PHYSIOLOGICAL STUDY ON MALAYSIAN TROPICAL TREE SPECIES
STUDY ON STORAGE AND GERMINATION OF LEGUMINOSAE AND DIPTEROCARPACEAE SEEDS

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One of the most difficult problems in the propagation of tropical rain forest species is that relating to seed production. Flowering and fruiting in the tropical rain forests are generally sporadic and irregular, as definite seasons do no exist. Also, premature abortion of the seeds is common because of frequent insect and fungus attacks to the seeds. It is difficult to predict a yield of sound seeds each year. Therefore, the successful storage of the tropical seeds is the key factor to enable silviculturists to plan systematic seedling production. However, methods of seed storage for the tropical rain forest species are not established yet, as some physiological characteristics of the seeds do not meet requirements for storage. For example, most of the seeds do not have dormancy properties, which include extensive dehydration and reduction of biological activity. Such non-dormant seeds encounter difficulties during the storage.

The present experiments deal with the storage and germination of legume and Dipterocarp seeds which represent important timber species in Malaysia. The legume seeds can be classified into two groups, one with soft seedcoat and the other with hard seedcoat. Moisture contents of the legume seeds are comparatively low even for the seeds with soft seedcoat. In contrast, Dipterocarp seeds are soft seeds with a high moisture content and are typically non-dormant seeds. In some cases, Dipterocarp seeds germinate even before the seeds are shed off from the mother tree (Fig. 1). These observations suggest that the storage and germination characteristics of Leguminosae and Dipterocarpaceae seeds are quite different.

Fig. 1 A seed of *Dryobalanops aromatica* germinated on the mother tree

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Storage and germination of Leguminosae seeds.

Storage: The legume seeds have low moisture contents, generally less than 20% of the dry weight (Table 1). The seeds are protected by a hard seedcoat except those of *Koompassia malacensis*. Seeds with a hard seedcoat can be stored at room temperature for a long time. *Parkia javanica, Intsia palembanica* and *Sindora coriacea* seeds have been surviving more than 3 years without any specific treatment. However, *Koompassia malacensis* seeds are susceptible to fungus infection, and they show a better survival after treatment with a fungicide (Table 2).

Germination: *Koompassia* seeds can easily germinate when imbibed. The other seeds with a hard seedcoat do not germinate without scarification on the seedcoat. Several methods of scarification were tested.

Table 1 Moisture contents of legume seeds

Species	Moisture content (%)
Intsia palembanica	7.7
Sindora coriacea	5.9
Dialium maingayi	12.0
Enterolobium cyclocarpum	12.2
Parkia javanica	20.5*
Koompassia malacensis	15.2**

* Newly collected seeds
** Soft seeds

Table 2 Storage of *Koompassia malacensis* seeds at room temperature

Days stored	% germination
4	90
6	90
20	80
34	60
54	100
84	80
119	10 Fungus infection
131	10 Fungus infection
181	60 Benlate 0.1% treatment

1 Filing on the seedcoat

Scratching the seedcoat with a file is an effective method of scarification. With the method, *Parkia javanica, Intsia palembanica, Sindora coriacea, Dialium maingayi* and *Enterolobium cyclocarpum* seeds germinated easily, but the method was tedious and time consuming for the treatment of a large quantity of seeds. Also, the method is suitable only for large size seeds such as those of *Intsia* and *Sindora* species. Another problem is the fungus infection from the wound. Dusting with a fungicide such as benlate and daconil was slightly effective to protect the seeds from fungus damage.

The most effective scarifying method for *Intsia palembanica* and *Sindora coriacea* seeds is to scrape off a small swelling point of the seedcoat at the opposite side of the hilum. The swelling point appears to result from the fusion of integuments at micropyle, and it is the weakest point of the seedcoat. A tiny hole made by scraping off the swelling point facilitates water uptake. Water

penetrates the seed from the hole and infiltrates through the seedcoat tissue along the edges of the seed. Within 30 minutes, the outer seedcoat is separated and ruptured (Fig. 2). This method has an advantage over the filing of the seedcoat as the embryo is not damaged. Also, the method is simple and does not require any specific skill.

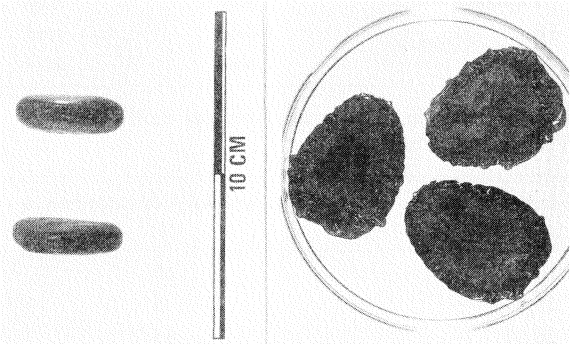

Fig. 2 Scarification and water imbibition of *Intsia palembanica* seeds

Water enters through the small hole made by scratching the swelling point of the seed. The top photograph shows control seed (right) and the scarified seed (left). The scarified seeds ruptured the seedcoat 30 minutes after imbibition (bottom photograph).

2 Treatment with concentrated H_2SO_4

Immersing the seeds into H_2SO_4 is effective to rupture the seedcoat of *Intsia, Parkia, Sindora,* and *Dialium* seeds. To determine the optimum duration of the treatment, the seeds were treated with the concentrated acid for various durations. After treatment with the acid, the seeds were taken out from the acid and washed thoroughly with running water to eliminate the remaining acid. The results show that *Intsia* and *Sindora* seeds need a prolonged treatment with the acid, whereas *Dialium* and *Parkia* seeds germinate after a short treatment (Table 3). Although the method is effective, its application for practical use may be limited.

Table 3 Treatment with concentrated H_2SO_4

Species	Time in H_2SO_4						
	Control	1 min.	5 min.	10 min. % germination	15 min.	30 min.	60 min.
Intsia palembanica	0	0	0	0	20	20	100
Sindora coriacea	0	10	20	20		10	80
Dialium maingayi	0	70	90	90	90	100	100
Parkia javanica	0		35	45	95	85	

3 Treatment with boiled water

The treatment with boiled water is sometimes used to remove waxy layers of the hard seedcoat. However, constantly boiling water killed even *Intsia* seeds within 5 minutes after immersion (Table 4). The best results were obtained by pouring boiled water over the seeds in a container. However, the results are sometimes inconsistent as the amount of water as well as the amount of seeds may affect the cooling rate of water.

It is comparatively easy to store the legume seeds, as most of the seeds can be stored at room temperature and can be germinated after scarification. However, some of the soft seeds need further study on storage at a low temperature, fungicide treatments, and storage under low humidity conditions.

Table 4 Boiling water treatment

Time in boiling water	*Intsia palembanica*	*Parkia javanica*
5 minutes	dead	dead
10	dead	dead
20	dead	dead

Seeds immersed in boiled water; water was cooled down at room temperature.

Time in hot water (minutes)	*Parkia javanica* seed germination (%)
10	10
20	5
30	15
60	30
Overnight	30

Storage and germination of Dipterocarpaceae seeds

In contrast to the legume seeds, Dipterocarpaceae seeds have high moisture contents. Unlike the legume seeds with low moisture contents, Dipterocarpaceae seeds continue to be very active biologically even after maturation. As indicated earlier, the seeds often germinate on the mother tree before they are shed off. Therefore, the storage of Dipterocarpaceae seeds is more difficult than that of the legume seeds.

Dipterocarpaceae consist of several genera and subgenera groups. Preliminary experiments showed some characteristic physiological distinctions of the seeds among genera or subgenera. Particularly, the survival rate of the seeds in a low temperature range could be grouped by genera or subgenera. In the following discussion, Dipterocarpaceae seeds will be classified into two groups, one with tolerance to a temperature of 4°C and the other requiring at least 15°C for survival.

1 Group of seeds which survived at 4°C

Anthoshorea (subgenus of *Shorea*)
Shorea talura seeds:

In 1975, *Shorea talura* trees at Field 19, Forest Research Institute, Kepong fruited at the end of March and shed all the seeds at the end of April. With these seeds, maturation, germination and storage were studied.

1) Seed maturation: The germination of *Shorea talura* seeds shows a significant increase with maturation. Germination tests in the laboratory as well as in the seed bed in the nursery indicate that the germinability of the seeds increases in a relatively short period toward the end of seed development close to the stage of seed shedding (Fig. 3). The moisture content of the seeds shows an inverse relation to the percentage of germination, with a gradual decline of the moisture content as the seeds mature (Fig. 4). Similarly, the lag time between the onset of water imbibition and emergence of radicles becomes shorter in the later seed collections (Fig. 5). Maximum durations of the storage appear to be prolonged for the mature seeds (Table 5). Therefore, it is important to collect completely mature seeds in order to obtain good results in storage.

Table 5 Storage of *Shorea talura* seeds collected at various stages of maturation

Date of collection	Original % germination	Moisture content (%)	Maximum storage period Days	% germination
Aug. 8, 1975	50	120.0	84	50
Aug. 12, 1975	80	105.9	145	30
Aug. 21, 1975	95	68.1	182	69

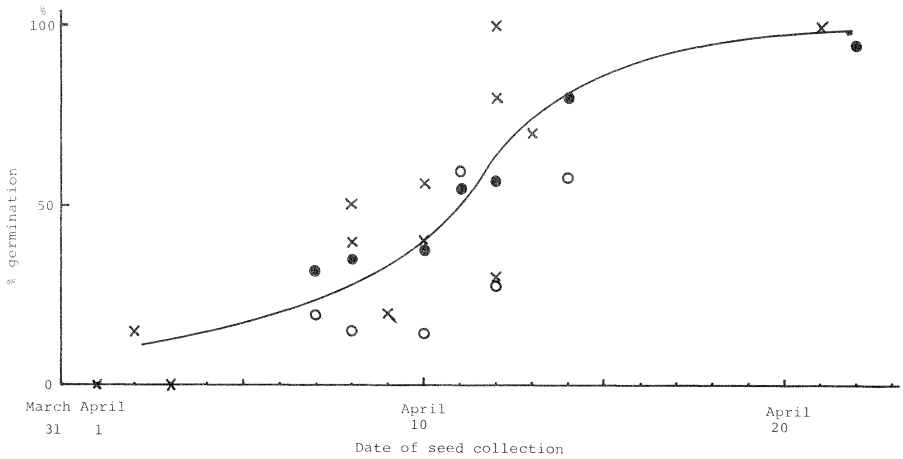

Fig. 3 Increase in germinability of *Shorea talura* seeds corresponding to maturation stages

[Germination percentages for large size seeds (●) and small size seeds (○) in the nursery bed as well as those in Petri dishes (×) are shown.]

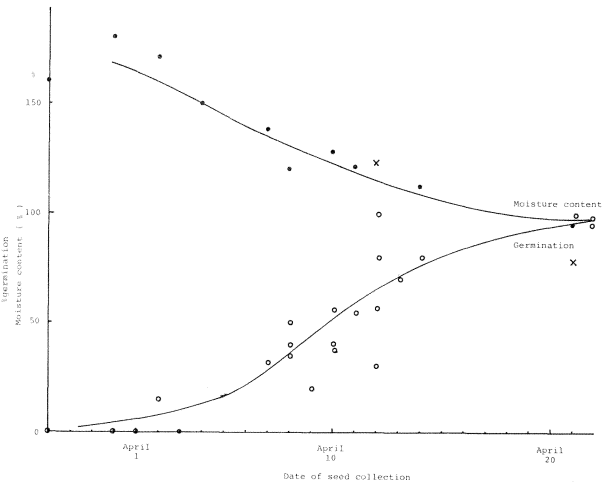

Fig. 4 Changes in moisture content and germinability of *Shorea talura* seeds in relation to maturation

[The gradual decline in moisture content of the seeds collected from the ground (●) and the tree (×) is compared with the gradual increase in germinability (○).]

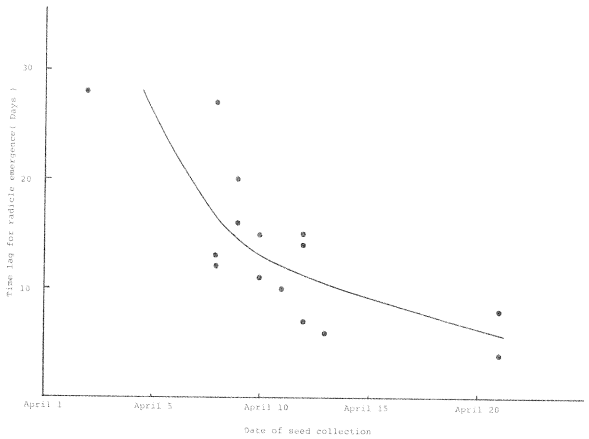

Fig. 5 Water imbibition period for *Shorea talura* seeds

[Time lag between the start of imbibition and the first radicle emergence is plotted against the time of the seed collection.]

The less mature seeds collected on April 9, 1975 showed a 50% germination at the time of collection. However, one month after they were stored at 4°C, the percentage of germination of the same seeds increased to 100%. This indicates possible evidence of after-ripening of the seeds during the storage at 4°C.

Probably related to the after-ripening of the seeds, a prechilling effect was observed in *Shorea talura* seeds. The seeds stored at 4°C in a closed polyethylene plastic bag germinated very quickly after a prolonged storage (Fig. 6). Therefore, the experiments were conducted to evaluate the effect of prechilling on *Shorea talura* seeds. The seeds were placed on moist paper in Petri dishes and kept in a cold room at 4°C. At various intervals, the prechilled seeds were taken out from the cold room and germinated at 25°C. The rate of germination of the prechilled seeds was markedly accelerated when the prechilling treatment was prolonged, whereas the seeds kept at 25° showed a gradual decline in the germination rate (Fig. 7). The results indicate that a certain degree of prechilling effect is present for *Shorea talura* seeds. However, extremely prolonged prechilling may be detrimental to the seeds, because slow infection with fungi and bacteria damages the seeds.

2) Critical moisture content for survival of seeds: *Shorea talura* seeds kept at 25°C in an open plastic bag showed a decline in moisture content by natural desiccation. These seeds lost their viability when the moisture content dropped to a level of 20% (Table 6a). In a similar experiment, the seeds kept at 21°C showed a loss of viability at 25% of the moisture content (Table 6b). Also, quick desiccation of the seeds killed the seeds. Therefore, the critical moisture content for the survival of *Shorea talura* seeds falls in a range between 20 and 25%.

3) Tolerance to low temperature: A temperature below the freezing point killed *Shorea talura* seeds. However, a remarkable phenomenon was the survival of *Shorea talura* seeds at a temperature as low as 4°C. As shown in Table 5, the seeds stored at 4°C survived for more than 6 months, provided that the moisture content was maintained at a level above the critical moisture content. Even after 6 months of storage at 4°C, the seeds did not develop chilling injury symptoms. The adaptation of *Shorea talura* seeds to the low temperature appears to be unique among Dipterocarp seeds.

Table 6a. Effect of moisture content on viability of *Shorea talura* seeds

Seeds collected on Aug. 12, 1975.					
Days	24	31	37	62	
Germination (%)	60	10	5	0	Seeds stored at 25°C.
Moisture content (%)	41.2	22.5	20.4	16.0	
Seeds collected on Aug. 21, 1975.					
Days	0	15	30	40	
Germination (%)	95	90	0	0	Seeds stored at 25°C.
Moisture content (%)	68.1	45.6	20.9	18.1	

Table 6b. Effect of moisture content on viability of *Shorea talura* seeds

Seeds collected on May 19, 1977.		Stored at 21°C.							
Days		0	7	20	32	46	69	88	122
Closed bag	Germination (%)	100	92	87	64	82	87	95	97
	Moisture content (%)	55.0	55.5	55.3	50.8	52.7	50.4	50.7	48.9
Open bag	Germination (%)	100	97	86	85	84	48	21	0
	Moisture content (%)	55.0	51.9	50.8	44.6	36.4	32.0	28.3	25.7

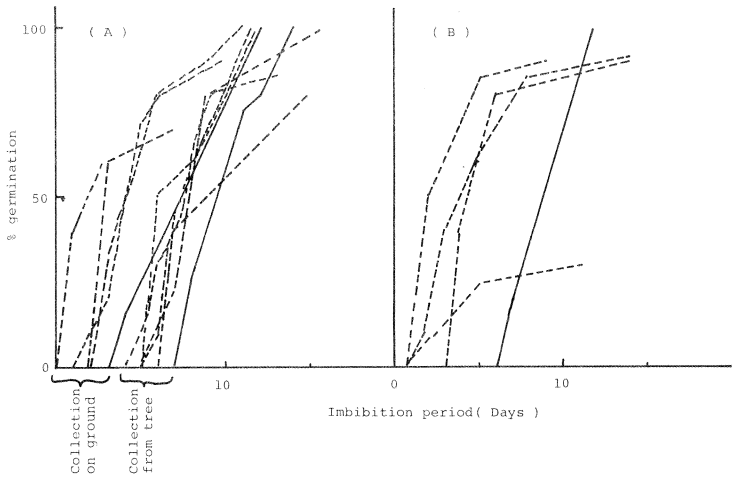

Fig. 6 Promotion of germination rates of *Shorea talura* seeds stored at 4°C.

[Solid lines indicate original germination rates. Broken lines indicate germination rates of the seeds stored at 4°C.]

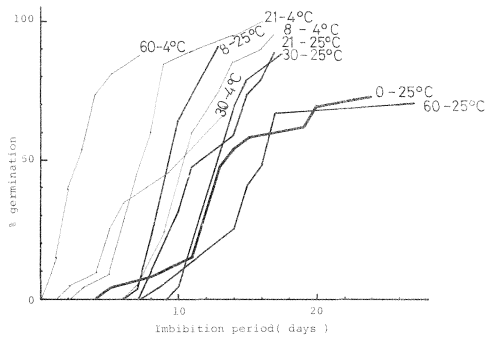

Fig. 7 Effect of prechilling on *Shorea talura* seed germination

[First numbers at the end of each line indicate the duration (day) of treatment.]

4) Storage trials: With a large quantity of seeds, storage experiments were carried out for *Shorea talura* seeds. Because of mechanical problems in the cold room, the seeds stored at 4°C lost their viability and the results of the 4°C-storage experiment can not be assessed fully. However, judging from the other test (Table 5), the seeds can be stored for more than 6 months at 4°C. For practical purpose, the storage at 21°C would be the best among the storage treatments (Table 7). Particularly the use of a plastic bag with slight ventilation through a chimney at the top appears to prevent the seeds from suffocating. As suffocation causes the fermentation of the seeds and secondary fungus infection, a ventilation system which would not reduce the moisture content needs to be developed.

Other white meranti seeds (*Anthoshorea* subgenus):

The seed maturation of *Anthoshorea* group appears to follow a pattern similar to that of *Shorea talura*. The seeds with a high moisture content above 100% did not survive in a long term storage.

Shorea assamica, Shorea bracteolata, Shorea hypochra, Shorea resinosa, and *Shorea sericeiflora* survived at 4°C, although the duration of the survival at 4°C varied among species. One of the factors for seed survival may be attributable to the maturation of the seeds, as fermentation and suffocation of the seeds were observed in relatively immature seeds with a high moisture content.

Hopea group:

Hopea ferrea, Hopea latifolia, Hopea odorata, Hopea nervosa, Hopea beccariana, Hopea sublata and *Hopea wightiana* survived at 4°C for various periods. However, the duration of the survival at 4°C did not exceed 3 months. Probably the tolerance to the cold temperature may not be the same as that of *Anthoshorea* group. *Hopea nervosa* seeds stored at 21°C survived more than 10 months, indicating that a long term storage is possible for some species of *Hopea* group.

Anisoptera group:

Only a few species were tested for tolerance to low temperature, but the group appears to tolerate low temperature, at least within the same range as that of *Hopea* group.

Table 7 Storage trials *Shorea talura* seeds

Seed source: collected in Perlis, April 20, 1977.
original seed germination 93%
original seed moisture content 56.7%

Storage temperature	Treatment	Maximum duration	Final germination	Final moisture content
		(months)	(%)	(%)
25°C	Closed bag	3	64	29.5
21°C	Closed bag	7 1/4	90	45.9
10°C*	Closed bag	5	5	62.5
4°C*	Closed bag	5	32	60.3
	Desiccator	5	42	55.7
	Closed bag	5	58	

Seed source: collected in Perlis, May 19, 1977.
original seed germination 100%
original moisture content 55.0%

Storage temperature	Treatment	Maximum duration	Final germination	Final moisture content
		(months)	(%)	(%)
25°C	Closed bag	2 1/4	70	25.7
21°C	Closed bag	10	33	46.2
17°C*	Closed bag	3	43	25.6
10°C	Closed bag	3	75	47.6
4°C	Closed bag	4	79	46.8

* Power failure and mechanical problems caused the deterioration of the seeds stored.

Dipterocarpus group:

Dipterocarpus oblongifolia seeds survived at 4°C at least for 2 months. Other unidentified species also showed a similar tendency. Probably *Dipterocarpus* seeds tolerate low temperature like those of *Anthoshorea* group.

Richetia group (subgenus of *Shorea*):

Shorea resina-nigra, Shorea multiflora, Shorea Faguetiana, and *Shorea hopeifolia* seeds survived at 4°C for about a month. However, slow development of chilling injury symptoms finally damaged the seeds.

Vatica group:

Only two species, *Vatica lowii* and *Vatica cinerea* were tested. These two species showed some degree of tolerance to low temperature, but the degree of the tolerance could not be evaluated fully because of immature seed sources. Probably the degree of the tolerance to the temperature may be similar to that of *Hopea* group, with a survival of no more than 3 months at 4°C.

Dryobalanops, Balanocarpus, and *Parashorea* groups:

The seeds of these species survived at a temperature below 10°C for one or two weeks. However, the survival at 4°C does not exceed more than one month.

Among Dipterocarp seeds, *Shorea talura* seeds show the highest tolerance to low temperature, and other *Anthoshorea* seeds appear to follow a similar pattern. *Dipterocarpus* seeds also tolerate the storage at low temperature. These *Anthoshorea* and *Dipterocarpus* seeds have common physiological characteristics as the main seed reserve consists of starch.

The low temperature tolerance of the seeds needs to be studied further in detail. Probably the tolerance is physiologically related to the dormancy processes, as after-ripening and prechilling effects could be demonstrated in *Shorea talura* seeds.

2 Group of species extremely susceptible to low temperature (below 15 °C)

Two subgenus groups of *Shorea, Rubroshorea* and *Eushorea* showed very high susceptibility to low temperature at 4°C. The seeds of the two groups developed chilling injury symptoms within a few days after exposure at 4°C, in some cases within 2 hours at 4°C. The chilling injury includes several symptoms. The cotyledon tissues lose their elasticity and become brittle. The color of the cotyledons changes from green or white to water-soaked, necrotic brown. The development of symptoms in radicles is delayed, but follows a pattern similar to that of the cotyledons. The color changes of the tissues do not take place when the seeds are placed in a freezer at −20°C, but the seeds lose their viability. This indicates that enzyme systems which cause the brown staining and which probably involve polyphenol oxidases are also susceptible to the subzero temperature. Microscopic observations suggest that the brown staining is present on the cell wall and that the cell membranes are ruptured by chilling injury.

Rubroshorea (subgenus of *Shorea*)
Shorea ovalis seeds:

The seeds were collected from the isolated tree. No other trees were fruiting nor flowering in the vicinity. The first collection of the seeds was made on Aug. 14, 1975. Thereafter, the seeds were collected on Aug. 18, 29, and finally on Sept. 8, 1975. Immediately after the last collection, all the remaining seeds on the tree were shed and the development of new leaves was observed.

1) Seed maturation: Although germination rates were high even in the earlier collections, other factors still indicate that maturation processes were progressing until the seeds were shed off from the tree (Table 8). For example, the moisture content decreased from over 100% on a dry weight basis to 53%. Also, the time for radicle emergence after imbibition became shorter in the last collection of the seeds. The decline in the moisture content appears to prolong the viability of the seeds during the storage, as fermentation and fungus infection can be suppressed in the drier seeds. However, the artificial drying of the seeds could not replace natural drying processes associated with maturation processes. When the seeds were dried artificially for various durations, germination ratios immediately after the drying treatment were reasonably high, depending on the degree of desiccation. However, storage for 30 days after artificial drying caused the deterioration of the seeds except those dried only for one day (Table 9). Such results illustrate that natural drying during maturation involves physiologically complex processes and not just simple desiccation. A typical pattern of increase in the percentage of germination and a decline in the moisture content was noted in *Shorea dasyphylla* seeds (Fig. 8). The moisture content decreased sharply as the germinability of the seeds increased. Probably biological dehydration was associated with the initial rapid drop of the moisture content, whereas physical desiccation was the main cause for the second slow decline of the moisture content.

The moisture content of the seeds is a good indicator of seed maturation as the moisture content decreases to a 50% range in mature seeds. However, the measurement of the moisture content is impossible in the field, and is tedious as well as time consuming even in the laboratory. Therefore, the moisture content as an indicator of maturation is unpractical for silvicultural use. A good indicator for judging the seed maturation is to examine browning and drying of the seed wings. The wings of typically mature seeds are brown in color down to the base of the wing. Only a narrow

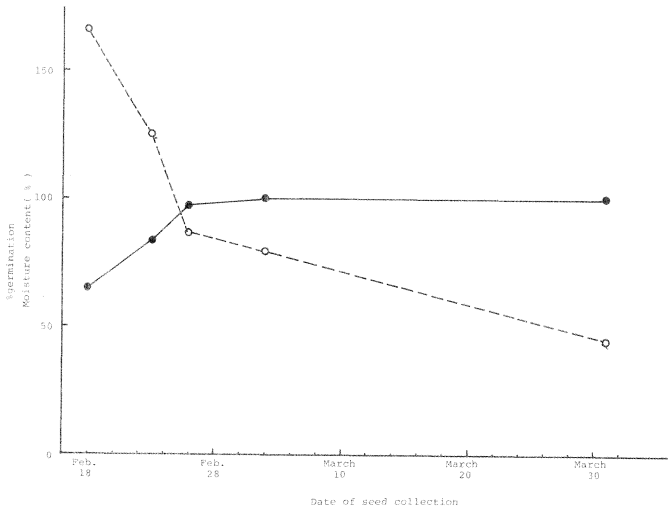

Fig. 8 Changes in germinability and moisture content for *Shorea dasyphylla* seeds during the maturation period

[Initial sharp decline in moisture content (○) and increase in germination ratios (●) are noted.]

Table 8 Date of seed collections in relation to maturation parameters for *Shorea ovalis* seeds

Date of collection	Aug. 14	Aug. 18	Aug. 29	Sept. 8
Germination (%)	100	100	100	100
Moisture content (%)	138.7	144.9	55.2	53.2
Duration of imbibition for seed germination (days)*	8	8	4	3

* The time lag between the start of imbibition and the first radicle emergence was expressed in days.

Table 9 Effect of artificial drying on viability of *Shorea ovalis* seeds

Drying period	Immediately after treatment		30 days after storage	
	Germination (%)	Moisture content (%)	Germination (%)	Moisture content (%)
Air dried for 1 day	100	96.1	75	74.8
Air dried for 2 days	85	47.4	0	32.5
Air dried for 3 days	40	39.2	0	19.7
Air dried for 4 days	10	15.1	0	11.7

bottom part of the wing base remains green and the portion immediately above the green tissue shows a distinct demarcation with the dark staining. The dark staining resembles a paper chromatographic front. The seeds with such wings contain a moisture content of 50% range. The demarcation of the dark staining can not be observed in the seeds desiccated artificially after shedding from the tree. Therefore, the wing browning is a good indicator of seed maturation and it can be used as a criterion in seed collections.

2) Storage trials: As indicated earlier, *Shorea ovalis* seeds can not tolerate temperatures below 15°C nor desiccation below 20%. Also, mature seeds are needed to obtain good results in storage. The best results were obtained by storage at 21°C in a closed polyethylene plastic bag. Although fungus infections were serious in the moist conditions, even after 4 months, 10% of the seeds still survived at 21°C. Fungicide dusting may be helpful for protecting the seeds from infection.

Since the seeds can not survive below 15°C, the optimum temperature for the storage of *Shorea ovalis* seeds should be within a range between 16 and 25°C.

Other *Rubroshorea* and *Eushorea* seeds:

Most of the seeds in the group showed the best results when stored at 21°C, with the maximum storage period not exceeding 3 months. Although these seeds can not survive in a low temperature range below 15°C, the duration of the survival at 4°C varied among species. For example, *Shorea dasyphylla* seeds survived at 4°C slightly longer than other seeds, with 15% survival after 3 days.

These observations suggest that physiological characteristics of Dipterocarpaceae seeds vary among species depending on the genera or subgenera. The seeds tolerant to low temperature such as *Anthoshorea* subgenus, and *Hopea* may be stored for a longer period than those of *Rubroshorea* and *Eushorea* subgenera.

Acknowledgement

The present report is a part of a physiological study on Malaysian tropical tree species, which was carried out at Forest Research Institute, Kepong, Selangor, Malaysia under the cooperative research program between Malaysia and Japan. The full report of the study is presented to Forest Research Institute, Kepong.

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Discussion

Arihara, M. (Japan): Is there any specification in the harvesting period of Dipterocarp seeds?

Answer: The last gregarious year of flowering occurred in 1976 and not since. It may take another two to three years at least to observe the next flowering year. However in between the gregarious flowering years, sporadic flowering can be observed. Also some species such as *Shorea talura, Hopea odorata, Hopea ferrea* may flower every year in December or January while seeds appear from April to May. Genetic factors may be responsible for such phenomenon.

Sambas W. (Indonesia): 1) Could you comment on insect attacks? 2) Are there any methods enabling to improve seed collection in the developing countries?

Answer: 1) Insect attack is a serious problem in seed production. However, repeated flower abortion is often followed by a period of flowering and sound seeds can be collected after maturation. Also in gregarious flowering years, insect attacks affect seeds less frequently that in sporadic flowering. The cause of flower or fruit abortion is not known, 2) There are some primitive methods such as that consisting of shaking the trees.!

Wawan K. (Indonesia): Are there any chemical methods enabling to store seeds?

Answer: Treatment of seeds with chemicals such as BA, GA, ABA, antiperspirant or substitution of water by glycol has not been successful. More research pertaining to physiological properties of seeds is required.