clone	Origin	Type	Perceived pod hardness	Pod surface
AMAZ15-15	Chalmers (1973)	Upper Amazon (UA) Forastero	Hard	Smooth
BAL244	Note 1[a]	Complex cross	Soft	Rough
BR25	JA Anselmi	Trinitario?	Average	Average
IMC23	Pound (1938)	UA Forastero	Average	Average
KKM22	Abdullah and Subali (1986)	Unknown	Soft	Smooth
PA13	Pound (1937)	UA Forastero	Average	Rough
PBC123	Chong and Shepherd (1986)	UA × Trinitario hybrid	Average-hard	Average-smooth
TAP1-2	Chalmers (1973)	UA Forastero	Soft	Rough

[a]A selection out of (UIT1 × NA32) × SCA12.

census, ten day harvesting intervals with thorough and complete harvesting and target pod spraying with synthetic pyrethroids and other insecticides using manual piston knapsack sprayers.

Each month, 100 newly ripened pods were collected from each clone, the ripeness standard being the first showing of pod colour (yellow or bright red for BR25 and PBC123). The mesocarp of each pod was shaved back to the sclerotic layer, to reveal the small entry holes and larger exit holes made by CPB larvae, which were then counted. Once the entry holes were counted, the pods were split and classified as apparently healthy, infested with CPB (I, infested regardless of severity), highly infested (HI, with 50% or more clustered beans unextractable by hand but extractable using a wooden scoop) and very highly infested (VHI, with 50% or more beans compacted, underdeveloped and unextractable). These categories were those used in the field census system. The wet cocoa beans from the infested and apparently healthy pods from each clone were weighed separately.

Pods were collected from November 1994 to November 1995. There were twelve missing samples, one from each clone except two from PBC123 and BR25 and three from KKM22. There were six samples of fewer than 100 pods. The shortfalls reflected the seasonal periodicity of cropping of the clones.

Data were subjected to estimation of variance components and analysis of linear mixed models by the method of residual maximum likelihood, using the REML directive in GENSTAT. The analyses were weighted by the number of pods per sample. Month was treated as a random effect and clone as a fixed effect.

In tests of pod hardness, a spring loaded ELE Pocket Penetrometer with a locally fabricated tip was mounted on a drill stand. Care was taken to mount freshly harvested freshly ripened pods from unstressed trees at right angles to the needle. On seven occasions, ten pods per clone were pierced on each of their five ridges and furrows, so there were 700 measurements per clone.

3. Results

A total of 8879 pods were examined, 1200 each of BAL244, IMC23 and PA13, 1186 of AMAZ15-15, 1138 of TAP1-2, 1100 of PBC123, 1023 of BR25 and 832 of KKM22. 36.8% of these were scored as "infested" when they were opened. Overall, the proportion of visibly infested pods ranged from 3.9% in November 1995 to 92.5% in May 1995. Among the clones, the range was 19.7% in BR25 and 55.3% in KKM22. The highest number of entry holes per pod was an average of 20.5 in PBC123 in March 1995.

The proportion of infested pods, and the number of entry and exit holes, were transformed to logarithm base e (proportion or number $+0.01$) for analysis in order to normalize the variance. The predicted mean proportions of infested pods for the clones are presented at Table 2. PA13 showed the smallest proportion and BAL244 the highest. AMAZ15-15, which was considered to have hard pods (Table 1), had a relatively high proportion of infested pods.

The number of entry holes per pod ranged from 1.05 holes per pod in November 1995 to 13.1 holes per pod in April 1995 (Fig. 1), and the number of exit holes per pod from 0.05 to 2.36 per pod in the same months. The proportion of larvae that exited, ranged from 1.2% in July 1995 to 17.7% in April 1995. The proportion of larvae that exited was positively correlated with the number that entered (Fig. 2), the regression of proportion on the number that entered accounting for 52% of the variance. Among the clones, the proportion of larvae exiting ranged from a high of 25.4% in BAL244 in February 1995 to several observations of zero percent.

The REML analyses of the complete datasets indicated that there were statistically significant ($p<0.001$) differences among the clones in the numbers of larvae entering and exiting from the pods and the proportion that was able to exit (Table 2). As would be expected from Fig. 2, there was agreement between the rankings of percentage infested pods and the number of entry holes (Spearman's $\rho = 0.812$, $p<0.05$).

Table 2
Predicted means for cocoa pod borer infestation, number of entry and exit holes per pod and proportion of larvae exiting

Clone	Infestation (log prop'$n+0.01$)	Entry holes per pod (log $n+0.01$)	Exit holes per pod (log $n+0.01$)	Ratio exit/entry holes (%)
AMAZ15-15	−1.358 (7)	1.250 (6)	−1.892 (4)	6.42 (3)
BAL244	−1.362 (6)	1.558 (8)	0.0775 (8)	12.58 (7)
BR25	−2.186 (1)	0.735 (1)	−1.934 (3)	9.33 (5)
IMC23	−1.491 (5)	1.031 (4)	−2.081 (2)	6.39 (2)
KKM22	−1.153 (8)	1.406 (7)	−1.034 (7)	12.72 (8)
PA13	−1.514 (4)	0.936 (2)	−1.824 (5)	9.50 (6)
PBC123	−1.567 (3)	1.210 (5)	−2.248 (1)	5.42 (1)
TAP1-2	−1.881 (2)	0.979 (3)	−1.762 (6)	8.21 (4)
SED (range)	0.2468–0.2895	0.1629–0.1912	0.3478–0.4081	1.772–2.077

Rank is given in brackets ( ).

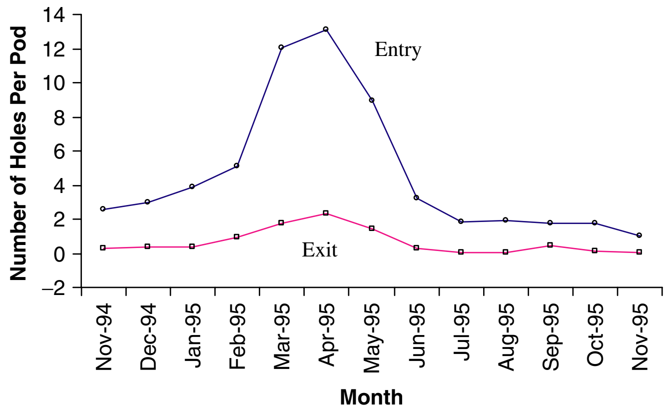

Fig. 1. Number of entry and exit holes with time.

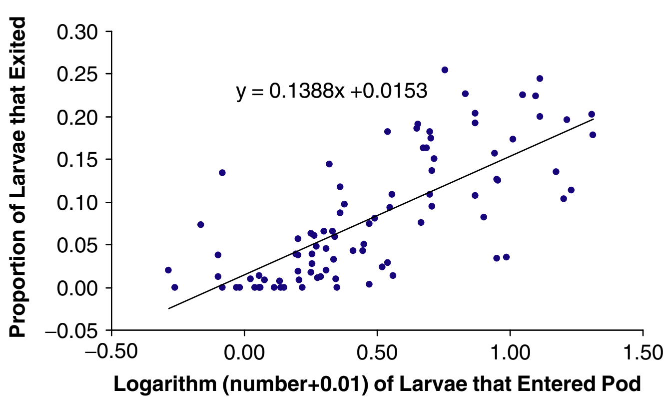

Fig. 2. Relationship between proportion of larvae exited and number of larvae that entered pod.

The lowest numbers of exit holes were found in PBC123, and the highest numbers in BAL244, a clone of Scavina origin with soft pod walls. The lowest proportion of larvae exiting was on PBC123 and the highest on KKM22. The rank correlation between the number of entry holes per pod and proportion of larvae exiting was $\rho = 0.24$, not significant.

Penetrometer readings from six of the clones are summarized in Table 3. There were no data from BAL244 and KKM22 with soft pod walls. IMC23 had the hardest pods, followed by PBC123, while BR25 had the softest ones among those that were measured. There was partial agreement between the rankings of the penetrometer values and the REML estimates of exit/entry ratio from Table 3, $r = 0.76$, $p < 0.05$; $\rho = 0.77$ (for $p = 0.05$, $\rho = 0.883$). The rank correlations with penetrometer values for the number of entry and exit holes per pod were -0.67 and 0.61, respectively. BR25 that had the softest pods had the lowest number of entry holes per pod.

Data on the weights of wet cocoa from all of the pods categorized as "infested" and apparently healthy pods are summarized in Table 4. In this statistical analysis the Wald statistic was significant at $p = 0.05$. Overall, the weight of wet cocoa per pod was reduced by 13.2%. The loss of fermented and dried cocoa would have been slightly less as the wet to dry weight conversion ratio is expected to be higher for beans from infested pods, although their quality may be lower. There were statistically significant differences among the clones in the weight of wet beans per pod from both apparently healthy and infested pods. However, there were negative variance components in all REML analyses of the reduction of wet weight. The raw data suggested that PBC123 was insensitive to infestation, whereas KKM22 showed a large reduction in weight of wet beans per pod.

Table 3
Penetrometer estimates of pod hardness (higher values indicate harder pods)

Clone	Value (arbitrary scale)
AMAZ15-15	322.8 (3)
PA13	290.5 (4)
IMC23	464.5 (1)
TAP1-2	255.0 (5)
PBC123	377.8 (2)
BR25	234.5 (6)

Rank is given in brackets ( ).

4. Discussion

It is unlikely that the lack of replication of the clones will have compromised the estimation of the ratio of exit to entry holes. Cocoa pod borer was present throughout the cocoa area at Golden Hope Research Centre, Sabah, and there is no reason to expect an interaction between clone and insect behaviour within one field. Observations were well replicated over time.

The eight clones varied in both the degree of infestation, which was correlated with the number of larval entry holes per pod and the proportion of the larvae that were able to exit from the pod. The variation in numbers of larvae entering is difficult to interpret without data on the numbers of eggs that were laid, as there is no means of separating uneven distribution of the moth or preference for oviposition from differential mortality of eggs and larvae before they penetrate the sclerotic layer. The growth habit of the clone may be a factor, as it is believed that there is less infestation of the pods of upright clones like BR25 (J.A. Anselmi, personal communication, 1987) that had the lowest number of entry holes per pod in this study. Even if there were genotypic effects on egg-laying preference, Day (1985) and others have pointed out that a negative preference that is expressed in a mixed stand would not necessarily be useful in a pure stand. The literature offers no evidence of differential mortality of first instar larvae.

Reducing the success of larval exit will directly reduce the moth population in successive generations, although it will have no perceptible effect on crop loss until there is a

Table 4
Effect of cocoa pod borer infestation on the weight of wet cocoa per pod

Clone	Weight of wet cocoa per pod (g)		% Reduction in weight (over all pods harvested)
	Apparently healthy	Infested	
AMAZ15-15	120.6	101.4	14.3
BAL244	147.1	128.0	24.9
BR25	116.6	105.6	13.5
IMC23	155.8	139.8	18.9
KKM22	147.5	124.9	12.7
PA13	114.3	108.1	15.6
PBC123	121.5	92.9	18.1
TAP1-2	115.0	97.7	4.8
Mean	129.8	112.3	13.2
SED (range)	9.472–10.71	9.472–10.46	

high enough proportion of resistant trees. If a female moth typically lays 150 fecund eggs, there is 15% predation, 23.6% parasitism (Lim's average figure) and 15% larval mortality in the pre-sclerotic layer (an approximate average from Day, 1985), no larval mortality between exit and pupation, and 40% of pupae preyed upon, then 3.85% must exit to maintain the moth population. If the number of eggs is taken as 102.5, the average of Day's (1985) and Azhar and Long's (1996) estimates, the proportion rises to 5.92%. The latter proportion is slightly larger than the 5.42% recorded for PBC123 from 1100 pods. In this clone, the proportion of larvae exiting exceeded 5.92% in two of the eleven months for which data are available, compared to nine out of ten months and twelve months for KKM22 and BAL244, respectively. If as Azhar and Lim (1987) suggest, a harder sclerotic layer is a greater barrier to larval entry, then this form of resistance will be even more effective.

Increasing larval survival at higher levels of infestation has not been reported previously. Indeed, Day (1985) concluded that larval survival declined at higher larval densities, due to competition. A biological explanation is that higher larval densities cause more damage inside the pod, which then ceases development with reduction in the final density of the sclerotic layer that is the main barrier to larval exit. Such an effect would enhance the effectiveness of the partial resistance shown by PBC 123. The current results may differ from Day's because the maximum number of larvae entering the pods was relatively low: with over 40 larvae per pod recorded on occasion, although not in this work (Chong-Lay Teh, unpublished observations).

The correlation between the penetrometer readings and the ratio of exit to entry holes suggests that the tool can be used as a screening technique in a clone selection programme. Inclusion of the two most susceptible clones, BAL244 and KKM22 that are known to have soft pods would most likely have increased the correlation with the exit/entry hole ratio. The tool requires refinement, in particular a standardized method of measurement has yet to be developed, with an absolute scale so that results can be shared between workers. It is envisaged that the tool would be used in an ortet selection programme, with a high weighting in the economic selection index.

The study of the effect of infestation on weight of wet beans per pod was inconclusive. In further work, the weight of usable and unusable beans should be recorded against the number of entry and exit holes. Observations at Golden Hope Research Centre, Sabah suggest that clones vary in their tolerance of pod borer infestation. Some, such as BR25 and KKM22 show relatively little internal damage despite relatively large numbers of larvae, while others like PBC123 seem to be more sensitive to infestation.

The work reported here provides evidence that there is effective resistance to CPB in cocoa, based on the sclerotic layer of the pod wall, and provides a starting methodology for use in a selection programme. Further controlled work is required on egg laying preference and differential survival of first instar larvae through the pod wall including the sclerotic layer in the hope of finding further useable forms of resistance.

Combining the PBC123 level of resistance, with tolerance of infestation if confirmed, desirable agronomic characteristics including upright growth habit, high yield and the flavour required by the consuming industry is a challenge. For example, BR25 yielded 132% of PBC123 in a large-scale trial and in another trial, the second and third most resistant clones, IMC23 and AMAZ15-15, yielded only 59% and 33% of BR25, respectively (Pang Thau Yin, in preparation). The Scavina material that is characterized by soft pods, provided the highest yielding material in the breeding programme at Golden Hope Research Centre, Sabah. Nonetheless, the financial cost of CPB due to crop loss and of minimizing it and the environmental and consumer risk of large scale use of pesticides warrant a high loading for CPB resistance and tolerance in an economic selection index, especially for smallholder production systems.

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