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Cryopreservation of *Coffea liberica* Seeds and Embryos Following Desiccation and Freezing Treatments

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Keywords: Cryopreservation, *Coffea liberica*, desiccation, freezing treatments

ABSTRAK

Pengeringan biji benih Coffea liberica *selama 6 hari di dalam bilik berhawa dingin mengurangkan kelembapan biji benih dan embrio tersambung daripada 52.58% dan 47.49% kepada masing-masing 14.58% dan 12.56%. Percambahan biji benih dan kemandirian embrio sambungan kekal pada tahap sederhana, iaitu masing-masing 66% dan 38%. Walau bagaimanapun, semua benih dan embrio sambungan yang telah di keringkan mati selepas pembekuan di dalam nitrogen cecair. Embrio terpotong yang dikeringkan di dalam "lamina flow cabinet" hilang kelembapannya dengan cepat, iaitu daripada 36.8% kepada 9.27% dalam masa 1.5 jam. Lebih daripada 70% embrio ini masih hidup selepas pengeringan. Malahan, pengeringan separa embrio kepada kelembapan 17.17% atau kurang mengakibatkan ketahanan embrio terhadap pembekuan. Di antara 35%-50% embrio tahan kepada pembekuan perlahan sehingga -38°C, tetapi kadar kemandirian ini berkurangan kepada 30% apabila ianya terus dimasukkan ke dalam nitrogen cecair. Pembekuan serta merta dengan memasukkan terus ke dalam nitrogen cecair mengakibatkan 10%-35% kemandirian. Pengeringan embrio kopi selama 0.5 jam kepada kelembapan 17.17% adalah optimum untuk kriopenyimpanan bagi sebarang kadar penyejukan. Analisis terma pembeza tisu biji benih menunjukkan bahawa ketiadaan air "freezable" merupakan satu faktor penting bagi menentukan kejayaan kriopenyimpanan embrio kopi. Walau bagaimanapun, kepentingan kecergasan awal, variasi kelembapan dan medium pemulihan juga dibincangkan.*

ABSTRACT

Desiccation of Coffea liberica *seeds for 6 days in an air-conditioned room reduced seed and attached embryo moisture from 52.58% and 47.49% to 14.58% and 12.56% respectively. Seed germination and viability of the attached embryo were maintained at moderate levels of 66% and 38% respectively. However, none of the desiccated seeds or embryos survived freezing in liquid nitrogen. Excised embryos desiccated in the lamina flow cabinet lost their moisture very rapidly from 36.8% to 9.27% within 1.5 hours. More than 70% of these embryos survived the desiccation. Moreover, partially desiccated embryos at 17.17% moisture or less survived subfreezing temperatures. Between 35% to 50% survived slow freezing to -38°C, but this was reduced to approximately 30% when they were subsequently plunged into liquid nitrogen. Fast freezing by direct plunge into liquid nitrogen also resulted in 10% to 35% survival. Desiccation of excised coffee embryos for 0.5h to 17.17% moisture was optimal for cryopreservation, irrespective of the speed of freezing. Differential thermal analyses of seed tissues suggest that the absence of freezable water is an important factor for successful cryopreservation of excised coffee embryos. However, the importance of initial vigour, moisture variation and recovery media is also discussed.*

INTRODUCTION

Coffea liberica Bull ex Hiern is one of more than twenty species of the genus *Coffea* (Leroy 1967). It is one of the species grown commercially in the tropics as a beverage. Coffee originates from tropical Africa, but as in many other crops the genetic resources of this crop are rapidly eroded through increased land development and deforestation in the tropics. Although pockets of germplasm are scattered in museums and

arboreta, these resources are also endangered by diseases, natural disasters and in some cases, inadequate management. These dangers were recognised by the International Board for Plant Genetic Resources (IBPGR) which listed coffee as one of the priority crops for research to provide more information on techniques for genetic conservation (IBPGR 1983).

However, literature on the storability of coffee seeds is conflicting especially on the effects of moisture and temperature. Owing to this, coffee was classified earlier as a recalcitrant seed and thereby a poor storer (King and Roberts 1979), but was later suggested to be orthodox (Roberts *et al.* 1984). More recently, Ellis *et al.* (1990) made a critical study of the effects of moisture and temperature on storage of coffee seeds and suggested that they should be classified as an intermediate between recalcitrant and orthodox. They further emphasised that the recommended conditions for long-term genetic conservation for seeds at 5% moisture and -18°C are not feasible for coffee.

Although presently there is no practical method for coffee seed conservation using conventional methods, cryopreservation can be an alternate means of genetic conservation. The method has been demonstrated to be successful for a number of orthodox seeds (Stanwood 1984) and even some recalcitrant seed species (Chin and Hor 1989; Hor, Chin and Murugaiah 1990). This study investigates the potentiality of cryopreservation as a means of conserving the genetic resources of coffee by assessing the effects of desiccation and freezing rate on seed and embryo survival in liquid nitrogen.

MATERIALS AND METHODS

Effects of Seed Desiccation

Seeds of *Coffea liberica* were extracted from ripening yellowish red berries and soaked in 0.1% w/w of a 1:1 benomyl-thiram solution for 15 minutes. The seeds were surface dried on clean towels and desiccated in an air-conditioned room at 22°C and 55% relative humidity. After 0, 2, 4 and 6 days of drying, batches of seeds were randomly removed to index seed germination and embryo viability before and after direct plunge into liquid nitrogen. Further samples of seeds were used to measure seed and embryo moisture.

Seed germination was carried out with 20 seeds per replicate using the in-sand method recommended by the International Seed Testing Association (ISTA 1985). For embryo viability, a further 20 seeds were surface sterilised for 10 min in 1% v/v chlorine before the embryos were aseptically excised and cultured in Murashige and Skoog (Murashige and Skoog 1962) medium. The medium was incorporated with 0.2% activated charcoal and 1mg/l each of kinetin, indoleacetic acid and gibberellic acid (GA_3). The embryos were cultured in a 22°C room supplied with 12 hours of light (2000 lux).

Seed and embryo moistures were measured separately by drying ten seeds or embryos in a 103°C oven for 16 h. All treatments were replicated three times.

Effects of Embryo Desiccation and Prefreezing Protocol

Seeds from ripening yellowish red berries of *Coffea liberica* were extracted as described above and surface sterilised for 10 min in 1% w/w chlorine. The sterilised seeds were rinsed twice in sterile water and the embryos excised aseptically in a lamina flow cabinet. The excised embryos were then desiccated on sterile filter papers in the flow cabinet for 0, 0.5, 1.0, and 1.5 h. After desiccation, the embryos were exposed to various freezing protocols. These included slow freezing in an alcohol bath (Julabo circulator, model F40-HC) to -38°C at a rate of -1°C/min, prefreezing in the alcohol bath to - 38°C followed by plunging into liquid nitrogen at -196°C, and fast freezing by direct plunge into liquid nitrogen. For control, desiccated embryos were also cultured directly in Murashige and Skoog (MS) medium without prefreezing or liquid nitrogen exposure. Survival was measured by the percentage of embryos that turned green and expanded in the modified MS medium described above. The experiment was factorial with a randomised complete block design and four replicates of ten embryos per treatment.

Differential Thermal Analysis of Coffee Seeds

The availability of freezable water in coffee seeds dried to different moisture contents was monitored by differential thermal analysis as described before (Hor, Stanwood and Chin 1990). Seeds were dried to various moisture contents in the air-conditioned room before they were wrapped individually with aluminium foil around a thermocouple. The seeds were then frozen at -1°C/min to -70°C after which their moisture content was measured.

RESULTS AND DISCUSSION

Seed Desiccation and Survival

Fresh coffee seeds and their enclosed embryos have similar high moistures of more than 47%. When desiccated in the air-conditioned room, both lost their moisture gradually (*Fig. 1*). However, unlike many other tropical seeds, the moisture contents of the embryonic axes were only slightly higher (2%-8% only) than the moisture content of the whole seed. The differential was further reduced with increasing hours of desiccation. At the end of six days the seed and embryo moisture contents were 14.58% and 12.56% respectively.

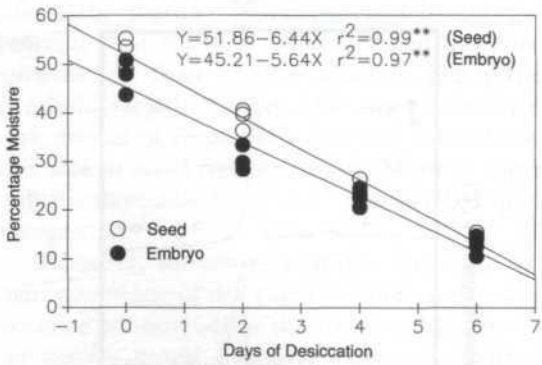

Fig. 1: Percentage moisture of seed and attached embryo after different days of seed desiccation in an air-conditioned environment.

Germination of fresh coffee seeds was above 90%, but with desiccation there was a gradual decrease in germination (*Fig. 2*). By the sixth day of drying when the moisture had decreased to 14.58%, germination was reduced to approximately 66%. The moderate survival at low moisture content of 14.58% confirms that coffee seeds are non-recalcitrant. However, none of the seeds survived exposure to liquid nitrogen within the moisture range of 52.58% to 14.58%.

Compared with seeds, embryos excised from dried seeds had lower viability especially with increased seed desiccation (*Fig. 2*). Embryos from fresh seeds had high viability (more than 90%), but after 6 days desiccation to moisture contents of 12.56%, viability was reduced to 38%. As in seeds, none of these embryos survived exposure to liquid nitrogen, irrespective of their moisture content.

Excised Embryos Desiccation and Survival

In contrast to attached embryos in seeds, excised embryos dried very rapidly in the lamina flow cabinet (*Fig. 3*). This was especially so in the first half hour when nearly 19% of moisture was lost (36.80% to 17.17%). After that there was a more gradual decrease to 9.27% moisture after 1.5 hours of drying.

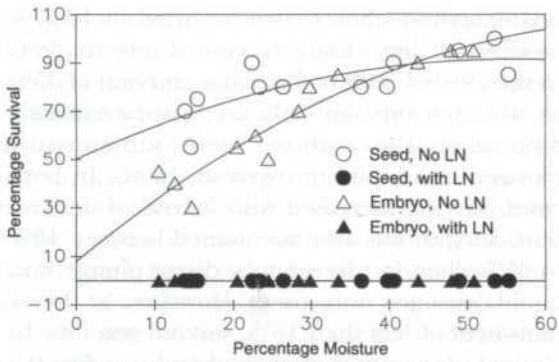

Fig. 2: Percentage survival of desiccated seeds and attached embryos with and without exposure to liquid nitrogen. The appropriate regression equations are :
*Y=50.14+1.50X-0.01X² r²=0.59** (SEED, NO LN)*
*Y=7.89+2.68x-0.02x² R²=0.90** (EMBRYO, NO LN)*

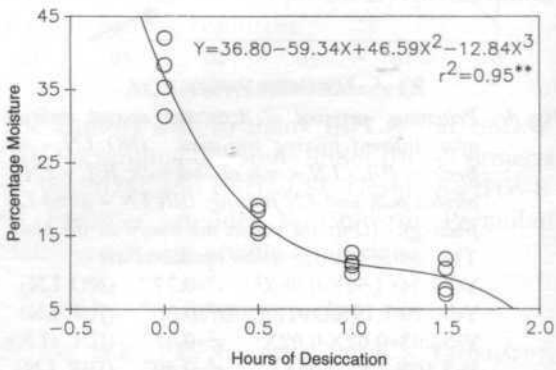

Fig. 3: Percentage moisture of excised embryo desiccated for different hours in the lamina flow cabinet.

Excised embryos desiccated to as low as 9.27% moisture had a good survival rate of more than 70% when they were not subjected to freezing temperatures (*Fig. 4*). Of greater significance is that many of these excised embryos were able to survive exposure to liquid nitrogen, indicating that excised desiccated embryos have potential for cryopreservation of coffee genetic resources. Survival of excised embryos in liquid nitrogen was dependent on their moisture content and freezing rate (*Fig. 4*). Fresh embryos at a high moisture of 36.8% did not survive freezing, but approximately 10% to 50% of the em-

bryos survived when they were dried to 17.17% moisture or less. Embryos cooled only to -38°C in the alcohol bath had a higher survival of 35% to 50%, but this was reduced to approximately 30% when the embryos were subsequently plunged into liquid nitrogen at -196°C. In both cases, survival increased with increased desiccation. Survival was also maintained between 10% to 35% when fast freezing by direct plunge into liquid nitrogen was used. However, at lower moistures of less than 10%, survival was low. In general, desiccation of excised embryos for 0.5 hour to 17.17% moisture was optimal for preservation in liquid nitrogen irrespective of the speed of freezing.

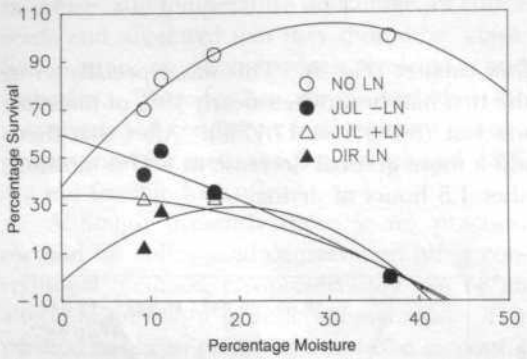

Fig. 4: Percentage survival of desiccated excised embryos after different freezing treatment. (NO LN = no freezing; JUL - LN = only alcohol bath; JUL = LN = alcohol bath and LN freezing; DIR LN = direct LN freezing). (Only the means are shown in the plot). The appropriate regression equations are :

$Y=37.54+4.52X-0.08X^2$	$r^2=0.77^{**}$	(NO LN)
$Y=59.29-1.19X-0.01X^2$	$r^2=0.56^{**}$	(JUL-LN)
$Y=33.93+0.02X-0.02X^2$	$r^2=0.47^{*}$	(JUL +LN)
$Y=3.16X-1.81-0.08X^2$	$r^2=0.49^{*}$	(DIR LN)

Differential Thermal Analysis of Seed Tissues

Seed tissues with moisture content ranging from 26.74% to 52.97% exhibited two exothermal peaks when cooled to -70°C at a rate of -1°C per minute (*Fig. 5*). The first exotherm was broad and minor and occurred at a temperature of approximately -4°C. The size of the exothermal peak is independent of the seed moisture suggesting that it is non-aqueous in origin. Becwar *et al.* (1983) reported similar peaks in *Coffea arabica* and suggested that they were caused by phase transition of the oils or lipids present in the seed tissues. The second peak was sharply spiked and occurred at temperatures between -12° to -31°C. The exotherm rose sharply at the higher temperature, suggesting that freezing commenced instantaneously and progressed homogeneously. As the size of the peak was directly related to the seed moisture, the exotherm was caused by the freezing of water in the seed tissues. This is further supported by the exothermal temperatures (-12°C to -31°C) which were similar to those reported for freezable water in other seeds (Hor, Stanwood and Chin 1990). In general there was a 0.62°C drop in exothermal temperature with every 1% decrease in seed moisture, resulting in lower exothermal

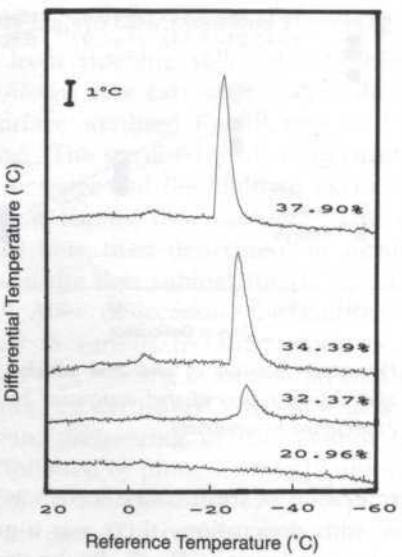

Fig. 5: DTA cooling profiles of coffee seeds dehydrated to different moisture contents.

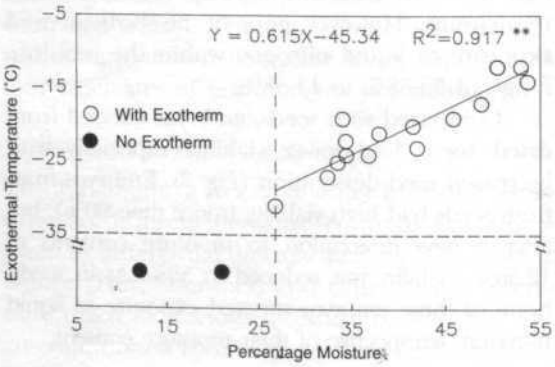

Fig. 6: Exothermal temperatures of Coffea liberica *seed as a function of seed moisture. (The vertical dotted line denotes the threshold moisture below which freezable water is absent).*

temperatures for tissues subjected to greater desiccation (*Fig. 6*). Seed tissues dried to less than 26.74% moisture did not exhibit an exotherm as freezable water within the tissues were removed.

Differential thermal analysis of seed tissues can provide a partial explanation for the survival of excised coffee embryos in liquid nitrogen. Freshly excised embryos did not survive liquid nitrogen exposure because their moisture content (36.80%) was higher than the threshold moisture (26.74%) below which all freezable water was removed from the seed. Freezable water remaining in the freshly excised embryos froze at approximately -12°C causing lethal injury to the tissues. However, excised embryos desiccated for 0.5 hour or longer had moisture contents less than 17.17% which is below the threshold moisture of 26.74%. Such embryos were devoid of freezable water and were therefore able to avoid freezing injury. Many of these embryos were able to survive exposure to liquid nitrogen.

However, the above postulate does not explain why many of the excised embryos dried to moisture content below the threshold levels did not survive liquid nitrogen exposure. Neither does it explain why embryos dried within the seeds to sub-threshold levels (after 6 days drying) did not survive in liquid nitrogen. It does emphasise, however, that although freezable water must be removed from tissues before they can be frozen, other factors are also important for survival in liquid nitrogen. One important factor may be the vigour of the excised embryos. Within a seedlot, vigour can vary depending on the size and maturity of seeds (Chin and Hor 1989). Smaller and more immature embryos may not have sufficient vigour to survive and recover from the liquid nitrogen treatment, although they can grow well in the absence of freezing. This can result in a proportion of the excised, partially desiccated embryos being killed in liquid nitrogen even though they were dried below the threshold level. Other factors, such as variation in moisture between individual embryos and different media requirement of cryopreserved embryos, may also lead to their reduced survival in liquid nitrogen.

Compared with excised embryos, embryos dried in the seeds can have their vigour further reduced. Desiccation of the embryo was slow within the confined, moist environment of the perisperm which can encourage microbial infection and inhibit aerobic respiration. The resulting decreased vigour coupled with the increased resistance of the partially dried perisperm can also prevent many of the embryos from emerging through the perisperm. Further, embryos from seeds dried for a few days were difficult to excise and many may have been injured. Collectively, these factors can be the cause for non-survival of embryos dried within the seeds.

Excising embryos is a useful method for conserving genetic resources of many tropical species in liquid nitrogen. Many of these species produce large seeds, which are impractical to conserve; they are likely to be killed by the freezing. On the other hand excised embryos survive better and are conveniently conserved in small cryovials as reported for other tropical species (Normah *et al.* 1986). Although in the present study the survival of excised coffee embryos in liquid nitrogen was found to be only moderate, further studies are in progress to evaluate the effects of other factors in increasing their survival levels. These studies hopefully will lead to a practical technique for cryopreservation of their genetic resources.

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REFERENCES

BECWAR, M.R., P.C. STANWOOD and K.N. LEONHARDT. 1983. Dehydration effects on freezing characteristics and survival in liquid nitrogen of desiccation-tolerant and desiccation-sensitive seeds. *J. Amer. Soc. Hort. Sc.* **108(4):** 613-618.

CHIN, H.F. and Y.L. HOR. 1989. Seed and *in vitro* storage of genetic resources. *Proc. of the 6th Internatl. Congr. of SABRAO* **6:** 79-84.

ELLIS, R.H., T.D. HONG and E.H. ROBERTS. 1990. An intermediate category of seed storage behaviour? *J. Expt. Bot.* **41(230):** 1167-1174.

HOR, Y.L., H.F. CHIN and S. MURUGAIAH. 1990. Preservation techniques of recalcitrant seeds. Third Progress Report. IBPGR Project 88/99. Rome: IBPGR.

HOR, Y.L., P.C. STANWOOD and H.F. CHIN. 1990. Effects of dehydration on freezing characteris-

tics and survival in liquid nitrogen of three recalcitrant seeds. *Pertanika* **13(2):** 309-314.

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES. 1983. Report of the first meeting, IBPGR advisory committee on in-vitro storage. Rome: IBPGR.

INTERNATIONAL SEED TESTING ASSOCIATION. 1985. International rules for seed testing. *Seed Sci. and Technol.* **13(2):** 299-355.

KING, M.W. and E.H. ROBERTS. 1979. The storage of recalcitrant seeds: achievements and possible approaches. Rome: IBPGR.

LEROY, J.F. 1967. Recherches sur les cafeiers. Sur la classification biologique des cafeiers et sur l'origine et l'aire du genre *Coffea*. *Comptes Rendus de l'Academie des Sciences, Paris.* **265:** 1043-1045.

MURASHIGE, T. and F. SKOOGE. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15:** 473-497.

NORMAH, M.N., H.F. CHIN and Y.L. HOR. 1986. Desiccation and cryopreservation of embryonic axes of *Hevea brasiliensis* Murr.- Arg. *Pertanika* **9(3):** 299-303.

ROBERTS, E.H., M.W. KING and R.H. ELLIS. 1984. Recalcitrant seeds: their recognition and storage. In *Crop Genetic Resources: Conservation and Evaluation.* Eds. J.H.W. Holden and J.T. Williams. London: George Allen and Unwin. p. 38-52.

STANWOOD, P.C. 1984. Cryopreservation of seeds. Report of second meeting. IBPGR Advisory committee on seed storage. Rome: IBPGR.

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The Relationship between Population Fluctuations of *Helopeltis theivora* Waterhouse, Availability of Cocoa Pods and Rainfall Pattern

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Keywords: Population fluctuations, *Helopeltis theivora*, cocoa pods, rainfall

ABSTRAK

Kajian mengenai faktor-faktor yang mempengaruhi fluktuasi populasi daripada Helopeltis theivora *yang berhubungan dengan sumber makanan dan curah hujan telah dilakukan. Hasil kajian menunjukkan bahawa fluktuasi populasi dipengaruhi oleh curah hujan dan jumlah buah-buah koko yang ada dimana terdapat korelasi positif antara mereka. Hasil kajian juga menunjukkan bahawa pengurangan yield yang nyata didapati bersamaan dengan kenaikan populasi mirid. Jumlah cherelles dan buah koko yang didapati pada satu pokok akan menentukan daya tarikannya terhadap infestasi mirid, disebabkan lebih banyak cherelles dan buah koko didapati pada pokok yang diinfestasi oleh mirid apabila dibanding dengan yang tidak diinfestasi.*

ABSTRACT

Investigation on factors affecting the population fluctuations of Helopeltis theivora *in relation to food supply and rainfall in the field was conducted. Results showed that population fluctuations seem to be dictated by rainfall and numbers of available pods as shown by positive correlations between them. The results also showed a significant yield decrease with increased mirid populations. The numbers of cherelles and pods on a tree determine its attractiveness, since significantly more cherelles and pods were found on infested trees than on uninfested trees.*

INTRODUCTION

Helopeltis theivora formerly known as *Helopeltis theobromae* Miller (Stonedahl 1991) is the major pest of cocoa in Peninsular Malaysia. Feeding by the active stages causes lesions on shoots and pods of all ages including the newly formed cherelles. There is some disagreement over factors responsible for seasonal fluctuations in *H. theivora*. In Malaysia, pod production peaks twice a year. The relative scarcity of pods at other times of the year can influence the population dynamics of the species; Azhar (1986) emphasised pod shortage as a limiting factor to population increase. While the fluctuation in pod numbers probably plays an important role in the ecology of *H. theivora* and of other mirids, this role is likely to be moderated or even masked by other factors such as weather. The importance of cocoa pods as a source of food and as an oviposition site for *H. theivora* has been stressed by many workers (Miller 1941; Tan 1974). Cocoa pods are the main source of food for development and play a vital role in the reproductive success of *H. theivora* (Rita and Khoo 1983). Young shoots are not as good as pods for nymphal instars, but can sustain them when pods are scarce. However, pods are critical to adults for optimum survival and reproduction (Alias *et al.* 1988). The feeding and oviposition preferences for pods may be attributed to the suitability of their food value and to their texture for egg laying (Rita 1992). Tan (1974) observed that an increase in the population of *H. theivora* coincided with both increasing numbers of pods and increasing rainfall. A decrease in the numbers of *H. theivora* followed a decrease in rainfall even though the pod numbers remained high. Betrem (1941) also observed that there was a decrease of *H. antonii* Sign. numbers following a dry season. Roepke (1916) also observed that hot and humid weather conditions with sufficient sunshine and intermittent, but not too heavy,

rainfall were optimum conditions for *H. antonii*.

In West Africa, *Sahbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) showed well-marked annual population fluctuations which coincided with the main rainy seasons to influence mirid population patterns (Marchart 1969). In Indonesia, *H. antonii* populations decreased following a dry season (Roepke 1916). The importance of cocoa pods and vegetative tissues as food has been stressed for *S. singularis* and *D. theobroma* (Williams 1954).

Food supply and rainfall no doubt contribute to the changes in the pattern of mirid populations. Studies were conducted in the field to further investigate factors which affect the population fluctuations of *H. theivora* to gain a better understanding of the mirid's ecology. These investigations covered a three-year study of population fluctuations in one plantation.

METHODS

The study was conducted over a period from July 1986 to October 1989 at Seafield Estate, Shah Alam on a block of 49.8 hectares of Sabah mixed hybrid cocoa intercropped with coconuts. In the first four months the area was subject to two rounds of insecticide spraying (Lindane 250 g ai/ha) which killed off most of the *H. theivora* population; subsequently no insecticides were applied. A central plot of 15 rows x 15 trees was used in the study. The numbers of *H. theivora* on each tree in the plot were assessed at monthly intervals by the method known as 'counting to hand height' (Williams 1954). In this method, the trees are individually numbered, and the numbers of mirids found infesting up to a height of about six feet are counted but not removed. Altogether, assessments were made on thirty-nine sampling occasions. At each count the numbers and sizes of cocoa cherelles and pods on each tree were recorded: sizes were divided into cherelles (<10 cm long), small pods (10-12 cm long), medium sized pods (12.1-14 cm long) and large pods (>14 cm long). Local rainfall and crop yield data (dry bean weight) derived from the 49.8 hectares block were recorded. Subsequently, data from the 15x15 central plot were computed based on the relationship between the numbers of *H. theivora*, the numbers of cocoa pods and yield as based on 'per 100 trees'. Association and tests of significance to determine correlations between the numbers of *H. theivora* per 100 trees, the numbers of cocoa pods and crop yield per 100 trees, and the rainfall were statistically compared using Spearman's rank correlations (Siegel and Castellan 1988). The data for the first 10 months were not included in the analyses to allow for the mirids to recover from the insecticide treatments.

Additional data were obtained for each sampling on the numbers of cherelles and pods on the infested trees, and on the uninfested trees surrounding the infested trees, as well as on the numbers of nymphs and adults of *H. theivora* on the infested trees. These were statistically analysed and the differences between individual means were tested using Duncan's Multiple Range Test. (Table 1).

TABLE 1
Number of pods and *H. theivora* per uninfested and infested tree from May 1987 to September 1989

	Mean number of pods per tree/ per sampling			Mean numbers of *H. theivora* per tree/per sampling		
	Cherelles and small pods	Medium and large pods	Total	Nymphs	Adults	Total
Uninfested tree	1.3 ± 0.2b	1.7 ± 0.2b	3.1 ± 0.3b	0	0	0
Infested tree	4.4 ± 0.6a	5.1 ± 0.4a	9.6 ± 0.8a	1.5 ± 1.6	0.8 ± 0.5	2.4 ± 1.7

Note: About 3% of the uninfested trees are without pods.
Any two means within the column followed by the same letter are not significantly different at 5% level based on Duncan Multiple Range Test

RESULTS

Fig. 1 shows the relationship between the numbers of *H. theivora* per 100 trees, the numbers of pods, the crop yield per 100 trees and the rainfall. In the first 10 months of sampling there were fewer *H. theivora* compared to subsequent periods. This was because it took time for the *H. theivora* population to build up after insecticide treatment was stopped. *Fig. 1* shows that, after the first ten months, the population fluctuated annually with two peaks, in June-July and December-January.

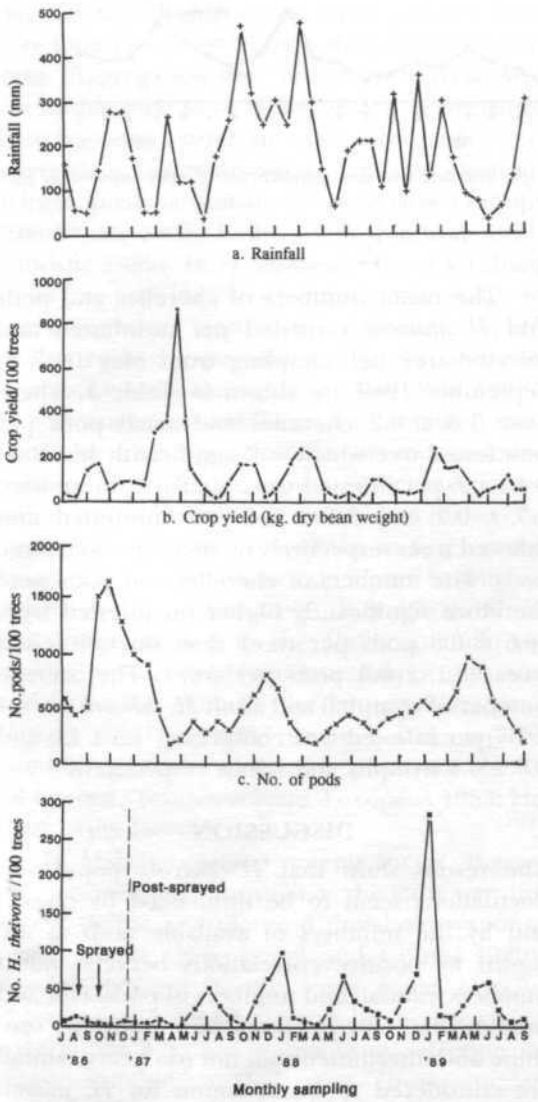

Fig. 1: Relationship between numbers of H. theivora, *numbers of pods, crop yield per 100 trees and the rainfall pattern between July 1986 to September 1989.*

Rainfall occurred throughout the year, but with sharp increases in April and May of each year. The wettest months usually occur towards the end of each year, usually from October to December, and may extend into January. The population peaks of *H. theivora* in December-January coincided with months of heaviest rainfall, although a one month gap was observed in the June-July peak. Some cherelles and pods were available throughout the year with most after the onset of the rainy season, especially between October and January. A flow chart of correlations is shown in *Fig. 2.*

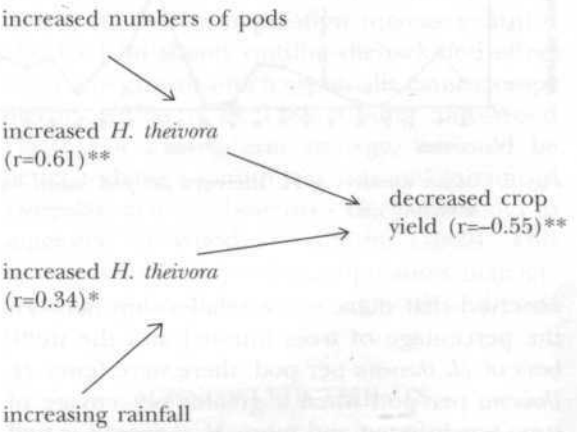

Fig. 2: Diagram showing relationship between H. theivora *population with availability of food (total numbers of pods), rainfall and crop yield*

Fig. 2 indicates that food in the form of numbers of pods available in the field and the increased rainfall were associated with an increase in the *H. theivora* population since positive correlations were obtained between these parameters. But the *H. theivora* population appeared to reduce the total crop yield at the same time since a negative correlation was obtained when decreased yield of harvested crop and increase in the *H. theivora* population were compared.

The numbers of *H. theivora* were calculated in terms of the numbers per pod, the mean numbers per infested tree and the percentages of infested trees (*Fig. 3*). At the population peaks, the mean numbers of *H. theivora* per infested tree varied from 3 to 10 and the percentages of trees infested during the June-July peak were between 10-20% but increased to about 40% in the December-January peak. It was

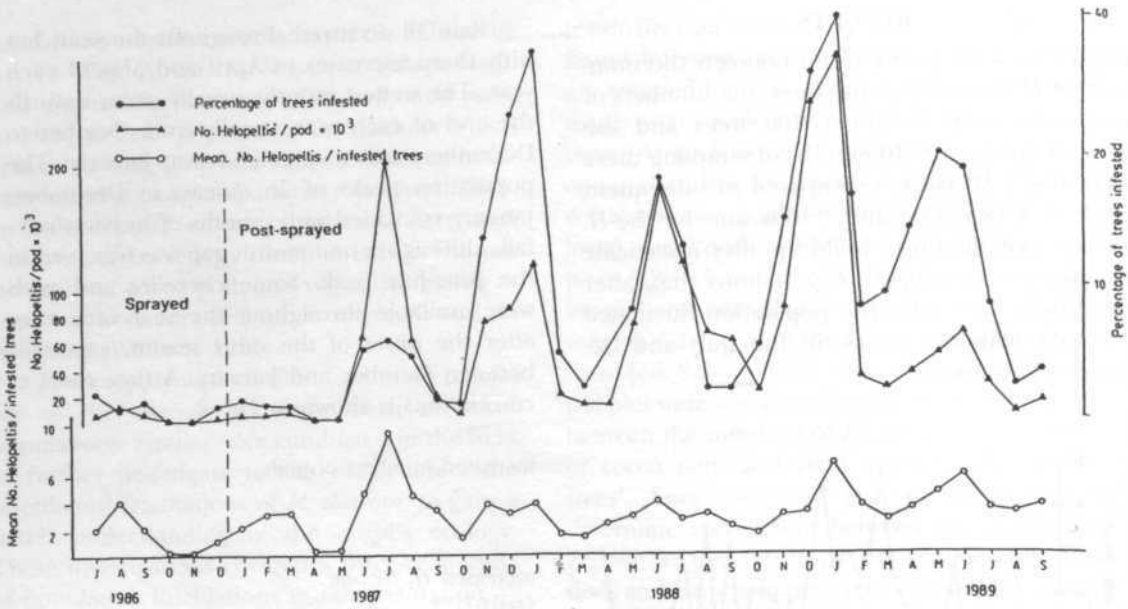

Fig. 3: Mean numbers of H. theivora *per pod, mean numbers per infested tree and percentages of trees infested at each sampling time*

observed that there was a relationship between the percentage of trees infested and the numbers of *H. theivora* per pod; there were fewer *H. theivora* per pod when a greater percentage of trees was infested and more *H. theivora* per pod when a smaller percentage of trees was infested. Although about similar percentages of infested trees (20%) and mean numbers per infested tree (4 insects per infested tree) were recorded in the June-July peaks in 1988 and 1989, about three times more per pod were recorded in 1988 than in 1989. This could be attributed to the fewer pods available during the 1988 peak which was nearly one-third of the numbers found in 1989. In June-July 1987, the mean numbers per infested tree was the highest (10 insects per infested tree) but with about similar numbers per pod as in 1988. This could be attributed to the the fewer trees which were infested (7%). In the December-January peaks of 1987 and 1988, similar percentages (about 40%) of trees were infested but the numbers per pod in 1987 were about one- third of those found in 1988. These differences were mainly due to the fact that the total numbers of *H. theivora* were about three times greater in 1988 than in 1987. The numbers of *H. theivora* per pod or per infested tree were directly related to the total numbers of *H. theivora* and the total numbers of pods available.

The mean numbers of cherelles and pods and *H. theivora* recorded per uninfested and infested tree per sampling from May 1987 to September 1989 are shown in Table 1. There were 1.3 ± 0.2 cherelles and small pods per uninfested tree which was significantly less than 4.4 ± 0.6 per infested tree. Similarly, there were 1.7 ± 0.2 and 5.1 ± 0.4 per uninfested and infested trees respectively on medium sized-large pods. The numbers of cherelles and pods were therefore significantly higher on infested trees (9.6 ± 0.8 pods per tree) than on uninfested trees (3.1 ± 0.3 pods per tree). The average number of nymphal and adult *H. theivora* was 2.4 ± 1.7 per infested tree comprising 1.5 ± 1.6 and 0.8 ± 0.5 nymphs and adults respectively.

DISCUSSION

The results show that *H. theivora* population fluctuations seem to be influenced by rainfall and by the numbers of available pods as are shown by positive correlations between mirid numbers, rainfall and numbers of pods. Hot and humid weather conditions with sufficient sunshine and intermittent but not too heavy rainfall are considered to be optimum for *H. antonii* (Roepke 1916). Pods play a vital role as feeding and oviposition sites for *H. theivora* (Alias *et al.* 1988). Their greatest role recurs in an annual

pattern when mirid species rely on them for food (Entwistle 1972). The *H. theivora* population was not directly affected by the availability of pods, or rainfall alone, but by an interaction of rainfall availability of food as suggested by Tan (1974). Our results also strongly suggest that *H. theivora* decreases yield, since a significant overall yield decrease was observed with increased mirid populations.

Patches, or pockets of *H. theivora*, have been observed, where individual trees harbour relatively large populations of *H. theivora*. The results show that the numbers of cherelles and pods on a tree determine its attractiveness, especially as significantly more cherelles and pods were found on infested trees than on uninfested trees. Aggregation also occurs with West African mirids such as *D. theobroma* and *S. singularis*. This happens either in open areas of cocoa where there are many young fans and chupons in incomplete parts of an otherwise closed canopy (Youdeowei 1965; Lodos 1969; Johnson 1971; Entwistle 1985). In *H. theivora*, a heavy localised attack on pods of certain trees is the result of heavy egg-laying and nymphal feeding by *H. theivora* related especially to cherelle and pod abundance at the time of infestation (Rita 1992). In this study, no evidence was obtained that particular trees have cherelles or pods that are resistant to or deter the pest. Sometimes a tree with many pods remained unattacked for several months; but this was probably because it was not found, since, once detected, it was heavily attacked as were other trees (Rita 1992). Mirid aggregation as well as the ability to build up rapidly to assume destructive proportions was evident in this study in the January 1989 peak (*Fig. 1*). This rapid build- up necessitates regular monitoring which can provide a basis for chemical control (Youdeowei and Toxopeus 1983; Ho 1986; Wills 1986).

In Malaysia, census systems for *H. theivora* have been formulated such as the early warning system (EWS) and plot and threshold response system (Wills 1986; Wood and Chung 1989). Results obtained in this study could help in improving the census systems by incorporating an additional criterion involving selection of certain suitable sampled trees. These sampled trees chosen in the census system, for example, might be those bearing large numbers of cherelles and small to medium sized pods as these will attract possible infestations. Knowledge of the population fluctuations of *H. theivora* could help in reducing the amounts of insecticide used. Populations of *H. theivora* have been shown to have two annual peaks coinciding with the wet seasons; insecticide spraying would normally be done preferably during these peak periods which coincide with peaks of cherelle production. Insecticide spraying in wet seasons is less effective and more difficult to organise, but it has been shown that spraying during peak periods is effective in reducing the mirid population (Chung and Wood 1989). However, damage can occur earlier at the cherelle stage; therefore to prevent loss, it would be better to spray before *H. theivora* population increases. Mirids also feed on shoots causing dieback and affect vegetative growth which eventually cause canopy dieback (Alias *et al.* 1988; Chung and Wood 1989). For a long-term strategy, it would be better to have a monthly census and to control *Helopeltis* activity above 10 - 15 % threshold as suggested by Wood and Chung (1989). This strategy could be used in population management to ensure healthy cocoa growth and good yield.

ACKNOWLEDGEMENTS

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REFERENCES

ALIAS AWANG, RITA MUHAMAD and K.C. KHOO. 1988. Comparative merits of cocoa pod and shoot as food sources of the mirid, *Helopeltis theobromae* Miller. *The Planter* **64**: 100-104.

AZHAR, I. 1986. Seasonal and daily activity patterns of *Helopeltis theobromae* Miller (Hemiptera: Miridae) and their implications in field control. *Proceedings of International Conference on Cocoa and Coconuts : Progress and Outlook*. Kuala Lumpur-Malaysia, pp.305-316.

BETREM, J.G. 1941. Verdere gegevens omtrent de bestrijding van *Helopeltis* middle van Derrispowder. (Additional data regarding control of *Helopeltis* with Derris powder). *Bergcultures* **15**: 238-249.

CHUNG, G.F. and B.J. WOOD. 1989. Chemical control of *Helopeltis theobromae* Mill and crop loss assessment in cocoa. *Journal of Plant Protection in the Tropics* **6 (1)**: 35-48.

ENTWISTLE, P.F. 1972. *Pests of Cocoa.* London: Longman Group Limited.

ENTWISTLE, P.F. 1985. Insects and cocoa. In *Cocoa,* (4th edn), ed. G.A.R. Wood and R.A. Lass), pp. 366 - 443. London: Longman Group Limited.

HO, C.T. 1986. Chemical control of mirid pests of cocoa. *Proceedings of the Second International Conference in Plant Protection in the Tropics.* Kuala Lumpur - Malaysia, pp. 41 - 43.

JOHNSON, C.G. 1971. The relation between capsids numbers, new damage and the state of the canopy and its significance in tactics of control. *Proceedings of the Third International Cocoa Research Conference.* Accra - Ghana.

LODOS, N. 1969. Mirid studies. *Report Cocoa Research Institute Ghana 1967 - 1968,* pp 44 - 45.

MARCHART, H. 1969. Capsid movement. *Report Cocoa Research Institute Ghana 1967 - 1968.* p. 58.

MILLER, N.C.E. 1941. Insects associated with cocoa (*Theobroma cacao*) in Malaya. *Bulletin of Entomological Research* **32**: 1-15.

RITA MUHAMAD and K.C. KHOO. (1983). A technique for rearing the cocoa mirid, *Helopeltis theobromae* Miller, in captivity. *Newsletter Malaysian Plant Protection Society* **7(11)**, Supplement, 8-10.

RITA MUHAMAD, A. 1992. Ecological and behavioral relationships between *Helopeltis theobromae* (Miridae) and its cocoa host plant. Doctoral Thesis. Imperial College of Science and Technology. University of London, 188pp.

ROEPKE, W. 1916. Het *Helopeltis* - vraagstuk, in het bijzonder met betreeking tot cacao. (The *Helopeltis* - problem with particular reference to cocoa). *Mededeelingen Proefstation Midden Java* **21:** 1-40.

SIEGEL, S. and N.J. CASTELLAN. 1988. Nonpara-metric statistics for the behavioral sciences. (2nd Edn). pp 1 - 399. Singapore: McGraw - Hill International Editions.

STONEDAHL, G.M. 1991. The oriental species of *Helopeltis* (Heteroptera: Miridae): a review of economic literature and guide to identification. *Bull. Entomol. Res.* **81:** 465-490.

TAN, G.S. 1974. *Helopeltis theivora theobromae* on cocoa in Malaysia. 1. Biology and population fluctuations. *Malayan Agriculture Research* **3**: 127-132.

WILLIAMS, G. 1954. Field observations on the cacao mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) in the Gold Coast. Part 1. Mirid damage. *Bull. Entomol. Res.* **44**: 101-119.

WILLS, G.A. 1986. Use of an early warning system for the control of *Helopeltis theivora theobromae* in cocoa. *Proceedings of Cocoa and Coconuts Conference: Progress and Outlook.* Kuala Lumpur - Malaysia, pp. 241 - 253.

WOOD, B.J. and G.F. CHUNG. 1989. Integrated management of insect pests of cocoa in Malaysia. *The Planter* **65 (762):** 389-418.

YOUDEOWEI, A. 1965. A note on the spatial distribution of the cocoa mirid *Sahlbergella singularis* Hagl. in a cocoa farm in Western Nigeria. *Nigerian Agriculture Journal* **2:** 66-67.

YOUDEOWEI, A. and H. TOXOPEUS. 1983. The *Helopeltis* census and management system (HCMS). *Bulletin Cocoa Advisory Group for Indonesia* **1:** 1-12.

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Urea as the Nitrogen Source in NFT Hydroponic System

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ABSTRAK

Unsur nitrogen dalam larutan nutrien hidroponik cara NFT (nutrient film technique) menjalani proses hidrolisis dengan pengeluaran ammoniam. Hidrolisis urea berlaku dengan pesat dari hari ketujuh dan tamat pada hari keduapuluh. Dalam masa yang sama jumlah ammoniam dalam larutan meningkat dan mencapai tahap maksima pada hari keduapuluh. Penitratan berlaku serentak dan kepekatan nitrat mencapai maksima pada hari keduapuluh juga. Berat kering tanaman pada peringkat matang tidak berbeza antara larutan rawatan urea dan rawatan nitrat. Kepekatan ammoniam dalam daun dan akar rawatan urea adalah tinggi dari rawatan nitrat. Kepekatan nitrat dalam daun dan akar tidak berbeza diantara kedua dua rawatan tersebut. Kajian menunjukkan urea dapat menggantikan nitrat sebagai sumber unsur nitrogen untuk tanaman dalam sistem hidroponik cara NFT.

ABSTRACT

Urea as the source of nitrogen in the nutrient solution in NFT (nutrient film technique) hydroponic system undergoes hydrolysis which results in the release of ammonium in solution. Urea hydrolysis was rapid from the 7th day onwards and ended by the 20th day. At the same time, ammonium concentration in solution increased and reached its maximum on the 20th day. Nitrification occurred simultaneously and peaked also on the 20th day. Plant dry matter weight at harvest was similar for both urea and nitrate treatments. Ammonium concentration in leaves and roots was higher in urea than in nitrate treatments. Nitrate concentration in leaves and roots was similar for both treatments. The study showed that urea can be substituted for nitrate as the nitrogen source in the NFT hydroponic system.

INTRODUCTION

The source of nitrogen in the nutrient solution for the growing of crops under the hydroponic system has been in the forms of $Ca(NO_3)_2$ and KNO_3 (Cooper 1975). Even though urea is increasingly being used as the main source of nitrogen fertilizer for crops grown on soil (Gould *et al.* 1986), its use as the N source for crops grown under the hydroponic system has yet to be evaluated. One of the reasons which could hinder the use of urea in hydroponics is that urea has to undergo hydrolysis with the release of NH_4^+ and subsequent formation of NO_3^- through nitrificaton, forms that are utilised by the plants. Hydrolysis of urea proceeds through enzymatic catalysis of urease (Vlek *et al.* 1980). In soils urease activity is located in the soil biomass and solution originating from decaying organisms (Skujins 1976; Vlek *et al.* 1980). There is also evidence that soil urease activity may be derived from plants (Frakenberger and Tabatabai 1982). It has also been shown that floodwater of tropical lowland rice soils has measurable amounts of urease activity (Sahrawat 1980), which could possibly originate from the soil.

Since the presence of urease has always been associated with soil, its presence in the nutrient solution in the hydroponic system devoid of soil therefore is of interest. Since bacteria can proliferate in nutrient solution and urease originates from bacteria cells, the possible presence of urease in nutrient solution media and hydrolysis of urea in the solution therefore is great.

The objective of this study was to examine the possibility of using urea as the nitrogen source in the nutrient solution in the hydroponic system by following its transformation in the nutrient solution and the performance of *Brassica chinensis* grown in it.

MATERIALS AND METHODS

The NFT (nutrient film technique) of soilless culture with plants grown in channels fed with recirculating nutrient solution was used (Cooper, 1975). The container which held the recirculating nutrient solution had a capacity of 50 l. The channels were 3.7 m long, 30 cm wide and 5 cm high. These channels were constructed from 1 cm thick plywood. The inner surface of the channels was covered with polyethene sheets along which the solution flowed and bathed the plant roots. A submersible pump in the container delivered the solution through 2 cm (diameter) PVC pipes from the container to the top of the channel, discharging the solution through a 5 mm tube at the rate of 2 l/min. The channels were elevated at the top end (10 cm higher) so that the solution would flow in a thin film on the surface of the channel to the bottom end and into the container and then recirculated. Each replicate of a treatment consisted of the channel, a container and a pump. Three replicates were used for each treatment. The experiment was run in the greenhouse in a complete randomized design. Mini pak choy (*Brassica chinensis* var Ching Chiang -Taiwan) seeds were sown on rockwool blocks 6 cm^2 and 40 cm in length for each block; nine blocks were used for each channel.These blocks were placed at the centre of the channels. Three seeds were placed at each point, 8 cm apart. Each rockwool block had 5 plants. Thinning was done on the tenth day leaving one plant per point. The total number of plants per channel at the start of the experiment was 45. Since the concentration of urea in the solution had to be monitored continuously for the whole period of the plant growth, elemental nutrients were added to the solution for plant need, based on the electrical conductivity (E.C.) of the solution; the element content of the solution was analysed on the 20th day to ensure that all elements with the exception of N were adequate for plant need. Plants were observed for deficiency symptoms. Cooper and Charlesworth (1977) concluded that addition of nutrients under the NFT based on the readings of the E.C. and the solution pH was adequate to produce equivalent tomato yield compared to nutrient addition based on nutrient analysis.

Treatments

The standard nutrient formulation used was based on Cooper (1975) (Table 1). The other three treatments were 100% N-urea (No.2), mixture of 50%N-nitrate and 50%N- urea (No.3) and 75%N-urea and 25%N-nitrate (No.4). An accompanying treatment (No.5) similar to (No.2) but without plants grown was included. $CaCl_2$, KOH and K_2SO_4 were substituted for the $Ca(NO_3)_2$ and KNO_3 for treatments containing urea, resulting in some treatments having higher sulphur and chloride contents which, however, did not exceed toxic levels. pH of the solution was maintained at between 5.5-6.5 by adding HCl or NaOH. Electrical conductivity (E.C.) of the solution was maintained at 2.4 dS m^{-1} until the plants were harvested. Solution E.C. which fell below this value was replenished with the stock solution of the respective treatments. Amounts added to each treatment are given in Table 2. Water loss through evapotranspiration was replaced with tap water to the 50 l mark daily.

Sampling

Solution samples were taken daily for the first seven days and thereafter on the 10th, 20th,

TABLE 1
Chemical make-up of the various treatments

Source	Treatments (g/1000)				
	1	2	3	4	5
urea	–	480	240	360	480
KH_2PO_4	272	272	272	272	272
KNO_3	808	–	808	404	–
$Ca(NO_3)_2$	943	–	–	–	–
$CaCl_2$	–	588	588	588	588
$MgSO_4$	492	492	492	492	492
KOH	–	224	–	112	224
K_2SO_4	–	348	–	174	348
$MnSO_4$	5.27	5.27	5.27	5.27	5.27
H_3BO_3	3.03	3.03	3.03	3.03	3.03
Na_2MoO_4	.027	.027	.027	.027	.027
$ZnSO_4$	.22	.22	.22	.22	.22
$CuSO_4$	.05	.05	.05	.05	.05
EDTA-Fe	19.48	19.48	19.48	19.48	19.48

TABLE 2
Nitrogen supplement during the period of plant growth

Treatments	NO_3-N (g)	Urea-N (g)
T1	14.79	–
T2	–	6
T3	19.78	8.7
T4	7.38	10.8
T5	–	–

30th and the 40th day. Plants were harvested on the 40th day. PMA (phenylmercuric acetate) was added to samples to retard hydrolysis of urea before analysis of the samples was done. Samples were analysed for NO_3^- and NH_4^+ (Bremner 1965) and urea (Douglas and Bremner 1970).

Plant samples were taken on the 20th, 30th and the 40th day. For the first two samplings, 9 plants (one from each rockwool block taken at random) were harvested from each channel. The tops and roots were separated and weighed. The final harvest comprised 27 plants. They were oven dried, reweighed and ground. For analysis of NH_4^+ and NO_3^- in the samples, 1 g sample (oven dry) was shaken with 50 ml distilled water for 1 hr and the extract filtered. 10 ml of the extract was determined for NO_3^- and NH_4^+ (Woolley *et al.* 1960).

RESULTS AND DISCUSSION

Results of the analysis of the nutrient solution on the 20th day (Table 3) indicated that the macronutrient element content was adequate (Cooper and Charlesworth 1977). The elements content in the leaves at harvest also indicated that their concentration in plants were adequate (Table 3). Visual observation of the plants indicated no apparent nutrient deficiency except that the 100% N-urea treatment had darker green leaves than those in the other treatments.

Urea concentration in solution of treatments containing urea dropped rapidly from the 7th day onwards which showed that it had been hydrolysed. By the 20th day all the urea was depleted from the nutrient solution (*Fig. 1*). In soils urea is found to be fully hydrolysed within 3 days after application mostly in the form of NH_4^+ and by the 14th day most are in the NO_3^- form (Gasser, 1964; Bundy and Bremner 1974). Decrease in the urea concentration of the solution in treatments containing urea was rapid from the 7th day onwards. Loss of urea in the 100% N- urea with plant treatment was greater compared to the same treatment without plants on the 10th day. It is possible that the urease activity was greater in solution where plants were present. Elliott (1986) found hydrolysis of urea in cropped media more rapid than in uncropped media. Between the 1st and 7th day the urea loss was about 5%. Between the 7th and 10th day, urea in solution of cropped treatments decreased by 30%. Most of the urea was hydrolysed between the 10th and 20th day. Ammonium in the solution was detected as early as the first day after the start of the experiment in all the treatments receiving urea, indicating that hydrolysis of urea occurred immediately.

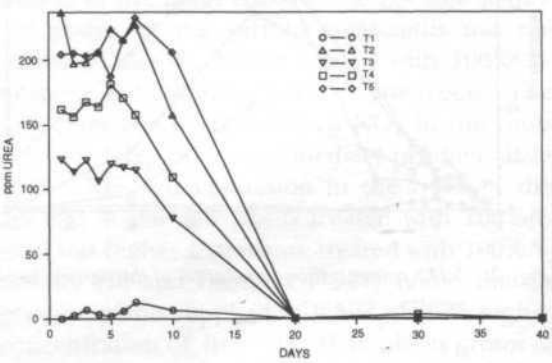

Fig. 1: Urea concentration in solution of the various treatments

Ammonium in Solution

Ammonium concentration in the nutrient solutions increased until the 20th day, thereafter its concentrations began to decline except in the treatment without plants (*Fig.2*). Plant uptake and the transformation of NH_4^+ to NO_3^- via nitrification are the possible reasons for the decline after the 20th day. Furthermore, its source, urea, had been depleted by that time. Ammonium concentration in solution depended on the amount of urea in the treatments. High urea levels resulted in high NH_4^+ concentrations. The highest NH_4^+ concentration was recorded in the 100% N-urea treatments. With plant growth the decrease was higher which could be due to plant uptake and increased nitrification. Decline in NH_4^+ concentration in the treatment without plants after the 20th day

was slight. 75% and 50% N- urea treatments attained maximum NH_4^+ concentration on the 20th day and the NH_4^+ concentrations were in proportion to the amount of urea added. It can therefore be said that rate of hydrolysis of urea was not affected by the amount of urea present within the amounts of urea used in the experiment. This showed that urease was a non-limiting factor. Several researchers have found that in soils the rate of urea hydrolysed by soil urease increased with increase in urea concentration until the amount of urea added is sufficient to saturate the enzyme with the substrate (Douglas and Bremner 1971; Tabatabai and Bremner 1972; Dalal 1975).

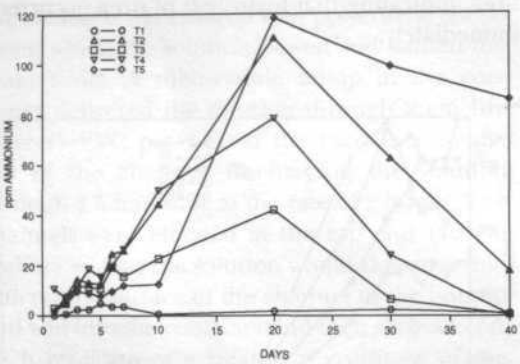

Fig. 2: NH_4^+ concentration in solution of the various treatments

Nitrate in Solution

The extent of nitrification occurring in the solution containing urea is shown in *Fig. 3.* Similar to the NH_4^+ buildup, NO_3^- increase in the nutrient solution was observed in the 100% N-urea treatments with and without plants; however the buildup started later (from the 7th day onwards). While treatments with 100% and 50% N-NO_3^- source declined for the period of 7- 10th day, treatments with 100%N-urea had NO_3^- concentration increased during the same period.

Maximum concentration of NO_3^- in the nutrient solution in the 100% N-urea treatments was attained on the 20th day. Subsequent periods showed no increase in nitrate concentration as shown by the 100% N- urea treatment without plants. At the 20th day the concentration of NH_4^+ was only one third of NO_3^-.

Ammonium buildup and nitrate formation in the treatments containing urea occurred simultaneously. While 100% N-urea treatment with plants had a NO_3^- concentration decrease after the 20th day, treatment without plants maintained the NO_3^- concentration until the 40th day. Nitrate concentration in treatments which contained urea decreased more sharply until the 40th day compared to plants in the 100% N-NO_3^- treatment.

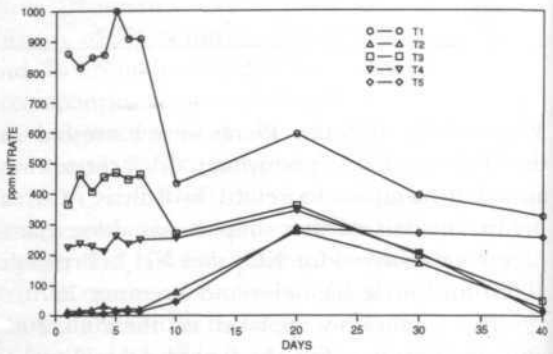

Fig. 3: NO_3^- concentration in solution of the various treatments

Total N in solution

Summation of the various forms of N (NO_3^-, NH_4^+ and urea) in the solution of the treatments is presented in *Fig. 4.* Total N remained the same for the first 7 days. From the 7th to 10th day total N of treatments 100% and 50% N-NO_3^- was lower compared to the others. From the 10th to the 20th day , 100% and 75 % N- urea treatments decreased in total N value. Further decline in total N occurred in all treatments except the one without plants from the 20th to the 30th day. At the 40th day, treatments which contained urea had lower values compared to 100% N- NO_3^- treatment. From the 20th day onwards the treatment without plants maintained its total N to the end.

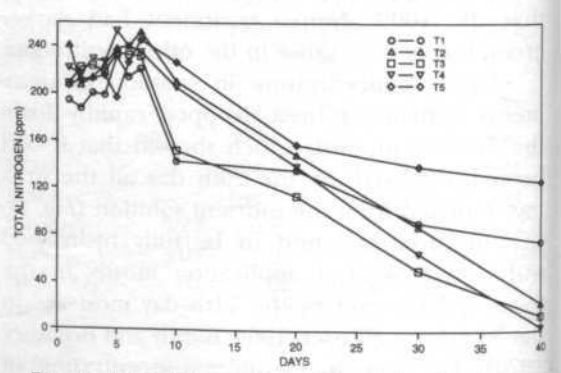

Fig. 4: Total N in solution of the various treatments

TABLE 3
Macronutrient concentrations in the solution (ppm) on the 20th. day and in the leaves at harvest (%)

Elements		Treatments			
		1	2	3	4
K	(solution-s)	306	332	249	263
	(leaves-l)	11.5	8.5	8.2	8.4
P	(s)	29	52	32	39
	(l)	1.33	1.10	1.04	1.22
Ca	(s)	175	214	227	200
	(l)	3.70	3.08	3.09	3.25
Mg	(s)	68	42	43	42
	(l)	.63	.56	.43	.50
N	(l)	4.97	5.51	5.18	4.4

Plant dry weight

Plant dry weight on the 20th day for 100% N-urea treatment was the lowest recorded and was significantly different ($P<0.05$) from those of 100% and 50% N-nitrate treatments (Table 4). This initial difference in the dry weight can be attributed to the time taken for urea to be hydrolysed before NH_4^+ and the subsequent release of NO_3^- in the solution for plant use. On the 30th day, the plant dry matter weight of the 100% N- nitrate treatment was not significantly different from that of 100%N-urea treatment. However, the two treatments with mixtures of urea and nitrate registered the highest value. Studies by Cox and Reisenauer (1973) have shown that mixtures of NH_4^+ and NO_3^- in solution give higher yield than those of NO_3^- or NH_4^+ supplied singly. Moneerat *et al.* (1982) reported that maximum dry weight was achieved with a ratio of 60: 40 (NH_4^+: NO_3^-) in the solution. Dry matter weight on the 40th day showed no significant difference among the four treatments (Table 4).

Total N Uptake by Plants

Total N uptake values normally follow dry matter weight figures. Total N uptake values of *Brassica* at harvest (40 days) were not significantly different among treatments. The 50% N-urea and 50% N-nitrate mixture recorded the highest N uptake value of 3.16 g. Baker and Maynard (1972) reported that maximum N absorption by plants was achieved when NH_4^+ and NO_3^- were combined in the nutrient solution. The amount of N taken up by the plants accounts for 80% of the N supplied in the solution.

Ammonium in Roots

The percent coefficient of variation (%C.V.) of the NH_4^+ content in the plant roots on the 20th day growth stage was high due to the problem of separating the roots from the rockwool media. At this stage the roots were very fine and were firmly embedded in the media making the task of separation difficult. At the 30th and 40th day, the roots were thicker and bigger and the rockwool was softer making the task of separation easier. This was reflected in the decreasing trend in the C.V. value as the plants matured. No significant difference in ammonium concentration in the plant roots of the 20- and 30-day-old plants of the various treatments was observed (Table 4). Plants treated with 100% N-nitrate did contain NH_4^+ in the roots. The reduction and assimilation of NO_3^- in the roots produce NH_4^+ as an intermediary product (Pate 1973). NH_4^+ concentration in the roots of the 30- and 40-day old plants treated with 100%N-urea was higher than those treated with 100%N-nitrate. Pill and Lambert (1977) noted that in general, plants supplied with NH_4^+ have a higher concentration of free NH_4^+ than plants grown at comparable levels of NO_3^-. NH_4^+ accumulation in the roots of the 100% N-urea treatment could also be due to the slow conversion of NH_3^- into glutamine which is a function of the carbohydrate supply (Vickery *et al.* 1936).

Ammonium in Leaves

Ammonium concentration in the leaves of 20-day-old plants treated with 100% N-urea was higher than that in plants in the 100% N- nitrate treatments (Table 4). Pate and Wallace (1964) found that the bulk of the NH_4^+ absorbed by roots is converted to amino acids by the metabolic system of the roots and only a small percentage of the NH_4^+ is found in the xylem exudate presumably transported to the leaves for assimilation. It is possible that in leafy vegetables, the leaves play a major role in the assimilation of NH_4^+ which thus results in concentration of NH_4^+ in leaves being comparable to that in the roots. On the 30th day , NH_4^+ concentration in the leaves in all the treatments was similar.

TABLE 4
Dry matter yield, total N, NH_4^+ and NO_3^- content of pak choy

Dry matter yield (g/9 plants)		C.V. (%)	Treatments 1	2	3	4
D	20	21.89	1.26 a	0.75 b	1.26 a	1.21 ab
a	30	30.70	16.5 ab	10.77 b	21.61 a	19.43 ab
y	40	19.95	135.18 a	147.69 a	184.91 a	187.38 a
Total N uptake (g/9 plants)						
Day	40	23.86	2.20 a	2.75 a	3.16 a	2.76 a
NH_4 (roots) ppm						
D	20	91.58	824.6 a	803.6 a	200.0 a	304.3 a
a	30	29.72	570.0 b	1247.8 a	1470.0 a	1320.0 a
y	40	14.92	2010.0 b	2910.0 a	2610.0 ab	2610.0 ab
NH_4 (leaves) ppm						
D	20	43.66	324.5 b	693.8 a	330.0 b	360.0 ab
a	30	28.13	1260.0 a	1620.0 a	1680.0 a	1350.0 a
y	40	23.79	1680.0 b	2790.0 a	2100.0 ab	2340.0 ab
NO_3 (roots) %						
D	20	54.60	2.13 a	1.10 ab	0.90 ab	0.73 b
a	30	47.99	0.30 a	0.40 a	0.36 a	0.20 a
y	40	46.41	0.10 a	0.09 a	0.12 a	0.10 a
NO_3 (leaves) %						
D	20	46.30	3.27 a	2.27 a	2.73 a	2.38 a
a	30	33.78	2.48 a	1.63 ab	1.55 ab	1.21 b
y	40	46.93	1.01 a	0.99 a	1.21 a	0.89 a

Means with the same letter in a row are not significantly different at 0.05 using DMRT.

This period coincides with the high concentration of NH_4^+ in the nutrient media. As the concentration of NH_4^+ in solution started to fall by the 40th day, NH_4^+ concentration in leaves of 100%N-nitrate plants decreased. Since 100% N-nitrate treatment plants had less than 5% NH_4^+ content in the nutrient solution, we can deduce that the NH_4^+ present in the the leaves was from the reduction of NO_3^-. The NH_4^+ concentration in the leaves increased as the plant matured.

Nitrate in Roots

Nitrate concentration in roots was in contrast with that of NH_4^+. Whilst the concentration of NH_4^+ increased with plant growth, NO_3^- concentration decreased. NO_3^-: NH_4^+ concentration ratio was >20 in the 20-day-old plants and was <1 in the 40-day-old plants. Increased NO_3^- assimilation to amino acids in plants as they mature could be the reason for the fall in NO_3^- concentration. No significant difference in % nitrate in roots of plants treated with 100% N-nitrate and 100% N-urea at the 20th, 30th and 40th day was observed.

Nitrate in Leaves

NO_3^- concentration in the leaves of pak choy was higher than in the roots. Pate(1973) found that

the reduction of NO_3^- concentration in the leaves is relative to the roots and varies widely among plant species. It is possible that NO_3^- assimilation in the leaves of pak choy is more intense than in the roots. In maize plants, the roots were found to reduce about 1/3 of the nitrate and the percentage of NO_3^- reduction decreased as the plant aged (Raghuveer 1977). No significant difference in % nitrate in leaves between treatments was observed at the three plant growth stages (Table 4). The amount of NO_3^- in the leaves decreased as the plant growth increased. Percent NO_3^- was ten-fold higher than NH_4^+ in the leaves, the ratio being greater when plants were young.

CONCLUSION

Urea as the source of N used in nutrient solution in the NFT hydroponic system undergoes hydrolysis with the release of NH_4^+ and the subsequent formation of NO_3^-. The urea was completely hydrolysed by the 20th day. There was no difference in either the plant dry weight in the 100% N-urea or 100% N-nitrate treatments at harvest. NH_4^+ concentration in roots and leaves was higher in the 100% N-urea treatment than in the 100% N-nitrate treatment. No difference in NO_3^- concentration in roots and leaves of 100% N-nitrate and 100% N-urea treatments was detected. The study showed the possibility of using urea as the N source for the NFT hydroponic system in crop production.

REFERENCES

BAKER, A.V and D.N. MAYNARD. 1972. Cation and nitrate accumulation in pea and cucumber plants as influenced by N nutrition. *J. Amer. Soc. Hort. Sci.* **97:** 27-30.

BREMNER, J.M. 1965. Inorganic Forms of Nitrogen. In C.A. Black (ed.). *Methods of Soil Analysis, Part* 2. 1179-1232. Madison, Wisconsin: Am. Soc. Agron.

BUNDY, L.G. and J.M. BREMNER. 1974. Effects of nitrification inhibitors on transformation of urea N in soils. *Soil Biochem.* **6:** 369-376.

COOPER, A.J. 1975. Crop production in recirculating nutrient solution. *Scientia Hortic.* **3:** 251-258.

COOPER, A.J. and R.R. CHARLESWORTH. 1977. Nutritional control of a nutrient-film tomato crop. *Scientia Hortic.* **7**: 189-195.

COX, W.J. and H.M. REISENAUER. 1973. Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. *Plant Soil* **38:** 363-380.

DALAL, R.C. 1975. Urease activity in some Trinidad soils. *Soil Biol. Biochem.* **7:** 5-8.

DOUGLAS, C.A. and J.M. BREMNER. 1970. Extraction and colorimetric determination of urea. *Soil Sci. Soc. Am. Proc.* **34:** 859-862.

DOUGLAS, C.A. and J.M. BREMNER. 1971. A rapid method of evaluating different compounds or inhibitors of urease activity in soils. *Soil Biol. Biochem.* **3:** 309-315.

ELLIOTT, G.C. 1986. Urea hydrolysis in potting media. *J. Am. Soc. Hort. Sci.* **6:** 862-866.

FRAKENBERGER, W. T. and M.A. TABATABAI. 1982. Amidase and urease activities in plants. *Plant and Soil* **64:** 153-166.

GASSER, J.K.R. 1964. Urea as a fertilizer. *Soils Fert.* **27**: 175-180.

GOULD, W.D., C. HAGEDORN and R.G.L. MCCREADY. 1986. Urea transformations and fertilizer efficiency in soil. *Adv. in Agron.* **40:** 209.

MONEERAT, P.H., P.D. CASTALLANE, J.L.C. ZAMBON, J.G. PANDUA and J.J.V. MULLER. 1982. Effect of ammonium to nitrate ratio on initial development and mineral composition of tomato, *Lycopersicon esculentum. Proceedings of Amer. Soc. Hort. Sci.* **25:** 451-455.

PATE, J.S. 1973. Uptake, assimilation and transport of nitrogen compounds by plants. *Soil Biol. Biochem.* **5:** 109-119.

PATE, J.S. and W. WALLACE. 1964. Movement of assimilated nitrogen from the root systems of the field pea (*Pisum arvense* L.) *Ann. Bot.* **28:** 83-99.

PILL, W.G. and V.N. LAMBERT. 1977. Effects of NH_4 and NO_3 nutrition with and without pH adjustments on tomato growth, ion compositions and water relations. *J. Am. Soc. Hort. Sci.* **102:** 78-81.

RAGHUVEER, P. 1977. Characteristics of nitrate uptake from nutrient solution and root nitrate reductase activity among corn genotype. Ph.D Dissertation. Univ. of Illinois, Urbana-Champaign.

SAHRAWAT, K.L. 1980. Urease activity in tropical rice soils and floodwater. *Soil Biol. Biochem.* **12:** 195-196.

SKUJINS, J.J. 1976. Extracellular enzymes in soils. *CRC Crit. Rev. Microbiol.* **4:** 383-421.

TABATABAI, M.A and J.M.BREMNER. 1972. Assay of urease activity in soils. *Soil Biol. Biochem.* **4:** 479-487.

VICKERY, H.B., G.W. PUCHEZ and H.E. CLARK. 1936. Glutamine metabolism of the beet. *Plant Physiol.* **11:** 413-420.

VLEK, P.L.G., J.M. STUMPE and B.H.BYRNES. 1980. Urease activity and inhibition in flooded soil systems. *Fert. Res.* **1:** 191-202.

WOOLLEY, J.T., G.P.HICKS and R.H. HAGEMAN. 1960. Rapid determination of nitrate and nitrite in plant material. *J. Agric. and Food Chem.* **8**: 481-482.

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Prevalence of an Entomopathogenic Fungus, *Hirsutella citriformis* on Leucaena Psyllid, *Heteropsylla cubana*, in Malaysia

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Keywords: ***Hirsutella citriformis, Heteropsylla cubana, Leucaena leucocephala***

ABSTRAK

Psilid petai belalang, Heteropsylla cubana *Crawford (Homoptera: Psyllisae) ialah perosak eksotik penting* Leucaena leucocephala *(Lam.) (Leguminosae) di Asia Tenggara, Kepulauan Pasifik, Hawaii dan Australia. Walaupun serangga ini telah merebak dengan meluas di Malaysia, tiada maklumat mengenai entomopatogen berkaitan dengan perosak ini telah direkodkan. Kajian ini melapurkan untuk pertama kali kehadiran kulat entomopatogen,* Hirsutella citriformis *Speare (Deuteromycotina: Hyphomycetes) pada psilid petai belalang,* H. cubana *di Malaysia. Keputusan dari penyampelan bulanan selama setahun menunjukkan bahawa psilid petai belalang boleh dijangkiti oleh kulat* H. citriformis. *Psilid mati didapati menjadi keras dan terlekat dengan miselia berwarna krim pada daun dan ranting pokok petai belalang. Keputusan juga menunjukkan bahawa psilid dewasa lebih mudah dijangkiti oleh kulat berbanding dengan nimf. Kadar purata jangkitan berlaku pada populasi dewasa ialah 20% manakala populasi nimf mengalami kurang daripada 2% kadar purata jangkitan.*

ABSTRACT

The leucaena psyllid, Heteropsylla cubana *Crawford (Homoptera: Psyllidae) is a serious exotic pest of* Leucaena leucocephala *(Lam.) (Leguminosae) in Southeast Asia, Pacific Islands, Hawaii and Australia. Even though the insect is already widespread throughout Malaysia, no information on the entomopathogens associated with this pest has been recorded. This study reports, for the first time, the occurrence of an entomopathogenic fungus,* Hirsutella citriformis *Speare (Deuteromycotina: Hyphomycetes) on the leucaena psyllid,* H. cubana *in Malaysia. Results from monthly sampling of the psyllid over a period of one year established that the leucaena psyllid,* H. cubana *was susceptible to infection by the fungus,* H. citriformis. *Dead psyllids were found mummified and cemented by cream-coloured mycelia to the leaves and branches of the leucaena plant. The results also showed that adult psyllids were more prone to fungal infection than nymphs. The adult population had an average infection rate of about 20% while nymphs had an infection rate of less than 2%.*

INTRODUCTION

Leucaena leucocephala (Lam.) (Leguminosae), an exotic fast-growing multipurpose species, is an important plant in the rural economy of the people in the Southeast Asia region. This perennial nitrogen-fixing plant, commonly found planted in farms with other crops such as maize, groundnut and tapioca, is widely used as animal feed, firewood and as a vegetable for human consumption. The plant has also been recognized as one of the most promising forage and tree crops for the tropics (Anon. 1977). In recent years, however, leucaena plantations in this region have been threatened with problems of frequent outbreaks of an introduced insect pest, the leucaena psyllid, *Heteropsylla cubana* (Crawford) (Homoptera: Psyllidae). This psyllid causes heavy defoliation and stunting of the plant. Repeated infestations can also lead to death of the branches and sometimes the whole plant. In Malaysia, the psyllid, which may have come earlier than 1986 when it was first re-

ported (Tho 1986), has spread widely throughout Peninsular Malaysia and Sabah and Sarawak.

Even though the psyllid can now be found essentially on any leucaena plant in Malaysia, few studies with respect to the biology and ecology of this pest have been undertaken (Lim *et al.* 1989). No information on its natural enemies, particularly its entomopathogens, has been documented. Studies elsewhere, however, have shown that this psyllid, like many homopterans, is prone to infection by a number of entomopathogenic fungi (Hsieh *et al.* 1987; Napompeth *et al.* 1989; Villacarlos *et al.* 1989). This paper reports for the first time the prevalence of an entomopathogenic fungus, *Hirsutella citriformis* Speare on the leucaena psyllid, *H. cubana* and its impact on the seasonal abundance of the psyllid population in Malaysia.

MATERIALS AND METHODS

Field Survey

A monthly field sampling, from October 1988 to September 1989, was carried out in two leucaena plots. These plots were located at the Universiti Pertanian Malaysia, Serdang and Ijok, Kuala Selangor, Selangor, Malaysia. Ijok is approximately 80 km from Serdang. From each locality, twenty leucaena plants were randomly selected and a shoot was randomly sampled from each plant. The chosen shoot was wrapped in a clear polythene bag and the shoot cut to a length of 15 cm. This was done quickly so as to minimize escape by the adult psyllids. The samples were kept in an ice chest and brought back to the laboratory for examination. The number of nymphs and adults, dead and alive, were recorded. Dead psyllids were examined under a dissecting microscope for fungal infection. Other dead individuals were examined under a scanning electron microscope. For this purpose, infected psyllids were air-dried, coated with gold-palladium alloy, and examined with a Stereoscan 360 Cambridge electron microscope. Samples of infected psyllids were also sent to the CAB-International Mycological Institute, Kew (U. K.) for identification.

Weather Data

Weather data were obtained from Serdang only since Ijok has no facility for recording weather. Local temperatures, precipitation and relative humidities were recorded.

RESULTS AND DISCUSSION

Population of Leucaena Psyllid

Mean population estimates of leucaena psyllid densities sampled over twelve months from two localities are presented in *Figs. 1* and *2*. The overall results show that the psyllid population densities varied greatly from month to month with adult population densities invariably lower than those of the nymphs. The population from Serdang fluctuated from two to 160 nymphs/shoot with a mean of 68 nymphs/shoot/month and numbers of adults fluctuated from four to 44 adults/shoot with a mean of 22 adults/shoot/month (*Fig. 1*). The population density recorded from Ijok was significantly higher than that from Serdang with numbers fluctuating from 47 to 442 nymphs/shoot with a mean of 131 nymphs/shoot/month and adults from three to 137 adults/shoot with a mean of 70 adults/shoot/month (*Fig. 2*).

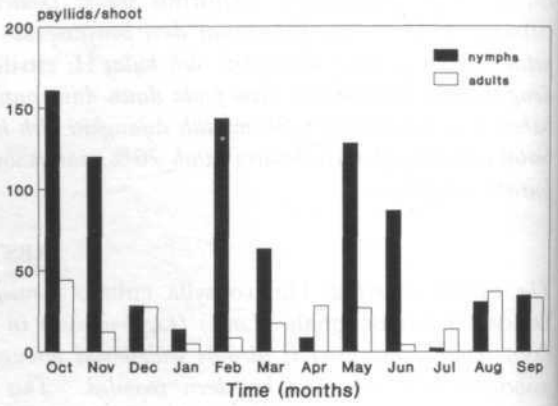

Fig. 1: Population densities of H. cubana *at Serdang in 1988/89*

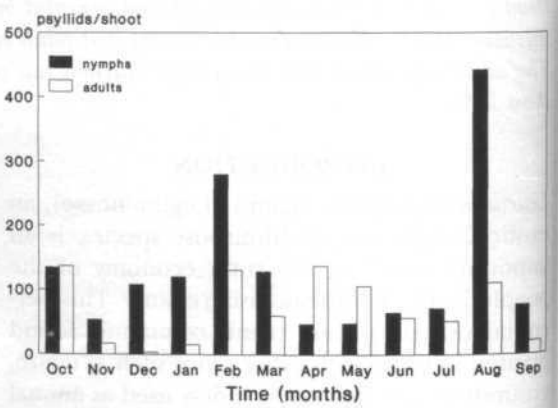

Fig. 2: Population densities of H. cubana *at Ijok in 1988/89*

Prevalence of H. citriformis*-infected Psyllids*

The leucaena psyllids were infected by *H. citriformis* in the two plots surveyed. Infected psyllids were mummified and cemented to leaves and branches of the leucaena plant. A thick mat of cream-coloured mycelia enveloped the psyllid and, in some instances, with synnemata growing from the mycelial substrate (*Plate 1*). This hyphomycete fungus is characterized by the presence of cylindrical synnematous hyphae which bear laterally-scattered phialides. The synnemata measure 114 - 200μm in width and 300-780 μm in length and are club-shaped. The phialides, clustered on the synnemata, measure about 20 μm in length, are cylindrical with globose bases tapering into a long neck towards the tip. The conidia which measure 0.33 x 1.00 μm are ellipsoidal in shape and are found singly at the pinnacle of the phialides (*Plate 2*).

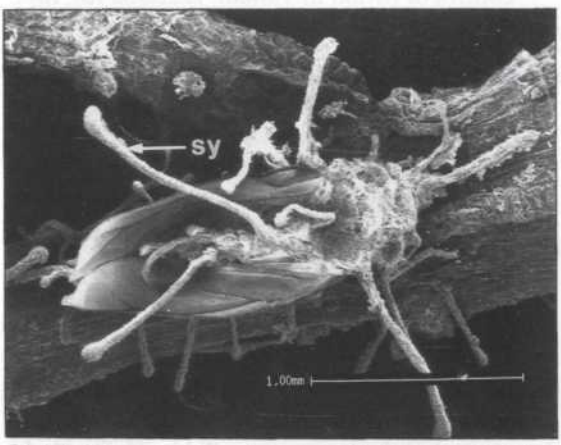

Plate 1 Heteropsylla cubana *adult infected and killed by* H. citriformis *fungus. sy = synnema*

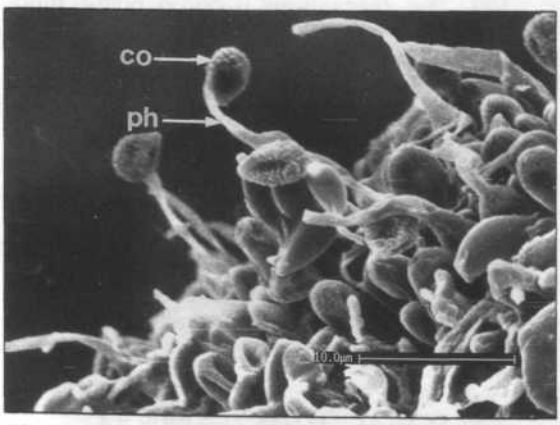

Plate 2 *Conidia of* H. citriformis *fungus formed at the tip of basally swollen phialides co = conidium; ph = phialide*

Although the psyllids were prone to infection by *H. citriformis*, the levels of fungal infection varied seasonally and from field to field. The psyllid population from Serdang had a mean number of infected nymphs and adults ranging from zero to 8 and zero to 12.2/shoot, respectively (*Fig. 3*). Samples from Ijok had a mean number of infected nymphs and adults ranging from zero to 7.5 and zero to 42.3/shoot, respectively (*Fig. 4*). These levels of infection constituted approximately 4.9 and 6.5% of the total monthly population densities sampled from Ijok and Serdang, respectively.

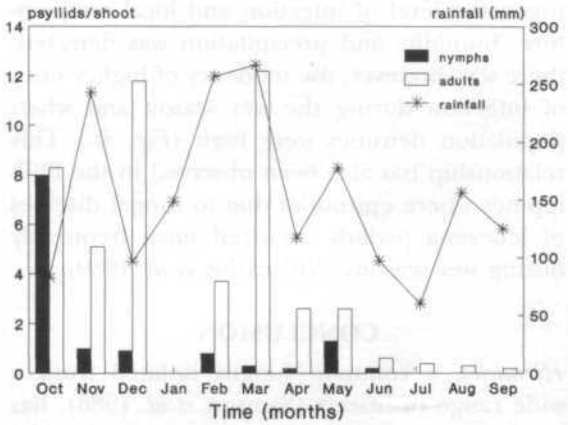

Fig. 3: *Population of* H. cubana *infected with* H. citriformis *fungus and rainfall recorded from Serdang in 1988/89*

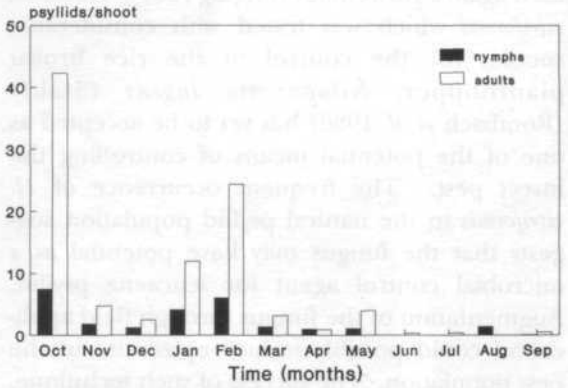

Fig. 4: *Population of* H. cubana *infected with* H. citriformis *fungus recorded from Ijok in 1988/89*

The prevalence of fungal-infected psyllid was apparently affected by the stage of psyllid development. Adults had a higher rate of infection than did the nymphs despite the fact that the proportion of nymphs in the population was

higher. The infection rates for adults ranged from zero to 77% with a mean of 20% in Ijok and from zero to 70% with a mean of 23% in Serdang compared with less than 2% in the nymphal population examined at both localities. The slow growth of the fungus relative to the frequent moulting and short duration of nymphal development may be in part the reason for the low rate of nymphal infection. On the other hand, long adult longevity could have permitted the process of successful fungal infection to occur and thereby contributed to a greater incidence of adult infection.

Even though no significant correlation between the level of infection and local temperature, humidity and precipitation was detected, there was, however, the tendency of higher rates of infection during the wet season and when population densities were high (*Fig. 3*). This relationship has also been observed in the Philippines where epizootics due to fungal diseases of leucaena psyllids occurred most frequently during wet seasons (Villacarlos *et al.* 1989).

CONCLUSION

Hirsutella, a common fungus isolated from a wide range of insects (Samson *et al.* 1988), has not been fully exploited for use in the microbial control of insects. Only one species, *H. thompsonii* Fisher, has been widely and effectively used against citrus mites (McCoy 1981). *Hirsutella citriformis* which was tested with considerable success for the control of the rice brown planthopper, *Nilaparvata lugens* (Stahl) (Rombach *et al.* 1986) has yet to be accepted as one of the potential means of controlling the insect pest. The frequent occurrence of *H. citriformis* in the natural psyllid population suggests that the fungus may have potential as a microbial control agent for leucaena psyllid. Augmentation of the fungus through field application could possibly induce epizootics in the pest population. The success of such technique, however, will be dependent on detailed information on the dynamics of the host, pathogen and disease (Carruthers and Hural 1990).

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REFERENCE

ANON. 1977. *Leucaena: Promising Forage and Tree Crop for the Tropics.* Washington D.C: National Academy of Sciences.

CARRUTHERS, R. I. and K. HURAL. 1990. Fungi as a naturally occuring entomopathogens. In *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases.* eds. R. R. Baker and P. E. Dunn. p. 115-138. New York: Alan R. Liss, Inc.

HSIEH, H. J., Y. C. CHANG and F. J. PAN. 1987. The potential of entomogenous fungi as a factor in control of psyllid in Taiwan. *Leucaena Res. Rept.* **7**: 81-83.

LIM, G. S., C. L. TAN and C. C. WONG. 1989. Studies on leucaena psyllid in Malaysia. In *Leucaena Psyllid: Problems and Management.* eds. B. Napompeth and K.G. McDicken p. 28-39. Proc. International Workshop. Bogor, Indonesia.

MCCOY, C. W. 1981. Pest control by the fungus *Hirsutella thompsonii.* In *Microbial Control of Pests and Plant Disease.* ed. H. D. Burgess, p. 499-512. New York: Academic Press.

NAPOMPETH, B., A. WINOTAI and P. SOMMARTYA. 1989. Utilization of natural enemies for biological control of the leucaena psyllid in Thailand. In *Leucaena Psyllid: Problems and Management.* eds. B. Napompeth and K.G. McDicken, p. 175-180. *Proc. International Workshop.* Bogor, Indonesia.

ROMBACH, M. C., R. M. AGUDA, B. M. SHEPARD and D. W. ROBERTS. 1986. Infection of the brown planthopper, *Nilaparvata lugens* (Delphacidae: Homoptera) by field application of entomogenous fungi (Deuteromycotina). *Environ. Entomol.* **15:** 1070-1073.

SAMSON, R. A., H. C. EVANS and J. P. LATGE. 1988. *Atlas of Entomopathogenic Fungi.* Berlin: Springer-Verlag.

THO, Y. P. 1986. The jumping plant lice in Peninsular Malaysia. *MAPPS Newsl.* **10:** 3-4.

VILLACARLOS, L. T., R. V. PAGLINAWAN and R. P. ROBIN. 1989. Factors affecting leucaena psyllid populations in Leyte, Philippines. In *Leucaena Psyllid: Problems and Management.* eds. B. Napompeth and K. G. McDicken. p. 122-129. Proc. International Workshop. Bogor, Indonesia.

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Growth and Yield Potential of Green Pepper as Affected by Nitrogen at Transplanting

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Keywords: Green pepper, *Capsicum annuum*, fruit set, nitrogen

ABSTRAK

*Anak benih cili hijau (*Capsicum annuum *L. cv. Lady Bell) yang berumur 7 minggu diubah ke ladang. Kadar N berikut telah digunakan: 112, 224, 336 dan 448 kg/ha. Kadar N yang tinggi diberikan ketika mengubah tidak merangsangkan pertumbuhan tampang sebaliknya telah merencatkan pertumbuhan pokok terutama sekali pada jangkamasa awal pertumbuhan. Pertambahan kadar N menyebabkan hasil buah awal dan jumlah buah yang kurang. Hasil awal berkorelasi positif dengan berat kering pokok. Menggandakan kadar N dari 112 kepada 224 kg/ha menambahkan 21% jumlah kudup bunga dikeluarkan tetapi peratus kejadian buah berkurangan dengan pertambahan kadar N. Kejadian buah mempunyai korelasi negatif dengan kandungan N dalam daun dan korelasi positif dengan berat kering pokok, menunjukkan bahawa kandungan N yang tinggi dalam pokok dan berat kering pokok yang rendah akan menjejaskan kejadian buah dan hasil cili hijau.*

ABSTRACT

*Green pepper (*Capsicum annuum *L. cv. Lady Bell) was grown for 7 weeks and transplanted into the field. The following rates of N were applied: 112, 224, 336 and 448 kg/ha. High N rates at transplanting did not stimulate vegetative growth but suppressed plant growth, particularly during the early growing period. As N rates increased, plants exhibited poor early growth and produced lower early and total fruit yields. Early yield positively correlated with plant dry weight. Doubling the N rate from112 to 224 kg/ha resulted in a 21% increase in flower buds, but the percentage of fruit set decreased as N rates increased. Fruit set correlated negatively with total leaf N and positively with plant weight, suggesting that a high leaf N content and a lower plant weight were detrimental to fruit set and yield of green pepper.*

INTRODUCTION

The problems of low fruit yield of green pepper are often related to poor fruit set. Poor fruit set may be due to incomplete pollination or effects of environmental factors such as moisture and nutrient stress. Nitrogen is known to be the most important nutrient affecting fruit yield in pepper. Nitrogen influences flower development of several vegetable crops including pepper, tomato and cucumber (Kinet *et al.* 1985). In tomato, high N increases flower formation but not fruit set (Garrison *et al.* 1967). However, the effect of N fertilizer upon flowering and fruit set of green pepper has produced contradictory results. Higher rates of N increased the number of blossoms formed and fruit set (Cochran 1933), but Maynard *et al.* (1962) found that although high N rates increased fruit set, flower production decreased as the plant reached physiological maturity. Schmidt (1983) found that increased N did not affect the number of flower buds or fruit set.

Early N availability appears necessary for plant growth, fruit size and yield of pepper. The yield was greater with a single N application than split applications when plants were grown under mulch (Locascio *et al.* 1985). Similarly, fruit weight was heavier with preplant than with split applications when slow-release N fertilizers were applied (Wiedenfeld 1986). Improved N

availability during early growth increased fruit size. However, plants receiving excess N produced excess foliage and decreased yield (Stroehlein and Oebker 1979). Thus, the purpose of this experiment was to determine the effects of high nitrogen rates at transplanting on growth, fruit set and yield of green pepper.

MATERIALS AND METHODS

Green pepper (*Capsicum annuum* L. cv. Lady Bell) seeds were sown in 5 cm^2 cell size containing peat:vermiculite (2:1 v/v) in the glasshouse. Seedlings received no supplemental light and were fertilized 4 weeks after emergence with 3g of 20:20:20 (N:P:K). When the plants were 5 weeks old, they were hardened for 2 weeks. Uniform, 7-week-old seedlings were transplanted into a Flanagan silty clay loam (fine Montmorillonitic Mesic, 5% organic matter, cation exchange capacity of 23.5 meq/100 g, and a pH of 6.1). Plants were spaced 1.2 m apart in and between rows in a randomized complete block design, with four replications consisting of four rows of 40 plants. Prior to planting, 50 kg/ha of P and 300 kg/ha of K were broadcast and incorporated by disking. Urea was applied in a single application 3 days after transplanting at 112 (the recommended rate, (Gerber and Swiader 1985)), 224, 336 and 488 kg N/ha. Plots were irrigated with 1.25 cm of water immediately after fertilizer application.

Weeds were controlled with a preplant application of trifluralin (α-trifluror-2-6-dinitro-N, N-dipropyl-p-toluidine) at 0.75 kg a.i./ha, with mechanical and manual cultivation as needed. Plots were sprinkler irrigated when necessary during the growing season.

The above-ground parts of 4 plants were sampled from each plot at three sampling dates during the growing season to determine plant growth response. Sampling dates were: 40 days after transplanting (40 DAT) when plants were setting fruits; after the second fruit harvest (70 DAT); and one week before the last harvest (100 DAT). Fresh weight and dry weight of plants (24 h, 70°C) were measured. At each sampling date, newly-expanded leaves were randomly collected for total N analysis. Leaves were washed with distilled water, dried, ground (100mg used), and total N (duplicate samples) determined by the method of Nelson and Sommers (1973).

Mature green fruits were harvested five times at 10-14 day intervals. The marketable fruits were counted, weighed and recorded. Diseased, chlorotic and small fruits were recorded as culls. Fruit yield data were based on 20 plants from each replication.

Three plants per replication were randomly selected to determine the number of flower buds produced and fruit set. Flower buds 3-4 mm in diameter were tagged and recorded for eight weeks. The number of marketable fruits, cull and small fruits left on the plant at last harvest were harvested and counted.

Data were analyzed by analysis of variance to test the effect of N levels on the various parameters. Comparisons between means were made by application of Fisher's least significant difference (LSD), where the F test indicated significant effects at 5% level. The relation between variables was determined using correlation coefficients.

RESULTS AND DISCUSSION

Plant Growth.

The effect of high N during early plant growth is critical. Once early growth was retarded, the plants did not recover very well as the season progressed. Plants grown with high N generally were relatively small at 3 sampling dates (Table 1), indicating that high N did not stimulate vegetative plant growth. Early plant growth, as shown by the first sampling date, was significantly suppressed by high N. At N rates higher than 224 kg/ha, the plant dry weights were significantly lower than those at lower N rates. Although plant height was not measured, we observed visually that plants grown with high N were short, bushy and very dark. Some leaves, particularly the lower ones, were chlorotic. Locascio *et al.* (1981) found that yield and dry weight of pepper plants were reduced when soluble N sources were broadcast at 308 kg/ha. A large amount of highly soluble N, when applied at transplanting, can be harmful to seedlings and reduce plant stand and early plant growth. Exessive N can result in excess soil salts, which probably is the major cause of plant growth reduction.

Plant dry weight increased as the season progressed (Table 1). There was no significant difference in plant dry weight measured at 100 days after transplanting at N rates lower than

336 kg/ha, indicating a slow recovery in growth for plants with high N at transplanting. Nitrogen may have been limiting at the lower N rates as the season progressed, whereas at higher N rates, more N was still available during the later part of the growing season.

TABLE 1
Effect of nitrogen levels at transplanting on dry weight of green pepper plants (g/plant)

N (kg/ha)	Days after transplanting		
	40	70	100
112	8.7 a	39.6 a	77.5 a
224	8.0 ab	39.8 a	76.5 a
336	7.9 b	34.2 b	75.6 ab
448	6.6 c	30.6 b	73.6 b

Values in a column followed by the same letter do not differ significantly at 5% by LSD test.

Fruit Yield.

Early fruit yield, as indicated by harvest 1 and 2, was significantly reduced by high N (Table 2). The number of marketable fruit decreased by 54 and 62% for the first harvest and by 26 and 53% for the second harvest as N was increased from 112 to 336 and 448 kg/ha, respectively.

Total fruit yield was significantly affected by N rates. Total marketable fruit weight per plant decreased by 0.5 kg per plant (Table 2) as N levels were increased from 112 to 448 kg/ha. Total fruit weight correlated positively with the number of fruit (r = 0.98). The low total fruit weight at high N was due to fewer fruit per plant. Although no statistical differences were observed, pepper fruits were smallest in size at the highest N levels. However, fruits were darker green as N levels increased. High N levels are known to increase N content in plant tissues and improve fruit colour (Miller 1960).

The reduction in pepper fruit yield was influenced by the effect of N levels on early plant growth. In general, plants which showed vigorous early growth produced greater yields. When early growth was vegetatively affected due to high N at transplanting, less fruit was produced at early harvests and consequently less total yield was realized. When large amounts of highly soluble N fertilizer were applied early, plant growth was retarded and fruit yield was low (Locascio *et al.* 1981; Wiedenfeld 1986; Hochmuth *et al.* 1987). Total fruit number from harvest 1 and 2 correlated positively (r = 0.73) with plant dry weight (Table 2). This indicates that more fruits were produced by plants which had greater dry weight.

Flowering and Fruit Set

Nitrogen rates influenced flower bud production (Table 3). Doubling the N rate from 112 to 224 kg/ha resulted in increased flower buds from 118.4 to 143.5 (or 21%) per plant. Nitrogen rates greater than 224 kg/ha did not result

TABLE 2
Effect of nitrogen levels at transplanting on marketable fruit yield of green pepper

N (kg/ha)	No. of fruits/plant		Total maketable fruits/plant		Av wt/ fruit
	Harvest 1	Harvest 2	No.	Wt(kg)	(g)
112	1.6 a	1.9 a	16.7 a	2.1 a	125.7 a
224	1.4 a	1.7 ab	16.9 a	2.2 a	130.2 a
336	0.7 b	1.4 b	15.8 ab	2.1 a	132.9 a
448	0.6 b	0.9 c	13.3 b	1.6 b	120.3 a

Values in a column followed by the same letter do not differ significantly at 5% by LSD test.

TABLE 3
Effects of nitrogen levels at transplanting on number of flower buds and fruits and fruit set per plant of green pepper

N (kg/ha)	Flower buds/plant (no.)	Fruits/plant (no.)	Fruit set (%)
112	118.4b	41.8a	35.3a
224	143.5a	38.4ab	26.9b
336	129.4b	35.0b	27.1b
448	144.6a	33.1c	22.8c

Values in a column followed by the same letter do not differ significantly at 5% by LSD test.
Total fruit includes marketable, cull and small fruits left on the plant after harvest.

TABLE 4
Effect of nitrogen levels at transplanting on total leaf nitrogen in green pepper

N (kg/ha)	Days after transplanting		
	40	70	100
		%N	
112	4.6c	4.4c	2.7d
224	4.7b	4.6b	2.8c
336	4.9a	4.9a	3.0b
448	4.9a	4.9a	3.1a

Values in a column followed by the same letter do not differ significantly at 5% by LSD test.

in a further increase in the number of flower buds.

Fruit set is defined as fruit number relative to flower bud number. The number of fruits produced did not increase as flower buds increased. The number of buds increased with increased N rates. However, fruit number and fruit set per plant significantly decreased as N rates were increased from 112 kg/ha to 448 kg/ha. This is a 35 % reduction in fruit set. A similar result was also found with tomato where flower formation (but not fruit set) increased with high N rates (Garrison *et al.* 1977).

The difference between flower bud formation and fruit production induced by high rates of N indicates that excessive N at transplanting promotes bud production but is detrimental to fruit set. A large number of flowers was lost at higher N rates. However, these losses may have been compensated for by plants producing more flower buds initially. The plants are capable of utilizing the additional N by producing more flower buds, but cease to produce fruit set and develop. Furthermore, since fruits were allowed to grow until they reached the mature green stage, the strong sink effects from developing fruits caused more flower abscission before fruits were set or induced more young fruits to abort.

Leaf N Content. Regardless of N treatment, total leaf N was high at early plant growth stage and gradually decreased over the season (Table 4). Total N ranged from 4.6 to 4.9% at 40 days and 2.7 to 3.1% at 100 days after transplanting. As the season progressed, plants increased in size, and N concentration declined due to its dilution with rapidly accumulating carbohydrate products of photosynthesis (Knavel 1977).

Thomas and Heilman (1964) reported that the critical level of leaf N was about 4% and N deficiency symptoms were observed when leaf N concentration was 3.8 to 4%. Leaf N was greater than 4.4% at the first two sampling dates for all treatments. However, it decreased markedly towards the end of the growing season. Some N deficiency symptoms were observed on plants at the low N rates at the final sampling date.

A negative correlation was found between leaf N concentration and the total number of fruits per plant ($r = -0.69$). The number of fruit at the first ($r = 0.65$) and second ($r = -0.64$) harvests correlated negatively but significantly with leaf N concentration sampled at 40 days. Fruit set correlated with leaf N content ($r = -0.57$) negatively and positively with plant dry weight ($r = 0.78$). This suggests that N concentration during early plant growth influenced early fruit production. External N levels were effective in modifying the internal N levels which apparently influenced the fruit set.

CONCLUSION

The study shows that application of high amounts of N at transplanting is not recommended for

pepper fruit production and could be a concern for pepper growers. Providing high N at the early plant growth stage was detrimental to fruit set and yield.

REFERENCES

COCHRAN, H.L. 1933. Factors affecting flowering and fruit setting in the pepper. *Proc. Amer. Soc. Hort Sci.* **29:** 434-437.

GARRISON, S.A., C.A. TAYLOR and W.O. DRINKWATER. 1967. The influence of nitrogen nutrition on flowering, fruit set and yield of processing tomatoes. *J. Amer. Soc. Hort. Sci.* **91:** 534-543.

GERBER, J.M. and J.M. SWIADER. 1985. Fertilizer guide for commercial vegetable growers. Univ. of Ill. at Urbana-Champaign, College of Agriculture. Coop. Ext. Service Cir. 1185.

HOCHMUTH, G.J., K.D. SHULER, R.L. MITCHELL and P.R. GILREATH. 1987. Nitrogen crop nutrient requirement demonstrations for mulched pepper in Florida. *Proc. Fla. State Hort. Soc.* **100:** 205-209.

KINET, J.M., R.M. SACHS and G. BERNIER. 1985. *The Physiology of Flowering.* Vol I. CRC Press Inc.

KNAVEL, D.E. 1977. The influence of nitrogen on pepper transplant growth and yielding potential of plants grown with different levels of soil nitrogen. *J. Amer. Soc. Hort. Sci.* **102:** 533-535.

LOCASCIO, S. J., J.G.A. FISKELL and F.C. MARTIN. 1981. Response of bell pepper to nitrogen sources. *J. Amer. Soc. Hort. Sci.* **106:** 628-632.

LOCASCIO, S.J., J.G.A. FISKELL and D.A. GRAETZ. 1985. Nitrogen accumulation by pepper as influenced by mulch and time of fertilizer application. *J. Amer. Soc. Hort. Sci.* **110:** 325-328.

MAYNARD, D. N., W.H. LACHMAN, R.M. CHECK and H.F. VERNELL. 1962. The influence of nitrogen levels on flowering and fruit set of peppers. *Proc. Amer. Soc. Hort. Sci.* **81:** 385-389.

MILLER, C.H. 1960. Some effects of different levels of 5 nutrient elements on bell pepper. *J. Amer. Soc. Hort. Sci.* **77:** 440-448.

NELSON, D.W. and L.E. SOMMERS. 1973. Determination of total nitrogen in plant material. *Agron. J.* **65:** 109-111.

SCHMIDT, J.C. 1983. Influence of sidedressed nitrogen on flowering and fruit set of bell pepper. M.S. Thesis, Univ. of Illinois, Urbana, Illinois, U.S.A.

STROEHLEIN, J.L. and N.F. OEBKER. 1979. The effects of nitrogen and phosphorus on yield and tissue analysis of chilli peppers. *Commun. Soil. Sci. Plant Anal.* **10:** 551-563.

THOMAS, J.R. and M.D. Heilman 1964. Nitrogen and phosphorus content of leaf tissue in relation to sweet pepper yields. *Proc. Amer. Soc. Hort. Sci.* **85:** 419-425.

WIEDENFELD R.P. 1986. Rate, timing, and slow-release nitrogen fertilizer on bell peppers and muskmelon. *Hort Science* **21:** 233-235.

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New Host Records of Parasites in the Malayan Red Jungle Fowl, *Gallus gallus spadiceus*

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Keywords: New host records, parasites, Malayan red jungle fowl.

ABSTRAK

Tiga puluh spesies parasit telah dijumpai di dalam tujuh ekor ayam hutan merah Malaya jantan dewasa Gallus gallus spadiceus *dari Ulu Langat, Selangor, Malaysia. Lima belas daripada spesies parasit adalah rekod baru bagi perumah ini, iaitu* Pseudolynchia canariensis, Eimeria *sp.,* Trichomonas gallinarum, Ascaridia galli, Capillaria annulata, Capillaria contorta, Cardiofilaria nilesi, Dispharynx spiralis, Gongylonema *sp.,* Heterakis gallinarum, Tetrameres fissispina, Hymenolepis cantaniana, Raillietina cesticillus, R. tetragona *dan* Postharmostomum gallinum. *Parasit yang paling biasa terdapati ialah spesies nematod* Heterakis *dan* Capillaria.

ABSTRACT

Thirty species of parasites were recovered from seven adult male Malayan red jungle fowls Gallus gallus spadiceus *from Ulu Langat, Selangor, Malaysia. Fifteen species of the parasites are newly recorded for this host; they are* Pseudolynchia canariensis, Eimeria *sp.*, Trichomonas gallinarum, Ascaridia galli, Capillaria annulata, Capillaria contorta, Cardiofilaria nilesi, Dispharynx spiralis, Gongylonema *sp.,* Heterakis gallinarum, Tetrameres fissispina, Hymenolepis cantaniana, Raillietina cesticillus, Raillietina tetragona *and* Postharmostomum gallinum. *The most common parasites are the nematode species of* Heterakis *and* Capillaria.

INTRODUCTION

The Malayan red jungle fowl, *Gallus gallus spadiceus* is one of the four species of jungle fowl found in the Indian subcontinent and South East Asia. It is regarded as the ancestor of the domestic fowl (*Gallus domesticus*) due to its widespread distribution. The other three species of jungle fowl are the grey jungle fowl (*Gallus sonneretii*), the Ceylonese jungle fowl (*Gallus lafayetti*) and the green jungle fowl (*Gallus varius*) (Beebe 1926; Nishida *et al.*1985).

There are several reports on parasites of the Malayan red jungle fowl (Chin *et al.* 1974; Dissanaike and Fernando 1974a, 1974b; Fernando and Dissanaike 1975; Amin-Babjee *et al.* 1985; Lee *et al.* 1985a, 1985b; Lee *et al.* 1989a, 1989b; Lee and Amin-Babjee 1990). In the case of the Ceylonese jungle fowl, *Ascaridia galli, Raillietina tetragona* and *Eimeria praecox* are the only parasites recorded (Rysavy *et al.* 1973; Long *et al.* 1974), whereas in the grey jungle fowl *Lemdana sonneretta* is the only parasite recorded (Ali 1969). The objective of this study was to compile a check-list of all species of parasites collected from the Malayan red jungle fowl.

MATERIALS AND METHODS

Seven red jungle fowls were obtained from Ulu Langat district, Selangor State, Malaysia. One thin blood film was prepared directly on a clean glass slide and about 2 ml of blood was collected in a heparinised tube from each bird. The thin films were dried at room temperature, fixed in methanol and stained in 10% Giemsa-buffer solution in a coplin jar. From the heparinised blood, direct wet smears were made and examined for any active parasites like microfilaria or trypanosome and if negative for microfilaria, Knotts blood concentration technique was performed to verify it.

After autopsy, the feathers were soaked in 70% alcohol for any ectoparasite. The intestines and organs were opened separately with scissors and soaked in normal saline (NS) for helminths. Helminths collected were rinsed twice in NS and relaxed in hot 70% alcohol (for nematodes); wrapped around glass slides (for cestodes) or placed between glass slides (for trematodes), tied loosely with rubber bands and fixed in hot 70% alcohol. The eyes, ears and skin were searched grossly for parasites. Rectal faeces were processed and floated with saturated sodium chloride for ova or oocysts.

TABLE 1
Parasites from the Malayan red jungle fowls

Parasite	Site	No of birds	
		examined	infected
ARTHROPODS			
Lipeurus caponis (lice)	plumage	7	1
Menopon gallinae (lice)	plumage	7	4
Megninia cubitalis (mites)	plumage	7	2
*Pseudolynchia canariensis** (hippoboscids)	plumage	7	1
PROTOZOA			
*Eimeria sp**	faeces	7	1
Plasmodium gallinaceum	blood	7	1
*Trichomonas gallinarum**	caecum	7	1
NEMATODES			
*Ascaridia galli**	small intestine	7	1
*Capillaria annulata**	crop	7	1
*Capillaria contorta**	gizzard	7	1
Capillaria obsignata	caecum	7	5
*Cardiofilaria nilesi**	body cavity	7	1
*Dispharynx spiralis**	proventriculus	7	1
*Gongylonema sp.**	crop	7	2
Heterakis beramporia	caecum	7	3
*Heterakis gallinarum**	caecum	7	5
Lemdana latifi	body cavity	7	3
Lemdana sonneretta	body cavity	7	1
Oxyspirura mansoni	eye	7	3
Pelecitus galli	base of leg	7	1
Strongyloides avium	small intestine	7	1
*Tetrameres fissispina**	proventriculus	7	1
CESTODES			
Amoebotaenia cuneata	duodenum	7	3
Davainea proglottina	duodenum	7	4
*Hymenolepis cantaniana**	small intestine	7	1
*Raillietina cesticillus**	small intestine	7	3
Raillietina echinobothridia	small intestine	7	4
*Raillietina tetragona**	small intestine	7	1
TREMATODES			
*Postharmostomum gallinum**	caecum	7	2
Tanaisia vietnamensis	kidney	7	1

* New host records

Parasites were gathered by using the stereomicroscope. Mites were mounted in Hoyers medium, nematodes in lactophenol and cestodes/trematodes stained with acid-alum-carmine for identification under a compound microscope.

RESULTS AND DISCUSSION

Thirty species of parasites have been recovered from the red jungle fowl. Fifteen of the species from the present study are considered new records for this host. The new host records are *Pseudolynchia canariensis, Eimeria* sp., *Trichomonas gallinarum, Ascaridia galli, Capillaria annulata, C. contorta, Cardiofilaria nilesi, Dispharynx spiralis, Gongylonema* sp., *Heterakis gallinarum, Tetrameres fissispina, Hymenolepis cantaniana, Raillietina cesticillus, R. tetragona* and *Postharmostomum gallinum.*

Twenty other species of parasites reported by Amin-Babjee *et al.* (1985) were not observed in this study. They are *Haemaphysalis wellingtoni, Neoschongastia gallinarum, Megninia cubitalis, Menopon gallinae, Lipeurus caponis* and *Gonoides dissimilis* (all arthropods); *Plasmodium gallinaceum, Plasmodium juxtanucleare* and *Leucocytozoon sabrazesi* (protozoa); *Heterakis berampora, Capillaria obsignata, Lemdana* sp., *Tetrameres crammi, Cheilospirura hamulosa, Syngamus trachea* and *Strongyloides avium* (nematodes), *Mediorhynchus gallinarum* (acanthocephala); *Raillietina echinobothrida, Amoebotaenia* sp. and *Davainea* sp. (cestodes); *Tanaisia vietnamensis* and Heterophyginae family (both trematodes). Their absence in this study may be due to the larger number of fowls studied (16) from a wider area (Banting, Dengkil and Kajang) by Amin-Babjee *et al.* (1985) as compared to only seven birds from Ulu Langat district in the present study.

There was only a single specimen of the fly *Pseudolynchia canariensis* encountered from one of the birds. This fly is commonly present in pigeons but may exist in some wild birds (Soulsby 1986).

The few oocysts seen in the rectal faeces of one of the jungle fowls were identified as that of *Eimeria* sp. Morphologically they were small, ovoidal and without micropyle. They may be similar to some of the species of coccidia found in domestic chicken since species of jungle fowl in Asia are considered to be the ancestors of the domestic fowl (Long *et al.* 1974).

The single nematode *Gongylonema* was not identified down to the species level because no male specimen was available for the purpose.

However, this study corresponds to an earlier study in which the most common parasites were the nematodes *Heterakis (H. beramporia* and *H. gallinarum)* and *Capillaria (C. annulata, C. contorta* and *C. obsignata)* (Amin-Babjee *et al.* 1985).

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REFERENCES

ALI, M.M. 1969. Observations on some filariid worms from Indian birds with a description of two new genera. *Zool. Anz.* **183:** 309-316.

AMIN-BABJEE, S.M., C.C. LEE and M. KRISHNASAMY. 1985. A preliminary survey of parasites of Malaysian red jungle fowl (*Gallus gallus spadiceus*). *Kaj. Vet.* **17:** 141-146.

BEEBE, W. 1926. *Pheasants: Their Lives and Homes.* Garden City, New York: Doubleday, Page & Co.

CHIN, H.C., A.S. DISSANAIKE and M.A. FERNANDO. 1974. *Leucocytozoon sabrazesi* - first record in the Malaysian jungle fowl, *Gallus gallus spadiceus. SEA J. Trop. Med. Publ. Hlth.* **5:** 455.

DISSANAIKE, A.S. and M.A. FERNANDO. 1974a. Filarioids and a trypanosome from the Malaysian jungle fowl, *Gallus-gallus spadiceus. SEA J. Trop. Med. Publ. Hlth.* **5:** 138.

DISSANAIKE, A.S. and M.A. FERNANDO. 1974b. *Pelecitus galli* n. sp. from the Malaysian jungle fowl *Gallus gallus spadiceus. J. Helminthol.* **48:** 199-203.

FERNANDO, M.A. and A.S. DISSANAIKE. 1975. Studies on *Plasmodium gallinaceum* and *P. juxtanucleare* from the Malaysian jungle fowl (*Gallus gallus spadiceus*). *SEA J. Trop. Med. Publ. Hlth.* **6:** 25-32.

LEE, C.C., S.M. AMIN-BABJEE and M. KRISNASAMY. 1985a. Report of *Mediorhynchus gallinarum* (Bhalerao, 1937) Van Cleave, 1947 in a new host, *Gallus gallus spadiceus* from Peninsular Malaysia. *Kaj. Vet.* **17:** 71-73.

LEE, C.C., M. KRISNASAMY, S.M. AMIN-BABJEE and J.D. DUNSMORE. 1985b. *Tanaisia (Tamerlania) zarudnyi vietnamensis* Odening, 1963 (Eucotylidae: Trematoda) from the red jungle fowl *Gallus gallus spadiceus* in Peninsular Malaysia. *Trop. Biomed.* **2:** 93-97.

LEE, C.C., S.M. AMIN-BABJEE and M. KRISHNASAMY. 1989a. *Lemdana latifi* n.sp. (Filarioidea: Onchocercidae) from the red jungle fowl (*Gallus gallus spadiceus*). *System. Parasitol.* **13:** 213-237.

LEE, C.C., S.M. AMIN-BABJEE and M. NOORDIN. 1989b. Heavy infestation of *Postharmostomum gallinum* in a crossbred jungle fowl. *J. Vet. Malays.* **1:** 61-62.

LEE, C.C. and S.M. AMIN-BABJEE. 1990. *Lemdana sonneretta* in the Malayan red jungle fowl (*Gallus gallus spadiceus*). *Malays. Appl. Biol.* **19:** 91-94.

LONG, P.L., M.A FERNANDO and O. REMMLER. 1974. Experimental infections of the domestic fowl with a variant of *Eimeria praecox* from the Ceylon jungle fowl. *Parasitology* **69:** 1-9.

NISHIDA, T., Y. HAYASHI, T. HASHIGUCHI and S. MANSJOER. 1985. Morphological identification and distribution of jungle fowls in Indonesia. *Jap. J. Zootech. Sci.* **56:** 598-610.

RYSAVY, B. V. BARUS and M.A. FERNANDO. 1973. Two helminth species from *Gallus lafayetti* (Galliformes) in Ceylon. *Folia Parasitol.* **20:** 39-40.

SOULSBY, E.J.L. 1986. *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th Edn. London: Bailliere Tindall. 809 pp.

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Preliminary Study of the Seagrass Flora of Sabah, Malaysia

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ABSTRAK

Suatu tinjauan terhadap komposisi, taburan serta biomas beberapa dataran rumput laut yang terdapat di sepanjang pantai Sabah telah dilakukan. Di dalam tinjauan ini, sejumlah enam genera yang terdiri daripada sembilan spesies rumput laut telah direkodkan. Lima daripada spesies tersebut iaitu Cymodocea rotundata *Ehrenb.* et *Hempr.* ex *Aschers.*, Cymodocea serrulata *(R. Br.) Aschers.* et *Magnus,* Halodule uninervis *(Forssk.) Aschers.,* Halodule pinifolia *(Miki) den Hartog dan* Syringodium isoetifolium *(Aschers.) Dandy yang belum pernah dilaporkan sebelum ini merupakan rekod baru bagi kawasan Sabah. Spesies yang kerap ditemui di stesen-stesen persampelan ialah* Halophila ovalis *(R. Br.) J.D. Hook dan diikuti oleh* Thalassia hemprichii *(Ehrenb.) Aschers. serta* Enhalus acoroides *(L.f.) Royle. Rumput laut telah diperhatikan wujud di kawasan intertidal sehingga ke kedalaman 2.5 m dan tumbuh di atas berbagai jenis substrat seperti pecahan karang, pasir dan pasir berlumpur. Walaupun taburannya tidak menunjukkan penzonan yang spesifik, namun terdapat dua zon yang boleh dibezakan berdasarkan spesies yang paling melimpah. Dataran rumput laut di Sabah juga di dapati menghasilkan biomas yang tinggi terutamanya habitat berlumpur yang membatasi kawasan pokok bakau (contohnya biomas total* E. acoroides *sehingga mencapai 468.5 g berat kering tanpa abu. m^2). Sementara dataran rumput laut di sekitar pulau yang di luar pantai seperti Pulau Sipadan,* T. hemprichii *(biomas 146 g berat kering tanpa abu. m^2) didapati merupakan pengeluar biomas yang utama.*

ABSTRACT

The species composition, distribution and the biomass of different seagrass beds along the coast of Sabah have been surveyed. Nine seagrass species belonging to six genera were recorded during the survey. Five of these i.e Cymodocea rotundata *Ehrenb.* et *Hempr.* ex *Aschers.*, Cymodocea serrulata *(R. Br.) Aschers.* et *Magnus,* Halodule uninervis *(Forssk.) Aschers.,* Halodule pinifolia *(Miki) den Hartog and* Syringodium isoetifolium *(Aschers.) Dandy have not previously been reported from this area, thus represent new records for Sabah. The most frequently encountered species at the sampling stations was* Halophila ovalis *(R. Br.) J.D. Hook followed by* Thalassia hemprichii *(Ehrenb.) Aschers and* Enhalus acoroides *(L.f.) Royle. Seagrasses were observed from intertidal zone down to 2.5 m depth on various substrate types such as coral rubble, sand to muddy-sand. There was no specific zonation in the distribution of seagrasses. However, two zones may be distinguished according to the most abundant species. The seagrass beds in Sabah were also found to produce very high biomass particularly in the muddy habitat bordering mangroves (e.g. total biomass* E. acoroides *amounted to 468.5 g AFDW m^2). Among the seagrass beds around the off-shore islands such as Pulau Sipadan,* T. hemprichii *(146 g AFDW m^2) was found to be a very important biomass contributor.*

INTRODUCTION

At least nine species of seagrasses are found in Malaysian waters (Fortes 1990). While information regarding recent local records of seagrass are available for the Malaysian Peninsula (Phang and Pubalan 1989), knowledge of the seagrasses in Sabah, however, is almost exclusively from the three previously reported species of *Halophila ovalis* (R. Br.) Hook. f., *Thalassia hemprichii* (Ehrenb.) Aschers. and *Enhalus acoroides* (L.*f.*) Royle (den Hartog 1970) collected from Labuan, Sandakan and Lahad Datu. Furthermore, no

direct studies on the local seagrass ecosystem have yet been done though it is an important habitat for coastal fishery resources. The objectives of this study were to survey for seagrass composition, their biomass and distribution along the coast of Sabah.

MATERIALS AND METHODS

Investigations on the seagrass ecosystem along the coast of Sabah were carried out from July 1991 to June 1992. *Fig. 1* depicts the geographical location of the study area. The composition and distribution of seagrass beds were studied by wading and snorkling; specimens were taken from near shore to a depth of about 1 - 2 m during low tides. The specimens were either dried and mounted or preserved in 4% formalin in seawater. The collections have been lodged with the Marine Science Museum of the Universiti Kebangsaan Malaysia (Sabah Campus). Nomenclature, at generic and specific level, follows den Hartog (1970).

At the sampling stations of Sg. Salut, Tanjung Mengayau, Bak-Bak and Pulau Sipadan, the seagrass distributions were studied along transects perpendicular to the coastline. The transects extended from the upper shore to the low water level and continued until only very similar communities were observed over a long distance. Samples containing the above and below ground parts of the seagrasses were also collected for biomass determination, using a PVC cylinder, height 1 m and surface area 0.02 m^2, placed vertically over the sampling plots. The plants were thoroughly rinsed in sea water and taken to the laboratory. They were then separated into seagrass species and soaked in 5% phosphoric acid to remove epiphytes and subsequently rinsed in tap water. The species were fractionated into leaves, shoots, rhizomes and roots. Leaves and shoots were regarded as aboveground biomass, rhizome and roots as belowground biomass. All samples were oven dried at 105°C to constant weight. The dry matter of samples of plant fractions were combusted at 550°C for 2h and weighed again to determine the ash content (Zieman and Wetzel 1980).

RESULTS AND DISCUSSION

Distribution of Seagrasses

During the present investigation, nine species of seagrasses belonging to six genera were identified from the coastal waters of Sabah (Table 1). The maximum number of seagrasses, except *Syringodium isoetifolium* (Aschers.) Dandy, were found in several beds in Sapangar Bay in the west coast. There were also large numbers of species present from a single seagrass meadow at Bak-Bak in the Sulu Sea. Dense meadows of seagrasses with one or two dominant species have also been located in the east coast. The finding of *Cymodocea rotundata* Ehrenb. *et* Hempr. *ex* Aschers., *Cymodocea serrulata* (R. Br.) Aschers. *et* Magnus, *Halodule uninervis* (Forssk.) Aschers., *Halodule pinifolia* (Miki) den Hartog and *S. isoetifolium* represents the first record for Sabah. This finding has brought to nine the total number of seagrass species presently known from this region.

From the nine species of seagrasses found in this survey, seven were widely distributed. The most frequently encountered species was *Halophila ovalis* (R. Br.) J.D. Hook. This species was followed, according to abundance, by *Thalassia hemprichii* (Ehrenb.) Aschers, *Enhalus acoroides* (L.*f.*) Royle, *C. rotundata*, *H. uninervis*, *H. pinifolia*, *C. serrulata*. The range of remaining species, *Halophila minor* (Zoll.) den Hartog and *S. isoetifolium*, were limited. *S. isoetifolium* was restricted to one site of Bak-Bak in Kudat. This species may also occur elsewhere; however, it was not observed in the course of this study. More extensive collections may reveal the true distribution of this species in Sabah.

Although *H. ovalis* was frequently encountered from the mid intertidal zone down to a depth of 1.5m, it was very sparse in the study areas. This species inhabited a range of substrates: fine, muddy sediments in mangrove lagoons as well as coarse sandy bottoms on reef flats. At Pulau Maganting and Pulau Tabawan reef flats, the plant occurred in a wide patch and was covered by sand. Different morphological types of this species were observed. Among them, the specimens from Pulau Sipadan and Pulau Maganting were larger with broad leaves.

T. hemprichii has been observed to grow from low water level to a depth of 2.5 m. A very large patch of almost pure stands occurred and formed the dominant seagrass species in the clear water around Pulau Sipadan and Pulau Bohay Dulang. The habitat at both localities was not uniform and consisted of patches of coral, coral sand and coarse sand. On the reef flat at Tanjung Mengayau, this species was frequently observed in association with algal beds

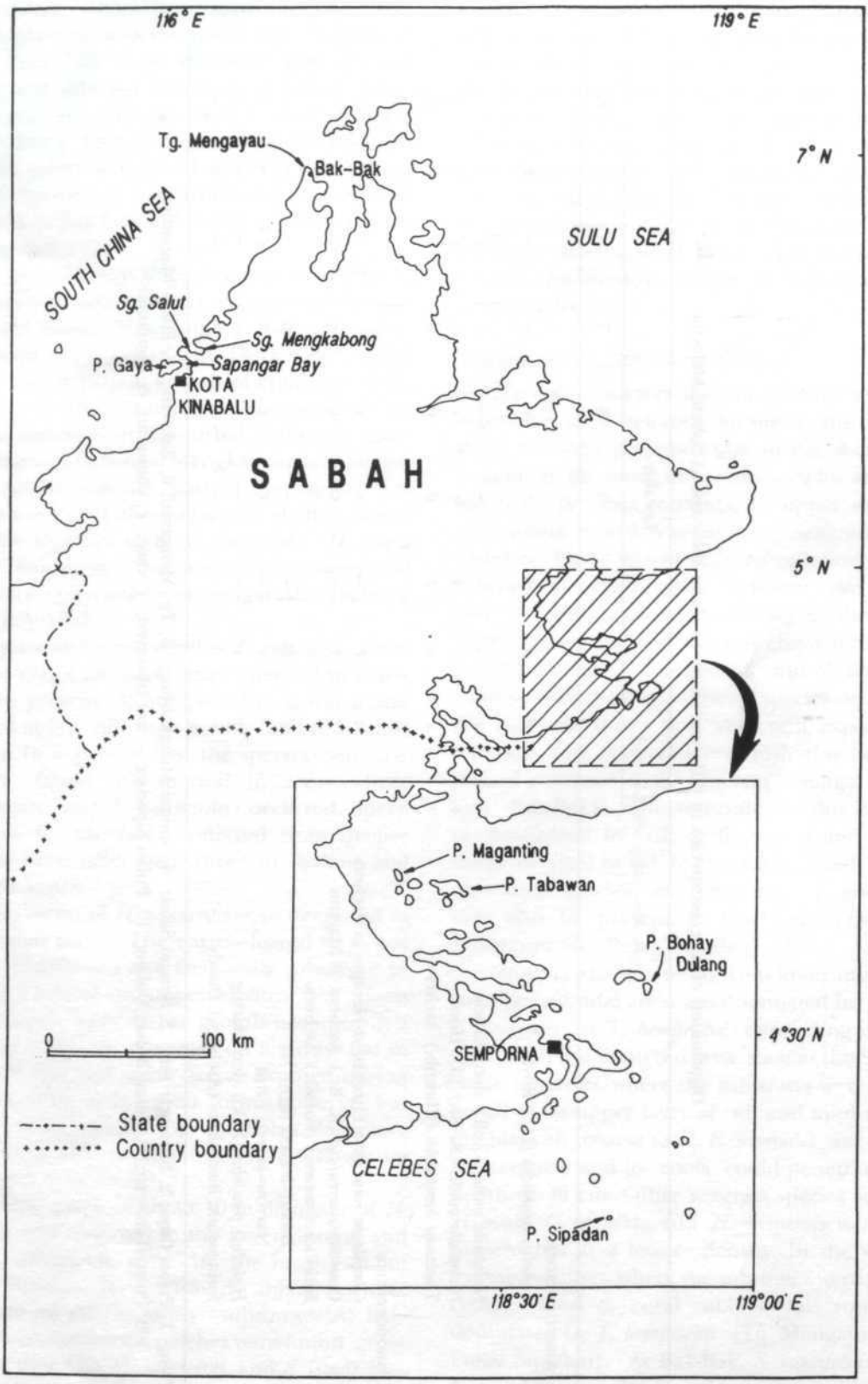

Fig. 1: Map of Sabah showing the study sites

TABLE 1

Distribution of seagrass species in different locations along the coast of Sabah, Malaysia

	Locality									
	1	2	3	4	5	6	7	8	9	10
Hydrocharitaceae										
Enhalus acoroides (L.*f.*) Royle	–	d	d	d	r	r	–	–	r	–
Halophila minor (Zoll.) den Hartog	–	r	–	–	–	–	r	r	–	–
Halophila ovalis (R. Br.) J.D. Hook.	r	c	c	r	r	r	c	c	r	r
Thalassia hemprichii (Ehrenb.) Aschers	r	c	c	r	d	d	d	–	d	d
Cymodoceaceae										
Cymodocea rotundata Ehrenb. *et* Hempr. *ex* Aschers.	–	c	c	–	r	r	–	r	–	c
Cymodocea serrulata (R. Br.) Aschers. *et* Magnus	–	r	r	–	c	c	–	–	–	–
Halodule uninervis (Forssk.) Aschers.										
Broad-leaved form	–	c	–	–	–	–	d	–	–	–
Narrow-leaved form	r	–	r	–	r	c	c	r	–	–
Halodule pinifolia (Miki) den Hartog	–	r	r	–	–	r	–	–	–	c
Syringodium isoetifolium (Aschers.) Dandy	–	–	–	–	–	c	–	–	–	–

Note: 1, Pulau Gaya; 2, Sapangar Bay; 3, Sungai Salut; 4, Sungai Mengkabong; 5, Tg. Mengayau; 6, Bak-Bak; 7, Pulau Maganting; 8, Pulau Tabawan; 9, Pulau Bohay Dulang; 10, Pulau Sipadan; –, not observed; r, rare; c, common; d, dominant

comprising *Sargassum* spp., *Hypnea* sp., *Acanthophora* sp. and *Gracilaria* spp. A patch of more than 100 m in diameter and exposed during low tide was found on a muddy sandy substrate of Sungai Salut and Sungai Mengkabong. This species was also recorded on a similar substrate type in Labuan by den Hartog (1970). Specimens with fruits and flowers were observed at Bak-Bak and Tanjung Mengayau on 16 February 1992.

E. acoroides beds were common in sheltered areas such as Sapangar Bay, estuaries of Sungai Salut and Sungai Mengkabong, from mid intertidal down to a depth of 2 m. Thick stands of the plants, with luxuriant growth of epiphytic algae, occurred on the sediments of fine mud rich in detrital materials in the turbid waters of mangrove lagoons of Sungai Mengkabong and Sungai Salut. At the seaward part of the lagoon, this species was found thriving on the shallow sandy substrate together with *C. rotundata*, *H. ovalis* and *T. hemprichii*. Specimens with flowers and fruit were collected from Sungai Mengkabong on 9 July 1991.

A pure and thick stand of *C. rotundata* community with a patch of more than 30 m diameter was present at Sungai Salut. It was found exposed at low tide on a muddy and fine sandy bottom. In Sapangar Bay, the species was frequently found interspersed in areas where *E. acoroides* and *T. hemprichii* occurred. Specimens of *C. rotundata* collected from deeper habitats were taller than those in shallow and exposed zones.

Two forms of *H. uninervis* were recorded in the present survey. The narrow-leaved form was widely distributed and frequently observed in the lower littoral and upper sublittoral of about 30 cm depth. Very dense growth occurred in a patch of > 8m in diameter on a sandy flat in Sapangar Bay and some were exposed during low tide. The wide-leaved form, however, was only found in Sapangar Bay and Pulau Maganting in the sublittoral of about 0.5 m. No flowering specimens were found.

A wide patch of about 10 m diameter of *H. pinifolia* was observed in the lower littoral and upper sublittoral zone in the north-east of Pulau Sipadan. It was found mixed with *C. rotundata* on coarse sandy substrates. At Bak-Bak, Kudat, occasional patches were found growing together with *H. uninervis*, and *S. isoetifolium* on coral sand under about 30 cm of water. In other localities it was rare.

C. serrulata was commonly found associated with *Thalassia* beds at Tanjung Mengayau on coarse sandy substrates and coral rubbles at depth < 30 cm. It was occasionally seen to occur at Bak-Bak, Sungai Salut and Sapangar Bay.

Other species such as *H. minor* and *S. isoetifolium* were not well distributed along the coast of Sabah. The species grew in the lower littoral and sublittoral down to about 1 m depth on sandy substrates. *H. minor* was also seen to inhabit the muddy bottom in Sapangar and formed pure beds.

Seagrass Distribution Along Transect

Fig (2a - d) illustrates the zonation of seagrass vegetation at 4 selected localities, distributed along transects perpendicular to the shoreline. In general, the most important area for seagrass was from the mid intertidal to upper subtidal zone where mixed vegetation of seagrass grow together. There was no specific zonation in the distribution of seagrasses. However, two zones may be distinguished according to the most abundant species. Zone 1 was characterized by the dominance of *C. rotundata* and *H. uninervis* (narrow-leaved form). These species occupied the intertidal zones and were well exposed at low tide and appeared brownish due to prolonged exposure to air and sun (Sungai Salut and Bak-Bak). The substrate on this zone is predominated by silt or fine sand and sometimes overlaid by soft to compacted mud. Other associated species, *H. ovalis* and *H. pinnifolia* may also be present in this zone (Tanjung Mengayau and Pulau Sipadan).

Zone 2, which extends from lower intertidal to upper subtidal areas was dominated by either *E. acoroides* or *T. hemprichii* depending on the habitat. In the sheltered area such as the Sungai Salut estuaries, where the substrates were composed of an upper layer of silt and mud overlying blackish coarse sand, *E. acoroides* was found to dominate and its roots could penetrate to a depth of 40 cm. Other seagrass species such as *H. ovalis*, *C. serrulata* and *H. uninervis* were also present but at a lower density. In the waved-exposed stations, where the substrates were sandy, coarse sand or coral rubbles, this zone was dominated by *T. hemprichii* (Tg. Mengayau and Pulau Sipadan). At Bak-Bak, *S. isoetifolium* and *C. serrulata* were also found to co-exist with equal density.

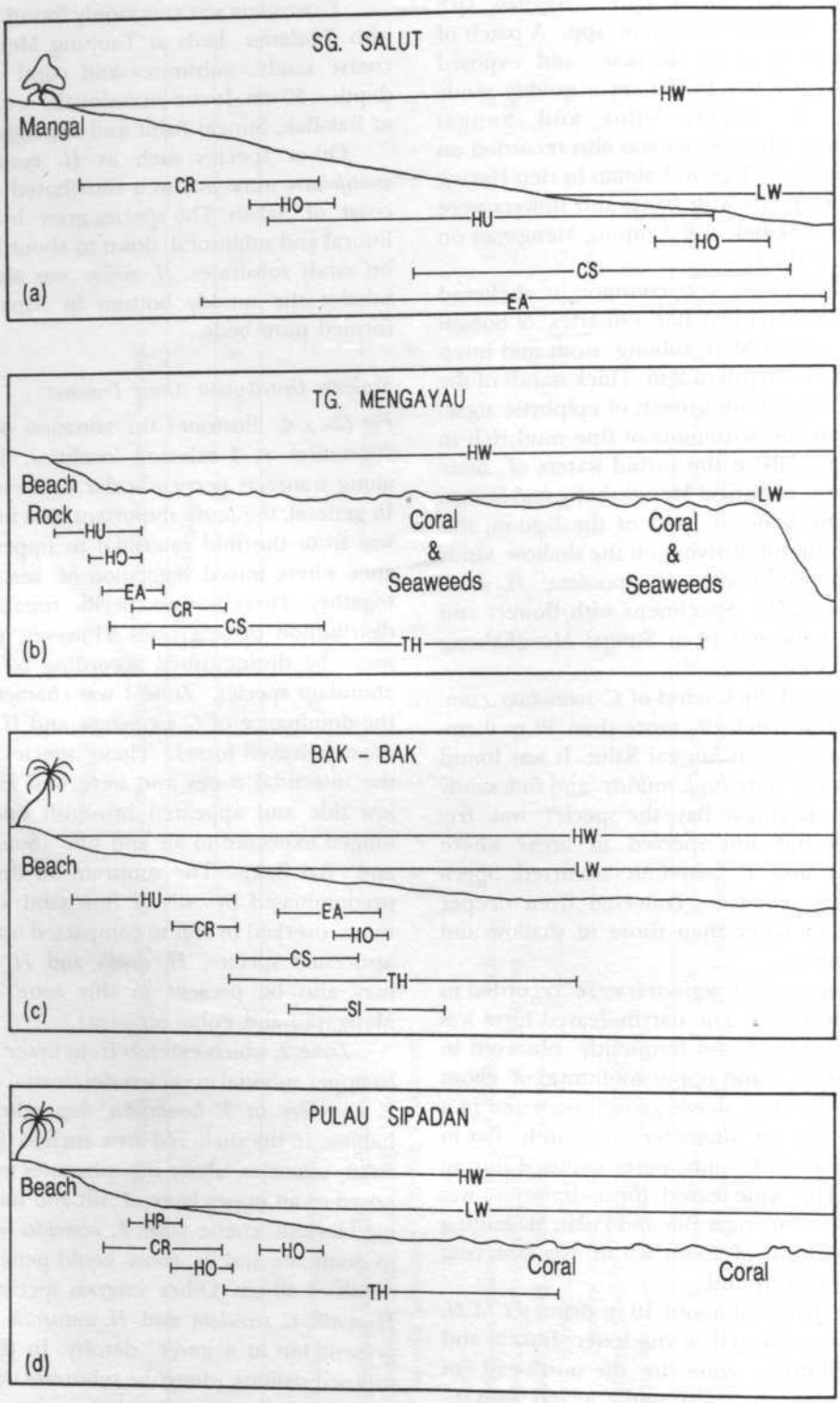

Fig. 2(a - d): Diagrammatic representation of the zonation pattern of seagrass vegetation along transects perpendicular to the shoreline at four different localities around Sabah. Approximate length of transects; Tg. Mengayau 300 m, Sg. Salut and Bak-Bak 200 m, P. Sipadan 150 m. Tidal amplitude (HW minus LW) approximately 1 - 2m. EA = Enhalus acoroides; *TH* = Thalassia hemprichii; *HO* = Halophila ovalis; *CS* = Cymodocea serrulata; *HU* = Halodule uninervis; *CR* = Cymodocea rotundata

TABLE 2

Biomass data of seagrass species in the quantitative samples (0.02 m^2) taken from Sg. Salut (23 - 25 Jan., 1992) and Pulau Sipadan (11 May, 1992)

Species	Locality	Type of vegetation	No. of samples	Mean AFDW gm^{-2} (± s.e.) AGB	BGB	Total	Ratio AGB : BGB
E. acoroides	Sg. Salut	Mixed	82	34.8± 3.5	433.8 ± 33.2	468.5± 33.7^{D}	1 : 12.5
C. rotundata	Sg. Salut	Pure stands	39	61.7± 4.5	144.5 ± 9.7	206.0± 12.9^{C}	1 : 2.3
C. rotundata	Sg. Salut	Mixed	30	17.2± 2.9	46.4 ± 8.1	63.6± 10.7^{B}	1 : 2.7
C. rotundata	P. Sipadan	Mixed	10	3.9± 1.8	11.4 ± 4.9	15.3± 6.6^{A}	1 : 2.9
C. serrulata	Sg. Salut	Mixed	24	6.8± 2.7	5.9 ± 2.1	12.8± 3.8^{A}	1 : 0.9
H. uninervis	Sg. Salut	Mixed	42	2.7± 0.4	6.9 ± 1.5	8.6± 1.4^{A}	1 : 2.6
H. ovalis	Sg. Salut	Mixed	13	0.6± 0.1	(entire plant)	0.6± 0.1^{A}	
T. hemprichii	P. Sipadan	Pure stands	10	34.7± 5.0	111.5 ± 16.2	146.2± 19.0^{B}	1 : 3.2

The values within the column with the same letter were not significantly different ($p < 0.05$) with the LSD Test.

Seagrass Biomass

No information is available on the biomass of seagrass in Sabah. The present studies on the biomass of leaves and shoot (above ground-biomass or AGB) as well as of rhizomes and roots (below-ground biomass or BGB), of seagrass species from Sg. Salut and Pulau Sipadan are shown in Table 2. Although biomass data for certain seagrass species from the Malaysian Peninsula are available (Mohd. Kusairi 1992), they may not be compared directly with the local data of this study, because different methods and data expression were used.

The results indicate that the seagrass beds particularly in the muddy habitat bordering mangroves were found to produce dense biomass. For example, the major biomass contributor in the habitat of Sungai Salut was *E. acoroides* (ANOVA), the largest seagrass species in the Indo-West Pacific (Table 2). This was followed by *C. rotundata* (pure stands and mixed vegetation). *E. acoroides* had a mean total (entire plant) biomass amounting to 468.5 g AFDW.m^{-2}, about 93% of which was contributed from its below-ground parts. However, this value is only 11.2% of the higher mean biomass of *E. acoroides* vegetation from the Flores Sea region (Nienhuis *et al.* 1989). The biomass of *C. rotundata* in pure stands was 206 g AFDW.m^{-2} which is comparable to the mean biomass (201 g AFDW. m^{-2}) of the species reported from Papua New Guinea (Brouns 1987). The largest proportion of the biomass values was attributed to the below-ground parts (about 70%). *C. rotundata* has a ratio of above-ground biomass (AGB) : below-ground biomass (BGB) of 1 : 2.3 in pure stands with the tendency of developing larger below-ground biomass in mixed vegetation (ratio 1: 2.7). *H. uninervis* studied from the mixed vegetation had a total biomass of 8.6 g AFDW. m^{-2}, with BGB about 3 times larger than AGB. Total biomass of *C. serrulata* and *H. ovalis* was 12.8 and 0.6 g AFDW.m^{-2}. These data of local *H. uninervis, C. serrulata* and *H. ovalis* are comparable to those for similar species from the Flores Sea, Indonesia (Nienhuis *et al.* 1989).

In contrast, the highest total biomass in the off-shore island of Pulau Sipadan in the Celebes Sea was recorded for *T. hemprichii* (146.2 g AFDW. m^{-2}). About 76% of the biomass of this species consisted of below-ground parts. The biomass of *C. rotundata* at this locality was significantly lower compared to that from Sg. Salut habitat (ANOVA). However, these data are within the observed range of those species from the Flores Sea, Indonesia (Nienhuis *et al.* 1989).

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REFERENCES

BROUNS, J.J.W.M. 1987. Aspects of production and biomass of four seagrass species (Cymodoceoideae) from Papua New Guinea. *Aquatic Botany* **27**: 333 - 362.

DEN HARTOG, C. 1970. *The Seagrasses of the World.* Amsterdam: North-Holland Publishing Co. pp. 275 .

FORTES, M.D. 1990. *Seagrasses: a Resource Unknown in the ASEAN Region.* ICLARM Education Series 5, Manila, pp. 46.

MOHD. KUSAIRI, M.R. 1992. Penemuan-penemuan kajian ekologi rumput laut di Semenanjung Malaysia. Paper presented at the Fisheries Research Seminar, Penang, Malaysia, 1992. Department of Fisheries, Ministry of Agriculture Malaysia.

NIENHUIS, P.H., J. COOSEN, and KISWARA, W. 1989. Community structure and biomass distribution of seagrasses and macrofauna in the Flores Sea, Indonesia. *Netherlands Journal of Sea Research* **23(2)**: 197-214.

PHANG, S.M. and R. PUBALAN. 1989. Seagrasses of Malaysia. Paper presented at the *First Southeast Asian Seagrass Resources Research and Management Workshop (SEAGRAM 1),* 17 - 22 January 1989. University of the Philippines, Diliman, Quezon City.

ZIEMAN, J.C. and R.G. WETZEL. 1980. Productivity in seagrasses: methods and rates. In: *Handbook of Seagrass Biology: an Ecosystem Perspective,* ed. R.C. Phillips and C.P. McRoy. pp. 88 - 115. New York: Garland STPM Press.

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Least-Cost Feed Formulation for Juvenile *Macrobrachium rosenbergii* (De Man) by Using the Linear Programming Technique

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Keywords: Feed formulation, linear programming, juvenile *Macrobrachium rosenbergii*

ABSTRAK

Pemprograman kos-terkecil linear telah digunakan ke atas juvana M. rosenbergii *untuk merumuskan satu formula makanan dengan menggunakan bahan-bahan asli (hampas ikan, hampas udang, kelapa kering, hampas kacang soya, tepung gandum dan minyak kelapa sawit). Berikut adalah kriteria yang digunakan: Kandungan asid amino perlu adalah hampir sama dengan kandungan asid amino perlu dalam juvana* M. rosenbergii, *lemak kasar 5-10% dan tenaga kasar 4,400 cal/g dengan kos yang minimum. Empat jenis makanan dihasilkan dengan julat protein di antara 25% hingga 50%. Tindakbalas kadar pertumbuhan juvana* M. rosenbergii *ke atas makanan yang dirumus ini, menunjukkan bahawa makanan yang mempunyai 40% kandungan protein (P40) memberikan kadar pertumbuhan dan nisbah penukaran makanan yang terbaik. Makanan P40 adalah disyorkan sesuai untuk juvana* M. rosenbergii.

ABSTRACT

Linear least-cost programming was used in juvenile M. rosenbergii *feed formulation using locally available feed ingredients (fish meal, shrimp meal, copra meal, soybean meal, wheat flour and palm oil). The following constraints were established: the essential amino acid contents were closely similar to those of juvenile* M. rosenbergii, *crude fat 5-10% and gross energy 4,400 cal/g with least cost. Four types of feed were produced with protein ranges from 25% to 50%. Growth responses of juvenile* M. rosenbergii *fed these formulated feeds showed that the 40% protein feed (P4O) supported the best specific growth rate and feed conversion ratio. P4O feed is recommended for juvenile* M. rosenbergii.

INTRODUCTION

Feed costs for *M. rosenbergii* production can account for 30-40% of total operation costs (Liao and Chao 1982). Formulation of a least-cost nutritionally balanced feed would offer an opportunity of reducing overall production cost as well as increasing production. The application of linear programming in least-cost feed formulation for livestock and poultry has gained wide recognition. Its use in fish diet formulation was described by Chow *et al.* (1980). The linear programming technique in feed formulation for *M. rosenbergii* has not yet been reported. There is a paucity of information on the nutritional requirements for prawns including *M. rosenbergii* in the literature (New 1976; Sick and Millikin 1983). Therefore, a study was undertaken to make use of the available information on prawn nutrition to formulate a least-cost and nutritionally balanced feed from locally available ingredients for *M. rosenbergii* juveniles.

The constraints for the linear programme were set as follows: (1) The amino acid content of the feed was similar to that obtained from the muscle of juvenile *M. rosenbergii.* Many studies have indicated that artificial diets containing essential amino acid profiles that were similar to those of the cultured prawns including *M. rosenbergii* produced better growth (Farmanfarmaian and Lauterio 1979 and 1980; Kanazawa 1985; Pascual 1989) (2) Crude fat content of the diet was confined between 5 to 10%. Diets which have 10 % or more lipid have an adverse effect on the growth and survival of penaeid prawns (Bautista 1986; Sheen and D'Abramo 1991); (3) The gross energy of 4,400

cal/g was based on the reports by Sedgwick (1979); and (4) Based on the wholesale price of the ingredients, the feed production cost was minimal.

MATERIALS AND METHODS

Proximate Analyses

Fish meal, shrimp meal, soybean meal, copra meal and wheat flour were the ingredients used for this study. Their moisture contents, crude fat and crude protein compositions were analyzed according to the methods of A.O.A.C. (1975). Gross energy content was determined by using a bomb calorimeter (Parr Adiabatic Calorimeter). For palm oil, only the crude fat and gross energy contents were determined. *M. rosenbergii* of two different sizes, i.e. 4.5-5.4 cm orbital length (1.35-1.55g) and 6.2-6.8 cm orbital length (1.92-2.20g) and other formulated feeds were also subjected to the same analyses. Six replicates were conducted on each sample.

Amino Acid Analysis

Amino acid contents in the ingredients, juvenile *M. rosenbergii*, and the formulated diets were analyzed following the method of Spitz (1973). A Technicon amino acid analyser (TSM) was used for the analysis with Sigma standard amino acid solution (A.A.S. 18) as a reference. Three replicates were run on each sample.

Linear programming for feed formulation

Four types of feed, i.e. P25 (25% protein), P30 (30% protein), P40 (40% protein), and P50 (50% protein) were formulated. The following constraints were established for the programming: essential amino acid contents were similar to those of the muscle of juvenile *M. rosenbergii*, crude fat 5-10%, gross energy 4,400 cal/g, and least-cost for the ingredients. The wholesale price for fish meal, shrimp meal, soybean meal, copra meal, wheat flour, and palm oil at the time of this study was RM 1.40, RM 1.00, RM 0.88, RM 0.35, RM 0.60, and RM 1.00 (Malaysian Ringgit) per kilogram, respectively. The linear programming was executed using the Functional Mathematical Programming System (FMPS) software package(Sperry Univac). Three per cent of mineral mix, 0.2% vitamin premix, 0.5% basfin (as binder) and 0.25% of calcium propionate (as fungicide) were included for the final feed preparation.The cost of the additives was added to the least-cost of the individual feed to give the final cost of the prepared feed.

Growth Response of Prawns Fed with the Formulated Feeds

Weight gains were used as the criterion for evaluation of the growth responses of prawns fed with the formulated feeds. The initial weight of prawns used for this study ranged from 0.014g to 0.336g. The prawns were fed at 5% body weight twice a day at 0900 and 2100. Triplicate experiments were conducted on each diet. The water quality parameters of the culture water were as follow: temperature 27-28 °C; dissolved oxygen 6.8-7.8 ppm; pH 6.9-7.3; total ammonium-nitrogen, < 0.02 ppm. Three culture systems were used for the experiments: the aquarium series, basin series and circular tanks. The aquarium series consisted of fifteen glass aquariums measuring 100cm x 60cm x 50cm (length, width and height). Each aquarium was partitioned off 1/3 of its length to form a biological filter section comprising limestone chips and cockle shells with a filtration rate of 6 litres of water per minute. Fifty postlarvae were randomly assigned to each aquarium with adequate shelters as hiding places.

The basin culture system consisted of a series of twelve basins receiving water from the same recirculating system. The flow rate of water into each basin was 500 ml per minute.The dimension of each culture unit was 49cm x 60cm x 30cm (bottom diameter, top diameter and height). The depth of water in each basin was 24 cm. Forty-two and fifteen postlarvae were randomly assigned to each basin in series 1 of 11 experiments respectively. For the circular tanks, the dimension of each unit was 150 cm diameter x 40 cm height (water depth). The culture system consisted of an undergravel filter 10 cm thick and the water flow rate was 1.5 litres per minute.

RESULTS

The proximate analyses of crude protein, fat, ash, moisture, and gross energy of the ingredients and the juvenile *M. rosenbergii* are shown in Table 1. The essential amino acid composition of the ingredients as well as that of the fresh muscle content of the juvenile prawns and the formulated feeds are shown in Tables 2 and 3

TABLE 1
Proximate analyses of feed ingredients and juvenile *M. rosenbergii*

	Ash (%)[1]	Crude fat (%)[1]	Crude protein (%)[1]	Gross energy (cal/g)	Moisture (%)
Ingredients					
Fish meal	26.90 ± 0.42[2]	9.95 ± 0.07	65.32 ± 0.09	4433.6 ± 43.4	11.91 ± 0.05
Shrimp meal	27.90 ± 0.24	2.26 ± 0.09	42.35 ± 0.81	3843.0 ± 35.2	16.29 ± 0.14
Soybean meal	6.09 ± 0.05	4.97 ± 0.05	45.14 ± 0.28	4808.5 ± 7.2	9.73 ± 0.14
Copra meal	6.28 ± 0.02	13.12 ± 0.15	24.97 ± 0.08	4926.5 ± 12.9	10.19 ± 0.12
Wheat flour	0.45 ± 0.00	1.17 ± 0.03	14.24 ± 1.11	4358.2 ± 14.0	3.11 ± 0.18
Palm oil	–[3]	100	–	9480.2 ± 0.0	–
M. rosenbergii					
6.20 – 6.82 cm[4] (1.92 - 2.20)[5]	18.87 ± 0.07	5.84 ± 0.08	63.32 ± 0.33	4609.6 ± 11.3	–
4.50 – 5.35 cm (1.35 – 1.55 g)	20.09 ± 0.09	5.28 ± 0.17	63.59 ± 0.12	4468.0 ± 30.9	–

1: On dry weight basis. 2: Mean ± S.D. (n ± 6). 3: Not determined
4: Post-orbital length. 5: Body weight

TABLE 2
Essential amino acid content of feed ingredients

	Fish meal	Shrimp meal	Soybean meal	Copra meal	Wheat flour
	% Protein (% dry weight)				
Histidine	2.43[1] (1.59)[2]	2.06(0.93)	3.11(1.40)	4.76(1.19)	5.75(0.82)
Arginine	6.02(3.93)	5.75(2.60)	6.82(3.08)	9.63(2.41)	2.41(0.34)
Valine	4.79(3.25)	4.36(1.97)	4.34(1.96)	3.62(0.90)	3.51(0.50)
Methionine	2.77(1.65)	3.06(1.38)	2.06(0.93)	1.28(0.32)	1.76(0.25)
Isoleucine	3.74(2.44)	2.64(1.19)	3.11(1.40)	2.27(0.57)	2.52(0.36)
Leucine	7.42(4.85)	5.29(2.39)	6.36(2.87)	4.81(1.20)	5.53(0.79)
Tyrosine	2.64(1.72)	7.29(3.29)	3.80(1.72)	1.29(0.32)	0.93(0.13)
Phenylalanine	3.65(2.38)	3.89(1.76)	5.35(2.41)	3.57(0.89)	3.93(0.56)
Lysine	7.82(5.11)	8.07(3.65)	6.67(3.01)	7.41(1.85)	10.85(1.55)
Threonine	4.58(2.99)	4.01(1.81)	3.41(1.54)	2.48(0.62)	2.35(0.33)

1: Calculated from % of essential amino acid on dry weight basis x (1/% crude protein on dry weight basis).
2: Mean value from three analyses, on dry weight basis.

TABLE 3
Essential amino acid content of the formulated feeds and juvenile *M. rosenbergii* (1.55g - 2.20g)

	M. rosenbergii	P25	P30	P40	P50
	% Protein (% dry weight)				
Histidine	1.61[1] (1.07)[2]	1.80(0.45)	2.31(0.69)	1.40(0.56)	1.23(0.61)
Arginine	3.84(2.55)	4.24(1.06)	4.63(1.39)	3.90(1.56)	3.72(1.86)
Valine	3.11(2.06)	3.20(0.80)	3.10(0.93)	3.09(1.24)	3.08(1.54)
Methionine	2.91(1.56)	2.66(0.67)	3.42(1.04)	2.06(0.81)	1.79(0.89)
Isoleucine	2.92(1.94)	2.92(0.73)	2.57(0.77)	2.69(1.08)	2.78(1.39)
Leucine	5.05(3.34)	6.52(1.63)	5.70(1.71)	6.05(2.42)	5.61(2.80)
Tyrosine	3.87(2.57)	1.48(0.37)	1.23(0.37)	1.63(0.65)	1.76(0.88)
Phenylalanine	3.04(2.02)	4.24(1.06)	3.73(1.12)	3.64(1.46)	3.13(1.56)
Lysine	6.41(4.26)	4.24(1.06)	4.10(1.23)	3.83(1.53)	4.50(2.25)
Threonine	2.50(1.66)	2.92(0.73)	2.03(0.61)	3.24(1.30)	2.72(1.36)

1: Calculated from % of essential amino acid on dry weight basis x (1/% crude protein on dry weight basis)
2: Mean value from three analyses

respectively. The ingredient composition, proximate analyses and cost of the formulated feeds are presented in Table 4. The growth responses of *M. rosenbergii* postlarvae and juveniles fed with the formulated feeds in the different culture systems are given in Table 5. The apparent digestibility of the feeds is given in Table 6.

DISCUSSION

Many studies have shown that prawns including *M. rosenbergii* require dietary sources of essential amino acids for growth (Watanabe 1975; NRC 1983; Kanazawa 1985). Depressed appetite and reduced growth rate of the prawns are usually reported when the prawns are fed with diets deficient in any of the essential amino acids. Farmanfarmaian and Lauterio (1979, 1980) reported that the Purina Marine Ration (M20) was insufficient in some of the essential amino acids such as isoleucine, lysine and arginine for juvenile *M. rosenbergii*. However, when it was adjusted to the amino acid pattern of the prawn, it became the effective source of protein.This clearly supported the phenomenon that the

TABLE 4
Ingredient composition, proximate analyses and cost of the formulated feeds

	Formulated feeds			
	P25	P30	P40	P50
Ingredients, kg/kg of feed.				
Fish meal	0.096	0.116	0.154	0.456
Shrimp meal	0.200	0.224	0.439	0.309
Soybean meal	0.050	0.200	0.100	0.100
Copra cake	0.010	0.114	0.150	0.013
Wheat flour	0.503	0.346	0.133	0.100
Palm oil	0.076	0.050	0.025	0.023
Fine sand	0.065	0.050	0.000	0.000
Proximate analyses (on dry weight basis)				
Crude protein (%)	25.37 ± 0.12[1]	30.34 ± 0.47	39.50 ± 0.07	49.48 ± 0.41
Crude fat(%)	8.09 ± 0.09	8.35 ± 0.09	6.91 ± 0.14	8.07 ± 0.03
Ash (%)	18.15 ± 0.11	17.52 ± 0.03	19.64 ± 0.00	22.03 ± 0.06
Gross energy (cal/g)	4283.6 ± 0.5	4229.3 ±46.2	4206.7 ± 39.2	4344.1 ± 5.5
Cost with additives (RM/kg)[2]	0.94	0.95	1.08	1.30

1: Mean ± S.D. (n = 6). 2: US $1 ± RM 2.5 (Malaysian Ringgit)

closer the amino acid composition of the feed to that of the prawn, the more effective is the protein source. Therefore, based on this phenomenon, four types of feeds with protein levels ranging between 25 % to 50 % where the amino acid content was similar to that of the whole fresh muscle of juvenile *M. rosenbergii* were formulated.

The nutrient contents of the formulated feeds were remarkably close to the constraints set by the linear programme (Table 4). The feeds were isocaloric with gross energy content of about 4,400 cal/g. The amino acid contents in the feeds, in terms of per cent protein, were very close to the values obtained from the fresh muscle amino acid content of juvenile *M. rosenbergii*, except for tyrosine which was about 50% lower. The reason for lower tyrosine levels in the feed is not known. Most probably, it was lost during the feed preparation. The water stability of the feeds was greater than 3 hours at 28°C.

The performance of the feeds was evaluated by using the growth responses and feed conversion ratio of postlarvae and juvenile *M. rosenbergii* fed with these feeds in the aquarium and basin culture systems for a period of 33-61 days. The results indicated that under both culture systems, all formulated diets produced specific growth rates that were significantly different ($P< 0.01$) where P40 feed supported the highest growth rate followed by P50, P30, and P25 feeds. In general, P40 feed also showed better feed conversion ratio (1.68-2.03) among all the feeds, except for P30 feed which was comparable or sometimes better (Table 5). The decline in growth rate and feed conversion ratio of P50 feed suggested that the optimal protein level in the diet for *M. rosenbergii* postlarvae and juveniles was around 40% with a gross energy of 4,400 cal/g, a protein:gross energy ratio of 1:110 and an amino acid profile similar to that of the prawn. The cost of ingredients required for the production of 1 kilogram of P40 feed was RM 1.08 (US$1.00 = RM 2.50).

It is difficult to compare the results of this study with those of other studies on the growth of juvenile *M. rosenbergii* on the pelleted feed because of the diverse culture systems, stocking densities, and different protein levels in the feed. However, it is interesting to note that the feeds used in this study seemed to give a higher specific growth rate and conversion ratio as compared to the studies conducted elsewhere with feeds of the same protein level, and under similar culture conditions. The better performance of the feeds used in the study may be due to the

TABLE 5
Growth responses of postlarvae and juvenile *Macrobrachium rosenbergii* fed on the formulated diets

Culture system	Type of feed	Stocking density (no/unit)	Initial weight (g)	Final weight (g)	Culture period (days)	Specific growth rate (% per day)	FCR	Survival rate (%)
Aquarium series	P50 (3)[1]	50 (111)[2]	0.014	0.541 ± 0.0393	61	5.99 ± 0.12	2.13 ± 0.03	80.67 ± 5.03
	P40 (3)	50 (111)	0.014	0.652 ± 0.017	61	6.30 ± 0.04	2.03 ± 0.09	82.00 ± 2.00
	P30 (3)	50 (111)	0.014	0.487 ± 0.18	61	5.82 ± 0.06	1.98 ± 0.05	82.67 ± 4.16
	P25 (3)	50 (111)	0.014	0.338 ± 0.22	61	5.22 ± 0.11	2.39 ± 0.24	83.33 ± 3.06
	RBS (3)	50 (111)	0.014	0.300 ± 0.028	61	5.02 ± 0.11	2.55 ± 0.06	80.67 ± 9.02
Basin series-I	P50 (3)	42 (221)	0.104 ± 0.006	0.347 ± 0.036	35	3.43 ± 0.22	1.89 ± 0.09	90.47 ± 8.60
	P40 (3)	42 (221)	0.103 ± 0.004	0.389 ± 0.024	35	3.66 ± 0.17	1.86 ± 0.07	82.80 ± 4.10
	P30 (3)	42 (221)	0.108 ± 0.011	0.327 ± 0.034	35	3.16 ± 0.18	1.81 ± 0.19	93.80 ± 4.42
	P25 (3)	42 (221)	0.100 ± 0.006	0.263 ± 0.034	35	2.72 ± 0.20	2.48 ± 0.16	83.30 ± 9.80
Basin series-II	P50 (3)	15 (79)	0.347 ± 0.008	1.842 ± 0.054	58	2.88 ± 0.07	1.89 ± 0.45	84.47 ± 3.87
	P40 (3)	15 (79)	0.336 ± 0.015	2.072 ± 0.169	58	3.13 ± 0.11	1.68 ± 0.02	82.23 ± 3.87
	P30 (3)	15 (79)	0.342 ± 0.013	1.543 ± 0.061	58	2.60 ± 0.02	1.67 ± 0.02	86.70 ± 0.00
	P25 (3)	15 (79)	0.345 ± 0.003	1.117 ± 0.030	58	2.11 ± 0.05	2.43 ± 0.07	80.00 ± 0.00
Circular Tank	P30 (1)	50 (28)	1.590 ± 0.050	4.822 ± 0.250	50	2.70	1.78	90.00
	P30 (1)	100 (56)	1.35		15.05		159	1.52 2.55
60.00								

1: Parenthesis indicates the number of replicates
2: Number of prawns per unit of culture system (Number of prawns per m^2); 3: Mean ± S.D.

TABLE 6
Apparent nutrient digestibility of P30 and P40 feeds by juvenile (0.495 g–1.271g) and adult (7.954 g–25.588 g) *M. rosenbergii*

	Apparent digestibility (%)[1]					
	Protein	Fat	Carbohydrate	Ash	Gross energy	Dry matter
P30 Feed						
Juvenile	66.02 ± 4.25[2]	83.48 ± 6.11	82.16 ± 1.83	27.27 ± 9.37	78.77 ± 6.03	62.83 ± 3.84
Adult	72.59 ± 2.37	84.12 ± 2.86	72.15 ± 4.07	41.34 ±12.61	70.89 ± 2.66	64.94 ± 2.15
P40 Feed						
Juvenile	66.50 ± 3.30	79.61 ± 0.69	63.81 ± 6.86	33.27 ± 4.49	65.77 ± 3.67	57.17 ± 3.23
Adult	75.93 ± 0.36	85.78 ± 0.51	70.35 ± 4.50	43.10 ± 3.47	74.44 ± 3.19	65.77 ± 1.13

1: On dry weight basis. 2: Mean ± S.D. (n = 6)

amino acid pattern in the feed which was similar to that of the muscle of the prawn. A specific growth rate of 5.22 % per day in the aquarium experiment using P25 feed was comparable to that of a 25% protein feed as reported by Willis *et al.* (1976). They obtained a specific growth rate of 5.71 % per day and a feed conversion ratio of 5.50, while we obtained a similar growth rate but a much better feed conversion ratio of 2.39.

Millikin *et al.* (1980) reported a specific growth rate of 2.59% per day for *M. rosenbergii* fed on a 40% protein feed and grown in basin. Using the similar culture system, we obtained a specific growth rate of 3.80 % per day with the P40 feed. In the basin experiment, our P25 feed produced a much better feed conversion ratio of 2.43 compared to that of 3.16-8.16 for 25% protein feed reported by Sandifer and Smith (1977). Even among the treatments in the aquarium experiments of this study, P25 feed produced a comparable higher specific growth rate and a better feed conversion ratio(5.22 % per day & 2.39) than the repelleted broiler starter (RBS) which had the same levels of protein, fat and gross energy (5.02% & 2.55).

In the circular tank experiments, the juvenile *M. rosenbergii* weighing 1.35 g achieved an average weight of 15.05 g after 159 days when fed with the P30 feed. The specific growth rate was 1.52 % per day and the feed conversion ratio was 2.55. These values were better than those obtained from juvenile *M. rosenbergii* that were fed with the Purina Marine 25 which was supplemented with fresh beef liver; the specific rate and conversion ratio were 0.81 % per day and 5.18 respectively (Farmanfarmaian and Lauterio 1979). These results seem to support the phenomenon that feeds containing protein having a similar amino acid pattern to the culture species will be a more effective source of protein for growth.

The findings of this study revealed that the 40 % protein formulated feed (P40) supported the optimal growth of *Macrobrachium rosenbergii.* In fact, many studies have also indicated that the optimal protein requirement for *M. rosenbergii* is about 40 % (Pandian 1989). Millikin *et al.*(1980) demonstrated that juvenile *M. rosenbergii* fed with a 40% protein diet had a better weight gain than those fed with 49%, 32% and 23% protein diets over a period of 14 weeks. The results of the protein requirement studies of juvenile *M. rosenbergii* by Chao (1979) suggested that a protein level in excess of 35% was required for optimum growth of the prawn. Willis *et al.* (1976) showed that a 40% protein diet (trout chow) produced better growth than the 25% protein diet of Purina Marine Ration 25. Natural productivity in the pond plays an important role in providing supplemental diets for the growth of *M. rosenbergii* fed on pelleted feed (Fair and Fortner 1981). Therefore, in practice, farmers found that pelleted feeds containing 15-25% protein were satisfactory for the culture of *M. rosenbergii* in earthern ponds that had been fertilized with manure (New 1976; New 1990). Feed cost accounts for a high percentage of the production cost for *M. rosenbergii.* To reduce the cost of production, P30 feed has been successfully used along with chicken manure fertilization of the pond for *M. rosenbergii* production (Maclean *et al.* 1989).

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REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. *Official Methods of Analysis.* Washington, D.C.: A.O.A.C. 1094 pp.

BAUTISTA, M.N. 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. *Aquaculture* **53:** 229-242.

CHAO, N.H. 1979. Freshwater prawn farming in Taiwan - the patterns, problems and prospects. *Proc. World Maricult. Soc.* **10:** 51-67.

CHOW, K.W., G. L. RUMSEY and P. W. WALDROUP. 1980. Linear programming in fish diet formulation. In: *Fish Feed Technology.* ADCP/REP/80/11, p. 241-286.

FAIR, P.H. and A. R. FORTNER. 1981. The role of formula feeds and natural producitivity in culture of the prawn, *Macrobrachium rosenbergii. Aquaculture* **24:** 233-243.

FARMANFARMAIAN, A. and T. LAUTERIO. 1979. Amino acid supplementation of feed pellets of the giant shrimp *M. rosenbergii. Proc. World Maricult. Soc.* **10:** 674-688.

FARMANFARMAIAN, A. and T. LAUTERIO. 1980. Amino acid composition of the tail muscle of *M. rosenbergii* - comparison to amino acid pattern of supplemented commercial feed pellets. *Proc. World Maricult. Soc.* **11:** 454-462.

KANAZAWA, A. 1985. Nutrition of Penaeid prawns and shrimps. In Y. Taki, J.H. Primavera, J.A. Llobrera (eds.), *Proc. 1st. Intl. Conf. on culture of Penaeid Prawns/Shrimps.* Iloilo City. Philippines. Aquaculture Dept. SEAFDEC, p. 123-130.

LIAO, I-C. and N. H. CHAO. 1982. Progress made in *M. rosenbergii* farming and its extension in Taiwan. In M.B. New (ed.), *Giant Prawn Farming.* Amsterdam: Elsevier. p. 357-381.

MACLEAN, M. H., K. J. ANG, J. H. BROWN and K. JAUNCEY 1989. The effect of organic fertilizer and formulated feed in pond culture of the freshwater prawn, *Macrobrachium rosenbergii* (de Man): prawn production. *Aquacult. Fish. Manage.* **20:** 399-406.

MILLIKIN, M.R., A. R. FORTNER and P. H. FAIR. 1980. Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn (*Macrobrachium rosenbergii*). *Proc. World Maricul. Soc.* **11:** 382-391.

NATIONAL RESEARCH COUNCIL. 1983. Nutrient Requirements of Warmwater Fishes and Shellfishes. Revised Edition. Washington. D.C. National Academic Press. 102 pp.

NEW, M.B. 1976. A review of dietary studies with shrimp and prawns. *Aquaculture* **9:** 101-144.

NEW, M.B. 1990. Freshwater prawn culture: a review. *Aquaculture* **88:** 99-143.

PANDIAN, T.J. 1989. Protein requirements of fish and prawns culture in Asia. In: S.S. De Silva, (ed.). *Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting.* Asian Fish. Soc. Spec. Publ. **4.** 166 pp. Manila. Philippines: Asian Fisheries Society.

PASCUAL, F.P.1989. Status of shrimp nutrition and feed development in Southeast Asia. In S.S De Silva (ed.), *Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting.* Asian Fish. Soc. Spec. Publ. **4.** 166 pp. Manila. Philippines: Asian Fisheries Society.

SANDIFER, P.A. and T.I.J. SMITH. 1977. Intensitive rearing of postlarval Malaysian prawns, *M. rosenbergii* in a closed cycled nursery system. *Proc. World Maricult. Soc.* **8:** 235-255.

SEDGWICK, R. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *P. merguiensis. Aquaculture* **16:** 7-10.

SHEEN, S.-S. and L.R. D'ABRAMO. 1991. Response of juvenile freshwater prawn, *Macrobrachium rosenbergii,* to different levels of a cod liver oil/corn oil mixture in a semi-purified diet. *Aquaculture* **93:** 121-134.

SICK, L.V. and M. R. MILLIKIN. 1983. Dietary and nutrient requirements for culture of the Asian prawn, *Macrobrachium rosenbergii.* In: J.P. McVey (ed.), *CRC Handbook of Mariculture. Vol. I. Crustacean Aquaculture.* Boca Raton, Fl: CRC Press, Inc. p. 381-389.

SPITZ, H.D. 1973. A new approach for sample preparation of protein hydrolysates for amino

acid analysis. *Anal. Biochem.* **56:** 66-73.

WATANABE, W.O. 1975. Identification of essential amino acids of the fresh-water prawn, *Macrobrachium rosenbergii.* Masters thesis. University of Hawaii. Honolulu. Hawaii, USA.

WILLIS, S.A., R. W. HAGOOD and G. T. ELIASON. 1976. Effects of four stocking densities and three diets on growth and survival of postlarval *M. rosenbergii and M. acanthurus. Proc. World Maricult. Soc.* **7:** *655-662.*

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Clay Minerals in the Weathering Profile of a Quartz-Muscovite Schist in the Seremban Area, Negeri Sembilan

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Keywords: Weathering profile, quartz-muscovite schist, clay minerals, kaolinite, illite, randomly interstratified illite-montmorillonite

ABSTRACT

Difraktogram-difraktogram pancaran-X menunjukkan bahawa mineral-mineral lempung ilit-montmorilonit berinterstratifikasi rawak dan kaolinit ditemui di dalam horizon-horizon morfologi atas profil peluluhawaan, semasa mineral lempng ilit ditemui di dalam horizon morfologi bawah. Difraktogram-difraktogram juga menunjukkan bahawa mineral-mineral lempung kaolinit, ilit dan ilit-montmorionit berinterstratifikasi rawak terdapat di dalam horizon-horizon morfologi perantaraan. Peningkatan kandungan kaolinit dan ilit-montmorilonit berinterstratifikasi rawak atas profil peluluhawaan dan pengurangan kandungan ilit mencermintkan penambahan kesan proses-proses peluluhawaan. Perkecaian dan perpisahan mineral-mineral muskorit dan serisit di dalam bahan batnuan asal mengakibatkan ilit, semasa ilit-montmorilonit berinterstratifikasi rawak dan kaolinit berasal daripada larutan lesap ilit.

ABSTRACT

X-ray diffractograms show that randomly interstratified illite-montmorillonite and kaolinite are the clay minerals present in the upper morphological horizons of the weathering profile, while illite is the only clay mineral present in the lowest morphological horizon. In the intermediate morphological horizons, the diffractograms show that kaolinite, illite and randomly interstratified illite-montmorillonite are the clay minerals present. Increasing amounts of randomly interstratified illite-montmorillonite and kaolinite up the weathering profile, and a corresponding decrease of illite, reflect increasing effects of weathering processes; disaggregation and disintegration of muscovites and sericites within the original bedrock material initially resulting in illite, followed by development of the randomly interstratified illite-montmorillonite and kaolinite through leaching of the illites.

INTRODUCTION

There is a general lack of published literature on the clay minerals of weathering profiles over quartz-mica schist bedrock in Malaysia, except for Yeow (1975) and Siti Zauyah (1986). Yeow (1975) studied two well-drained weathering profiles; one over a quartz-phengite schist (exposed at a 8 m high slope cut), and the other over a graphitic muscovite-quartz schist (exposed at a 10 m high slope cut). In the profile over the quartz-phengite schist, Yeow (1975) concluded that kaolinite formed where rapid leaching of potassium and iron from the phengite occurred, though where the rate of removal of these ions was slow, a mixed layer phengite-montmorillonite was formed. In the profile over the graphitic muscovite-quartz schist, Yeow (1975) concluded that muscovite altered to kaolinite and halloysite, though the rate of decomposition was slow. Siti Zauyah (1986) investigated a well-drained weathering profile (exposed at a 8 m high slope cut) over a graphitic quartz-sericite schist and concluded that sericite altered to kaolinite.

In studying the characterisation (for engineering geological purposes) of a weathering profile over a quartz-muscovite schist bedrock, samples were collected at various depths and their clay fractions investigated by x-ray diffraction studies. Results of these studies are presented in this paper together with a discussion

on the origins of the clay minerals present in the weathering profile.

SAMPLING SITE - GEOLOGICAL SETTING

The selected weathering profile is exposed at a slope cut, excavated between 1974 and 1975, located on the north side of the Kuala Lumpur - Seremban Highway at Km 67.9 (*Fig. 1*). The highway here cuts across a low hill and trends in a general west to east direction across an undulating terrain of low hills and flat-bottomed, alluviated valleys. The cut is of an approximately symmetrical shape with a length of about 150 m along its base and a maximum vertical height of 20 m at its centre. The cut, which has an overall angle of 40°, is benched, with the benches of some 2.75 m vertical height and face angles of 50°, separated by horizontal berms of variable width. The lowest bench, however, is about 6 m high with a face angle of 80°.

At this cut a weathering profile developed over an original bedrock mass is exposed consisting mainly of light grey to buff coloured, quartz-muscovite schists inter-layered with thin bands and lenses of dark grey, graphitic-quartz-muscovite schist. These schists, which contain several quartz veins and pods, are strongly folded with variable strikes and dips and have been correlated with the Lower Palaeozoic Dinding Schist of the Kuala Lumpur area (Khalid 1972).

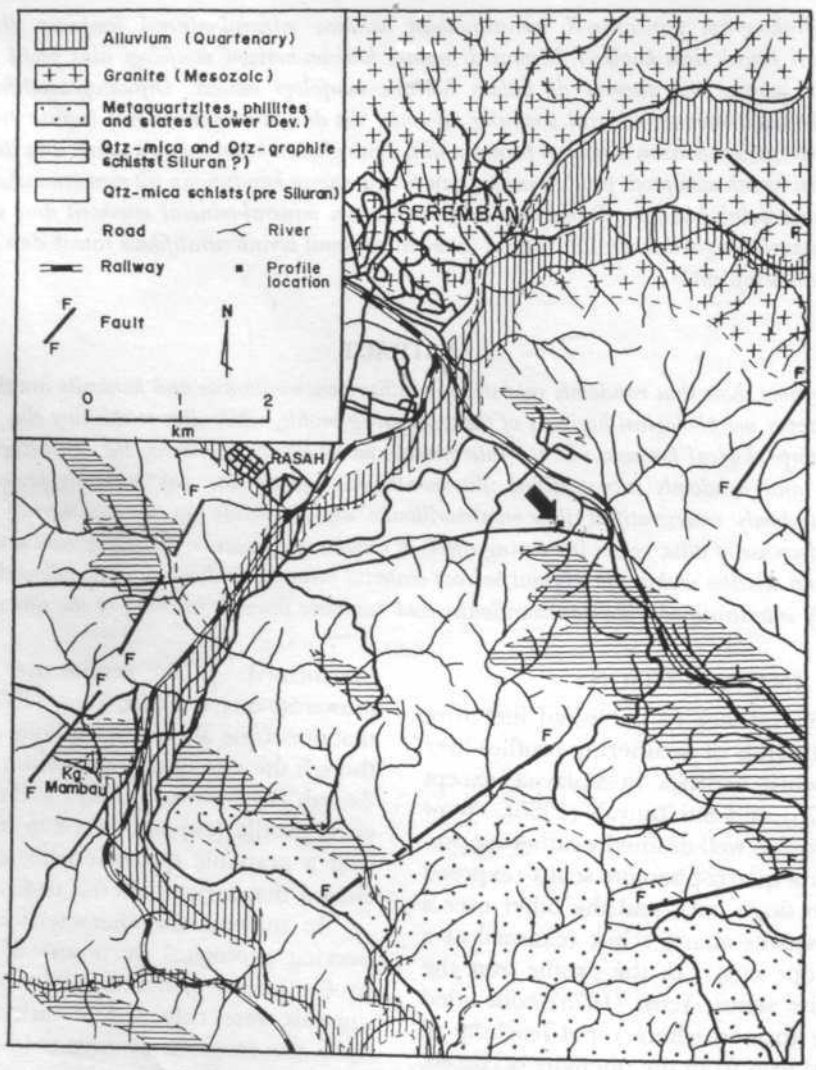
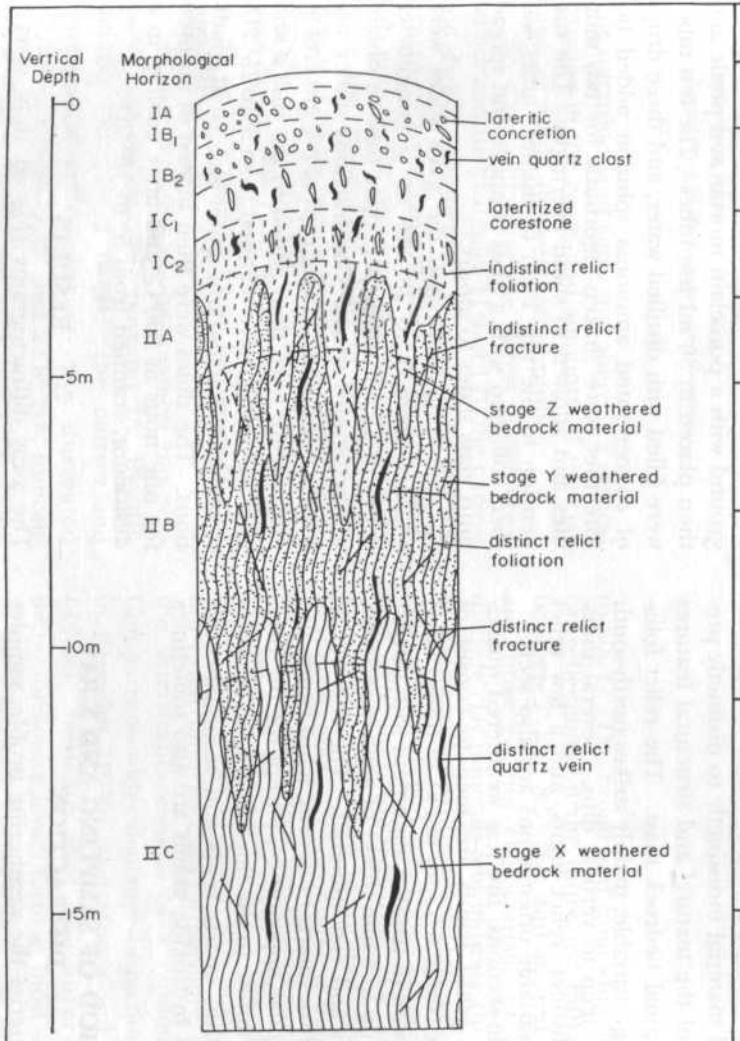


Fig. 1: Geological sketch map of the Seremban area. (After Khalid 1972)



MORPHOLOGICAL HORIZON	DESCRIPTION
IA	Yellowish red, firm clay with subangular blocky structure and some roots; boundary wavy, clear.
IB_1	Red, firm clayey sand with abundant gravel sized lateritic concretions and some roots; boundary irregular, diffuse.
IB_2	Red, firm clayey sand with abundant gravel sized lateritic concretions and vein quartz clasts; some roots and some lateritized corestones; boundary irregular, diffuse.
IC_1	Reddish yellow, stiff clayey sand with some yellow mottles; some gravel sized vein quartz clasts and lateritized corestones; boundary irregular, diffuse.
IC_2	Reddish yellow, firm clayey silt with some yellow mottles (Stage Z weathered bedrock material); many gravel sized vein quartz clasts and lateritized corestones; distinct relict quartz veins and pods; indistinct relict foliation; boundary irregular, diffuse.
IIA	Thick bands and wedges of reddish yellow, firm clayey silt (Stage Z weathered bedrock material) with indistinct relict foliation alternating with thin bands of pinkish to grey, firm silt (Stage Y weathered bedrock material) with distinct relict foliation; distinct relict quartz veins and pods; some secondary iron oxide and hydroxide concretions and stains along relict structural planes; boundary broken, diffuse.
IIB	Thick bands of pinkish to grey, firm silt (Stage Y weathered bedrock material) with distinct relict foliation alternating with thin bands and wedges of reddish yellow, firm clayey silt (Stage Z weathered bedrock material) with indistinct relict foliation; distinct relict quartz veins and pods and distinct relict fracture planes; some secondary iron oxide and hydroxide stains and concretions along relict structural planes; boundary irregular, diffuse.
IIC	Thick bands of white to light grey, stiff silt (Stage X weathered bedrock material) with distinct relict foliation alternating with thin bands and wedges of pinkish to grey, firm silt (Stage Y weathered bedrock material) also with distinct relict foliation; distinct relict quartz veins and pods as well as distinct relict fracture planes are present.

Fig. 2: Schemicatic sketch, and field description, of morphological horizons within the weathering profile over the quartz-muscovite schist. Note - Stages of weathering are defined in Table 1

TABLE 1
Later stages of weathering of the quartz-muscovite schist bedrock material

Stage	Description
Z	Reddish yellow, firm clayey silt with yellow mottles and instinct relict foliation. Material slowly disaggregates when dry samples are soaked and agitated in water. Dry density ranges from 1.7 to 1.85 gm/cc, while porosity ranges from 32 to 38%. Coarse-grained fraction consists largely of sericite flakes with some quartz and secondary iron oxide grains. (Most weathered bedrock material).
Y	Pinkish to grey firm silt with distinct relict foliation. Material readily disaggregates when dry samples are soaked and agitated in water. Dry density ranges from 1.75 to 1.85 gm/cc, while porosity ranges from 32 to 34%. Coarse-grained fraction consists mainly of serifite flakes with some quartz and secondary iron oxide grains. (Less weathered bedrock material).
X	While to light grey, stiff silt with distinct relict foliation. Material disaggregates when dry samples are soaked and agitated in water. Dry density ranges from 1.80 to 1.92 gm/cc, while porosity ranges from 29 to 39%. Coarse-grained fraction consists mainly of sericite flakes with some quartz grains. (Least weathered bedrock material).

The exposed weathering profile can be subdivided into a number of morphological horizons, each of which is characterised by the lateral similarity of morphological features (*Fig. 2*). Completely unweathered bedrock material is, however, not exposed at the cut, though the weathered material indistinctly to distinctly preserves all of the textural and structural features of the original bedrock mass. The relict foliation, though variable, mainly strikes north-south with very steep to vertical dips. Several indistinct to distinct, relict joints, and a few relict faults, of variable orientations are also seen.

In thin-sections, the less weathered quartz-muscovite schist bands are seen to consist of thin layers (some 0.5 mm thick) of fine-grained quartz crystals in parallel alignment with thicker layers (of up to 5 mm thick) of aligned sericites, muscovites and clay minerals. The less weathered graphitic-quartz-muscovite schist bands also show a similar appearance, except for the presence of graphite in the thick layers. In the thin-sections, thin quartz veins and secondary iron oxide and hydroxide grains are also sometimes seen.

METHOD OF SAMPLING AND X-RAY DIFFRACTION

To characterise the weathering profile, samples of the weathered materials were collected at various depths (*Fig. 3*) using thin-walled, cylindrical brass rings of 7.6 cm internal diameter and 4 cm height. Moisture contents of these samples were determined, following which they were air dried and separated into smaller fractions using a sample splitter. Fractions of samples for the x-ray diffraction studies were gently ground with a porcelain mortar and pestle and then placed in 30 ml test tubes. The test tubes were filled with distilled water, and three drops of concentrated ammonia solution added before they were shaken vigorously for two minutes and allowed to stand overnight. The suspension in the top 1 cm of the test tubes was then collected with a glass dropper and spread onto glass slides to air-dry.

Following air-drying, the glass slides were scanned from 5° to 28° 2Θ- at a goniometer speed of 1°/min using a copper tube to obtain diffractograms of the clay fractions under untreated conditions. Two drops of 6% glycerol in ethyl alchol were then added to the slides, and after air-drying, were scanned from 5° to 15° 2Θ- to obtain diffractograms under glycolated conditions. The slides were then heated in an oven for one hour at 500°C, and after cooling in a desiccator, scanned from 5° to 15° 2Θ-.

RESULTS

The x-ray diffractograms (*Fig. 4*) show several reflections that indicate the presence of a number of clay minerals. These reflections are of vari-

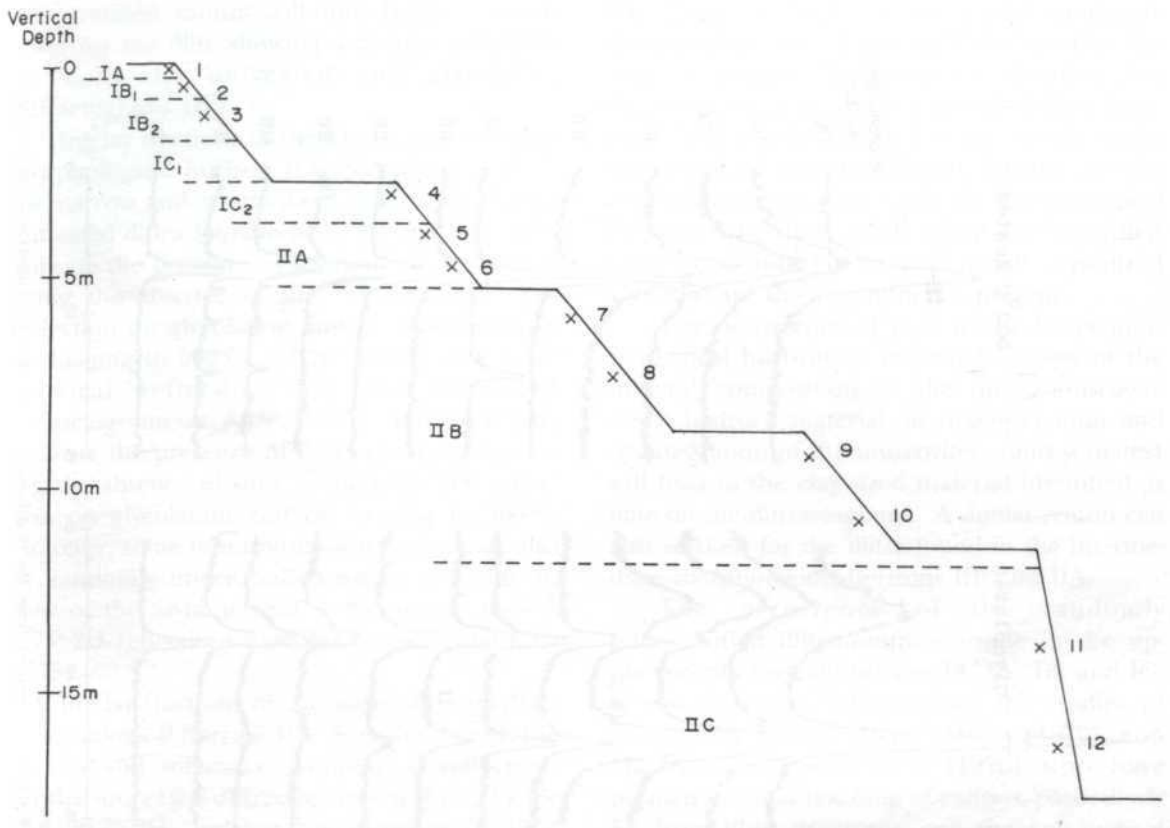


Fig. 3: Sample locations, and lateral extensions of morphological horizons, within the weathering profile over the quartz-muscovite schist.
Note (x^1 - sample number and location).

able intensities and show that there is a vertical variation in the types, and amounts, of the clay minerals within the weathering profile.

In clay fractions of the lowest morphological horizon IIC (Samples 11 and 12), the narrow and slightly asymmetrical reflections on the untreated diffractograms at 8.75°, 17.75° and 26.7° 2Θ-, indicate the presence of illite; confirmation being the absence of shifts of the 8.75° 2Θ- reflection on glycolation and on heating to 500°C (*Fig. 4*). The term 'illite' is here used in the sense proposed by Grim, Bray and Bradley (1937) i.e. as being a general name for mica-like clay minerals.

In clay fractions of the top-most morphological (or pedological) horizons IA, IB_1, IB_2 and IC_2 (Samples 1 to 4), the narrow and symmetrical reflections on the untreated diffractograms at 12.25° and 24.8° 2Θ-, indicate the presence of kaolinite, confirmation being the absence of shift of the 12.25° 2Θ- reflection on glycolation and its disappearence on heating to 500°C. The broad and somewhat asymmetrical reflections on the untreated diffractograms between 7° and 8.5° 2Θ-, and around 17.8° 2Θ- are, however, not characteristic of individual discrete clay minerals and indicate the presence of an interstratified (or mixed-layered) clay mineral. As the broad reflections between 7° and 8.5° 2Θ- shift towards low 2Θ- angles on glycolation, and drop to around 8.5° 2Θ- on heating to 500°C, it is considered that this clay mineral is an interstratified illite-montmorillonite (Moore and Reynolds 1989). The absence of other reflections at lower 2Θ- angles on the untreated diffractograms furthermore, shows that the interstratification is of a random nature. Comparisons with calculated diffraction patterns in Reynolds (1980), and Moore and Reynolds (1989), indicate that the interstratified montmorillonite layers form at most some 10% of the randomly interstratified clay mineral. In the clay fraction of morphological horizon IC_2 (Sample 4), however, the content of the

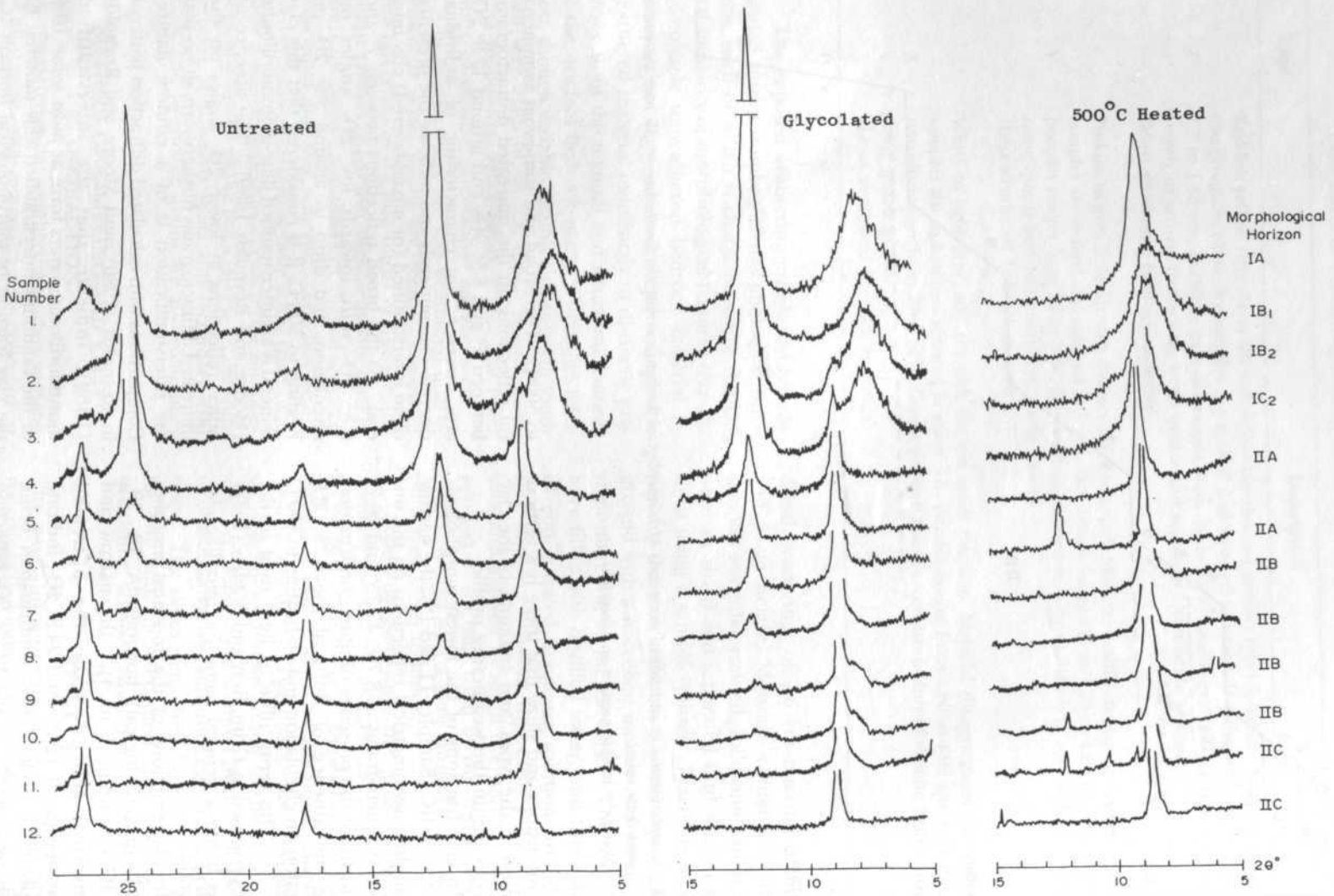


Fig. 4: Untreated, glycolated and 500°C heated X-ray diffractograms of the clay fractions of samples from the weathering profile over the quartz-muscovite schist

interstratified montmorillonite layers is much lower for the illite showing a distinct reflection on both the untreated and glycolated diffractograms (*Fig. 4*).

In clay fractions of the upper, intermediate morphological horizon IIA (Samples 5 and 6), the narrow and symmetrical reflections on the untreated diffractograms at 12.25° and 24.8° 2Θ-, indicate the presence of kaolinite, confirmation being the absence of shift of the 12.25° 2Θ- reflection on glycolation and its disappearence on heating to 500°C. The narrow and asymmetrical reflections on the untreated diffractograms at 8.75°, 17.75° and 26.7° 2θ-, indicate the presence of illite, confirmation being the absence of shift of the 8.75° 2Θ- reflection on glycolation and on heating to 500°C. However, some montmorillonite layers may also be randomly interstratified within the illite in view of the asymmetrical (towards low angles) 8.75° 2Θ- reflections (von Reichenbach and Rich 1975).

In clay fractions of the lower, intermediate morphological horizon IIB (Samples 7 to 9), the narrow and somewhat symmetrical reflections on the untreated diffractograms at 8.75°, 17.75° and 26.7° 2Θ-, indicate the presence of illite, confirmation being the absence of shift of the 8.75° 2Θ- reflection on heating to 500°C. There is, however, the possibility that there are some montmorillonite layers randomly interstratified within the illite, as the low angle part of the 8.75° 2Θ- reflection shifts slightly on glycolation and disappears on heating to 500°C (von Reichenbach and Rich 1975). The narrow to broad, symmetrical reflections on the untreated diffractograms at about 12.25° and 24.8° 2Θ-, indicate the presence of kaolinite, confirmation being the absence of shift of the 12.25° 2Θ- reflection on glycolation and its disappearance on heating to 500°C. In the clay fractions of Samples 9 and 10 it is very likely that the kaolinite present is poorly crystallized or very fine grained as it shows low and somewhat broad reflections on the untreated diffractograms.

DISCUSSION

From the results, it can be seen that there is a vertical variation in clay mineralogy within the weathering profile. In the lowest part of the weathering profile (in morphological horizon IIC), illite is the only clay mineral present, while in the top-most part (in pedological horizons IA, IB_1, IB_2 and IC_2), kaolinite and randomly interstratified illite-montmorilonite are the clay minerals present. At intermediate depths within the weathering profile, in morphological horizon IIA, kaolinite and illite (with some interstratified montmorillonite layers) are the clay minerals present, while in morphological horizon IIB, illite (with some interstratified montmorillonite layers) and poorly crystalized kaolinite are the clay minerals present.

The occurrence of illite in the lower morphological horizons is expected in view of the mineral composition of the quartz-muscovite schist bedrock material, as disaggregation and disintegration of the muscovites (and sericites) will lead to the clay sized material identified as illite on the diffractograms. A similar reason can also account for the illites found in the intermediate morphological horizons IIB and IIA.

The occurrence of the randomly interstratified illite-montmorillonite in the upper morphological horizons IA, IB_1, IB_2 and IC_2 is also expected, following on the studies of Droste and Tharin (1958), Millot (1970), and MacEwan and Ruiz-Amil (1975) who have pointed out that leaching of cations, particularly K^+, from illite structures, and the entrance of water, give rise to randomly interstratified illite-montmorillonite. The presence of some randomly interstratified montmorillonite layers within the illites of the intermediate morphological horizons IIA and IIB can also be attributed to these processes. Increasing effects of these processes within the weathering profile are clearly shown in the diffractograms (*Fig. 4*) with the gradual broadening and asymmetry of the 8.75° 2Θ- reflections up the profile. Interestingly the randomly interstratified illite-montmorillonite only becomes clearly discernible in the diffractogram of the clay fraction from pedological horizon IC_2; this horizon constitutes the *solvum* (or parent material) for the overlying pedological soil horizons.

The occurrence of kaolinite within the weathering profile is somewhat unexpected as the mineral composition of the quartz-muscovite schist is bedrock material. Increasing amounts of kaolinite up the weathering profile, and its absence in the lower morphological horizon IIC, however, show that it has developed as a result of weathering processes. In the intermediate morphological horizons IIB and IIA broadening of the 8.75° 2Θ- illite reflection is seen to correspond with

an increase in the heights of the 12.25° 2θ-kaolinite reflection and indicates that the development of the kaolinite is associated with leaching of the illite. Such an origin for kaolinite has been proposed by several other workers including Loughnan (1969), Weaver and Pollard (1973), Yeow (1975) and Siti Zauyah (1986).

CONCLUSION

It is concluded that randomly interstratified illite-montmorillonite and kaolinite are the clay minerals present in the upper morphological horizons of the weathering profile, while illite is the only clay mineral present in the lowest morphological horizon. In the intermediate morphological horizons, kaolinite, illite and randomly interstratified illite-montmorillonite are the clay minerals present. It is also concluded that increasing amounts of kaolinite and randomly interstratified illite-montmorillonite up the weathering profile, and a corresponding decrease of illite, reflect increasing effects of weathering processes; disaggregation and disintegration of muscovites and sericites within the original bedrock material initially result in illite, followed by development of the randomly interstratified illite-montmorillonite and kaolinite, through leaching of the illites.

ACKNOWLEDGEMENTS

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REFERENCES

DROSTE, J.B. and J.C. THARIN. 1958. Alteration of clay minerals in Illinoian till by weathering. *Bull. Geol. Soc. America* **69(1)**: 61-68.

GRIM, R.E., R.H. BRAY and W.F. BRADLEY. 1937. Mica in argill-aceous sediment. *Amer: Min.* **22** 813.

KHALID BIN NGAH. 1972. Brief geology of the Seremban area, Sheet 103. *Ann. Rept. Geol. Survey Malaysia 1972*: 86-93.

LOUGHNAN, F.C. 1969. *Chemical Weathering Of The Silicate Minerals.* New York: Elsevier Publishing Co. Inc, U.S.A. 154p.

MACEWAN, D.M.C. and A. RUIZ-AMIL. 1975. Interstratified clay minerals. *In Soil Components*, ed. J.E. Gieseking, Vol. 2, p.265-334. Berlin: Springer-Verlag.

MILLOT, G. 1970. *Geology Of Clays.* (Translated by W.R.Farrand and H. Paquet). Paris: Masson et Cie.

MOORE, D.M. and R.C. REYNOLDS. 1989. *X-ray Diffraction and the Identification and Analysis of Clay Minerals.* New York: Oxford University Press. 331p.

REYNOLDS, R.C. 1980. Interstratified clay minerals. In *Structures of Clay Minerals And Their X-Ray Identification*, ed. G.W. Brindley and G. Brown. p.249-303. London: Mineralogical Society.

SITI ZAUYAH DARUS. 1986. Characterisation of some weathering profiles on metamorphic rocks in Peninsular Malaysia. D. Sc. Thesis, State University of Ghent, Belgium. 388 p.

VON REICHENBACH, H.G. and C.I. RICH. 1975. Fine grained micas in soils. In *Soil Components*, ed. J. E. Gieseking, Vol. 2. p. 59-95. Berlin: Springer-Verlag.

WEAVER, C.E. 1958. The effects and geologic signifiance by potassium "fixation" by expandable clay-minerals derived from muscovite, biotite, chlorite and volcanic material. *Amer. Min.* **43:** 839-889.

WEAVER, C.E. and L.D. POLLARD. 1973. The Chemistry of Clay Minerals. *Dev. in Sedimentology*. Netherlands: Elsevier Publishing Co. p.213.

YEOW YEW HENG. 1975. Weathering of rocks in humid tropical conditions. Ph.D. Thesis, Universiti of Malaya. 155 p.

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The Penetration of CCA Preservative on Four Under-utilized Malaysian Hardwood Species

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Keywords: CCA preservative, vacuum pressure treatment, Malaysian hardwood

ABSTRAK

*Kajian kebolehrawatan telah dijalankan ke atas empat jenis kayu kurang digunakan di Malaysia iaitu Mempening (*Lithocarpus spp.*), Nyatoh Nangka Kuning (*Pouteria malaccensis *(Clarke) Baehni), Pauh Kijang (*Irvingia malayana*)(*Oliv.*) dan Petai (*Parkia speciosa *Hassk). Dua species kayu terkenal iaitu Jelutong (*Dyera costulata *Hk.*f) *dan Merbau (*Intsia palembanica *Miq.) digunakan sebagai kawalan. Semua spesimen kering diawet secara tekanan-vakum menggunakan larutan pengawet kuprum arsenat berkromat (CCA) berkepekatan 3%. Kandungan lembapan dan ketumpatan kayu-kayu berkenaan ditentukan dahulu sebelum diawet. Kajian menunjukkan terdapat korelasi umum di antara ketumpatan kayu dengan penembusan pengawet CCA. Petai memberikan penembusan sisi paling dalam (5) diikuti oleh Jelutong (4.9), Nyatoh Nangka Kuning (3.6), Mempening* (1.75), *Pauh Kijang (1.5) dan Merbau (0). Spesies berketumpatan rendah seperti Jelutong, Nyatoh Nangka Kuning dan Petai memperlihatkan penembusan pengawet lebih dalam dibanding dengan spesies yang mempunyai ketumpatan tinggi* (Merbau, Mempening dan Pauh Kijang). *Penembusan sisi di antara enam spesies yang dikaji memberikan perbezaan yang bererti. Kajian menunjukkan keempat-empat jenis kayu yang kurang digunakan ini dapat diawet menggunakan pengawet CCA.*

ABSTRACT

*Four under-utilized Malaysian hardwood species, namely Mempening (*Lithocarpus *spp.), Nyatoh Nangka Kuning (*Pouteria malaccensis *(Clarke) Baehni), Pauh Kijang (*Irvingia malayana Oliv.*) and Petai* (Parkia speciosa *Hassk.) were selected for the treatability study. Two popular timber species, namely Jelutong (*Dyera costulata *Hk.* f.) *and Merbau (*Intsia palembanica *Miq.) were included as controls. The moisture content and density of all the selected timbers were determined prior to the treatment. All the seasoned specimens were vacuum-pressure treated with three per cent copper-chrome-arsenate (CCA) preservative (Celcure "A") solution. There is a general correlation between the density (specific gravity) of wood and the penetration of CCA-preservative. Petai exhibited the deepest side penetration (5.0) followed by Jelutong (4.9), Nyatoh Nangka Kuning (3.6), Mempening (1.75), Pauh Kijang (1.5) and Merbau (0). Lower density timber species (Jelutong, Nyatoh Nangka Kuning and Petai) attained deeper penetration when compared to higher density timber species (*Mempening, Merbau and Pauh Kijang). *Significant differences in side penetration were found among the six species. The four under-utilized Malaysian hardwood species are amenable to the vacuum-pressure treatment with CCA preservative.*

INTRODUCTION

The Malaysian tropical rain forest is richly endowed with approximately 3,000 species of trees, of which 677 species of timber trees in 168 genera can attain a girth of 1.2 metre at breast height. Of these, 402 species are considered commercially important in the timber market. The remaining 275 species, distributed in 101 genera, are classified as under-utilized or lesser-known species and have been left unexploited

(Kochumen 1973). Studies in the natural forest of Sabah showed that the under-utilized species form about 16.8 per cent of the total timber volume (Udarbe 1974). In Sarawak, a similar situation prevails. Out of the 890 species of timber trees, 142 are classified as the under-utilized species (Anon 1983).

With the rapid development and economic growth of the country, the demand for durable timber has increased greatly. One of the possible ways to overcome the impending timber shortage is to promote greater utilization of the under-utilized species. The quality and serviceability of non-durable timber can be improved by means of chemical preservation. However, treatability studies of Malaysian under-utilized timber species are limited (Mohd. Dahlan 1983 Pers. comm.). Kempas (*Koompassia malaccensis*) which was once considered as a naturally non-durable species, is now an important commercial timber through chemical preservation. Its service life can be extended by six times its normal life span by preservative treatment (Stubbs 1967).

There is a need to optimize the utilization of these forest resources. The penetration study of four under-utilized species was conducted with the following objectives:

i. To evaluate the penetration of copper-chrome-arsenate within and between selected timber species.
ii. To determine the factors which influence the penetration of this preservative.

MATERIALS AND METHODS

Timber Species

Four under-utilized species namely Mempening (*Lithocarpus* spp.), Nyatoh Nangka Kuning (*Pouteria malaccensis*), Pauh Kijang (*Irvingia malayana*) and Petai (*Parkia speciosa*) and two commercially well-known species, namely Jelutong (*Dyera costulata* Hassk.) and Merbau (*Intsia palembanica* Miq.) were used in this study. These species were selected based on their potential as utility timber and their availability in volume from mixed-dipterocarp forest. Jelutong and Merbau were included as control.

Preparation of Specimens

The average diameter of the selected trees was about 45 cm. Good sound logs were selected and transported to the sawmill for conversion into scantling sizes of 3 m lengths. The scantlings were cut into 5 cm × 5 cm × 60 cm specimens. Twenty specimens were prepared from each species. These specimens were dried for one month in a dehumidified room at a temperature of 25°C and relative humidity of 50%. The moisture content and specific gravity of each species were determined in accordance with Malaysian Standard MS 3.38 (Anon 1976).

Anatomical Study

Anatomical properties (pore diameter, pore density and lumen percentage) of these species were examined from prepared slides using a light microscope. 30 cross-sectional fields were measured for each species at 40x magnification. The measurements were made and recorded automatically with an image analyser model Quantimet Q520+.

Chromated-Copper-Arsenate Preservatives (Celcure A)

CCA product, Celcure 'A' was used to treat the wood samples. The preservative solution was prepared at 3% concentration. The wood specimens were tied together and placed in a treatment vessel of 60 cm diameter and 3 m length. The specimens were then treated with Celcure 'A' solution using the full-cell treatment process in accordance with schedule 3 of the Malaysian Standard, MS 3.39 (Anon 1976).

Penetration Study

Two freshly-cut discs of 15 mm thickness were obtained from each specimen (*Fig. 1*). Chrome-

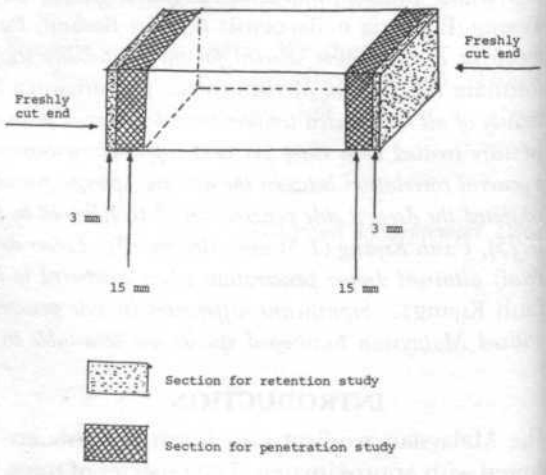

Fig 1: Cutting of specimen for penetration and retention studies.

Azurol-S solution was used to detect the presence of copper. The solution was sprayed evenly over the two freshly-cut treated wood surfaces. A deep-blue colour revealed the presence of copper. The depth of penetration was then measured with vernier calipers. The grading of side penetration was classified into six grades from no penetration (0) to complete penetration (5)(Koh 1979).

RESULTS AND DISCUSSION

Moisture Content and Density of the Treated Specimens

The final moisture content and the density of the six selected timber species are shown in Table 1. The moisture content ranged from 13.3 per cent for Nyatoh Nangka Kuning to 17.5 per cent for Jelutong. Results reveal that the density of the four under-utilized species varied. Pauh Kijang which is classified under the very heavy group, recorded the highest of 1201.6 kg/m^3 followed by Mempening (858.4 kg/m^3), Nyatoh Nangka Kuning (644.4 kg/m^3) and Petai (625.7 kg/m^3). The control samples, Jelutong had (457.2 kg/m^3) and Merbau 822 kg/m^3, are classified as light and heavy hardwood respectively. The moisture content level was well within the 25 per cent value as recommended for well seasoned timber for wood preservation treatment (Anon 1974, 1976).

TABLE 1
Average percentage of moisture content and densities of the six selected timber species

Species	Moisture content (%) Mean ± S.D	Density (kg/m^3) Mean ± S.D
Jelutong (*Dyera costulata*)	17.5 ± 0.3	457.2 ± 3.5
Petai (*Parkia speciosa*)	15.3 ± 0.2	625.7 ± 7.1
Nyatoh N.K. (*Pouteria malaccensis*)	13.3 ± 0.2	644.4 ± 7.1
Merbau (*Intsia palembanica*)	15.4 ± 0.3	822.0 ± 8.0
Mempening (*Lithocarpus* spp.)	17.2 ± 0.2	858.4 ± 9.2
Pauh Kijang (*Irvingia malayana*)	16.4 ± 0.2	1201.6 ± 8.9

S.D. : Standard deviation

TABLE 2
Average penetration rating for the six selected timber species

Species	Average penetration rating (mean ± S.D.)	Multiple comparisons by the Quade-test
Merbau (*Intsia palembanica*)	0	0 d*
Pauh Kijang (*Irvingia malayana*)	1.5 ± 0.14	1.5 c
Mempening (*Lithocarpus* spp.)	1.75 ± 0.14	1.75 c
Nyatoh Nangka Kuning (*Pouteria malaccensis*)	3.6 ± 0.13	3.6 b
Jelutong (*Dyera costulata*)	4.9 ± 0.07	4.9 a
Petai (*Parkia speciosa*)	5.0 ± 0	5.0 a

Grade: 0= No penetration 5= Full penetration
S.D:= Standard deviation
* Means with the same letter in this column are not significantly different at t = 0.05

Side Penetration

Table 2 shows the average visual ratings of the side penetration for the six selected timber species, pressure treated with CCA. The results indicate that the absorptive capabilities among these timber species varied. Among the four under- utilized species, Petai exhibited the deepest penetration (5), followed by Nyatoh Nangka Kuning (3.6), Mempening (1.75) and Pauh Kijang (1.5). By comparison, the side penetration for the controls, i.e. Jelutong and Merbau, were 4.9 and 0, respectively.

The rankings for the side penetration within and among the six selected timber species were tested using Kendall's coefficient of concordance (W-test) and were found to be unbiased. The differences in the side penetration of the CCA preservative among these species were examined using Quade's multiple comparison test.

The results show that there was no significant difference ($P > 0.05$) between the penetration rating for Petai (5) and Jelutong (4.9) and also between Mempening (1.75) and Pauh Kijang (1.5). The penetration of Merbau (0) and Nyatuh Nangka Kuning (3.6) were significantly different from the rest (Table 2).

Generally, sapwood can be treated more easily than heartwood (Anon, 1956; Jurazs and Wellwood 1965). In heartwood, extractives can infiltrate completely into the cell walls or they may occur as surface deposits or plugs in cell lumina. Their presence in wood affects the permeability and physical properties of wood (Hunt and Garratt 1967; Panshin and de Zeeuw 1980). This is probably one of the reasons which explains the untreatable nature of Merbau which contained yellowish deposits in the vessels of the seasoned specimen. A dark brown, sticky paste-like substance was observed on the specimens after the impregnation process. On the other hand, the higher absorptive capability of Jelutong, Mempening, Nyatoh Nangka Kuning, Pauh Kijang and Petai could probably be attributed to their low content of deposits or extraneous materials in the cell lumina (Wong 1976). The duration of treatment and intensity of pressure applied are among the factors which can influence the penetration and retention of preservatives (McLean 1960; Rak 1975). Lee and Ong (1972) observed that the penetration and salt retention of CCA preservatives in Meranti Tembaga (*Shorea leprosula* Miq.) could be increased by increasing the pressure and the period of impregnation.

The ability of a preservative to penetrate deeply into the structure of wood is important for satisfactory preservation (Rak 1975). For effective protection of wood against bio-degrading agents, the preservative must be evenly distributed in sufficient concentration throughout the vulnerable portions of wood. Preservative penetration could be more critical in the performance of treated wood than in preservative retention (Blew *et al.* 1970). Gersonde (1967) found that shallow penetration of preservative, though at high concentration levels of preservative in the outer zone, did not provide adequate protection to the treated wood. In other words, deep and uniform penetration of preservatives should be considered since achievement of minimum retention does not necessarily guarantee the effectiveness of any treated timber products against biodegradation (Hunt and Garratt 1967; Hickin 1972).

The results of penetration studies on these timber species indicate that some samples did not show any sign of being penetrated by CCA preservative, yet they possessed certain levels of net dry salt retention. This could be due to the salt deposited on the timber surface. Jalaluddin (1980) and Francis (1981) also observed similar results from their treatability studies on other Malaysian hardwoods.

Preservative penetration is influenced by various factors, particularly the anatomy of wood (Siau 1971; Nicholas 1973; Rak 1975; Yata *et al.* 1979, 1981, 1982). The longitudinal preservative flow path in hardwood species is mainly through the vessels. The lateral movements of preservatives to the adjacent element is through the small bordered or half-bordered pit pairs (Hunt and Garratt 1967; Nicholas 1973; Greaves 1974). The polar liquid was found to pass readily along the vessel in hardwoods (Anon 1964). The rate of movement is dependent on the liquid's polarity and temperature as well as the structure and density of the wood. This movement is of great practical importance in treating species of high density such as Mempening and Pauh Kijang.

Pore size (Siau 1971) and available flow passage of liquid (Panshin and de Zeeuw 1980) were reported to have a significant effect on the permeability of wood. The results show that Petai attained the deepest penetration followed by Jelutong, Nyatoh Nangka Kuning, Mempening, Pauh Kijang and Merbau, respectively. Such differences could be attributed to the variation in pore size, pore density and the percentage of lumen between the species concerned (Table 3). One should realise the complexity of the factors governing the penetration of the preservative into the wood. Deep penetration in Petai, Jelutong and Nyatoh Nangka Kuning may be due to a combination of factors involving pore diameter, pore density and higher lumen percentage. The number of pore per sq. mm of Petai and Nyatoh Nangka Kuning is about double that of Merbau and Mempening; and could have accounted for to the extent of the preservative penetration (Table 3). The relationship between percentage of lumen and degree of penetration is further illustrated in *Fig. 2.*

Kumar and Sharma (1982) reported that the penetration of preservatives is influenced by

TABLE 3
Average vessel (pore) diameter, pore density, lumen percentage and density of six Malaysian hardwood species

Species	Pore diam. (μm)	Pore/mm^2	Lumen percentage %	Density (kg/m^3)
Pauh Kijang	178.42	3.92	25.09	1201.60
Merbau	219.96	3.33	31.34	822.00
Mempening	206.59	3.00	41.61	858.40
Nyatoh N.K.	80.48	7.33	43.36	644.40
Petai	198.76	6.48	47.64	625.70
Jelutong	167.96	3.90	58.00	457.20

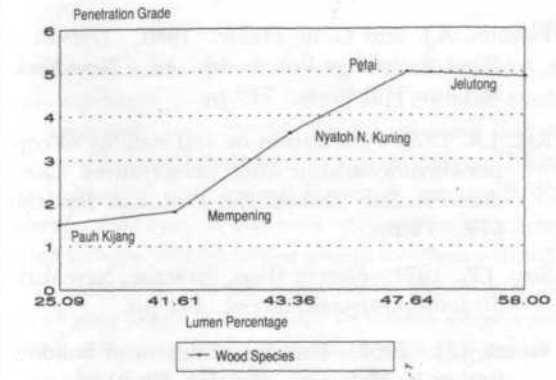

Fig. 2: Lumen percentage vs CCA penetration relationship of 5 Malaysian hardwood sepcies

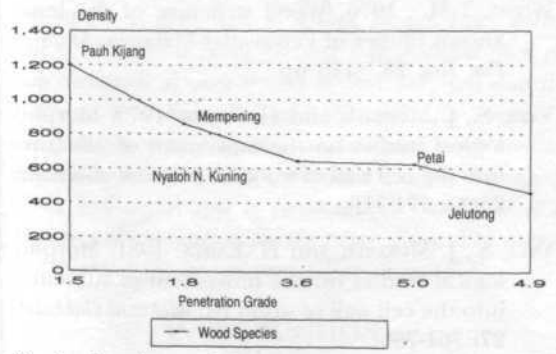

Fig. 3: Density vs CCA penetration relationship of 5 Malaysian hardwood species

the density of the wood. The relationship between the density and the penetration grades of these species is illustrated in *Fig. 3.* A lower specific gravity means less cell wall material and a correspondingly higher void volume; and a higher void volume corresponds to a higher amount of preservative that could be absorbed. Observations in this study reflect a general correlation between the density of wood and the penetrability of the solution. Species with a lower density such as Jelutong, Petai and Nyatoh Nangka Kuning exhibited good penetration, while those with a higher density, such as Mempening, Merbau and Pauh Kijang showed poor penetration. Similar observations were observed by Cheow (1978) and Koh (1979).

CONCLUSION

This study shows that Petai and Nyatoh Nangka Kuning exhibited better penetrability to CCA preservative than Mempening and Pauh Kijang. There is a general correlation between the density of the wood and the penetration of the solution, i.e. higher density species tend to absorb less preservative. To justify the durability and performance of these species, more extensive studies such as grave-yard tests should be carried out. The serviceability of timber can be improved through proper preservation techniques. Our study describes one such technique which could enhance wider utilization of the under-utilized species, particularly the light hardwoods, which could help overcome the impending shortage of durable timber in Malaysia by supplementing traditional resoures.

REFERENCES

ANON. 1956. Timber preservation. British Wood Preserving Association, London. 38 pp.

ANON. 1964. Timber preservation: cooling towers. Extract from Reprint for Forest Products, Australia.

ANON. 1974. Wood preservation plant. Operator's manual. Kuala Lumpur, Malaysia: Hickson and Timber Products Ltd.

ANON. 1976. Malaysian Standard MS 3.38. Specification for treatment of timber and plywood with copper-chrome-arsenic preservatives. Standards and Industrial Research Institute of Malaysia. 63 pp.

ANON. 1983. Under-utilized timber species of Sarawak. Paper presented in Bengkel National Mengenai Penggunaan Kayu-kayan Kurang Terkenal, Kuala Lumpur, Malaysia.

BLEW, J.O., E. PANEK and H.G. ROTH. 1970. Vacuum treatment of timber. *For. Prod. Jour.* **20:** 40-47.

CHEOW, L.T. 1978. Studies on chemical preservation of five lesser- known species. B.S. (For.) project report, Universiti Pertanian Malaysia 46 pp.

FRANCIS, G. 1981. A survey on the practices and effectiveness of treating solid timber with wood preservative by pressure treatment method. B.S. (For.) Project report, Universiti Pertanian Malaysia 126 pp.

GERSONDE, M. 1967. Testing and permanence of preservatives for constructional timber in the fungus cellar. *Master. U: Organ. Berl.* **2:** 249-261.

GREAVES, H. 1974. A review of the influences of structural anatomy on liquid penetration into hardwoods. *Jour. Inst. Wood Sci.* **6(6):** issue 36.

HICKIN, N.E. 1972. *The Wood Worm Problem.* 2nd. ed. London: Hutchinson, Co. Ltd. 123 pp.

HUNT, G.M. and G.A. GARRATT. 1967. *Wood Preservation.* 3rd. edition. New York: McGraw Hill Press. 433 pp.

JALALUDDIN, H. 1980. A survey on the practice and effectiveness of treating solid timber with wood preservatives by pressure treatment method. B.S. (For.). Project report, Universiti Pertanian Malaysia 109 pp.

JURAZS, P.E. and R.W. WELLWOOD. 1965. Variation in penetrability of western red cedar. *For. Prod. Jour.* **15:** 17-80.

KOCHUMEN, K.M. 1973. Lesser known timber trees of Malaysia. *Proc. Symp. Bio. Res and Nat. Dev.* 123-129.

KOH, H.L. 1979. Chemical preservation on three lesser-known wood species. B.S. (For.). Project report, Universiti Pertanian Malaysia 98 pp.

KUMAR, S. and R.P. SHARMA. 1982. Pressure impregnation of hardwood treatment schedules for easy to treat wood species. *Jour. Timb. Dev. Assoc.* (India) **28:** 24-29.

LEE, Y.H. and T.H. ONG. 1972. Interim report on high pressure treatment of Meranti Tembaga (*Shorea leprosula*). *Mal. For.* **35:** 60-63.

MCLEAN, J.D. 1960. Preservative treatment of wood by pressure methods. USDA. Agricultural Handbook 40. Revised Edition.

NICHOLAS, D.D. 1973. *Wood Deterioration and its Prevention by Preservative Treatments.* Vol. I and II. Syracuse, New York: Syracuse Uni. Press. 380 and 400 pp.

PANSHIN, A.J. and C. DE ZEEUW. 1980. *Textbook of Wood Technology* Vol. I. 4th. ed. New York: McGraw Hill Press. 719 pp.

RAK, J.R. 1975. Penetration by and stability of copper-chrome- arsenic wood preservatives. *Canadian For. Serv. Eastern. For. Prod. Lab. Rep. Op.* 87E. 12 pp.

SIAU, J.F. 1971. *Flow in Wood.* Syracuse, New York: Syracuse University Press. 131 pp.

STUBBS, J.H. 1967. The pre-treatment of building timber in Malaysia. *Mal. For.* **30:** 21-37.

UDARBE, M.P. 1974. The potential of the lesser known species in Sabah. *Mal. For.* **37:** 224-232.

WONG, T.M. 1976. Wood structure of the lesser known timber of Peninsular Malaysia. *Malayan For. Rec.* **28.** 115 pp.

YATA, S., J. MUKUDAI and H. KAJITA. 1979. Morphological studies on the movement of substance into the cell wall of wood II. *Mokuzai Gakkaishi* **25(3):** 171-176.

YATA, S., J. MUKUDAI and H. KAJITA. 1981. Morphological studies on the movement of substance into the cell wall of wood III. *Mokuzai Gakkaishi* **27:** 761-766.

YATA, S., J. MUKUDAI and H. KAJITA. 1982. Morphological studies on the movement of substance into the cell wall of wood V. *Mokuzai Gakkaishi* **28:** 10-16.

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Effects of Paclobutrazol and its Method of Application on the Growth and Transpiration of *Acacia mangium* Seedlings

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Keywords: Growth regulator, planting stock, plant water relations, triazole

ABSTRAK

Anak benih A. mangium *berumur 10 minggu telah dikenakan pembantut tumbesaran, paclabutrazol (PP333), pada kepekatan 0, 0.5, 1, 4 dan 12 g/l. Empat kaedah telah diuji iaitu S, membasahkan tanah di minggu 0; F1, semburan daun (tanah tabung dihalang dari terkena kimia) diminggu 0; F2, semburan daun (tanah tabung dihalang dari terkena kimia) di minggu 0 dan 6; dan S + F, semburan tanah dan daun (tanah tabung tidak dihalang dari terkena kimia) di minggu 0. Pokok-pokok dituai selepas 12 minggu untuk pengukuran beberapa parameter tumbesaran. Ketinggian dan keluasan daun, transpirasi dan konduksian stomata diukur setiap minggu. Paclobutrazol didapati berkesan untuk mengurangkan tumbesaran akar dan pucuk, transpirasi dan konduksian stomata anak benih. Pokok yang dikenakan kimia tersebut mempunyai perimbangan akar: pucuk yang tinggi. Kesan kimia bertambah dengan pertambahan kepekatan dan kekerapan semburan. Kimia itu paling berkesan apabila digunakan menerusi tanah samada melalui pembasahan tanah (S) atau semburan daun dan tanah (S+F).*

ABSTRACT

Ten-week-old potted A. mangium *seedlings were subjected to a growth retardant, paclobutrazol (PP333), at concentrations of 0, 0.5, 1, 4 and 12 g/l. Four methods of application were tested namely S, soil drenching at week 0; F1, foliar spray (potting soil protected from chemical) at week 0; F2, foliar spray (potting soil protected from chemical) at week 0 and 6; and S+F, soil and foliar spray (potting soil exposed to chemical spray) at week 0. Plants were harvested after 12 weeks for various growth measurements. Height and leaf area increments, transpiration and stomatal conductance were monitored at weekly intervals. Paclobutrazol was found to be effective in reducing root and shoot growth, transpiration and stomatal conductance of the seedlings. Treated plants had higher root to shoot ratios. The effects of the chemical increased with increasing concentration and frequency of application for the foliar spray. The chemical was most effective when applied through the soil either by drenching (S) or soil and foliar spray (S+F).*

INTRODUCTION

Malaysia has a programme to establish plantations of fast-growing hardwood species, principally *Acacia mangium* Willd. for general utility timber totalling 500,000 hectares by the year 2000. The success of such a large-scale plantation establishment depends on an efficiently-managed nursery to produce high-quality planting stock which can survive and grow rapidly when outplanted. Presently, potted seedlings about 3 months old and averaging 30 cm in height are transplanted from the nursery to the field. Survival and growth of these seedlings are reported to be good, but mortality sets in when transplanting is delayed and the seedlings overgrow in size (Abod and Abun 1989). The mortalities have often been attributed to unfavourable root to shoot ratios resulting in desiccation post-transplanting. Delays in transplanting often occur because of climatic factors which affect the timing of site preparation and its synchronization with field planting. This study was conducted in an attempt to develop an effective manipulative technique for controlling the growth of *A. mangium* seedlings and improving their establishment in the field.

Researchers have for many years sought reliable, effective and safe methods of controlling shoot growth of tree species using chemical growth retardants. Several new compounds have become available for testing in the last few years, all inhibitors of endogenous gibberellin biosynthesis. One of the most potent and long-lasting, the triazole paclobutrazol, has shown great efficacy in reducing height growth of many temperate fruit species and cultivars (Webster and Quinlan 1984).

At certain concentrations and spray volumes, paclobutrazol is reported to reduce shoot but not root growth and, in some instances, to improve plant water relations (Rademacher *et al.* 1984; Wang *et al.* 1986; Atkinson 1986). Swietlik and Miller (1983) reported that root length was stimulated by paclobutrazol applications at low to moderate concentrations. Moreover, Steffens *et al.* (1983) showed increases in fibrous root length, root: shoot ratio and unsuberised root diameter of apple (*Malus domestica* Borkh.) seedlings after treatment with paclobutrazol. Higher concentrations, however, may reduce root growth (Swietlik and Miller 1983). Increases in root: shoot ratios stimulated by plant growth retardant treatment should, in theory, also improve plant water relations and increase tolerance to drought (Turner and Begg 1981). Where growth retardants are applied at concentrations sufficient to control shoot growth it is likely that most of their effects on the water economy of the plant will be attributed to the indirect effect of reduced leaf number and total leaf area (Atkinson and Crisp 1982; Asamoah and Atkinson 1985). However, both Atkinson and Crisp (1982) and Dubravec *et al.* (1986) suggested an additional anti-transpirant effect of paclobutrazol, in addition to the indirect influence on water relations by reduction in leaf area.

Several methods have been used for applying paclobutrazol to trees. These include foliar sprays, soil surface sprays, trunk drenches, soil injection in narrow bands, incorporation into potting soil and pressure injection into the vascular system of woody stems. Foliar sprays with paclobutrazol have been reported to give immediate control of apple tree growth in the United Kingdom(Quinlan and Richardson 1984),but several applications are required in a single season to obtain effective growth control (Lever *et al.* 1982). Application as a soil drench is more effective than foliar spray in the long term (Curry and Williams 1983) mainly because the chemical can persist in the soil for longer periods and is readily absorbed by the roots and translocated acropetally through the xylem to the meristematic regions (Richardson and Quinlan 1986).

MATERIAL AND METHODS

A. mangium seeds were obtained from plantations in Sabah, Malaysia that were initially established from seeds imported from Queensland, Australia.

Seeds were pretreated in boiling water for 30 seconds, soaked in tap water for 24 hours and then germinated in a greenhouse. Four weeks after germination, each seedling was transplanted into a plastic pot measuring 19 cm in diameter and 14 cm in height. The potting medium was a mixture of soil, sand and peat in a ratio of 7:3:2. Each pot contained 3 kg of the medium. To every 100 kg of the potting medium, 2 kg of triple superphosphate fertilizer was added.

The experiment was conducted in a greenhouse. Plants were selected 10 weeks after germination. Seedlings with uniform shape and measuring 20 cm tall were chosen from a large number of available plants.

Paclobutrazol (PP333) was supplied by Imperial Chemical Indusries (ICI) in aqueous suspension at a concentration of 250 g/l with an active ingredient content of 22.0% w/w. Its trade name is Cultar and chemical formula [2RS 3RS) -1-(4-chlorophenyl)] -4, 4-dimethy1-2- (1H-1,2,4 triazol-1-yl) pentan-3-o1). The chemical was diluted in distilled water to give concentrations of 0.5, 1, 4, and 12 g/l. A surfactant (Du Pont agricultural surfactant) also supplied by ICI was added at a concentration of 2.0 ml/l.

The experiment tested the effects of 5 concentrations (inclusive of control at 0 g/l)and 4 methods of application as follows:

- S : Soil drenching at week 0 (i.e. at the start of the treatment)
- F1 : Foliar spray at week 0 (potting soil protected from chemical)
- F2 : Foliar spray at week 0 and 6 (potting soil protected from chemical)
- S+F : Foliar spray at week 0 (potting soil exposed to chemical)

The surfaces of the pots in the F1 and F2 treatments were covered with plastic sheets to

shield the soil from the foliar sprays. A knapsack sprayer was used to spray the aerial parts of plants to run-off.

A total of 102 plants were used at 6 replicates per treatment. Only 6 unsprayed plants were used as a common control for all treatments.

Plants were harvested 12 weeks after treatment and measurements were taken for height (i.e. length of the main stem from the soil to the shoot apex), root collar diameter, number of branches, total leaf area, total root length, root and shoot dry weights. Roots were washed over a sieve of mesh pore size less than 1.0 mm square using pressurized tap water. Total root length was measured by a Comair root length scanner (Abod and Webster 1989; 1991). Leaf area was measured by a portable leaf area meter.

Increments in height and leaf area were monitored at weekly intervals from 3 plants selected randomly from each treatment. Measurements of transpiration and stomatal conductance were also made on these plants. Transpiration was measured gravimetrically at weekly intervals and expressed as gram water use (or water loss) per unit leaf area per week. The plants were watered to field capacity (as determined by a soil moisture meter - a voltaic probe manufactured by Plant Cove Ltd. UK) from the base by placing the base of each pot in a shallow dish of water. The pot and soil surface were then enclosed within a polythene bag, sealed around the stem base and weighed. Soil moisture status was monitored using the soil moisture meter and the plants maintained near field capacity by watering via the dish twice each day. The amount of water provided per week per plant was recorded and the pots were re-weighed at the end of each week.

By estimating approximate values for plant fresh weight increase per week, from values at planting and at harvest, and using the records of both water use and leaf area, transpiration per week was estimated.

Measurements of stomatal conductance, commencing in the first week following spraying were made on the abaxial surface of selected leaves using a steady-state porometer. This was similar to that described by Jones and Norton (1979) which measured the difference in relative humidity between inlet (maintained at zero) and outlet air flowing at a constant rate (2.5 ml/s) through a chamber enclosing 1.76 cm^2 of leaf surface. Measurements were taken between noon and 1400 hours on one fully-expanded, unshaded leaf at the approximate mid-length of the main shoot of each plant.

The experiment was designed to test the effects of paclobutrazol and its method of application in a 4 x 5 factorial using 6 replications in a completely randomized design.

RESULTS

Root and Shoot Growth

Increments in height, diameter, leaf area and number of branches, and total root length of the treated plants were statistically lower ($P < 0.05$) than the unsprayed control 12 weeks after treatment. On the other hand, the ratios of root length to leaf area and root to shoot dry weight of treated plants were significantly greater ($P<0.01$) than the control (Table 1). The effects of the chemical increased from 0.5 to 12 g/l. These patterns of response were similar at each of the 12 weekly measurements monitored for increments in height (*Fig. 1a*) and leaf area (*Fig. 2a*). Differences between treatments increased from week 1 to 12.

Soil drenching (S) or soil and foliar spray (S+F) gave similar and statistically significant reductions ($P<0.05$) in the height, diameter, leaf area and total root length and increased the ratios of root length to leaf area and root to shoot dry weights compared to the foliar spray alone at either one (F1) or two (F2) frequencies (Table 1). The number of branches formed was not significantly affected by the method of application. Two frequencies of foliar spray (F2) at week 0 and 6 gave a statistically greater reduction ($P<0.05$) in height and also increased the root length to leaf area ratio compared to the single spray at week 0. The effects of frequency of foliar spray were not significant on the other parameters measured. These patterns of response were also similar when monitored at weekly intervals for height (*Fig. 1b*) and leaf area (*Fig. 2b*) increments. Differences between the methods of application increased with time after treatment.

Statistically significant ($P<0.05$) interactions between the method of application and chemical concentration were recorded for height and leaf area increments, total root length and the ratio of root length to leaf area.

TABLE 1
Main effects of paclobutrazol on the growth of *Acacia mangium* seedlings 12 weeks after treatments for factor I (method of application) and factor II (concentration).

		Height Increment Factor (cm)	Diameter Increment (cm)	Leaf Area Increment (cm^2)	No. of Branches Increment	Total Root Length (m)	ln Ratio Root Length (cm) / Leaf Area (cm^2)		ln Ratio Root Dry Weight (g) / Shoot Dry Weight (g)	
Method of Application	Soil	4.5	0.14	88.9	3.2	22.8	3.02	(20.56)	-0.49	(0.60)
	Foliar F1	7.2	0.16	169.1	4.2	33.7	2.64	(14.12)	-0.65	(0.52)
	Foliar F2	6.1	0.16	159.1	3.7	29.4	2.78	(16.16)	-0.61	(0.53)
	Soil & Foliar	4.4	0.13	88.4	3.5	21.9	3.08	(21.88)	-0.52	(0.59)
	Sed	0.11	0.005	4.50	0.9	1.04	0.022		0.040	
F-Test		**	**	**	ns	**	**		**	
Concentration (mg/1)	0	13.3	0.20	268.7	8.3	44.3	2.44	(11.55)	-0.78	(0.45)
	.5	5.0	0.16	128.5	3.3	26.8	2.83	*17.00)	-0.64	(0.52)
	1	4.3	0.14	99.8	2.8	25.2	2.91	(18.49)	-0.57	(0.56)
	4	2.7	0.13	71.5	2.1	20.3	3.06	(21.52)	-0.41	(0.66)
	12	2.5	0.12	63.3	1.8	18.2	3.15	(23.51)	-0.46	(0.63)
	Sed	0.12	0.006	5.03	0.44	1.16	0.025		0.044	
F-Test		**	**	**	**	**			**	

Error df=40 *p<0.05 **p<0.01 ns: not significant

Bracketed means are retransformed values.

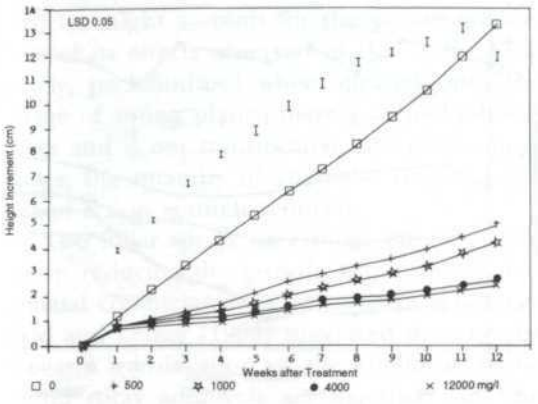

Fig. 1a: Effect of concentration of paclobutrazol on the height increment of Acacia mangium *seedlings*

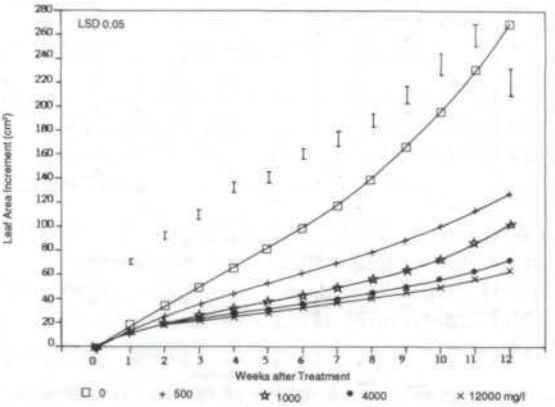

Fig. 2a: Effect of concentration of paclobutrazol on the leaf area increment of Acacia mangium *seedlings*

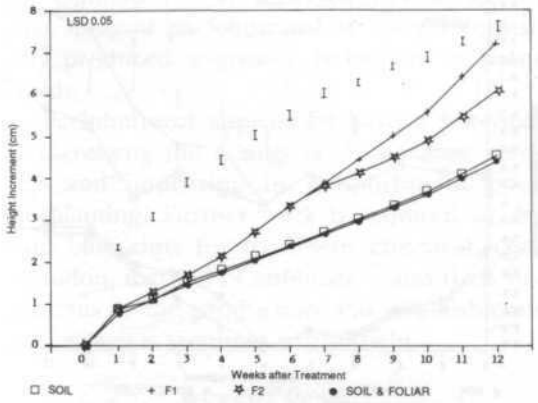

Fig. 1b: Effect of method of applying paclobutrazol on the height increment of Acacia mangium *seedlings*

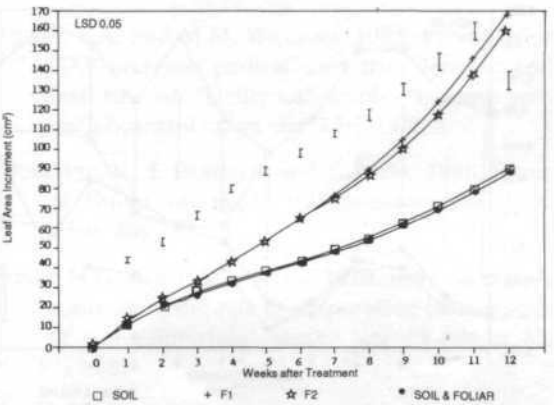

Fig. 2b: Effect of method of applying paclobutrazol on the leaf area increment of Acacia mangium *seedlings*

Transpiration and Stomatal Conductance

Both transpiration (*Fig. 3a*) and stomatal conductance (*Fig. 4a*) of treated plants were lower than the control throughout the 12 weekly measurements. Complete recovery was not apparent for either parameter at any of the concentrations 12 weeks after treatment. Stomatal conductance, however, appeared to recover to higher values from week 7 and this was most apparent at 4 and 12 g/l (*Fig. 4a*). Lowest transpiration and stomatal conductance were recorded at 12g/l and the values increased with decreasing chemical concentrations. Differences in values between the 4 and 12 g/l concentrations for either transpiration or stomatal conductance were generally not statistically significant, but the two higher concentrations induced significantly lower values than the lower concentrations (i.e. 1 and 0.5 g/l) at all sampling times.

Soil drenching (S) or soil and foliar spray (S+F) proved to be equally effective methods in reducing the transpiration (*Fig. 3b*) and stomatal conductance (*Fig. 4b*) compared to the foliar sprays at either one (F1) or two frequencies (F2) at all times of measurement. Compared to F1, the second spray in F2 significantly reduced both transpiration and stomatal conductance from week 7 to 12. Differences between the methods of application generally increased with time for both transpiration (*Fig. 3b*) and stomatal conductance (*Fig. 4b*).

Except at week 10 and 11, no statistically significant interactions were recorded between the method of application and concentration for transpiration. On the other hand, highly significant ($P<0.01$) interactions occurred between the two factors at each of the 12 weekly measurements for stomatal conductance.

DISCUSSION

Paclobutrazol was effective in retarding the root and shoot growth, transpiration and stomatal

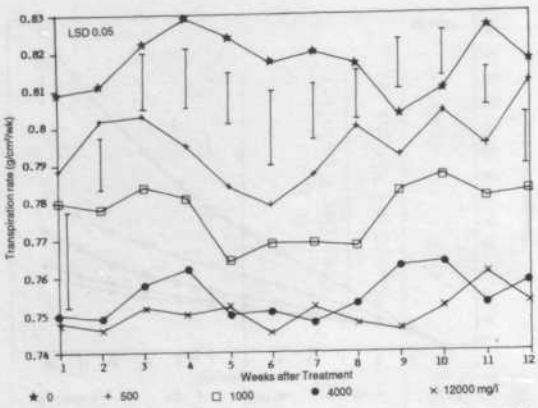

Fig. 3a: Effect of concentration of paclobutrazol on the weekly transpiration rate of Acacia mangium *seedlings*

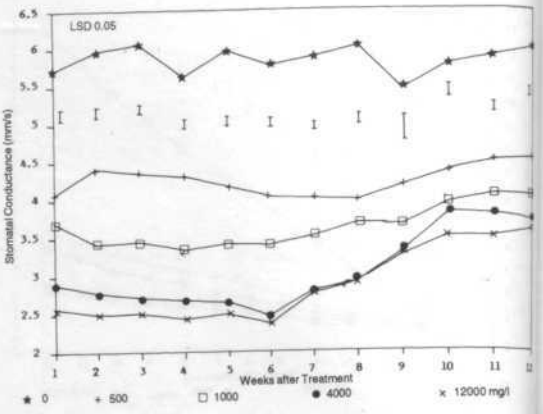

Fig. 4a: Effect of concentration of paclobutrazol on the stomatal conductance of Acacia mangium *seedlings*

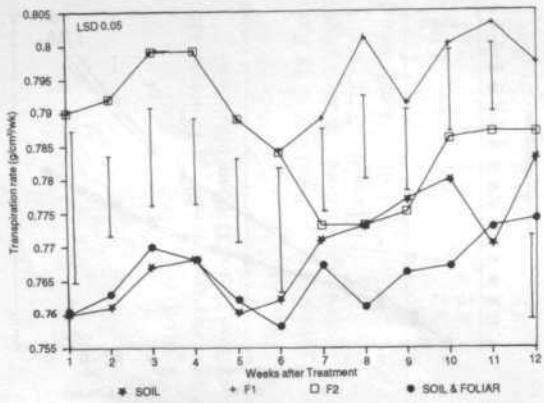

Fig. 3b: Effect of method of applying paclobutrazol on the weekly transpiration rate of Acacia mangium *seedlings*

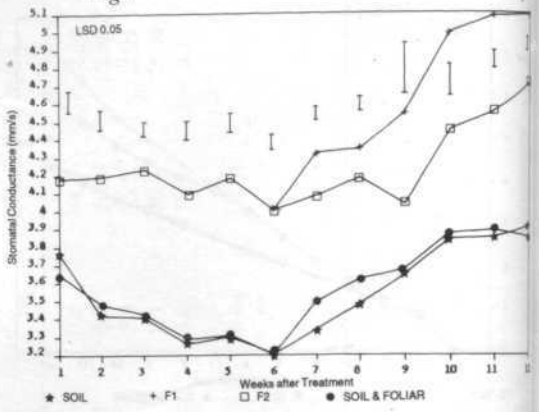

Fig. 4b: Effect of method of applying paclobutrazol on the stomatal conductance of Acacia mangium *seedlings*

conductance of *A. mangium* seedlings. The effects of the chemical increased from 0.5 g/l to reach a maximum at 12 g/l over the range of concentrations tested. No treatment damaged or deformed any seedling. Weekly measurements of height and leaf area increments and transpiration and stomatal conductance revealed the differences in values of treated plants compared to the control increased with time. There was no complete recovery for any of the parameters even at the lowest concentration tested 12 weeks after treatment. A lower concentration than 0.5 g/l appears necessary to obtain an earlier recovery from the chemical effect. The results also revealed that the soil needs to be exposed to the chemical during foliar sprays for more effective control of growth, transpiration and stomatal conductance.

Paclobutrazol-treated *A. mangium* seedlings had higher root to shoot ratios. Asamoah and Atkinson (1985) attributed such a response to a shift in assimilate partitioning from shoots to roots. The chemical also reduced the rate of transpiration. This reduction correlated well with reduced stomatal activity and decreased leaf area. Treated plants had higher root length to leaf area ratios. Atkinson and Thomas (1985) and Abod and Webster (1989) claimed that plants with these attributes have a greater chance to survive following transplanting because of improved plant water relations.

Paclobutrazol was found to be most effective in retarding the growth, transpiration and stomatal conductance of *A. mangium* seedlings when applied to the soil. This may be attributed to the chemical being readily absorbed by the roots and translocated almost exclusively in the xylem acropetally to the meristematic regions (Richardson and Quinlan 1986). The binding nature of the chemical with the soil colloids

particles might account for the greater persistence of its effects observed in this study. Conversely, paclobutrazol when sprayed onto the foliage of young plants, merely accumulates in leaves and is not translocated into other shoot tissues; the quantity of chemical reaching the sites of action is often reduced.

Two foliar sprays were more effective than one in reducing the growth, transpiration and stomatal conductance of *A. mangium* seedlings. Abod and Leong (1989) suggested that the uptake and translocation of the chemical at the second spray additively act together with the remaining triazole compounds from the previous application. In *Prunus domestica* L., Webster and Quinlan (1984) observed that a second foliar spray of paclobutrazol at 1.5 g/l consistently produced a greater reduction in shoot growth.

Paclobutrazol appears to have a potential for increasing the quality of *A. mangium* seedlings and improving its establishment post-transplanting. Further work is required to develop blueprints for treatment chemical, concentration, method of application and their implications to the production and establishment of *A. mangium* seedlings in the field.

REFERENCES

ABOD, S.A. and J. ABUN. 1989. Root and shoot growth and their manipulations of *Acacia mangium* seedlings. Paper presented at an International Symposium on Recent Development in Tree Plantations of Humid/Subhumid Tropics of Asia. Universiti Pertanian Malaysia. Serdang. 5-9 June, 1989.

ABOD, S.A. and C.K. LEONG. 1989. Physical and chemical control of the growth of *Acacia mangium* seedlings for plantation establishment in Malaysia. Paper presented at an International Symposium on *Efficiency of Stand Establishment Operation*, Rotorua. New Zealand. 11-15 Sept., 1989.

ABOD, S.A. and A.D. WEBSTER. 1989. The influence of foliar sprays of tetcylasis or paclobutrazol on the growth and water use by transplanted *Malus, Tilia* and *Betula* stocks. *J. Hort. Sci.* **66(1):** 85-94.

ASAMOAH, T.E.O. and D. ATKINSON. 1985. The effects of (2RS, 3RS) -1- (4-chlorophenyl) 4, 4-dimethyl - 2 - (IH-1, 2, 4 triazol-1-yl) and root pruning on the growth, water use and response to drought to Colt cherry rootstocks. *Plant Growth Regulation* **3:** 37-45.

ATKINSON, D. 1986. Efffects of some plant growth regulators on water use and uptake of mineral nutrients by tree crops. *Acta Horticulturae* **179:** 395-404.

ATKINSON, D. and C.M. CRISP. 1982. Prospects for manipulating tree root systems using plant growth regulators - Some preliminary results. Proc. 1982 British Crop Protection Conf. - *Weeds:* 593-599.

ATKINSON, D. and C.M.S. THOMAS. 1985. The influence of cultural methods on the water relations of fruit trees. *Acta Horticulturae* **171**: 371-382

CURRY, E.A. and M.M. WILLIAMS. 1983. Promalin or GA3 increase pedical and fruit length, and leaf size of "Delicious" apples treated with paclobutrazol. *Hort. Sci.* **18:** 214-215.

DUBRAVEC, K., I. DUBRAVEC and K. MYRA. 1986. Effect of Cultar on apple leaf transpiration. *Hort. Abstr.* **56:** 717.

JONES, H.G. and T.A. NORTON. 1979. Internal factors controlling the rate of evaporation from fronds of some intertidal algae. *New Phytologist* **83:** 771-781.

LEVER, B.G., S.J. SHEARING and J.J. BATCH. 1982. PP333 a new broad spectrum growth retardant. Proc. 1982. British Crop Protection Conf. - *Weeds* **1:** 3-10.

QUINLAN, J.D. and P.J. RICHARDSON. 1984. Effects of paclobutrazol on apple shoot growth. *Acta Horticulturae* **146:** 105-111.

RADEMACHER, W., J. JUNG., J.E. GRAEBE and L. SCHWENEN. 1984. On the mode of action of tetcyclasis and triazole growth retardants. Proc. of British Plant Growth Regulator Group Meeting "Biochemical aspects of synthetic and naturally occuring plant growth regulators" Monograph No. 11.

RICHARDSON, P.J. and J.D. QUINLAN. 1986. Uptake and translocation of paclobutrazol by shoots of M. 26 apple rootstock. *Plant Growth Regulator* **4:** 347-356.

SWIETLIK, D. and S.S. MILLER. 1983. The effects of paclobutrazol on growth and response to water stress of apple seedlings. *J. Amer. Soc. for Hort. Sc.* **108:** 1076-1080.

STEFFENS, G.L., S.Y. WANG, C.L. STEFFENS and T. BRENNA. 1983. Influence of paclobutrazol (PP333) on apple seedling growth and physiology. *Proc. of the Plant Growth Regulator Society of America* **10:** 195-205.

TURNER, N.C. and G.E. BEGG. 1981. Plant water relations and adaptation to stress. *Plant and Soil* **58:** 97-103.

WANG, S.Y., G.L. STEFFENS and M. FAUST. 1986. Effect of paclobutrazol on accumulation of carbohydrates in apple wood. *Hort. Sc.* **21:** 1419-1421.

WEBSTER, A.D. and J.D. QUINLAN. 1984. Chemical control of tree growth of plum (*Prunus domestica* L). I: Preliminary studies with the growth retardant paclobutrazol (PP333). *J. Hort. Sc.* **59(3):** 367-375.

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ISSN: 0126-6128

COMMUNICATION I

Kesan Penggabungan Germplasma Jagung dari CIMMYT (Rattray Arnold) dengan Germplasma Jagung Tempatan (Jagung Kumpit)

ABSTRAK

Tujuan kajian ini ialah untuk menentukan kesan penggabungan germplasma jagung putih 'dent' dari CIMMYT dengan jagung tempatan. Penentuan tersebut dibuat melalui perbandingan ciri-ciri morfo-agronomi di antara progeni kacukan dengan induk-induknya. Kajian dimulakan dengan membuat kacukan di antara populasi Rattray Arnold dari CIMMYT dengan populasi Jagung Kumpit. Seterusnya, biji benih kacukan, populasi Rattray Arnold dan Jagung Kumpit ditanam di plot percubaan dan cerapan dibuat ke atas ciri-ciri morfo-agronomi pada setiap pokok dari setiap populasi. Daripada analisis statistik ke atas data-data yang telah dicerap didapati progeni kacukan mencapai peringkat kematangan bunga staminat, kematangan bunga pistilat dan kematangan fisiologi yang lebih awal dari induk-induknya. Selanjutnya, progeni kacukan mempunyai nilai min tinggi pokok dan berat kering keseluruhan yang lebih rendah dari Rattray Arnold tetapi tidak berbeza dengan Jagung Kumpit. Dari segi keupayaan pengeluaran hasil pula, progeni kacukan mempunyai min berat tongkol dan saiz tongkol yang lebih baik dari Jagung Kumpit tetapi tidak berbeza dengan Rattray Arnold.

ABSTRACT

The objective of this study was to determine the effect of combining the white dent corn germplasm from CIMMYT with the local corn germplasm. The determination was done by comparing the morpho-agronomic characters between the progenies of the cross and the parents. The study was initiated by making the cross between Rattray Arnold, the population from CIMMYT with "Jagung Kumpit", the local population. Subsequently, seeds of the cross, Rattray Arnold and Jagung Kumpit,were planted on the experimental plot and observations were made on the morpho-agronomic characters of each plant in every population. The statistical analyses of the observed data showed that the progenies of the cross reached the stage of tasselling, silking and maturity earlier than the parents. The progenies of the cross had lower means in plant height and total dry weight than Rattray Arnold but did not differ significantly from "Jagung Kumpit". With respect to yielding ability, the progenies had significantly greater means of ear weight and ear length than the means compared to those of "Jagung Kumpit" but they did not differ from Rattray Arnold.

PENGENALAN

Crossa dan Gardner (1987) mencadangkan bahawa peningkatan kepelbagaian genetik di dalam sesuatu populasi boleh diperoleh melalui introgresi germplasma asing. Kacukan di antara populasi-populasi yang ingin diperbaiki dengan germplasma asing, eksotik atau liar akan menyediakan kepelbagaian genetik yang boleh digunakan oleh pembiakbaka untuk melakukan penabiran dan pemilihan di dalam program biakbaka tumbuhan. Di Argentina, introgresi germplasma elit jagung 'dent' dari Amerika Syarikat jagung hibrid 'flint' didapati telah meningkatkan prestasi pengeluaran hasil jagung hibrid 'flint' (Burn dan Dudley 1989). Dengan itu penggunaan germplasma dari negara-negara lain merupakan satu pendekatan untuk meluaskan takungan germplasma (Crossa dan Gardner 1987) dan meningkatkan heterosis serta prestasi pengeluaran hasil (Paterniani dan Lonquist 1963).

Di Sabah, Jagung Kumpit adalah populasi jagung tempatan yang digunakan sebagai makanan ringan. Jagung ini mempunyai isirong jenis 'dent' yang berwarna putih dan bersifat prolifikasi di mana setiap pokok mengeluarkan lebih dari satu tongkol jagung tetapi saiz tongkol adalah lebih kurang 8 cm sahaja (Lai 1990). Oleh yang demikian beberapa pendekatan perlu dilakukan untuk memperbaiki populasi Jagung

Kumpit. Di dalam kajian ini germplasma jagung putih 'dent' dari CIMMYT digunakan untuk memperbaiki populasi Jagung Kumpit. Secara khususnya, objektif kajian ini ialah untuk membandingkan ciri-ciri morfologi dan agronomi di antara tiga populasi jagung, iaitu Jagung Kumpit (populasi tempatan), populasi Rattray Arnold (jagung putih 'dent' dari CIMMYT) dan progeni kacukan di antara Jagung Kumpit dengan Rattray Arnold.

BAHAN DAN KAEDAH

Bahan

Biji benih yang digunakan di dalam kajian ini ialah biji benih dari populasi Jagung Kumpit (populasi tempatan), Rattray Arnold (1)8321 (populasi jagung putih 'dent' dari CIMMYT, Thailand) dan biji benih kacukan di antara Jagung Kumpit (induk jantan) dengan populasi Rattray Arnold dari CIMMYT (induk betina).

Kaedah Penanaman

Penanaman telah dilakukan pada 29 Ogos 1991 di Plot Percuban, Stesen Penyelidikan Pertanian, Tuaran. Rekabentuk eksperimen yang digunakan ialah rekabentuk rawak lengkap dengan bilangan pokok di dalam setiap populasi sebagai replikasi. Setiap populasi jagung ditanam di dalam plot seluas 2.25 m x 3.5 m. Biji benih ditanam dengan kedalaman 3 - 5 cm dan jarak 75 cm di antara barisan dan 25 cm di antara pokok di dalam barisan. Satu barisan sempadan ditanam pada setiap sisi plot untuk menyeimbangkan kesan persaingan. Bagi setiap titik penanaman tiga biji benih disemai di dalam tiga lubang yang berasingan. Pada peringkat tiga daun, dua anak benih dicabut dan meninggalkan satu yang terbaik.

Pembajaan dilakukan pada masa penanaman dengan menggunakan kadar pembajaan 50 N : 60 P_2O_5 : 60 K_2O kg/ha. Pada minggu kelima, tambahan 50 kg/ha N dilakukan. Pengairan, kawalan rumpai dan makhluk perosak dilakukan apabila perlu.

CIRI-CIRI YANG DIUKUR

Kesemua ciri-ciri yang berikut diukur pada setiap pokok. Cerapan dibuat pada seiap pokok semasa tumbesaran sehingga pokok-pokok jagung mencapai peringkat kematangan fisiologi. Berikut ialah ciri-ciri yang diukur dan kaedah pengukurannya:-

Tempoh kematangan staminat (TKS)

Tempoh kematangan staminat ialah bilanga hari dari penanaman hingga spikelet-spikele pada 'tessel' mengeluarkan stamen.

Tempoh kematangan pistilat (TKP)

Tempoh kematangan pistilat ialah bilangan ha dari penanaman hingga ovari (shoo mengeluarkan stigma (silk).

Tempoh kematangan fisiologi (TKF)

Tempoh kematangan fisiologi ialah bilangan ha dari penanaman hingga ciri-ciri berikut dicera

i. Daun menjadi kering
ii. Daun tongkol berubah kepada war cokelat
iii. 'silk' berwarna cokelat tua
iv. Bijirin mengandungi lapisan hita (black layer)

Ketinggian pokok (KP)

Ketinggian pokok ialah ukuran dalam cm da paras tanah hingga ke ruas yang paling ata Pengukuran dibuat dua minggu selep kematangan pistilat.

Bilangan tongkol per pokok (BT)

Bilangan tongkol bagi setiap pokok ialah jumla tongkol yang berisi pada peringkat matan fisiologi.

Berat kering tongkol (BKT)

Pada peringkat matang fisiologi, semua tongko yang berisi dituai dari setiap pokok. Tongko tongkol dikeringkan pada suhu 40°C selama 4 jam. Seterusnya tongkol-tongkol yang tela dikeringkan ditimbang dalam unit g da direkodkan sebagai berat tongkol berserta dau tongkol. Seterusnya daun tongkol dibuang da tongkol ditimbang dalam unit g dan direkodka sebagai berat kering tongkol.

Panjang tongkol (PT)

Tongkol yang paling atas dari setiap pokok da yang telah dikeringkan diukur dari dasar hingg ke hujung dalam cm dan direkodkan sebag panjang tongkol.

Berat kering keseluruhan pokok (BKKP)

Pokok-pokok yang telah dituai tongkolny ditebang pada paras tanah, dikeringkan pad suhu 80°C selama 48 jam dan ditimbang dala

unit g. Seterusnya nilai berat kering pokok dijumlahkan dengan nilai berat kering tongkol berserta dengan daun tongkol dan direkodkan sebagai berat kering keseluruhan pokok.

Indeks tuaian (IT)

Indeks tuaian diperoleh dengan formula berikut:-

$$IT = \frac{BKT}{BKKP} \times 100\%$$

KEPUTUSAN DAN PERBINCANGAN

Analisis varians menunjukkan ada perbezaan yang bererti di antara populasi bagi ciri-ciri tempoh kematangan staminat, tempoh kematangan pistilat, tempoh kematangan fisiologi, ketinggian pokok, berat kering tongkol, panjang tongkol, berat kering keseluruhan pokok dan indeks tuaian, dan tidak ada perbezaan yang bererti untuk ciri bilangan tongkol per pokok (Jadual 1). Selanjutnya perbandingan min-min di antara setiap dua populasi dilakukan dengan menggunakan ujian Student t pada paras keertian 5%.

Bagi ciri kematangan staminat, populasi Jagung Kumpit yang mencapai kematangan staminat pada hari ke-70 adalah berbeza secara bererti dari populasi Rattray Arnold yang mempunyai min KMS 67 hari. Progeni kacukan pula mempunyai min tempoh kematangan staminat yang lebih awal berbanding dengan kedua-dua induk, iaitu pada hari ke-61 selepas penanaman (Jadual 2).

Populasi Jagung Kumpit juga adalah lewat mencapai kematangan pistilat berbanding dengan populasi Rattray Arnold, iaitu dengan min masing-masing 74 dan 71 hari. Sama seperti ciri kematangan staminat, progeni kacukan juga adalah lebih awal dari kedua-dua induk bagi tempoh kematangan pistilat, iaitu secara puratanya pada hari ke-63.

Walau pun Jagung Kumpit adalah lewat mencapai kematangan staminat dan pistilat, tetapi ianya adalah lebih awal mencapai kematangan fisiologi berbanding dengan populasi Rattray Arnold. Jadual 2 menunjukkan bahawa Rattray Arnold mencapai kematangan fisiologi pada hari ke-100, manakala populasi Jagung Kumpit pula pada hari ke-94. Sama seperti ciri kematangan staminat dan pistilat, progeni kacukan mencapai kematangan staminat yang lebih awal dari kedua-dua induknya, iaitu secara puratanya pada hari ke-88.

Bagi ciri ketinggian pokok, populasi Rattray Arnold mempunyai min ketinggian pokok yang lebih tinggi dari Jagung Kumpit, iaitu dengan min masing-masing 125 cm dan 115 cm. Progeni kacukan mempunyai min ketinggian pokok 113 cm dan berbeza secara bererti dengan populasi Rattray Arnold tetapi tidak berbeza secara bererti dengan populasi Jagung Kumpit (Jadual 2).

JADUAL 2
Min tempoh kematangan staminat (TKS), tempoh kematangan pistilat (TKP) tempoh kematangan fisiologi (TKF) dan ketinggian pokok (KP) tiga populasi jagung

Populasi (hari)	TKS (hari)	TKP (hari)	TKF (cm)	KP
Rattray Arnold	67[a]	71[a]	100[a]	125[a]
Jagung kumpit	70[b]	74[b]	94[b]	115[b]
Progeni Kacukan	61[c]	63[c]	88[c]	113[b]

Huruf yang sama menunjukkan tidak ada perbezaan yang bererti

JADUAL 1
Min kuasadua analisis varians bagi ciri-ciri yang telah dicerap di dalam tiga populasi jagung

Sumber variasi	dk	TKS	TKP	TKF	KP	BT	BKT	PT	BKKP	IT
Populasi	2	460**	668**	682**	831**	0.23	6172**	36**	25548**	20**
Ralat	56	8	13	12	178	0.13	362	3	1257	1

**bererti pada 1%.

Secara puratanya, populasi Jagung Kumpit mengeluarkan 1.24 tongkol per pokok, manakala semua pokok Rattray Arnold hanya mengeluarkan 1.00 tongkol per pokok. Bagi progeni kacukan, terdapat pokok-pokok yang mengeluarkan 2.00 tongkol per pokok yang memberikan purata 1.12 tongkol per pokok. Walau bagaimanapun, ketiga-tiga populasi ini tidak berbeza secara bererti bagi ciri bilangan tongkol per pokok (Jadual 1 dan 3).

Populasi Rattray Arnold mempunyai min berat tongkol 70 g yang jauh lebih berat berbanding dengan Jagung Kumpit yang mempunyai min berat kering tongkol 30 g sahaja (Jadual 3). Progeni kacukan mempunyai nilai min berat tongkol 47 g iaitu nilai perantara dari kedua-dua induk dan berbeza secara bererti dengan nilai min kedua-dua induk (Jadual 3). Dari segi kepanjangan tongkol, populasi Rattray Arnold juga mempunyai min tongkol yang lebih panjang dari Jagung Kumpit, iaitu dengan min masing-masing 11.46 cm dan 8.48 cm. Progeni kacukan pula mempunyai min panjang tongkol 10.34 cm yang tidak berbeza secara bererti dengan populasi Rattray Arnold tetapi berbeza secara bererti dengan populasi Jagung Kumpit.

Bersesuaian dengan ciri ketinggian pokok dan berat tongkol, populasi Rattray Arnold ialah populasi yang mempunyai min berat keseluruhan pokok yang lebih tinggi dari Jagung Kumpit iaitu dengan nilai min 158 g. Progeni kacukan dan Jagung Kumpit mempunyai nilai min berat kering keseluruhan pokok yang sama iaitu 89 g (Jadual 3).

Jadual 3 juga menunjukkan populasi Rattray Arnold tetap lebih baik dari Jagung Kumpit bagi ciri indeks tuaian, iaitu mempunyai min indeks tuaian 44% manakala Jagung Kumpit hanya 35% sahaja. Progeni kacukan pula menonjolkan nilai min indeks tuaian yang mengatasi kedua-dua induknya, iaitu 53%.

JADUAL 3
Min bilangan tongkol (BT), berat kering tongkol (BKT) panjang tongkol (PT), berat kering keseluruhan pokok (BKKP) dan indeks tuaian (IT) tiga populasi jagung

Populasi	BT	BKT (g)	PT (cm)	BKKP (g)	IT (%)
Rattray Arnold	1.00[a]	70[a]	11.46[a]	158[a]	44[a]
Jagung kumpit	1.24[a]	30[b]	8.48[b]	89[b]	35[b]
Progeni Kacukan	1.12[a]	47[c]	10.34[a]	89[b]	53[c]

Huruf yang sama menunjukkan tidak ada perbezaan yang bererti.

Secara keseluruhannya, populasi Rattray Arnold mempunyai prestasi yang lebih baik dari Jagung Kumpit. Keputusan ini adalah dijangkakan memandangkan fakta bahawa populasi jagung dari CIMMYT adalah merupakan populasi yang telah diperbaiki, sedangkan populasi Jagung Kumpit masih belum diperbaiki.

Progeni kacukan yang mengandungi 50% germplasma Rattray Arnold dan 50% germplasma Jagung Kumpit didapati menunjukkan ekspresi heterosis bagi beberapa ciri yang dicerap. Bagi ciri tempoh kematangan staminat, pistilat dan fisiologi, didapati progeni kacukan menunjukkan sifat keawalan, iaitu lebih awal dari Rattray Arnold bagi kematangan pistilat dan staminat dan lebih awal dari Jagung Kumpit bagi kematangan fisiologi. Dari segi heterosis induk lebih baik, didapati progeni kacukan menunjukkan nilai heterosis -9.1% bagi ciri kematangan staminat, -11.4% bagi kematangan pistilat dan -6.5% bagi kematangan fisiologi.

Progeni kacukan juga mempunyai sela masa di antara kematangan staminat dan pistilat yang pendek iaitu 2 hari berbanding 4 hari bagi kedua-dua induknya. Bagi sela masa kematangan pistilat hingga kematangan fisiologi pula, didapati sela masa bagi progeni kacukan ialah 25 hari, iaitu lebih pendek dari Rattray Arnold yang mempunyai sela masa 29 hari tetapi lebih panjang dari Jagung Kumpit yang mempunyai sela masa 20 hari sahaja. Oleh itu dari segi tempoh pengisian biji, progeni kacukan didapati mempunyai tempoh yang lebih panjang dari Jagung Kumpit.

Bagi ciri pertumbuhan vegetatif, progeni kacukan adalah lebih menyerupai Jagung Kumpit iaitu tidak menunjukkan perbezaan bagi ciri ketinggian dan berat keseluruhan pokok. Walau bagaimanapun untuk ciri hasil, iaitu dari segi berat kering tongkol dan panjang tongkol, didapati progeni kacukan lebih baik dari Jagung Kumpit tetapi tidak mengatasi nilai min Rattray Arnold. Progeni kacukan didapati tidak menunjukkan ekspresi heterosis bagi kedua-dua ciri ini. Keputusan yang serupa juga didapati bagi ciri bilangan tongkol, iaitu ketiga-tiga populasi tidak berbeza dari segi jumlah tongkol yang dikeluarkan oleh setiap pokok. Keputusan

ini bersesuaian dengan kenyataan Sinha dan Khana (1975) bahawa tidak ada kesan heterosis bagi ciri bilangan tongkol per pokok dalam progeni kacukan F1.

Keadaan pertumbuhan vegetatif yang sama seperti Jagung Kumpit tetapi pengeluaran hasil yang lebih tinggi telah menyebabkan progeni kacukan mempunyai nilai indeks tuaian yang tinggi iaitu lebih tinggi dari Rattray Arnold, dengan nilai heterosisnya 22%.

KESIMPULAN

Secara keseluruhannya, Jagung Kumpit adalah kurang produktif berbanding dengan Rattray Arnold. Rattray Arnold yang menunjukkan pertumbuhan vegetatif yang lebih baik dari Jagung Kumpit juga didapati mencapai peringkat berbunga lebih awal dan mempunyai tempoh pengisian biji yang lebih lama. Sifat-sifat ini mungkin telah menyebabkan keupayaan pengeluaran hasil Rattray Arnold lebih baik dari Jagung Kumpit. Selanjutnya, gabungan di antara germplasma Jagung Kumpit dengan germplasma Rattray Arnold memberikan harapan untuk pembentukkan populasi yang lebih baik, iaitu progeni kacukan telah menunjukkan tempoh berbunga dan tempoh kematangan yang lebih awal dari kedua-dua induk. Bagi ciri pengeluaran hasil pula, progeni kacukan walau pun tidak mengatasi keupayaan pengeluaran hasil Rattray Arnold tetapi ternyata lebih baik dari Jagung Kumpit. Progeni kacukan juga menunjukkan penampilan yang baik dengan nilai indeks tuaian yang melebihi nilai kedua-dua induk, iaitu progeni kacukan lebih baik dari induk-induknya dari segi keupayaan untuk pemindahan hasil fotosintat dari tisu vegetatif kepada tisu reproduktif.

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RUJUKAN

BURN, L.E. and J.W. DUDLEY. 1989. Breeding potential in the USA and Argentina of corn populations containing different proportions of flint and dent germplasms. *Crop Sci.* **29:** 570-577.

CROSSA, J. and C.O. GARDNER. 1987. Introgression of exotic germplasm for improving an adapted maize population. *Crop Sci.* **27:** 187-190.

LAI, C.M. 1990. Pembaikan Jagung Kumpit Dengan Menggunakan Kaedah Pilihan Berulang Fenotip. Tesis Sarjana Muda Sains (Kep.), Jabatan Biologi, FSSA, UKM Kampus Sabah.

PATERNIANI, E. and J.H. LONNQUIST. 1963. Heterosis in interracial crosses of corn (*Zea mays* L.). *Crop Sci.* **3:** 504-507.

SINHA, S.K. and R.KHANA. 1975. Physiological, biochemical and genetic basis of heterosis. *Avd. Agron.* **27:** 123-174.

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COMMUNICATION II

The Use of Antibody-sensitized Latex to Detect *Cymbidium* Mosaic Virus in Orchids

ABSTRAK

Ujian agglutinasi lateks (L) dan satu modifikasi ujian lateks, bersalut protein A (PAL) telah dibandingkan untuk sensitisasi dengan antibodi kepada Cymbidium *mosaic virus' (CyMV). Satu siri kepekatan globulin telah diuji terhadap CyMV tulin. Kepekatan virus minimum yang boleh dikesan adalah sama untuk kedua-dua reaksi L dan PAL iaitu 0.104* µg/ml. *Titik akhir yang boleh dikesan di sap orkid* Oncidium *Gower Ramsey adalah serupa dipencairan 1/2560. PAL didapati lebih sensitif dari L di pencairan globulin lebih tinggi iaitu 1/512. PAL juga menunjukkan kesensitifan lebih tinggi dari L berasaskan kepada lima hibrid orkid dan menunjukkan persamaan dengan ujian mikroskop elektron.*

ABSTRACT

Latex agglutination test (L) and a modification of the latex test, protein A-coated latex (PAL) were compared for sensitization with antibodies to Cymbidium *mosaic virus (CyMV). A series of globulin concentrations was tested against purified CyMV. The minimum detectable virus concentration was similar for both L and PAL reactions at 0.104* µg/ml. *The detectable end points in infected* Oncidium *Gower Ramsey orchid sap were similar at a dilution of 1/2560. PAL was found to be more sensitive than L at a higher globulin dilution of 1/512. The PAL test showed higher sensitivity than L based on five orchid hybrids and compared favourably with electron microscopy test.*

INTRODUCTION

Commercially produced orchids in Malaysia are frequently infected with *Cymbidium* mosaic virus (CyMV) and to a lesser extent with *Odontoglossum* ringspot virus (ORSV) (Abdul-Samad 1985, 1986, 1989a, 1989b). Detection and identification of plants infected with CyMV or ORSV or both have been difficult because the infected plants may be symptomless or they may show some chlorotic or necrotic patterns pending on the species or hybrids. At present there is no available method of detection for use in orchid viruses locally. Imported orchids are rarely tested for the presence of viruses. Therefore a simple and quick method of testing is needed that combines high specificity and sensitivity with practical value, especially in quarantine programs, and for the development and testing of virus-free stocks. The most obvious choice would be a serological test.

The latex agglutination test (L) and the protein A-coated latex (PAL), a modification of the latex test (Querfurth and Paul 1979) have been used widely for the detection of plant viruses in their naturally occurring host as well as in herbaceous plant indicator species (Bercks 1967; Abu Salih *et al.* 1968; de Sequera and Lister 1969; Mumford 1977; Khan and Slack 1978; Koenig and Bode 1978; Thomas 1980; Torrance 1980; Demski *et al.* 1986). Due to their simplicity and the fact they do not require elaborate instruments, they can be used to test large numbers of samples in the laboratory or in field situations. Therefore this study was undertaken to determine the sensitivity of L and PAL and their potential use in detecting CyMV in orchids. A comparison between L, PAL and electron microscopy was also carried out.

MATERIALS AND METHODS

Virus and Antiserum

Cymbidium mosaic virus (CyMV) isolate was originally obtained from the orchid *Cattleya* sp. (Abdul-Samad 1985) and propagated on *Dendrobium* sp. The virus was purified according to the method used by Wisler *et al.* (1982). Systemically infected leaves were homogenised in 0.05M phosphate buffer, pH 7.5 containing

0.125M Na_2SO_3. The extract was clarified by adding chloroform (1:1, v/v) and the virus was concentrated with polyethylene glycol 6000 (PEG 6000). It was further purified through one cycle of differential centrifugation and a sucrose density gradient. The virus zone was fractionated, collected and concentrated by ultracentrifugation and the purified virus was resuspended in the same buffer.

Antiserum was produced by immunising rabbits using a series of two intravenous, one subcutaneous and one booster injection. Approximately 250 µg of viral antigen was administered intravenously to each animal at the first injection. The second intravenous injection was given one week later with 500 µg of antigen. Four weeks later, 1 mg of antigen emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously at several sites on the flank of the animal. Booster injection was given subcutaneously with 450 µg of antigen emulsified with Freund's complete adjuvant one month later. The rabbits were bled at weekly intervals starting one week after the last injection. Blood samples were collected from the ear veins and the serum recovered after clotting and removal of cellular material by centrifugation at 2000 g for 10 mins.

Preparation of Globulin and Sensitization of Latex

Globulin fractions were obtained from antiserum by precipitation with ammonium sulphate (equal volumes of saturated $(NH_4)_2SO_4$ with 0.5 ml antiserum diluted with 9.5 ml of distilled water). Solutions were incubated at room temperature for half an hour and centrifuged at low speed for 15 min. The precipitates were resuspended in saline and diluted to one-half of the original diluted antiserum and stored at 4°C after adding sodium azide to a concentration of 0.2% (Hill 1984). Antibody-sensitised latex (Sigma latex beads LB-8) and protein A-coated latex were prepared by the method described by Hill (1984).

Test Procedures

All tests were carried out in U-shaped microtiter plates (polystyrene) with 400µl capacity. Two-fold serial dilutions of the purified virus or infected plant sap to be tested were made with 0.05M Tris-HCl buffer, pH 7.2 containing 0.02% PVP 44,000. The latex was sensitised at a two-fold dilution of the globulin. For PAL the protein A solution was diluted 1:200 with glycine buffered saline and then mixed with the latex suspension which had been diluted with saline. Equal volumes (c. 50 µl) of L or PAL and the test samples were placed in wells. The plates were then shaken on a rotary shaker for 1 hr before observing agglutination reactions. The detection end point was recorded as the highest antigen dilution at which agglutination was visible (Ball 1974).

Electron Microscopy

A small piece of infected leaf tissue about 2-3 mm^2 was squashed on a glass slide in two drops of phosphotungstic acid (PTA), pH 6.8. A drop of sap mixture was placed on a 400 mesh carbon-strengthened Formvar coated grid, drained and dried and observed in a Philips HMG 400 electron microscope.

RESULTS AND DISCUSSION

Purified virus preparations and crude sap extracts reacted positively by forming visible loose aggregates with the latex-sensitised serum with or without protein A treatment. No agglutination of the sensitised latex preparations occurred against the control preparations where buffer and healthy orchid sap were used. The optimum antiserum dilution, which detected the highest dilution of antigen, for each serum globulin preparation for sensitising the latex particles for both L and PAL was determined by using purified virus. The dilution for both L and PAL was 1/512 in terms of the original globulin preparation. The minimum detectable virus concentration for the L and PAL reactions was similar at 0.104 µg/ml. Tests at higher globulin and antigen dilutions gave variable results. Determination of the detectable end-points by L and PAL was carried out by using crude sap extract from CyMV-infected *Oncidium* Gower Ramsey orchid. There was an increased detectable dilution end point from 1/128 to 1/512, a one-fold dilution between L and PAL. The PAL method showed higher sensitivity at a globulin dilution of 1/512. Therefore this would allow greater economy of use of antiserum globulins. No difference in sensitivity was detected when Tween 20 (Torrance 1980) was added to the extraction buffer for PAL. At a plant sap dilution of 1/2560 the virus could still be detected by L and PAL.

TABLE 1
Detection for CyMV in plants using L, PAL and electron microscopy

Orchid species/hybrid	L*	PAL*	E.M.#
Phalaenopsis Natasha	0	1+	+
Phalaenopsis sp.	1+	2+	+
Oncidium sp.	0	1+	+
Aerides Lorengga x *Vanda* Sanderiana	1+	2+	+
Dendrobium sp.	1+	3+	+
Vanda sp.	–	5+	+
Dendrobium utai	–	5+	+
Cattleya sp.	–	2+	+
Oncidium sp.	–	3+	+
Oncidium sp.	–	2+	+
Healthy sap (*Dendrobium* sp.)	0	0	0

* Tests were carried out using sample dilution at 1/320 and globulin dilution at 1/128. A minimum of three samples were taken from matured leaves

\# + Virus particles seen in at least three fields of view randomly selected at the illuminated viewing screen at an indicated 35,000 x magnification.

– Not tested

Agglutinations were ranked as follows:
0 = no reaction, 1+ = barely visible , 2+ = slight
3+ = moderate , 4+ = heavy , 5+ = very heavy

Both L and PAL were then used to test various orchid species and hybrids for presence of CyMV (Table 1) and sensitivity was compared with electron microscopy. Tests were carried out at sample dilution of 1/320 and globulin dilution at 1/128. The PAL test showed higher sensitivity than L, based on five orchid hybrids and compared favourably with electron microscopy.

The results from this study indicate that L and PAL constitute a sensitive, simple, rapid and reliable procedure for diagnosis of CyMV in orchids. Another advantage is that the sensitised latex has a long shelf-life when stored at 4°. Our test results showed that it could still be used after three months of storage without loss of sensitivity.

These techniques can be used in routine testing by growers advisory services for certification of healthy stocks. The technique does not require expensive equipment and biochemicals and has wider specificity where an antiserum to a single strain would be able to detect other strains of the same virus (Koenig *et al.* 1979). This technique could save cost, time and labour. The PAL method is very practical and particularly useful in the field even though it is not as sensitive as ELISA for detecting CyMV (N. Abdul-Samad, unpublished).

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REFERENCES

ABDUL-SAMAD, N. 1985. Leaf necrosis caused by *Cymbidium* mosaic virus in *Cattleya* orchid. MAPPS Newsletter **9:** 3-4.

ABDUL-SAMAD, N. 1986. Diagnosis of orchid diseases caused by *Cymbidium* mosaic virus (CyMV) and *Odontoglossum* ringspot virus (ORSV). In *Proc. Int. Conf. Pl. Prot. in the Tropics* 2 p. 356.

ABDUL-SAMAD, N. 1989a. A viral complex affecting *Aranthera* Beatrice Ng orchid. *Pertanika* **12:** 149-151.

ABDUL-SAMAD, N. 1989b. Orchid viruses and their management. *Mal. Agric. Digest* **7:** 21-25.

ABU SALIH, H.S., A.F. MURANT and M.J. DAFT. 1968. The use of antibody-sensitised latex particles to detect plant viruses. *J. Gen. Virol.* **3:** 299-302.

BALL, E.M. 1974. Serological tests for the identification of plant viruses. St. Paul, MN: Am. Phytopathol. Soc., 31 pp.

BERCKS, R. 1967. Methodische Untersuchungen uber den serologischen Nachweis pflanzen pethogener Viren mit dem Bentonit-Flockungstet, dem Latex-Test und dem Bariumsulfat-test. *Phytopath. Z.* **58:** 1-17.

DE SEQUERA, O.A. and R.M. LISTER. 1969. Applicability of latex flocculation serological testing to apple viruses. *Phytopathology* **59:** 572-574.

DEMSKI, J.W., D.C. BAYS and M.A. KHAN. 1986. Simple latex agglutination test for detecting flexuous rod-shaped viruses in forage legumes. *Plant Dis.* **70:** 777-779.

HILL, S.A. 1984. *Methods in Plant Virology. Vol.I. Methods in Plant Pathology.* London: Blackwell Scientific Publications.

KHAN, M.A. and S.A. SLACK. 1978. Studies on the sensitivity of a latex agglutination test for the serological detection of potato virus S and potato virus X in Wisconsin. *Am. Potato J.* **55:** 627-637.

KOENIG, R. and O. BODE. 1978. Sensitive detectio of Andean potato latent and Andean pota mottle viruses in potato tubers with serologic latex tests. *Phytopath Z.* **92:** 275-280.

KOENIG, R., C.E. FRIBOURG, and R.A.C. JONES. 197 Symptomatological, serological an electrophoretic diversity of isolates of Ande potato latent virus from different regions the Andes. *Phytopathology* **69:** 748-752.

MUMFORD, D.L. 1977. Application of the late flocculation serological assay to curley top rus. *Phytopathology* **67:** 949-952.

QUERFURTH, G. and H.L. PAUL. 1979. Protein coated latex-linked antisera (PALLAS): ne reagents for a sensitive test permitting the u of antisera unsuitable for the latex tes *Phytopath. Z.* **94:** 282-285.

THOMAS, B.J. 1980. The detection by serologic methods of viruses infecting the rose. *An Appl. Biol.* **94:** 91-101.

TORRANCE, L. 1980. Use of protein A to impro sensitisation of latex particles with antibod to plant viruses. *Ann. Appl. Biol.* **96:** 45-50.

WISLER, G.C., F.W. ZETTLER and D.E. PURCIFU 1982. A serodiagnostic technique for detecti *Cymbidium* mosaic virus and *Odontoglossu* ringspot viruses. *Phytopathology* **72:** 835-837.

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Proceedings
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