SEVERITY OF *CONOPOMORPHA CRAMERELLA* (SNELLEN) INFESTATION: OBSERVATION OF THE DAMAGE USING MALAYSIAN COCOA CLONES

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ABSTRACT - *Plant is generally considered resistant to an insect when the insect able to inflict greater damage to other plants of the same species under similar environmental conditions. Therefore, observation of the severity of 28 Malaysian Cocoa Clones from four different classes (I, II, III, and IV) towards Cocoa pod borer, Conopomorpha cramerella (Snellen) were evaluated at the Cocoa Research and Development Center (CRDC) Jengka, Pahang. One hundred mature cocoa pods were selected as a sample per clone, and the slicing technique was conducted to observe the entry hole, exit hole, and damage category. The best clones based on the lowest entry holes were observed at MCBC 8 (1.300), MCBC 1 (3.370), MCBC 10 (4.800), KKM 22 (6.360), and DESA 1 (6.470). Five best clones for observation on the exit holes were MCBC 8 (0.310), MCBC 10 (0.880), MCBC 1 (1.100), PBC 221 (1.170) and QH 22 (1.440). MCBC 8 was recorded as the top clone after assessing the ADSI (0.470), followed by MCBC 1 (0.910), PBC 221 (1.010), MCBC 10 (1.020) and KKM 22 (1.220). MCBC 8 and MCBC 1 were categorized into Healthy to Slight Infested category. Meanwhile, PBC 221, MCBC 10, and KKM 22 were categorized into Slight to Light Infested category. Based on the screening result, it can be summarized that four clones from Class I performed as the best clones in terms of lowest entry and exit holes, and ADSI (KKM 22, MCBC 1, MCBC 10 and MCBC 8). These clones suffered less from CPB infestation compared with the other 24 clones.*

Keywords: Average Damage Severity Index (ADSI), Cocoa, Cocoa pod borer, *Conopomorpha cramerella*, screening

INTRODUCTION

The cocoa pod borer *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae) is the most devastating pest of cocoa, and become the primary threat to cocoa-growing countries in the Southeast Asia region (Saripah, 2019). *C. cramerella* is widely distributed throughout Malaysia, Indonesia, the Philippines, and Papua New Guinea. This pest becomes one of the significant threats contributed to the declining production of cocoa in Malaysia since the first massive establishment at Tawau, Sabah, in late 1980 (Ooi *et al.*, 1987). Cocoa beans were hardened and clumped together, which lead to difficulty in extracting from the pod husk and mucilage. Uneven yellowing or premature ripening due to *C. cramerella* tunneling inside the pod may lead to the total yield loss.

C. cramerella has the potential to lay more than 300 eggs during their maturity stage (Lee *et al*., 2013). The female appeared to deposit their eggs started in the early development of cocoa pods as early from 70mm (Saripah, 2019), through the complete maturity of pods (150mm and above). The egg stage lasts for 2 to 7 days, and the symptoms of successful egg hatching are determined by the presence entry holes after the epicarp of cocoa pods were peeled off. The larvae feed on the mucilage and placenta, and the entire stage is in the pods. This larval stage is almost fully protected from any control approach. The larval stages may take 14 to 20 days, and larva will tunnel out from the pods, leaving a visible exit hole on the pod surface. The pre-pupa will spin the cocoon immediately, either on the cocoa pods, the dry leaves, or leaf litter on the ground. If cocoa pods were treated with insecticide or treatments applied on the surface, there was a tendency of the pre-pupa to wander away, thus subsequently pupate on the other material, especially on the dry cocoa leaves (Saripah *et al*., 2019). The pupation stage usually takes 6 to 8 days, and the entire metamorphosis stages will be completed with the emergence of adult moths that generally lives for about a week. This multivoltine lepidopteran then will continue to deposit their eggs, and the highest mean of eggs and entry holes usually recorded at pod lengths more than 150mm (Saripah, 2019).

As a main host for *C. cramerella*, cocoa, *Theobroma cacao* (Linnaeus) (Malvales: Sterculiaceae) is an important crop with 22 known species from the genus *Theobroma*. However, only *T. cacao* is widely cultivated in various countries (Hebbar *et al*., 2011). Since the mid 20th century, *T. cacao* varieties have been classified into three general groups: Criollo, Forastero, and Trinitario. Criollo refers to a group of genetically similar trees that produce lightly pigmented seeds. Criollo was cultivated in Central America, and Amelonado (the most common type of Forastero) was widely planted in the Amazon Basin. Forastero refers to any trees that are not Criollo and usually produced deep purple seeds. The cultivars of Criollo, Trinitario, and Amelonado originated in Venezuela, Trinidad, and Brazil, which were introduced during the colonial period to Africa and Asia. The third group, which was resulting from natural hybridization between Criollo and Amelonado Forastero, Trinitario, was originated in Trinidad (Motamayor *et al*. 2003). Trinitario refers to a hybrid between Criollo and Forastero trees.

Cocoa is planted from a hybrid or clone, and the method produces different criteria of growth pattern. The choice of planting material in cocoa cultivation is essential and ultimately determines the yield potential, bean quality, and tolerance to the pest and disease. The clonal tree is propagated from cuttings or budded seedling, whereas hybrid plants from the cocoa seed (Azhar & Long, 1993). A hybrid cocoa tree bears a vertical main trunk or chupon, and grows up to 15 meters. Clonal has replaced hybrid cocoa due to the former's superior agronomic characteristics. Clones that suitable for commercial planting must-resembles characteristics such as high yielding and uniform beans. Clones should preferably adapt to a wide range of agro-climatic conditions as well as tolerance to significant pest and disease (Azhar *et al*., 2009).

The sustainability and future of cocoa production are hinged on the diversity of genetic resources conserved and utilized in the development of planting materials for farmers (Olasupo & Aikpokpodion, 2019). Therefore, most of the cocoa-growing countries were conserved the genetic materials, for example developing cacao germplasm collections in Trinidad with 2,300 cacao accessions in their field collections (Bekele, 2012). Olasupo & Aikpokpodion (2019) discussed the cocoa genetic resources conservation and utilization for sustainable cocoa production in Nigeria from its first introduction in 1874 using Amelonado. There were international projects in conserving cocoa germplasm that had implemented in multiple cocoa-growing countries, including Brazil, Cameroon, Cote D'Ivoire, Ecuador, Ghana, Nigeria, Papua New Guinea, Trinidad and Tobago, Venezuela and Malaysia (IPGRI, 2004).

Malaysia, as one of the cocoa-producing countries in the Southeast Asia region, also appreciates the diversity of the planting materials by conserving the germplasm collection and release of new cocoa clones. Currently, there were 14 clones released by the Malaysian Cocoa Board (MCB), with the set of MCB 1 to 14 (MCB 2005; 2009, 2012 and 2013). Conserving the germplasm, maintaining the selection, and releasing new potential clones are fundamental importance in the sustainability of cocoa production. Olasupo & Aikpokpodion (2019) concluded that the diversity of planting materials provides necessary adaptation to the current biotic and abiotic environmental challenges, and later on enabling changes in the genetic composition to cope with changes in the environment. After releasing 14 cocoa clones, and conserving other progenies and clonal materials, MCB embarked on the action by releasing technical information of each clone. The booklet was released with information on the potential yield, pod and bean characteristics, plant characteristics, tolerance towards the diseases of the 53 Malaysian cocoa clones (MCB, 2012). However, little information on the current scenario of pest incidence, especially *C. cramerella* was documented. Therefore, this study's objective was to evaluate the severity of *C. cramerella* infestation by observing the damage caused by this neonate larva using 28 Malaysian cocoa clones.

MATERIALS AND METHODS

Screening of 28 Malaysian cocoa clones from four different Classes I, II, III, and IV were conducted at the Cocoa Research and Development Center (CRDC), Malaysian Cocoa Board, Jengka, Pahang, Malaysia (Longitude 100° 30' 31.64" E, Latitude 3° 36' 59.73° N). Clones selected were based on the booklet of Malaysian Cocoa Clones (MCB, 2012), Release of new Cocoa Clones (MCB, 2005), Release of New Cocoa Clones 2nd Series (MCB, 2009) and Released of New Cocoa Clones 3rd Series (MCB, 2013). The full technical information on each clone, including the potential yield, pod and bean characteristics, disease tolerance, self-compatibility, plant vigor, and growth habit were obtained from MCB (2012). Six clones evaluated from Class I, which were KKM 22, MCBC 1, MCBC 10, MCBC 8, PBC 123 and QH 1003. There were eight clones from Class II, which were BR 25, KKM 1, KKM 5, PBC 112, PBC 137, PBC 221, QH 22, and BAL 244. Clones Class III were DESA 1, KKM 27, KKM 28, MCBC 4, MCBC 6, MCBC 7, PBC 159, and QH 1176. Six clones were evaluated from Class IV, which were MCBC 3, MCBC 5, PBC 130, PBC 131, PBC 140 and QH 968.

The study was commenced from January through October 2019. One hundred mature cocoa pods were sampled from each clone. These destructive samplings were carried out for approximately ten months to complete the data collection. The data collected were *C. cramerella* entry holes, exit holes, and ADSI values. The exit hole, which is a sign of successful mature larva tunneling out from the cocoa pods, can be recognized by a 2-3mm hole surrounded by the dark circle on the cocoa pod surface. Meanwhile, the entry holes, which are the entrance symptoms of successful egg penetration to the ectocarp were examined using a slicing technique. The ectocarp of the pod was carefully peeled off using a sharp knife. The entry hole can be recognized as a small black pinhole size and only can be distinguished after the ectocarp was removed. Generally, the size of the entry holes is smaller than the exit holes. The category of *C. cramerella* infestation was divided into five, which was healthy, slight, light, medium, and heavy (Saripah, 2014), as described in Table 1. Average Damage Severity Index (ADSI) values were calculated, referring to Azhar (1995). The ADSI formula is as follows:

[(0xn1) + (1xn2) + (2xn3) + (3xn4) + (4xn5)] /N, where:

n1 : number of pods in category 0 (Healthy)
n2 : number of pods in category 1 (Slight)
n3 : number of pods in category 2 (Light)
n4 : number of pods in category 3 (Medium)
n5 : number of pods in category 4 (Heavy)
N : total number of pods examined

Table 1. Description of *C. cramerella* infestation

Score	Category	Description
0	Healthy	Healthy (no larva penetrated the sclerotic layer (SCL) and all beans extricable)
1	Slight	Slight damage (Larvae penetrated the SCL with a sign of infestation inside the pod, such as the frass and cell growth on the inner endodermis, but all beans are extricable)
2	Light	Light damage (<20% of the beans are inextricable)
3	Medium	Moderate damage (21-50% of the beans are inextricable)
4	Heavy	Heavy damage (>50% of the beans are inextricable)

Data of entry holes, exit holes, and ADSI values obtained from each clone were arranged separately in Microsoft® Excel 2007. They were subjected to statistical analysis using Analysis of Variance (ANOVA) and PROC GLM, SAS software from SAS® Version 8.

RESULTS AND DISCUSSION

Throughout the observation, the number of 2,800 cocoa pods was completely screened for *C. cramerella* entry holes, exit holes, and ADSI values. The results obtained for entry holes (Figure 1) show most of Class I clones recorded the lowest mean, which observed from MCBC 8 (1.300 ± 4.123 kl), MCBC 1 (3.370 ± 4.943 jk), MCBC 10 (4.800 ± 6.179 ijk), KKM 22 (6.360 ± 5.679 hijk). DESA 1 (Class III) also categorized into the five best clones, which harbored the lowest entry holes (6.470 ± 4.691 hijk). Among sampled clones, the five highest means of entry holes were recorded from MCBC 7 (43.480 ± 49.107 a), MCBC 6 (36.850 ± 34.615 b), PBC 112 (22.970 ± 20.428 c), PBC 137 (21.970 ± 22.440 c) and PBC 131 (21.070 ± 18.759 c).

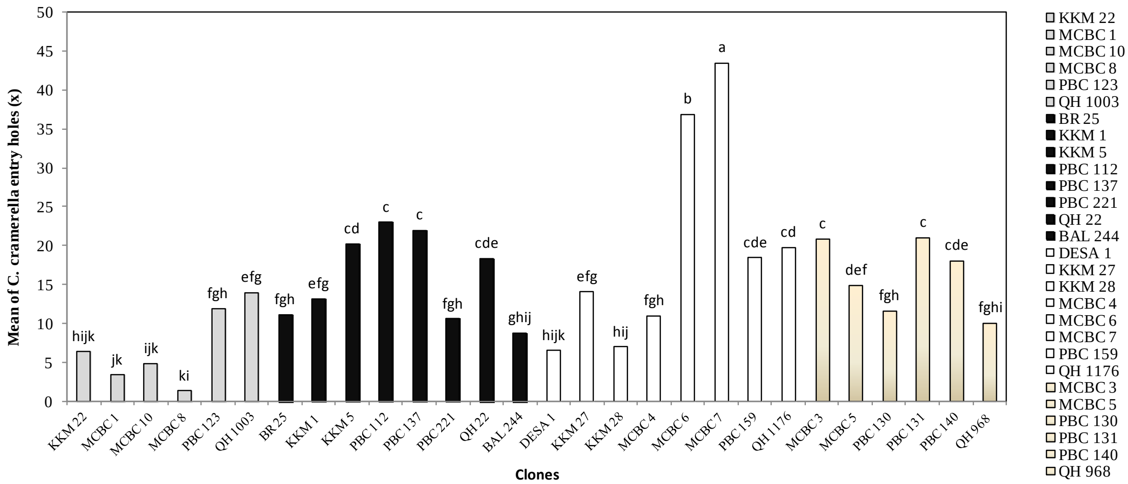
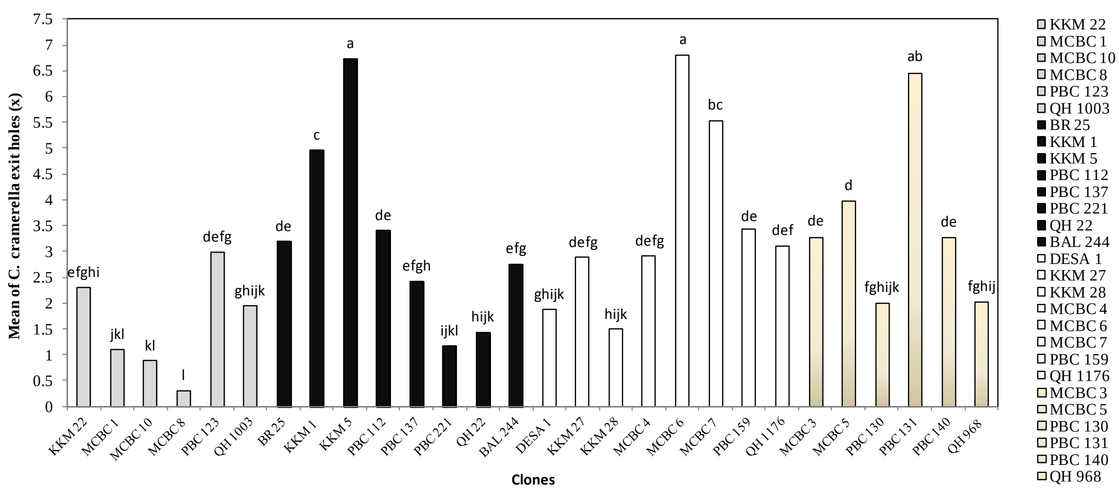


Figure 1. Mean of *C. cramerella* entry holes

Screening on the exit holes similar result with entry h clones were categorized into five best clones, which recorded the lowest holes compared to the other assessed clones. The clones were MCBC 8 (0.310 ± 0.825 l), MCBC 10 (0.880 ± 1.513 kl) and MCBC 1 (1.100 ± 1.767 jkl). Two clones from Class II successfully listed among the five best clones were

kl) and QH 22 (1.440 ± ther assessed clones, the highest mean of exit holes denoted from KKM 5 (Class II, 6.720 ± 7.459 a), MCBC 6 (Class III, 6.800 ± 6.148 a), PBC 131 (Class IV, 6.470 ± 7.170 ab), MCBC 7 (Class III, 5.540 ± 3.953 bc) and KKM 1 (Class II, 4.960 ± 4.987 c).

Figure 2. Mean of *C. cramerella* exit holes



II

Based on the ADSI values calculated using the formula derived from Azhar (1995), MCBC 8 was performed as the top clone after assessing the value of 0.470 ± 0.969 n, and classify into Healthy to Light infested category (Figure 3). Throughout the data collection, the other clone with classifying into the previous category was MCBC 1 (0.910 ± 1.102 m). Two other Class I clones recorded the best ADSI values, which were MCBC 10 (1.020 ± 1.340 lm) and KKM 22 (1.220 ± 1.010 klm). One clone from Class categorized into five best clones was PBC 221 (1.010 ± 0.948 lm). PBC 221, MCBC 10, and KKM 22 were categorized into Slight to Light Infested category. The most susceptible clones towards the *C. cramerella* infestation were MCBC 7 (Class III, 3.720 ± 0.805 a), PBC 112 (Class II, 3.160 ± 1.277 b), PBC 159 (Class III, 3.050 ± 1.266 b), MCBC 6 (Class III, 2.980 ± 1.287 b) and MCBC 5 (Class IV, 2.900 ± 1.411 bc).

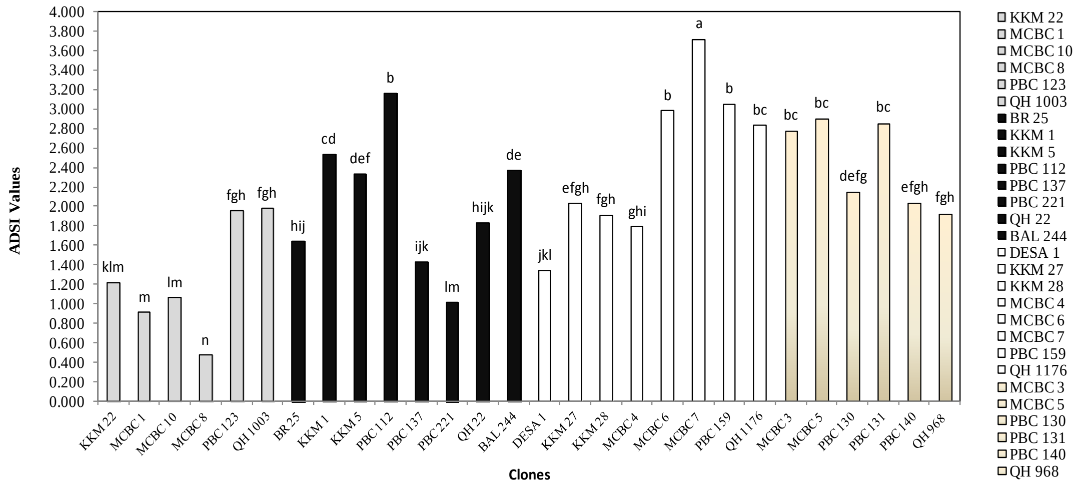


Figure 3. ADSI value

The result obtained from these screening assessments shows four clones from Class I performed as the best clones in terms of lowest entry and exit holes and ADSI values. The clones were KKM 22, MCBC 1, MCBC 8 and MCBC 10. These clones suffered less from *C. cramerella* infestation, compared with the other 24 clones. In summary, 2.70 % of the clones were classified as Healthy to Slight, 12.43% as Slight to Light, 11.39% (Light to Medium), and 3.11 % as Medium to Heavy infested clones (Figure 4).

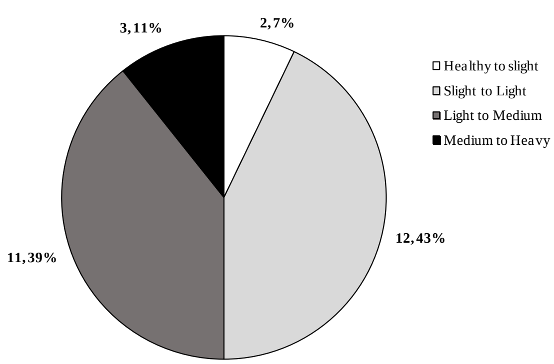


Figure 4. Category of *C. cramella* infestation

Several clones were reported to have traits of resistance to *C. cramerella*, as reported by Lim and Phua (1984). These were the clones of PA7, UA 30, UA 12, UA 9, and NA 34 after comparison was made using 59 clones. These clones showed the lowest dry bean loss among the clones evaluated under natural conditions. Meanwhile, indifferent observation, Azhar and Lim (1987) examined the severity of infestation on five clones (ICS 98, UIT 5, PA 7, UA 37, and LAFI 7) and denoted that LAFI 7 was the most tolerant. There were three components in resistance to *C. cramerella* that were suggested, which involve non-preference, tolerance, and antibiosis. Important points derived from their research where clones with the thickest sclerotic layer also suffered bean loss when infestation occurred before it had adequately developed. The resistant planting material is believed to be one of the sustainable methods; however, based on the report from MCB-CFC-ICCO (2011), there was no clone utterly resistant to *C. cramerella.*

In the field study conducted in Malaysia, there was a relationship between the number of eggs and the ADSI values, suggesting that the number of eggs laid by the female will influence the ADSI values later on (MCB-CFC-ICCO (2011). Their results confirmed that the increasing number of entry and exit holes would also increase the damage of bean or vice versa. Their finding suggests that the variety of both visible holes can be useful if used in the screening protocol to identify tolerant clones. Tolerant clones will have ADSI below 2.5, while the most tolerant varieties ranging from 0.7-1.5. Therefore, the results obtained in this study were in agreement with previous studies, where all the five best clones (KKM 22, MCBC 1, MCBC 8, MCBC 10, and PBC 221 harbored ADSI values lower than 1.300.

Saripah (2019) denoted that both entry and the exit holes gradually increased with the pod increment, where pods more than 140mm in length tend to have more exit holes than smaller-sized pods. It was believed that adult *C. cramerella* repeatedly deposit their eggs at the same pods, thus increased the cumulative number of exit holes through their maturity. It was proven that if the larvae of *C. cramerella* were able to spend more time inside the pod and allow them to complete their larval stages, the risk of pupae's successful emergence is high. The higher number of successful matures larvae to tunneling out from older pods may increase the number of exit holes later on, as found in this study. MCB-CFC-ICCO (2011) reported that some clones were suffering from the infestation only at 3 to 4 months old (NA 33 and T85/799) and concludes that the survival of larvae increased when an infestation occurs at the right age stage of the pod.

CONCLUSIONS

Screening of cocoa clones towards the *C. cramerella* infestation is crucial, due to the substantial incidence of this pest infestation not only in Malaysia, but also to the cocoa-growing countries. From this study, clones from Class I were categorized as tolerant clones to *C. cramerella* compared to the other classes. This information might provide necessary information on the severity of 28 Malaysian cocoa clones to the most devastating pest of cocoa in the Southeast Asian region. Screening of other clones will provide more precise and comprehensive result, furthermore later on, this information can be disseminated to the cocoa growers in Malaysia.

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