Diaspore Traits and Inter-tidal Zonation of Non-viviparous Mangrove Species

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Abstract: Diaspore traits and germination of four non-viviparous mangrove species in Hong Kong, *Lumnitzera racemosa* (Jack.) Voigt., *Heritiera littoralis* (Drgand.) Ait., *Excoecaria agallocha* L. and *Acanthus ilicifolius* L., were investigated. *L. racemosa* fruits planted immediately after collection failed to germinate but those stored in wet condition for 35 or 50 d were successfully germinated. This suggested that *L. racemosa* had endogenous and morphological seed dormancy, with embryos continued to develop during the dormant period. Germination rates of *L. racemosa* decreased with increasing salinity and no germination was found at salinities over 25 ppt (ppt, parts per thausand). *H. littoralis* seeds were easily germinated if the fruit coat was artificially removed. Fruit dissection significantly shortened time for root initiation and leaf expansion. *E. agallocha* and *A. ilicifolius* seeds were also easy to germinate, initiating roots within 2 and 3 d, respectively. In terms of germination, *A. ilicifolius* had more tolerance to high salinity than *L. racemosa*. The four species exhibited three types of adaptation to unstable environments: (1) prolonged diaspore longevity as shown in *L. racemosa* and *H. littoralis* ; (2) shortened rooting time as in *E. agallocha* and *A. ilicifolius* ; and (3) produced sinking diaspores in *L. racemosa*. Diaspore buoyancy was one of the most important factors in determining inter-tidal zonation of non-viviparous mangrove species. Among the four species, *L. racemosa* was distributed in the most seaward zones because its diaspores were sinkers while diaspores of *H. littoralis*, *E. agallocha* and *A. ilicifolius*, more abundant at backshore locations, were floaters. Root initiation was also important in influencing the inter-tidal zonation of the three species whose diaspores were floaters. *H. littoralis* with the longest rooting time, as compared to *E. agallocha* and *A. ilicifolius*, was distributed in the most backshore zone. None of other factors including salinity of seawater, animal predation, diaspore size and seedling dimension could account for inter-tidal zonation of these species.

Key words: *Lumnitzera racemosa* ; *Heritiera littoralis* ; *Excoecaria agallocha* ; *Acanthus ilicifolius* ; mangrove; inter-tidal zonation; seed dormancy

Mangrove swamps are unique inter-tidal wetland eco-systems found in sheltered tropical and subtropical shores. They maintain a rich and diverse biological resource with significantly ecological, environmental, social and economic values. However, mangrove habitats are decreasing around the world. Losses in many tropical countries exceed 1% of mangrove areas every year due to human activities (Spalding *et al*., 1997). In Hong Kong and other cities along southeast coasts of China, mangroves have been reclaimed and used as agricultural fields, fishponds, new town and infra-structural developments (Tam and Wong, 2000; 2002). In recent years, ecological restoration of mangroves has become increasingly important. Replanting of mangroves by governmental authorities and local green groups has taken place in the areas where natural mangrove swamps were destroyed (Tam and Wong, 2000).

Vegetative propagation is uncommon in mangroves (Tomlinson, 1986) and mangrove replanting depends on the survival and growth of the established seedlings. Due to the availability of seedlings, mangrove replanting in China was mainly done for viviparous mangrove species including *Kandelia candel*, *Bruguiera gymnorrhiza*, *Aegiceras corniculatum* and *Rhizophora stylosa* (Liao *et al*., 1996; 1998; Mo and Fan, 2001), and only those in *Sonneratia* were replanted for non-viviparous mangrove species (Zan *et al*., 2003). In Hong Kong, eight true mangrove species, namely *K. candel*, *B. gymnorrhiza*, *A. corniculatum*, *Avicennia marina*, *Lumnitzera racemosa*, *Heritiera littoralis*, *Excoecaria agallocha*, and *Acanthus ilicifolius*, are naturally distributed along the coastlines (Tam *et al*., 1997; Tam and Wong, 2002). However, only the first four species have been replanted because of their viviparous characteristics, large-sized propagules, abundance, and easy germination. The germination

techniques of non-viviparous species, namely *L. racemosa*, *H. littoralis*, *E. agallocha*, and *A. ilicifolius*, have not been established, despite the fact that these species are classified as rare ones and must be conserved in Hong Kong (Tam *et al.*, 1997; Tam and Wong, 2002). Natural regeneration of these four species is low and only a few seedlings are found in mangrove swamps. Although techniques on vegetative propagation were developed for some mangrove species difficult to germinate such as *E. agallocha* (Rao *et al.*, 1998; Basak *et al.*, 2000), *Laguncularia racemosa* (Elster and Perdomo, 1999) and *Heritiera* (Basak *et al.*, 2000), it is still important to understand their seed germination and explore successful germination methods because vegetative propagation will eventually decrease the genetic diversity of mangrove populations and hinder mangrove conservation.

Transplantation success is dependent on the supply of healthy and young seedlings, which is affected by the size, availability and lifespan of the diaspore (i.e. any spore, seed, fruit or plant part able to produce a new plant when dispersed), storage and germination methods. Tomlinson (1986) stated that most mangrove species do not have innate dormancy and will germinate immediately after maturity. However, recent studies by Clarke *et al.* (2001) found that all *L. racemosa* seeds failed to germinate, probably due to seed dormancy.

The adaptation of mangrove species to the very unstable environments and their distribution along the shore are controlled by effects of tidal action on the dispersal, lifespan and germination of diaspores. Inter-tidal zonation of mangrove species has been noted for a long time and many hypotheses on "tidal sorting and zonation" were proposed that mangrove zonation is controlled by tidal sorting of the diaspores according to dispersal characteristics such as size and buoyancy and by differential ability of diaspores to establish in deep water (Rabinowitz, 1978a; 1978b; Jimenez and Sauter, 1991). However, confusions and failure in zonation explanation were also reported, and factors related to diaspore buoyancy and size, root initiation, and environmental factors are unable to give a complete account for some mangrove zonation (Bunt, 1996; Ellison *et al.*, 2000; Clarke *et al.*, 2001). In Hong Kong, the four non-viviparous mangrove species, *L. racemosa*, *H. littoralis*, *E. agallocha* and *A. ilicifolius*, are naturally distributed at the back of the mangrove swamps, but *H. littoralis* is found only at the very high tide position (Tam and Wong, 2000). Whether such difference in inter-tidal zonation is related to the "tidal sorting and zonation" hypothesis or due to different reproduction and germination

pattern is unknown.

The present study therefore attempts (1) to investigate the germination of four non-viviparous mangrove species in Hong Kong aiming to shorten the germination time and provide seedlings for mangrove replanting, and (2) to compare diaspore traits of these species to explain their inter-tidal zonation.

1 Materials and Methods

1.1 Collection and dispersal characteristics of diaspores

Mature diaspores of four non-viviparous mangrove species, *Lumnitzera racemosa* (Jack.) Voigt., *Heritiera littoralis* (Drgand.) Ait., *Excoecaria agallocha* L., and *Acanthus ilicifolius* L. were collected from Kei Ling Ha Hoi, one of the typical mangrove swamps in Hong Kong with permission of the Agriculture, Fisheries and Conservation Department, Hong Kong Government. All of the eight true mangrove species present in Hong Kong are found in this swamp, with four non-viviparous species distributed at the backshore and the viviparous species, *Avicennia marina*, *Kandelia candel*, *Bruguiera gymnorrhiza* and *Aegiceras corniculatum*, in seaward zones. Few seedlings were found for non-viviparous species while a large number of young seedlings of the viviparous species were observed in this swamp. Diaspores of *L. racemosa*, *E. agallocha* and *A. ilicifolius* were considered mature if they fell from parent trees by gently shaking the branches. Mature diaspores of *H. littoralis* were collected on the forest floor. The shape, weight, size and other traits of mature fruits were measured from 20 diaspores for each species immediately after collection. Buoyancy of diaspores was tested in tanks filled with 15 ppt (ppt, parts per thousand) seawater. For storage experiments, diaspores of *H. littoralis*, *E. agallocha* and *A. ilicifolius* were stored at room temperature without any pretreatment and effects of storage on *L. racemosa* were described as follows.

1.2 Germination experiments

Some freshly collected fruits of *L. racemosa* were planted directly in a greenhouse by four different treatments, each in triplicate: (1) 40 fruits in sandy soil collected from Sai Keng mangrove swamp, at the southwest of Kei Ling Ha Hoi; (2) 40 fruits in 15 ppt seawater; (3) 40 fruits in silty soil collected from Mai Po mangrove swamp, another typical mangrove in western Hong Kong; and (4) 40 fruits were immersed in beakers containing 15 ppt seawater for about 10 h everyday for different days to promote germination prior to planting in silty soil. The fruits were placed on soil substrate in the same orientation as those naturally floated onto tidal water. They were irrigated by 15 ppt seawater on

the first day and any evaporation loss was compensated by daily addition of tap water. Percentages of surviving seedlings were recorded.

For storage experiments on *L. racemosa*, fruits were kept in sealed plastic bags, with 100 fruits per bag. The bags were stored in three conditions, each in triplicate: (1) in a laboratory cabinet at room temperature of about 25 (dry room storage); (2) in a cold room of around 5 (dry cold storage); and (3) in shade at room temperature after wetting the fruits with tap water (wet storage). In addition, some fruits (100 fruits per replicate and also prepared in triplicate) were buried in wet sand at a depth of about 2 cm on which some water was daily sprinkled (sand buried). The appearance of the fruit and the embryo were examined at regular time interval during a 35-d storage period. At the end of the storage period, fruits were planted in plastic pots containing sand and were irrigated with tap water (0 ppt salinity) for germination. Each pot had ten fruits and six replicate pots were prepared. The percentage germination, time of root initiation and time for expansion of the first pair of leaves were recorded.

Some fruits of *L. racemosa* were kept under "wet storage" for 50 d, then germinated in pots containing sand. They were irrigated with water of different salinities: 0 (fresh water), 5, 15, 25, and 35 ppt. Each pot had ten fruits. Three replicate pots for each salinity treatment were prepared. The percentage germination, time for root initiation and time for expansion of the first pair of leaves were recorded. A parametric one-way analysis of variance (ANOVA) test was used to examine the effect of salinity on percentage germination. Difference in percentage germination between the two storage time (35 d *vs* 50 d) under fresh water irrigation was examined by a student *t*-test.

The fruits of *H. littoralis* were divided into two portions: one dissected to remove the hard fruit coat and the second left intact. The dissection was done by carefully cutting along the "keel" of the fruit with a sharp knife to avoid damaging the embryo. Both intact and dissected fruits were germinated in two soil types, namely sandy mangrove soil collected from Sai Keng mangrove swamp and terrestrial soil mixed with compost, with one fruit per plastic bag. When planted, half of the fruit was buried in soil with the "keel"

upward and the embryo in the downward side. For each soil type and each fruit treatment (dissected or non-dissected), 24 bags were prepared. Due to limited availability of the fruits, salinity experiment for this species was not set up.

Fruits of *E. agallocha* were dissected to remove their fruit coats, planted in pots containing wet sand (25 fruits per pot), and irrigated with tap water. Nine replicate pots were set up. The percentage germination and early seedling growth in each pot were observed. Due to limited availability of fruits, salinity experiment for this species was not set up.

Fruits of *A. ilicifolius* were dissected to remove their coats, and seeds were germinated in pots containing sand. They were irrigated with water of different salinities: 0 (fresh water), 5, 15, 25, and 35 ppt (ten seeds per pot). Three replicate pots for each salinity treatment were prepared. The percentage germination, time of root initiation and time for expansion of the first pair of leaves were recorded. ANOVA test was used to examine the effect of salinity on percentage germination.

2   Results

2.1   Diaspore traits

Every adult *L. racemosa* tree produced thousands of fruits. Mature fruits were small, with an average weight of about 0.1 g (Table 1). The seed was well protected by a hard layer of sclerenchyma tissue inside the outer corky layer of fruit wall that was very difficult to remove. The mature fruits were spindly with pale green embryos and sank in seawater. After the fruits were kept in seawater for more than 20 d, their embryos died.

In Kei Ling Ha Hoi mangrove swamp, natural recruitment of *H. littoralis* was less successful than for other true mangrove species. Only seven seedlings were found in a natural *H. littoralis* stand of about 3 hm^2. Mature fruit was ellipsoidal, large and heavy with an average length, width and height of about 5.5, 4.0 and 3.5 cm, respectively, and unit weight of about 30 g (Table 1). Over 90% of the mature fruits fallen on the forest ground was eaten by insects and dead before germination despite their fruit coats were hard and thick (about 0.5 cm thick). The fruits had low density,

Table 1   Traits of mature diaspores of the four non-viviparous mangrove species in Hong Kong (*n* = 20)

Species	Type	Length (cm)	Weight (g)	Buoyancy	Viable period in 15 ppt seawater (d)	Coat
Lumnitzera racemosa	Fruit	1.5 ± 0.4	0.10 ± 0.03	Sinker	<20	Green, thin but hard, difficult to dissect
Heritiera littoralis	Fruit	5.5 ± 1.2	30 ± 6	Floater	>150	Brown, thick and hard, difficult to dissect
Excoecaria agallocha	Seed	0.35 ± 0.03	0.03 ± 0.00	Floater	<7	Brown, thin, easy to dissect
Acanthus ilicifolius	Seed	1.5 ± 0.3	0.02 ± 0.00	Floater	<11	Grey, thin, easy to dissect

ppt, parts per thousand.

consistently floated and kept viable in seawater for over three months.

Among the four non-viviparous species, the weights of diaspores were in the descending order of *H. littoralis* > *L. racemosa*, > *E. agallocha* > *A. ilicifolius* (*P* < 0.001, by one-way ANOVA) (Table 1). The last two species had very small diaspores (seeds) with average fresh weights of only 0.03 and 0.02 g for *E. agallocha* and *A. ilicifolius*, respectively. The former species had spherical seeds while the latter seeds were flat. Mature seeds of both species were floaters and kept floating in seawater at all times. However, all seeds of *E. agallocha* in seawater died within 7 d while that of *A. ilicifolius* survived for 11 d. Under dry conditions, the stored seeds of *E. agallocha* lost water rapidly and died within 2 d. Similarly, seeds of *A. ilicifolius* turned to black and became rotten within two weeks under dry storage. The order of longevity of diaspores of the four non-viviparous mangrove species was *H. littoralis* > *L. racemosa* > *A. ilicifolius* > *E. agallocha* while the buoyancy was in the order of *H. littoralis* = *A. ilicifolius* = *E. agallocha* > *L. racemosa*.
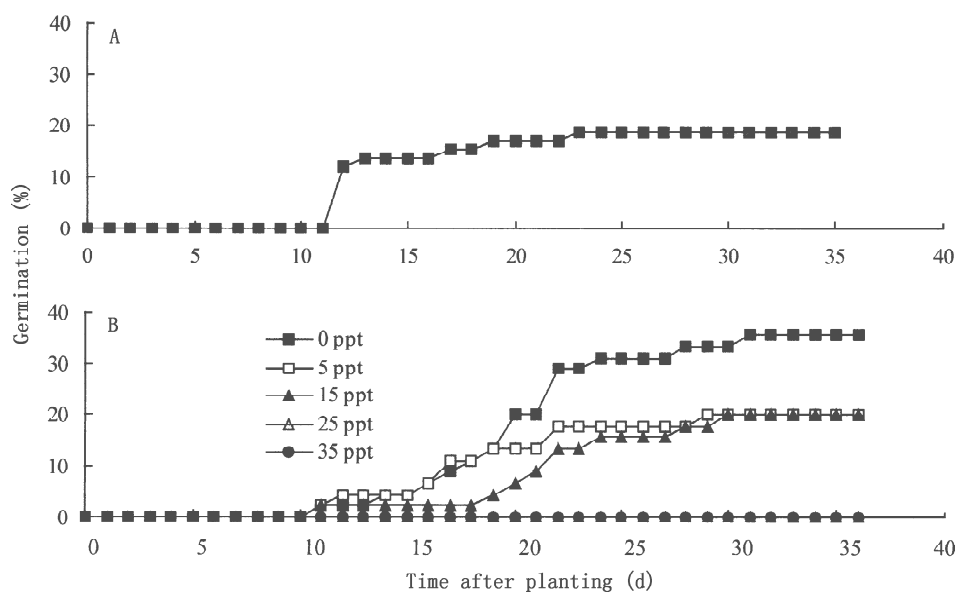
2.2 Germination

All mature fruits of *L. racemosa* planted in seawater, sandy soil (from Sai Keng mangrove) and silty soil (from Mai Po mangrove) immediately after collection as well as those planted in silty soil after promoting sprouting in seawater for several days failed to germinate and the embryos were found dead two months after planting. This observation suggested that freshly collected diaspores were unable to germinate.

When *L. racemosa* fruits were stored under dry conditions at either room temperature or in a cool room, all became black in color with dead embryos after one month of storage, and seeds shrunk and disappeared. However, about 30% of the fruits buried in wet sand (sand buried) had "good" seeds with a size double the original and consisted of viable deep-green colored embryos. When fruits were under "wet storage", all became black in color after 35- and 50-d storage, and about 95% of the stored fruits had "good" seeds with double-sized and deep green embryos, similar to those of "sand buried". In both "sand buried" and "wet storage" treatments, the sclerenchymatous fibers at the tip of the fruit were exposed. These results suggested that fruits of *L. racemosa* must be stored for a period of time under wet conditions to break their dormancy prior to germination.

After "wet storage" for 35 d, *L. racemosa* fruits started to germinate with roots initiated within 12 d of planting (Fig.1A; Table 2). The average time needed to expand the first pair of leaves was about 19 d. The cotyledons were out of the sands while germinating but the fruit coat was still in the sands after seedling establishment. The final percentage germination, about 20% germination on 23 d, was still low despite the effort to break the dormancy.

The salinity of irrigation water during planting had significant effects on germination of "wet stored" fruits of *L. racemosa* (Fig.1B; Table 2). The germination rates decreased with increased salinities (*P* < 0.001 according to one-way ANOVA), and fruits were unable to germinate at salinities over 25 ppt. According to *t*-test, the diaspores

Fig.1. Germination pattern of *Lumnitzera racemosa* diaspores (**A**) after 35-d "wet storage" under 0 ppt (ppt, parts per thousand) salinity and (**B**) after 50-d "wet storage" under different salinities.

Table 2   Germination indicators of the four non-viviparous mangrove species in Hong Kong

| Species	Plantation method	Salinity (ppt)	Time for root initiation (d)	Time for leaf expansion (d)	Final germination (%)
Lumnitzera racemosa	Wet stored for 35 d, sand	0	12.0 ± 0.0 (n = 6)	19.2 ± 1.8 (n = 6)	18.7 ± 9.9 (n = 6)
	Wet stored for 50 d, sand	0	14.7 ± 3.2 (n = 3)	27.3 ± 7.5 (n = 3)	35.6 ± 10.2 (n = 3)
		5	13.3 ± 3.2 (n = 3)	21.0 ± 4.6 (n = 3)	20.0 ± 6.7 (n = 3)
		15	19.7 ± 8.5 (n = 3)	27.5 ± 5.0 (n = 3)	20.0 ± 11.5 (n = 3)
		25	-	-	0
		35	-	-	0
Heritiera littoralis	Dissecting coats, terrestrial soil	0	24.7 ± 8.6 (n = 24)	62.8 ± 10.8 (n = 24)	100
	Dissecting coats, mangrove soil	0	62.5 ± 19.7 (n = 24)	85.0 ± 20.5 (n = 24)	100
	Not dissecting coats, terrestrial soil	0	71.2 ± 24.2 (n = 12)	104.2 ± 17.0 (n = 12)	50.0
	Not dissecting coats, mangrove soil	0	108.5 ± 17.2 (n = 4)	143.8 ± 16.8 (n = 4)	16.7
Excoecaria agallocha	Sand	0	2.0 ± 0.0 (n = 9)	11.6 ± 0.5 (n = 9)	81.3 ± 5.0 (n = 9)
Acanthus ilicifolius	Sand	0	3.0 ± 0.0 (n = 3)	14.7 ± 0.6 (n = 3)	100.0 ± 0.0 (n = 3)
		5	3.0 ± 0.0 (n = 3)	15.3 ± 0.6 (n = 3)	100.0 ± 0.0 (n = 3)
		15	3.7 ± 0.6 (n = 3)	18.3 ± 0.6 (n = 3)	100.0 ± 0.0 (n = 3)
		25	6.7 ± 0.6 (n = 3)	31.3 ± 2.3 (n = 3)	91.7 ± 8.3 (n = 3)
		35	7.7 ± 0.6 (n = 3)	42.3 ± 2.5 (n = 3)	52.8 ± 4.8 (n = 3)

Mean ± *SD* are shown. Number of replicates per treatment was shown in bracket as n value.

stored for 50 d had significant higher percentage of germination at 0 ppt than those stored for 35 d ($P = 0.049$).

Dissected *H. littoralis* fruits had 100% germination regardless of whether they were planted in mangrove or terrestrial soils (Fig. 2A; Table 2). More rapid germination was found in terrestrial soil than that in Sai Keng mangrove soil for both dissected and non-dissected fruits (Fig.2A, B). Dissected fruits had much more rapid germination than non-dissected fruits. Almost all dissected fruits planted in terrestrial soil had root initiations took place 25 d after planting and the first leaf expansion time was about 63 d, while

the corresponding time of root initiation and leaf expansion for fruits planted in mangrove soil were 63 and 85 d, respectively. The non-dissected fruits planted in terrestrial and Sai Keng soils had average root initiation time of 71 and 109 d, respectively, and the respective time for the first leaf expansion were 104 and 144 d. These results suggested that dissecting fruits planted in terrestrial soil mixed with compost not only enhanced the germination success but also shortened the germination time.
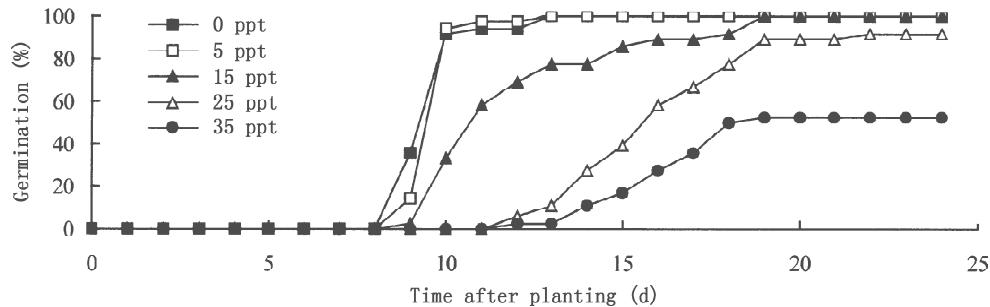
The diaspores (seeds) of *E. agallocha* had a relatively high germination percentage (about 81%), and rapid root
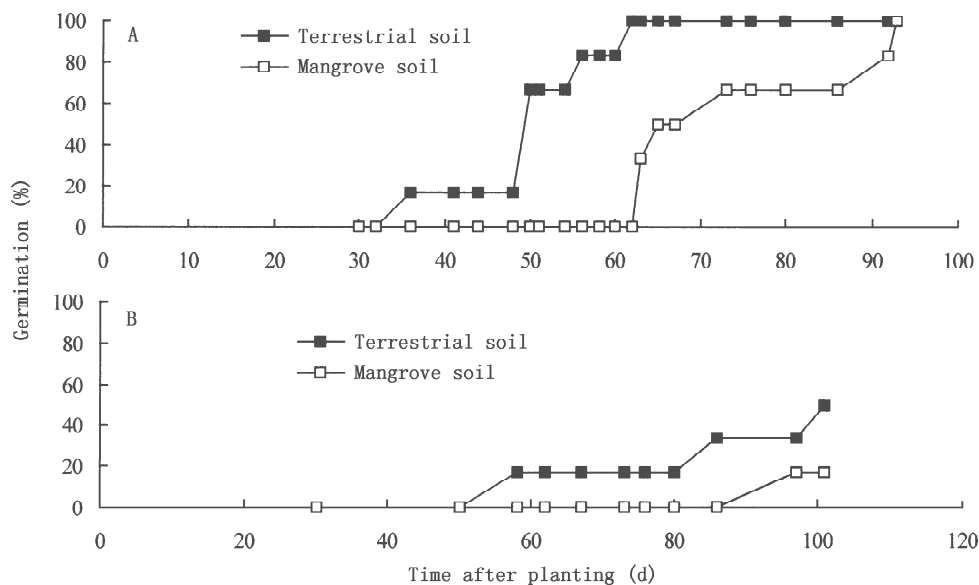
Fig.2.   Germination pattern of *Heritiera littoralis* diaspores planted in terrestrial soil and Sai Keng mangrove soil with (**A**) fruit coats dissected and (**B**) fruit coats non-dissected.
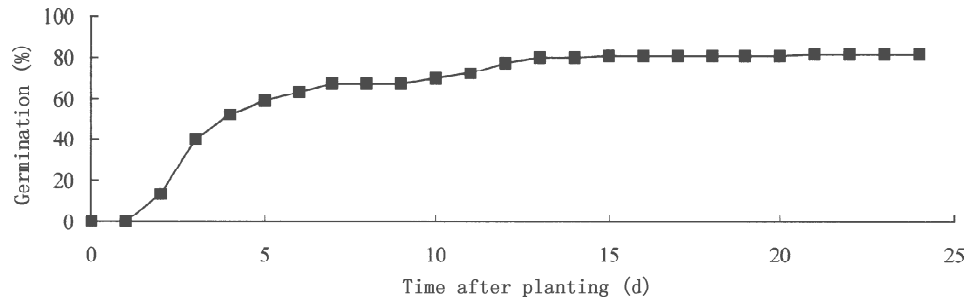
Fig.3.   Germination pattern of *Excoecaria agallocha* diaspores.

Fig.4.   Germination pattern of *Acanthus ilicifolius* diaspores under different salinities. ppt, parts per thousand.

initiations of 2 d after planting (Fig.3; Table 2). Similarly, germination of *A. ilicifolius* was very successful with 100% germination at salinities at and less than 25 ppt (Fig.4; Table 2). Salinities had significant effects on germination of *A. ilicifolius* (one-way ANOVA test, $P < 0.05$). The seeds had 100% germination and initiated roots 3- 4 d after planting at salinities below 15 ppt. At higher salinities, rooting and leaf expansion rates and percentage germination dropped. As compared to *L. racemosa*, *A. ilicifolius* had higher tolerance to salinity as the latter species had percentage germination of 52.8% and 91.7% at salinities of 25 and 35 ppt, respectively, while the former species was unable to germinate at these two high salinity levels.

Among the four species, *L. racemosa* had the lowest seedling survival percentage (<10%). The survived seedlings also had the slowest early growth in terms of increments in stem height (average value of 0.02 cm/d) and diameter (1.50 mm) compared to the other three species (Table 3). On the other hand, nearly 100% of the germinated

seedlings of *H. littoralis*, *E. agallocha* and *A. ilicifolius* survived. *H. littoralis* seedlings had the most rapid growth rate, followed by that of *E. agallocha*, and *A. ilicifolius*. This is probably because more nutrients were available from the large-sized seeds of *H. littoralis* for early seedling growth. There was no significant difference in seedling growth rates between the dissected and non-dissected *H. littoralis* fruits as well as between those planted in terrestrial and mangrove soils, with height increments of the dissected fruit in terrestrial and mangrove soils of 0.30 and 0.31 cm/d, respectively, while the respective values for the non-dissected fruits were 0.22 and 0.29 cm/d.

3   Discussion

3.1   Traits of diaspores and germination of non-viviparous mangrove species

Mature fruits of *L. racemosa* were small (about 0.1 g) and the seeds, even smaller, were protected by a hard layer of sclerenchyma inside the outer corky layer of the fruit

Table 3   Early growth indicators of seedlings of the four non-viviparous mangrove species in Hong Kong

Species	Time (d)	Height (cm)	Growth rate in height (cm/d)	Stem base diameter (mm)
Lumnitzera racemosa*	118	2.6 ± 0.2 (n = 4)	0.02	1.50 ± 0.12 (n = 4)
Heritiera littoralis**	132	34.3 ± 3.8 (n = 48)	0.30	3.94 ± 0.30 (n = 48)
Excoecaria agallocha	98	10.9 ± 1.6 (n = 121)	0.11	2.76 ± 0.39 (n = 121)
Acanthus ilicifolius	78	6.3 ± 0.2 (n = 180)	0.08	2.39 ± 0.21 (n = 180)

Mean ± *SD* are shown. Number of replicates per treatment was shown in bracket as n value. *, only four of all seedlings successfully germinated after 35 d and 50 d wet storage survived; **, only the seedlings germinated from dissected fruits were measured due to long germination period for non-dissected ones.

wall, thus root initiation took a relatively long time, 12 and 15 d after planting fruits stored for 35 and 50 d under wet conditions (Table 2). This implies that in natural field conditions, the embryos might be damaged by drought or other unfavorable circumstances before germination. Field observation showed that the quantity of seedlings in natural community was small. Many naturally recruited seedlings were found growing in the habitats with a thick growth of grasses or in areas where the fruits were maintained wet but not permanently waterlogged, such as stone lacunas. This indicated that the diaspores of *L. racemosa*, the sinkers as described by Clarke *et al.* (2001), required some kind of protection from tidal flushing to germinate. They could be viable for less than 20 d before germination under conditions without any shelters. Therefore, accurately simulating sheltered micro-circumstances is very important for this species to germinate successfully. Although Tomlinson (1986) classified the germination of *L. racemosa* as "modified hypogeal", we considered it epigeal because the cotyledons were out of the sediment while germinating although the fruit coat was still in the sediment after seedling establishment, in accord with the description by Clarke *et al.* (2001). Compared with the diaspores of other non-viviparous mangrove species, mature fruits of *H. littoralis* were large but took a long time to complete germination because of the hard fruit coats (Table 1). Under natural conditions, prolonged germination would enhance the chances of insect infestation, drought damages and other adverse environments leading to a low germination success. Field observation showed that over 90% of the mature fruits were damaged due to insect infection, animal grazing or microbial degradation. Therefore, it is important to know that the fruits have viable seeds when collecting and germinating. Viable fruits could be judged preliminarily by their weight (the heavier the better), color (the deeper brown color the better), and integrity (the more the intact the better, without any sign of infection). The fruit could also be examined by dissection and the removal of fruit coat to show whether it consists intact seed or not. The present study also demonstrated that fruit coat removal by dissection significantly shortened the rooting and leaf expansion time without affecting the germination percentage (Table 2).

E. agallocha is a dioecious species with poor seed set and germination, probably due to the predominance of male trees over female trees (Rao *et al.*, 1998). Therefore, some fruits might not bear any fertile seeds. Moreover, the timing of collecting mature fruits was very crucial for *E. agallocha*. Once the fruits reach maturity, they open and disperse the seeds that then lose the viability quickly if the seeds have not a chance of germination. We observed that almost all of the seeds on the forest ground lost their viable embryos. Mature fruit with brown coats and readily germinated seeds must be collected within a short period of time (less than 10 d).

3.2   Seed dormancy of *L. racemosa*

Reduction in seed dormancy was associated with increased seed weight, efficient spatial seed dispersal and long-lived species (Rees, 1994; 1997). The present study proved that *L. racemosa* was indeed a dormant species although Tomlinson (1986) stated that mangrove species have no innate dormancy. And all mature fruit planted immediately after collection failed to germinate under various conditions (silty soil, sandy soil or seawater) and the embryos died two months later. However, if the fruits were appropriately stored, they were successfully germinated even after 50-d of "wet storage". The embryos of *L. racemosa* were pale green when collected but turned to deep green with the embryo size increased to almost double after "wet storage". These results reveal that the embryos in mature fruits picking from parent trees were under-developed and would continue to develop during the dormant period. The dormancy was therefore endogenous and belonged to morphological dormancy according to the classification of seed dormancy types by Baskin and Baskin (1998). Clarke *et al.* (2001) also regarded *L. racemosa* as a dormant species as they found that the diaspores never showed signs of root or shoot development after planting but 60% of the fruits still had viable embryos. Nevertheless, Clarke's studies did not give sufficient evidence to conclude the seed dormancy of *L. racemosa*. There had not been any other report on the dormancy type and *L. racemosa* seemed to be the only innately dormant plant among all mangrove species. Further studies on the dormancy of mangrove plants are needed.

Because of the dormancy, suitable ways to store fruits of *L. racemosa* must be identified. From the present results, wet storage under room temperature or buried in wet sand were possible, and the "wet storage" seemed to be more convenient because the latter required daily irrigation and had a lower percentage of viable seeds. The time needed for the 35-d stored fruits to have their root and first leaf expansion were similar to those stored for 50 d, but the former fruits had lower percentage germination than the latter one (Table 2). Therefore, "wet storage" for 50 d was more preferable. However, the maximum storage time and other methods for dormancy interruption should be further explored because the highest germination percentages obtained were less than 40% (Table 2), still far from

satisfactory.

3.3 Reproductive adaptations of non-viviparous species to habitats

Mangroves have two reproductive types, namely viviparous (including true- and crypto-viviparous) and non-viviparous. The focus of mangrove reproduction was mainly on the viviparous species because it was considered as one of the important characteristics of mangrove plants (Tomlinson, 1986). For the viviparous species, the embryo produced from normal sexual reproduction has no dormancy but grows to seedling while still attached to the parent plant. Therefore, their diaspores are neither seeds nor fruits but seedlings. For viviparous species especially the true-viviparous ones, the diaspores with special shapes and morphology can readily anchor the sediment and escape from tidal flushing. On the other hand, the mature diaspores of the non-viviparous species, the fruits or seeds but not seedlings, generally cannot anchor the sediment immediately after falling from parent trees and are subject to tidal flushing. It is interesting to know how these diaspores adapt to mangrove habitats during the inundation periods.

The present study suggested that the four non-viviparous mangrove species in Hong Kong had three different mechanisms to adapt the very unstable and ever changing environment. They were: (1) prolonged longevity and viability of diaspores as in *L. racemosa* and *H. littoralis* by seed dormancy and hard fruit coat, respectively; (2) shortened rooting time for rapid establishment in soil as in *E. agallocha* and *A. ilicifolius*, with roots initiated within several days of germination; and (3) produced diaspores of sinkers as in *L. racemosa*. These mechanisms allow the diaspores to have a rapid escape from the adverse circumstances especially tidal flushing. In addition to these three mechanisms, the diaspores of *Sonneratia*, another non-viviparous genus, had different adaptive mechanism. In this genus, the entire seed capsule containing several hundreds of seeds falls from parent trees when the diaspores (seeds) ripe, sinks very quickly and releases individual diaspores which initiate roots and anchor the sediments very rapidly (Smith, 1992). The sinkable capsule protects the diaspores from tidal flushing, allowing them to routinely colonize the lowest inter-tidal zone in spite of their small size which is only 1.0- 1.5 cm in length (Tomlinson, 1986).

3.4 Inter-tidal zonation of non-viviparous species

Inter-tidal zonation has been considered as a common feature in mangrove swamps, which is often attributed to dispersal characteristics, animal predation and establishment capacity of diaspores, seedling dimensions, and physico-chemical factors (Rabinowitz, 1978a; Smith, 1987;

1992; McKee, 1993; 1995; Clarke *et al.*, 2001). However, it has also been proposed that none of the factors including rooting time, animal predation, buoyancy and size of diaspores, and salinity give a satisfactory account for the zonation of all mangrove species (Bunt, 1996; Ellison *et al.*, 2000; Clarke *et al.*, 2001). Bunt (1996) summarized that the distribution pattern of mangrove species across an inter-tidal mudflat was highly variable, influenced by many factors and often occurred as multiple distribution centers. In Hong Kong, the four non-viviparous mangrove species, distributed at the more backshore locations, shared some similar diaspore characteristics as the viviparous species which colonized in the more seaward regions such as larger diaspores in *H. littoralis*, sinker diaspores in *L. racemosa*, rapid root initiations in *E. agallocha* and *A. ilicifolius*. Clarke *et al.* (2001) also suggested that viviparous and non-viviparous mangrove species had similar buoyancy, seed weight and rates of root and shoot initiation, as well as early growth and salinity tolerance. These suggested that zonation explanation for both viviparous and non-viviparous mangrove species is complicated and it is difficult to explain why the non-viviparous species were more landward distributed than the viviparous ones.

Nevertheless, the obvious zonation among four non-viviparous species from seaward to landward in the order of *L. racemosa*, *E. agallocha*, *A. ilicifolius*, and *H. littoralis* might be explained by the diaspore characteristics. Rabinowitz (1978a) suggested that diaspore characteristics especially buoyancy determined mangrove zonation, and sinking diaspores would be restricted to lower elevations while consistently buoyant diaspores were only established at higher positions in the inter-tidal zone. The present study also showed that diaspore buoyancy could partially explain the zonation of non-viviparous species in Hong Kong. *L. racemosa* with sinking diaspores had relatively seaward distribution but the other three non-viviparous species with buoyant diaspores were more at the back of the shore.

Root initiation is an important factor, secondary to diaspore buoyancy, in determining shoreline zonation of non-viviparous mangrove species. Clarke *et al.* (2001) reported that time taken for a species to develop roots and anchor sediments, i.e. the obligate dispersal period, corresponded with the period when diaspores were unable to derive resources from sediments, and considered that backshore mangrove species had diaspores that were slow to initiate roots and shoots. This was similar to *H. littoralis*, the most backshore species in natural mangrove swamps in Hong Kong, took an extremely long period to initiate their roots

(109 d on average) even under favorable conditions. However, it was strange to find that *L. racemosa*, the most foreshore species among the four non-viviparous ones, still needed more than 10 d for root initiation even after a necessary long dormancy period (35 d "wet storage"), while the other more backshore non-viviparous species, *E. agallocha* and *A. ilicifolius* had their root initiations occurred in 2 and 3 d, respectively, even more rapid than the foreshore viviparous species. Rabinowitz (1978a) also observed that *Avicennia* and *Laguncularia* restricted in higher inter-tidal zones took 5- 7 d to initiate their roots, while the foreshore genera, *Rhizophora* and *Pellciera* needed 11- 15 d to become rooted, indicating that the zonation did not necessarily relate to the rooting time of mangrove diaspores. Therefore, root initiations could not completely explain mangrove zonation.

Rabinowitz (1978b) hypothesized that zonation in Panama mangrove forest was controlled by the size of the diaspores due to tidal action, and the distribution of mangrove species from low to high inter-tidal zones were highly related to the size of their diaspores. She found that *Avicennia* and *Laguncularia* were restricted to high inter-tidal zone because their small diaspores would be carried to the farthest land by high tides while species having large diaspores such as *Rhizophora* and *Pellciera* would not be carried into the high tide areas. The "tidal sorting" hypothesis was resurrected by Jimenez and Sauter (1991) in their study on zonation of *R. racemosa* and *R. bicolor* in Costa Rica. However, observations on species distribution in Australia and elsewhere indicated that "tidal sorting" was not a mechanism influencing mangrove zonation, and the genera *Aegiceras* and *Avicennia*, typically abundant in low inter-tidal areas had small diaspores (Bunt and Williams, 1981; Wells, 1982). Saenger (1982) investigated seedling recruitment in mangrove forests at Port Curtis in central Queensland coast (Australia) and found that *R. stylosa* with the largest diaspore among all species was distributed in all portions of the inter-tidal zone. Smith (1992) also rejected the "tidal sorting" hypothesis based on the study that *Sonneratia* species routinely colonized the lowest inter-tidal zone had very small diaspores in comparison to other mangrove plants. Results from the present study showed that *H. littoralis* distributed in the highest inter-tidal zone had heavier diaspores (with average weight of about 30 g) while the two non-viviparous species, *E. agallocha* and *A. ilicifolius* with very small diaspores were more towards to seaward zones. These suggested that mangrove zonation in Hong Kong was not related to the size of diaspores.

McKee (1995) suggested that physico-chemical factors influenced mangrove shoreline zonation in Twin Cays and 74% of the variation was explained by intensity of salinity-related stresses. The present study showed that the inhibitory effects of high salinities on seed germination were stronger in *L. racemosa* than that in *A. ilicifolius*, but the former species naturally colonizes in more seaward zone with higher salinity. This suggested that salinity could not explain non-viviparous mangrove shoreline zonation in Hong Kong.

Animal predation of diaspores was an important factor influencing the zonation of mangrove species because predators (mainly grasped crabs) appears to be the least in the lowest inter-tidal zone and increases to maximum amounts in the high inter-tidal zone (Osborne and Smith, 1990). However, over 90% diaspores of *H. littoralis* distributed in high inter-tidal zone were attacked and killed by insect predators. For the other three non-viviparous species distributed in more seaward zones, mortality of diaspores was not due to animal predations. Therefore, predation was not sufficient to account for the distribution pattern of non-viviparous species in mangrove forests of Hong Kong.

McKee (1993; 1995) reported that seedling dimensions after establishment would also influence mangrove zonation, and seedlings with small height and diameter had backshore distribution because the small stem diameter had a lower cross-sectional area for oxygen diffusion and limited aeration of their root systems. However, *H. littoralis* distributed in the highest inter-tidal zone had the largest seedling dimensions among four non-viviparous mangrove species in Hong Kong, while *L. racemosa* distributed in the lowest inter-tidal zone had the smallest seedling dimensions. These indicated that seedling dimensions also could not well explain the shoreline zonation of non-viviparous mangrove species in Hong Kong.

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