A METHOD FOR VEGETATIVE PROPAGATION OF *DRYOBALANOPS LANCEOLATA* (DIPTEROCARPACEAE) BY CUTTINGS

Pedro H. Moura-Costa & Lister Lundoh

RBJ-Face Foundation Rainforest Rehabilitation Project, Innoprise Corporation Sdn. Bhd., Danum Valley Field Centre, PS 282, 91108 Lahad Datu, Sabah, Malaysia

Received December 1992

MOURA-COSTA, P.H. & LUNDOH, L. 1994. A method for vegetative propagation of *Dryobalanops lanceolata* (Dipterocarpaceae) by cuttings. Two-node cuttings were prepared from the apices of juvenile seedlings of *D. lanceolata*. Leaves were trimmed reducing leaf area to approximately 30 cm^2 and the basal end of each cutting was treated with a fungicide solution (0.1 % w/v Benlate) followed by 3 % NAA powder. Cuttings were rooted in a closed chamber mist propagator. After roots formed, cuttings were potted in 7 × 21 *cm* polybags and weaned for two weeks in high humidity chambers. Percentage rooting after 12 weeks was 80 %. Mortality in the weaning-off stage was around 6 %.

Key words: Vegetative propagation - cuttings - dipterocarps - *Dryobalanops lanceolata*

MOURA-COSTA, P.H. & LUNDOH, L. 1994. Satu kaedah bagi pembiakan tampang *Dryobalanops lanceolata* (Dipterocarpaceae) secara pemotongan. Keratan dua-nod disediakan dari apeks anak benih muda *D.lanceolata*. Daunnya dipepat sehingga tertinggal keluasan daun yang berukuran kira-kira 30 cm^2. Hujung pangkal setiap keratan dirawat dengan larutan fugisid (0.1% berat/isipadu Benlate) diikuti dengan 3% serbuk NAA. Keratan-keratan ini dibiarkan berakar di dalam kebuk penyembur air yang tertutup. Setelah berakar, keratan-keratan tersebut diletakkan di dalam beg poli berukuran 7 × 21 *cm* dan dibiarkan di dalam kebuk yang mempunyai kelembapan yang tinggi. Peratus akar setelah 12 minggu ialah 80%. Peratus kematian apabila keratan-keratan tersebut dikeluarkan daripada kebuk yang mempunyai kelembapan tinggi lebih kurang 6%.

Introduction

Dipterocarps are the most abundant timber trees of the Malaysian rain forests, in some areas accounting for 80% of the canopy trees (Ashton 1982). *Dryobalanops lanceolata* Burck (kapur, Dipterocarpaceae) is an important hardwood tree species common in the dipterocarp rain forests of Malaysia, particularly the East coast of Sabah (Meijer & Wood 1964). It reaches heights of up to 77 *m*, with clear boles of 38 *m* or more, and timber volume of 64 m^3 (Burgess 1966). In some areas these trees represent up to 20 % of the total timber volume extracted from the forest (Silam Forest Products Sdn. Bhd., timber extraction tables for the Ulu Segama Region, Sabah, Malaysia, 1978-1981).

Dipterocarps exhibit erratic fruit setting, taking 2 to 10 years between seeding years (Ashton *et al.* 1988), and the seeds are recalcitrant preventing long term storage (Sasaki 1976, 1980, Boontawee & Nutivijarn 1989, Tompsett 1989). Vegetative propagation by cuttings has been investigated as an alternative method of supplying planting material of dipterocarps (Momose 1978, Hallé & Hanif-Kamil 1981, Srivastava & Penguang Manggil 1981, Smits 1983, 1986, Smits *et al.* 1987, 1990, Aminah Hamzah 1990 a, b, c, Kantarli 1993 b, c). However, dipterocarps are considered difficult to root (Chouffot-Struycken 1986).

To our knowledge, there is no report on vegetative propagation of *Dryobalanops lanceolata* in the literature. This paper describes a successful method for vegetative propagation of *D. lanceolata* by cuttings and the procedure for weaning-off in the nursery.

Materials and methods

Origin of stockplants

Stockplants were wild seedlings of *D. lanceolata* collected from the forest at the Ulu Segama region (Sabah, Malaysia) and potted in 7×21 *cm* poly-bags containing 79 cm^3 forest top-soil. The age of wildings at the time of collection was approximately three months. Plants were grown in the Danum Valley Field Centre nursery for four months before cuttings were taken for these experiments.

Preparation of cuttings

Two-node cuttings, *ca.* 7 to 10*cm* length, were prepared according to the method used by Smits (1983, 1986). The main stems of stockplants were cut across the third node down the apex, using pruning scissors. Leaves were trimmed, reducing the leaf area to *ca.* 30 cm^2, approximately 1/3 of the original area (Figure 1), based on the methods of Leakey *et al.* (1982), Smits (1983) and Aminah Hamzah (1990 c). The basal end of cuttings were dipped briefly in a fungicide solution (0.1% w/v Benlate) and treated with a commercial formulation of naphthalene acetic acid (NAA) powder (Trihormone, 3 % w/w NAA). To minimise stress, cuttings were inserted into the mist chamber immediately after they were prepared. Two batches of cuttings were prepared, containing 396 and 648 cuttings respectively, on two different occasions. Cuttings were grouped in blocks of 18 and blocks were randomly distributed around the rooting beds.

Design of propagator and environmental conditions during the rooting phase

Cuttings were rooted in a closed chamber mist propagator unit. Misting frequency was controlled by an electronic leaf controller connected to a control unit (MacPenny, UK). In order to increase air humidity around cuttings, beds were covered with transparent plastic supported by a wooden frame, forming a closed

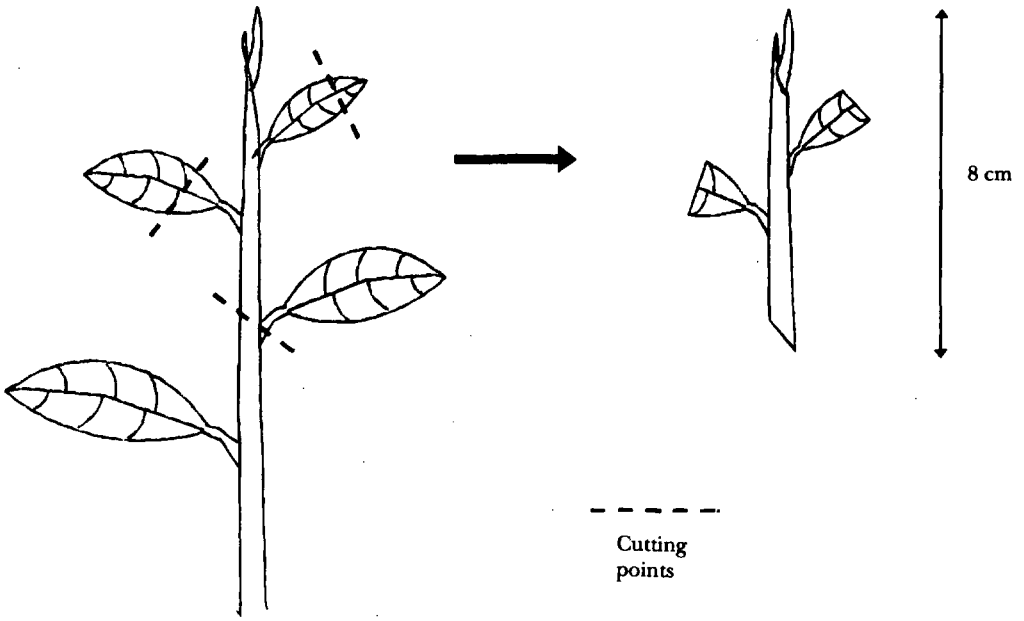

Figure 1. Procedure for cutting preparation

chamber 60 *cm* high (Figure 2). The whole mist unit was covered by 30 % shade netting. Rooting medium was river sand (particle size 313 ± 32 μm) boiled for 20 *min* to eliminate possible pathogens. Sand was placed on a layer of gravel (1.0 to 3.0 *cm* diameter), in order to allow free drainage. Mist was generated from rain water, with pH 6.5. Relative humidity inside the misting chamber, measured with a hygrometer, was about 94 %. Light intensity inside the chambers was measured using a portable infra-red gas analyzer connected to a Parkinson leaf chamber (ADC LCA-3, Hoddeston, UK), giving values around 290 $\mu mol\ m^{-2}\ s^{-1}$, 26 % of full direct radiation. Air temperature inside the chamber ranged from 22 to 36°*C* and temperature of the rooting medium from 24 to 34°*C*.

Weaning of rooted cuttings

After forming roots at least 0.5 *cm* long, cuttings were potted in 7×21 *cm* polybags containing 79 cm^3 forest top-soil and transferred to the shade house. About *ca.* 2 cm^3 of mycorrhizal inoculum was mixed with the soil in each bag. Inoculum consisted of soil from bags containing wildings of *D. lanceolata* in which mycorrhyzal associations were identified (Willie Smits, Tropenbos/Kalimantan Forestry Research Project). Plants were watered twice daily using a hose pipe. The shade house was covered by two layers of shade netting, and radiation at ground level was around 190 $\mu mol\ m^{-2}\ s^{-1}$, 18 % of full direct radiation. In order to increase air humidity around plants and reduce the stress caused by potting and transfer of plants from the misting unit, beds were covered with transparent plastic

supported by a wooden frame, forming closed chambers 30 *cm* high (Figure 3). Plants were kept inside these closed chambers for two weeks when the plastic covers were removed. Radiation inside the chambers was 180 $\mu mol\ m^{-2}\ s^{-1}$, 16 % of full direct radiation. Minimum relative humidity inside chambers was 64 % and outside 50 %, at 14:00*h*. Photoperiod was 12 *h*. Air temperature inside chambers ranged between 24 and 40°*C* and outside between 22 and 34°*C*.

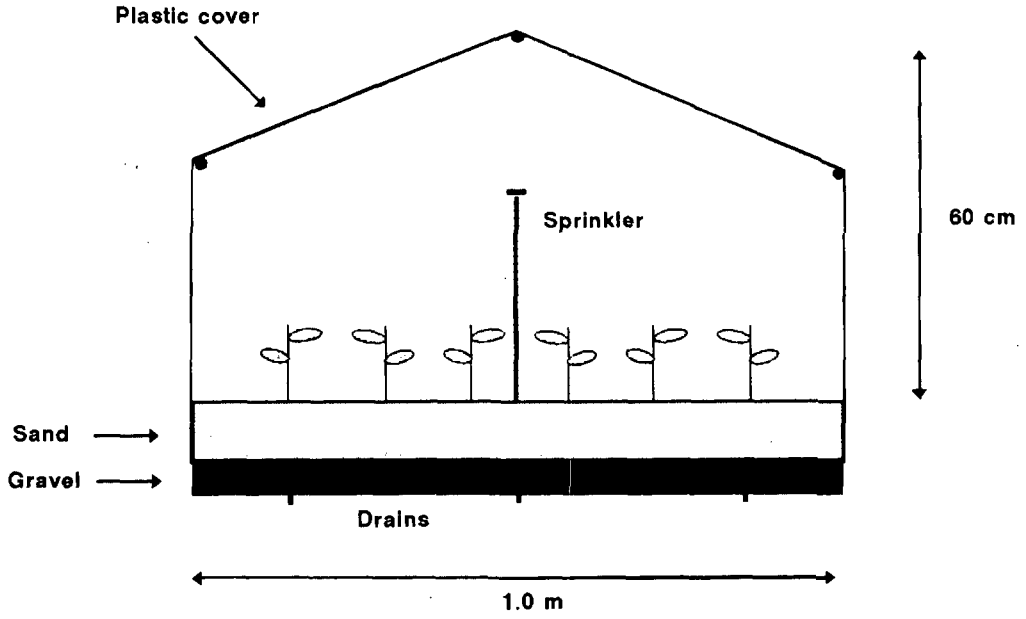

Figure 2. Design of the closed chamber mist propagator used in Danum Valley Field Centre

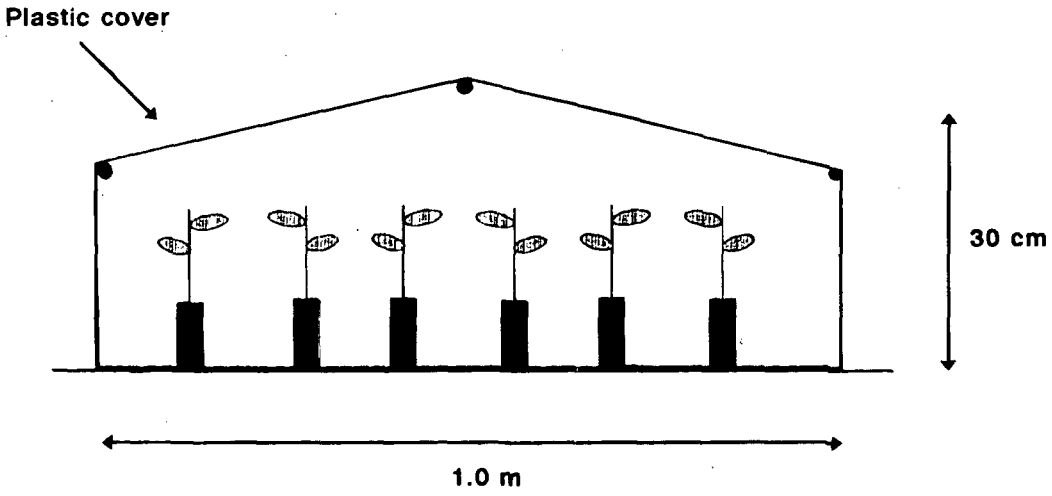

Figure 3. Design of the weaning-off chambers used in Danum Valley Field Centre

Assessments

Assessments of rooting were carried out after cuttings had been in the mist unit for 4, 8 and 12 weeks. Cuttings were scored as rooted if roots at least 0.5 *cm* long were formed. Assessments of mortality during the weaning stage were carried out four weeks after transfer of cuttings out from the mist unit. Plants were considered dead if they had shed their leaves and their stems were either dry or blackened.

Results and discussion

A high percentage of cuttings rooted after 12 weeks in the misting unit (73.09 to 88.25 % of cuttings, Table 1). It appeared that 12 weeks was the minimum time necessary for reaching a high percentage of rooting, since a much lower percentage was observed after 8 weeks. However, cuttings not rooted after 12 weeks in the mist unit were either dead or had not form root initials. It appeared more advantageous to replace them by new cuttings than to maintain them in the mist unit for a longer time. Approximately 6 % of rooted cuttings died during the weaning phase, reducing the percentage of plants recovered from cuttings to *ca.* 76%.

Table 1. Percentages of rooting, mortality and recovery of plants from rooted cuttings of *Dryobalanops lanceolata*. Means are followed by standard errors

	1st batch (396 cuttings)	2nd batch (648 cuttings)	Mean
Cumulative percentage of rooted cuttings after:			
4 weeks	11.52 ± 2.05	na	11.52
8 weeks	na	55.83 ± 2.10	55.83
12 weeks	88.25 ± 1.61	73.09 ± 1.93	80.67
Percentage death during weaning off			
	6.59 ± 1.62	5.33 ± 1.32	5.9
Percentage of plants recovered from cuttings initially prepared			
	82.43 ± 2.24	69.19 ± 1.70	75.81

na = not available.

The rooting percentages of dipterocarps achieved using this method were considerably higher than results from previous reports. In work with *Shorea talura*, *Vatica wallichii* and *Anisoptera scaphula*, Momose (1978) obtained an overall rooting percentage below 50%. Rooting of six dipterocarp species exposed to different types and concentrations of auxins was negligible, except for *Vatica pauciflora* treated with 0.2 or 0.3% w/v indole-3-butyric acid (IBA) solutions (Hallé & Hanif-

Kamil 1981). Srivestava and Penguang Manggil (1981) reported rooting percentages between 8 and 56 % for *Anisoptera scaphula, Shorea leprosula* and *Dipterocarpus chartaceus* raised in different propagator beds and exposed to a range of concentrations of IBA (0 to 2000 *ppm*). They reported high percentages of rooting of *Shorea bracteolata* (91.2 %) after 3 to 5 months, but the number of replicates used (between 2 and 4) was not large enough to allow substantiated conclusions.

The high percentages of rooting achieved in our study may have been due to a combination of factors, but we attribute special importance to the use of juvenile material (see review by Hackett 1985). Mature tissues of woody plants tend to have lower levels of endogenous auxins, are more differentiated (and therefore less prone to dedifferentiation), and may have thicker layers of lignin which creates a physical barrier to root development (Hartmann *et al.* 1990). Higher levels of rooting inhibitors may also explain lower rooting ability of mature tissues (Paton *et al.* 1970). It was postulated that phenols can act as auxin cofactors or synergists in root initiation (Haissig 1974), and the concentration of phenols in mature tissues of certain plants tends to be lower than their juvenile forms (Girouard 1967). The use of mature material might have been the reason for an overall low percentage of rooting achieved in the experiments described by Momose (1978), and Hallé and Hanif-Kamil (1981). All cuttings Momose prepared from mature tissues failed to root. On the other hand, high percentages of rooting were observed when juvenile material was used by Smits (1983) and Aminah Hamzah (1990a).

Other important factors contributing to the high percentages of rooting in this experiment were the design of the propagation unit and the concern for preventing water loss from cuttings. The rationale behind any strategy for vegetative propagation by cuttings is to minimize evapotranspiration rates until a root system forms. Water loss occurs when there is a negative water vapour gradient from leaves (V_{LEAF}) to the atmosphere (V_{AIR}) (Hartmann *et al.* 1990). It is necessary to reduce this gradient by either decreasing V_{LEAF} or by increasing V_{AIR}. The closed chamber mist propagator used in this study addresses both these processes simultaneously. The mist causes a reduction in leaf temperature, reducing V_{LEAF}, while V_{AIR} is much increased by using a closed chamber. Using this system, relative humidity inside the mist chamber was maintained above 90 %. The low rooting percentages achieved by Srivastava and Penguang Manggil (1981) may have been due to the low relative humidity inside their propagation chambers, which ranged from 66 to 68%. Water loss was also reduced by reducing leaf area and thus potential evapotranspiration. Further studies showed that percentage rooting of *D. lanceolata* cuttings was significantly higher when leaf area was reduced to *ca.* 30 cm^2, compared to cuttings with intact leaves (Moura-Costa & Lundoh, in preparation). Cuttings were taken in the morning between 7:30 and 10:00, before temperatures become too high and evapotranspiration rates increase. Cuttings were inserted into the mist unit immediately after preparation, and the stockplants were located in a shade house located a few meters from the mist unit. In this way the time between excision of plant material from stockplants until insertion into the misting unit was less than 10 *min*, reducing the period of potential water loss. Stock

plants were healthy and well watered, not suffering from any apparent stress.

Although cuttings were treated with NAA, further studies showed that exogenous auxins did not improve rooting of juvenile cuttings of *D.lanceolata* (Moura-Costa & Lundoh, submitted).

The plant mortality observed during the weaning-off stage (6 %) may have been caused by the relatively low humidity inside the weaning-off chambers (64%), since the plastic covers were not air-tight. It may also have been due to damage to the small young roots during potting.

The method described in this paper provides an alternative to seed propagation for multiplying planting stock of *D. lanceolata* and is being tested for the propagation of other dipterocarp species. Tissue culture techniques for *in vitro* propagation of dipterocarps are also being investigated (Moura-Costa, in press). Vegetative propagation can also be used for cloning selected genotypes of dipterocarps, a subject under study in Danum Valley Field Centre (Moura-Costa, in press). We are attempting to reduce the number of operations required for the production of rooted cuttings, thus reducing costs. Associated with the use of stockplants managed for continuous production of orthotropic shoots (Leakey 1983, Leppe & Smits 1988, Kantarli 1993 a), this method may be used for the large scale production of planting stock at a low cost.

Acknowledgements

This work was possible through the support of Innoprise Corporation Sdn. Bhd., Malaysia, and The Royal Society, London. The authors are grateful to the Forestry Department of Sabah for providing the propagation facilities at DVFC, and the Danum Valley Research and Training Programme. We would also like to thank Albert Ganing for providing the stockplants for this study, Willie Smits for the mycorrhyzae identifications, Martin Barker for advice on light measurements and Ruth Nussbaum for comments on an early version of the manuscript.

References

ASHTON, P.S. 1982. Dipterocarpaceae. *Flora Malesiana ser.1, Spermatophyta* 9 : 251 - 552.

ASHTON, P.S., GIVNISH, T.J. & APPANAH, S. 1988. Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *The American Naturalist* 132(1): 44 - 66.

AMINAH HAMZAH. 1990 a. A note on the rooting of *Shorea bracteolata* stem cuttings. *Journal of Tropical Forest Science* 3(2): 187 - 188.

AMINAH HAMZAH. 1990 b. A note on growth behaviour of branch cuttings of *Hopea odorata*. *Journal of Tropical Forest Science* 3(3): 303 - 305.

AMINAH HAMZAH. 1990 c. A note on the effect of leaf number on rooting of *Hopea odorata* stem cuttings. *Journal of Tropical Forest Science* 3(4): 384 - 386.

BOONTAWEE, B. & VUTIVIJARN, T. 1989. The germination of some dipterocarp seeds under different storage periods. Pp. 451-454 in Soerianegara, I., Tjitrosomo, S.S., Umaly, R.C. & Umboh, I. (Eds.) *Fourth Round-table Conference on Dipterocarps*. Biotrop Special Publication 41, Bogor.

BURGESS, P. F. 1966. *Timbers of Sabah*. Forest Department, Sabah. 501 pp.

CHOUFFOT-STRUYCKEN, B. 1986. Contribuition à l'étude des capacités de multiplication végétative du *Shorea curtisii* Dyer ex King. Dipterocarpacée de Malaisie. Thèse de l'Université Louis Pasteur. Strasbourg. 136 pp.

GIROUARD, R.M. 1967. Physiological and biochemical studies of adventitious root formation. Extractable rooting cofactors from *Hedera helix. Canadian Journal of Botany* 45(5): 687 - 699.

HACKETT, W. P. 1985. Juvenility, maturation and rejuvenation in woody plants. *Horticultural Reviews* 7: 109 - 155.

HAISSIG, B. E. 1974. Influences of auxins and auxin synergists on adventitious root primordium initiation and development. *New Zealand Journal of Forest Science* 4 : 311 - 323.

HALLÉ, F. & HANIF-KAMIL 1981. Vegetative propagation of dipterocarps by stem cuttings and air-layering. *The Malaysian Forester* 44(2 & 3): 314 -318.

HARTMANN, H.T., KESTER, D. E. & DAVIES, F.T. 1990. *Plant Propagation. Principles and Practices.* Fifth edition. Prentice-Hall International Editions, New Jersey. 645 pp.

KANTARLI, M. 1993 a. Hedging ability of 5-year-old *Hopea odorata* donors under selected light and fertilizer regimes. Technical Publication. ASEAN-Canada Forest Tree Seed Centre Project, Saraburi. (In press).

KANTARLI, M.1993 b. *Vegetative Propagation of Dipterocarps by Cuttings in ASEAN Region.* Review Paper No. 1. ASEAN-Canada Forest Tree Seed Centre Project, Saraburi. 58 pp.

KANTARLI, M. 1993 c. *Vegetative Propagation of Hopea Odorata by Cuttings: a Low-Cost Technology.* Technical Publication No. 16. ASEAN-Canada Forest Tree Seed Centre Project, Saraburi. 7 pp.

LEAKEY, R.R.B. 1983. Stockplant factors affecting root initiation in cuttings of *Tryplochiton scleroxylon* K. Schumm., an indigenous hardwood of West Africa. *Journal of Horticultural Science* 58 : 277 - 290.

LEAKEY, R. R. B., CHAPMAN, V.R. & LONGMAN, K.A. 1982. Physiological studies for tree improvement and conservation. Factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum. *Forest Ecology and Management* 4 : 53 - 66.

LEPPE, D. & SMITS, W.T. M., 1988. Metoda Pembuatan dan Pemeliharaan Kebun Pangkas Dipterocarpaceae. Forestry Research Institute Samarinda, Indonesia. 49 pp.

MEIJER, W. & WOOD, G. H. S. 1964. *Dipterocarps of Sabah* (North Borneo). Sabah Forest Record No 5. Forest Department, Sabah. 344 pp.

MOMOSE, Y. 1978. Vegetative propagation of Malaysian trees. *The Malaysian Forester* 41 (3): 219 - 223.

MOURA-COSTA, P. Nursery and vegetative propagation techniques for genetic improvement and large scale enrichment planting of dipterocarps. In Primack, R.B. & Lovejoy, T. (Eds.) *Ecology, Conservation and Management of Southeast Asian Rainforests.* Yale University Press, Connecticut. (In press).

MOURA-COSTA, P.H. & LUNDOH, L. The effects of auxins (IBA, NAA and 2,4-D) on rooting of *Dryobalanops lanceolata* (kapur, Dipterocarpaceae) cuttings. *Journal of Tropical Forest Science.* (Submitted).

PATON, D.M., WILLING, R.R., NICHOLS, W. & PRYOR, L.D. 1970. Rooting of stem cuttings of *Eucalyptus*: a rooting inhibitor in adult tissue. *Australian Journal of Botany* 18 : 175-183.

SASAKI, S. 1976. The physiology, storage and germination of timber seeds. Pp. 111-116 in Chin, H.F., Enoch, I.C. & Raja Harun, R.M. (Eds.) *Seed Technology in the Tropics.* Universiti Pertanian Malaysia, Serdang, Selangor.

SASAKI, S. 1980. Storage and germination of dipterocarp seeds. *Malaysian Forester* 43(3): 290 - 308.

SMITS, W.T.M. 1983. Vegetative propagation of *Shorea* cf *obtusa* and *Agathis dammara* by means of leaf-cuttings and stem-cuttings. *Malaysian Forester* 46(2): 175 - 185.

SMITS, W.T.M. 1986. Vegetative propagation and possibilities for its use with Dipterocarpaceae. In Wirakusumah, S. (Ed.). *Diskusi Tebatas Beberapa Aspek Pembangunan Hutan. Menelusuri Cara-Cara Inovatif Reboisasi di Indonesia I.* Jakarta. INHUTANI I. 8 pp.

SMITS, W.T.M., OLDERMAN, R.A.A. & LIMONARD, T. 1987. Mycorrhizae and Dipterocarpaceae in East Kalimantan rain forests. Pp. 67-78 in *Annual Report for the Netherlands Foundation for the Advancement of Tropical Research, 1986.*

SMITS, W.T.M., YASMAN, I., LEPPE, D. & NOOR, M. 1990. Summary of results concerning vegetative propagation of Dipterocarpaceae in Kalimantan, Indonesia. Pp. 449-450 in Gibson, G.L.,

Griffin, A.R. & Matheson, A.C. (Eds.) *Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry. Proceedings of the IUFRO Conference, Pattaya, Thailand.* Oxford Forestry Institute, Oxford.

SRIVASTAVA, P.B.L. & PENGUANG MANGGIL 1981. Vegetative propagation of some dipterocarps by cuttings. *Malaysian Forester* 44(2 & 3): 301-313.

TOMPSETT, P.B. 1989. A review of the literature on storage of dipterocarp seeds. Pp. 427 - 446 in Soerianegara, I., Tjitrosomo, S.S., Umaly, R.C. & Umboh, I. (Eds.) *Fourth Round-Table Conference on Dipterocarps. Biotrop Special Publication* 41, Bogor.