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Frugivory by insects on mangrove propagules: effects on the early life history of *Avicennia marina*

Abstract This study investigates how herbivory by larval insects on fruit of the mangrove *Avicennia marina* affects the subsequent establishment, growth, and survivorship of its seedlings. Research was done in a temperate mangrove forest in Australia where the larvae of two species of insects were the dominant frugivores: the mangrove fruit fly *Euphranta marina* and the mangrove plume moth *Cenoloba obliteralis*. Larvae consumed the cotyledons of fruit, but not their embryonic axes. Damage to the cotyledons of fruit while on the tree was obvious in abscised propagules and the cotyledons of seedlings, indicating that larvae within fruit continue to consume the cotyledons and develop while the propagule disperses and the seedling establishes. We found that 53% of fruit, 69% of abscised propagules, and 80% of the cotyledons of seedlings had been attacked by larval insects. The degree of damage to the cotyledons was positively related to the densities of larvae within the fruit and the number of emergence or exit holes in the surface of the cotyledons. Consequently, the number of exit holes could be used as a proxy for the amount of damage to the cotyledons. An experiment in which we placed propagules with different levels of damage (zero, one, two, or three exit holes) on the forest floor revealed that frugivory did not influence the establishment of seedlings. In another experiment, we monitored the performance of newly established seedlings with cotyledons that had zero exit holes, one or more exit holes, or where we had removed their cotyledons. In both experiments, the growth of seedlings as measured by their heights and number of leaves was negatively related to the degree of frugivory. Consumption of the cotyledons by larvae apparently reduces the energy reserves available for the initial growth of seedlings. The early mortality of seedlings in both experiments was minimal and appeared to be independent of differences in size due to frugivory. Experimentally removing the cotyledons of seedlings produced extremely short seedlings with increased mortality, confirming the importance of cotyledonary reserves to the initial growth and survivorship of seedlings of *A. marina*. This study highlights how the early life history of plants may be impacted by conditions experienced by the propagules before they disperse from the parental plant.

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Introduction

Herbivory by insects on the developing flowers, seeds, and fruit of plants can have significant demographic and population consequences (Janzen 1971; Stephenson 1981; Cavers 1983; Crawley 1989; Louda 1989; Fenner 1992; Chambers and MacMahon 1994). Herbivorous insects can decrease the output of propagules and damaged propagules may not be viable. Consequently, herbivory on propagules before they disperse from the parental plant can be an important factor limiting the supply of propagules and recruitment of seedlings (e.g., Louda and Potvin 1995). Although mostly documented for plants in terrestrial habitats (Crawley 1989, 1992; Louda 1989), herbivory by insects may also be an important factor structuring populations of plants in marine habitats such as salt marshes (Bertness et al. 1987) and mangrove forests (Robertson 1991).

Mangrove trees produce large fruit that would be expected to attract a diverse assemblage of herbivores (Robertson et al. 1990; Farnsworth and Ellison 1997). Research on herbivory of propagules in mangrove for-

ests has, however, largely focused on the impacts of post-dispersal herbivores, particularly crabs. Crabs are often voracious consumers of mangrove propagules and can influence the supply of propagules throughout the forest, small-scale patterns of seedling recruitment, and perhaps even the patterns of tree abundance (Smith 1987a, 1987b, 1992; Smith et al. 1989; Osborne and Smith 1990; Robertson 1991; McKee 1995a, 1995b; McGuinness 1997a, 1997b; Sousa and Mitchell 1999).

In addition to such conspicuous post-dispersal herbivores, mangrove forests contain a suite of smaller, less obvious herbivores, particularly specialist insects such as moths, beetles, and flies, which attack the developing flowers and fruit on the trees (Onuf et al. 1977; Rabinowitz 1977; Hutchings and Recher 1982; Murphy 1990; Robertson et al. 1990; Clarke 1992; Farnsworth and Ellison 1997). Relatively few studies have examined the influence of herbivorous insects on the population dynamics of mangrove trees, but some studies have shown that there can be negative consequences (see review by Robertson 1991). For example, insects can decrease the production of fruit and promote the abscission of premature propagules (Clarke 1992; Farnsworth and Ellison 1997). Infestation of propagules by larval and adult insects can also reduce the establishment success and growth of seedlings (Onuf et al. 1977; Robertson et al. 1990). A recent survey by Farnsworth and Ellison (1997) documented that the consumption of mangrove propagules by herbivorous insects is common globally, but the general importance of this factor to subsequent population dynamics of mangroves is unknown.

We found that many fruit of the mangrove *Avicennia marina* in a temperate forest near Sydney, Australia, were infested by larval insects (see also Hutchings and Recher 1974). This intense frugivory had been previously observed by Clarke (1992), and when he excluded larval insects from developing fruit, twice as many grew to maturity. We observed that some fruit chewed extensively by larval insects and riddled with bore holes could still develop to maturity and disperse naturally (see also Clarke 1992). Consequently, damage by larval insects to fruit while they are on the tree is passed on to the subsequent life history stages of the plant. How this frugivory affects the establishment, growth, and survivorship of seedlings of *A. marina* is not known (but see Robertson et al. 1990 for studies in the tropics).

Here, we examined the effect of frugivory by larval insects on the early life history of *A. marina* in a temperate mangrove forest near Sydney, Australia. First, we quantified how damage by insects to fruit is transferred to the propagule and seedling stages. To do this, we surveyed the incidence and severity of damage by herbivorous insects on fruit, abscised propagules, and the cotyledons of recently established seedlings. At the same time, we counted and identified the larval insects. Second, we designed two experiments to test hypotheses that frugivory by insects negatively affects the (a) establishment, (b) growth, and (c) survivorship of seedlings.

Materials and methods

Location studied

The study took place in the temperate mangrove forest surrounding Woolooware Bay, which is located on the south side of Botany Bay near Sydney, New South Wales, Australia. Access to the study area on the northeast side of the bay is restricted because it forms part of the Towra Point Nature Reserve. The mangrove forest in this area ranges in width from 50 to 150 m and is bordered on its landward side by salt marsh and on its seaward side by mud flats and seagrass beds. Two species of mangrove trees are present at Woolooware Bay: *A. marina*, which extends throughout the forest forming a virtual monoculture, and *Aegiceras corniculatum*, which is irregularly interspersed among *A. marina* primarily along the landward edge of the forest. Tides are semi-diurnal with a maximal range of ≈2 m.

The mangrove *A. marina*

In temperate mangrove forests at this latitude, *A. marina* fruit develop over the winter into late spring (Duke 1990; Clarke and Myerscough 1991a). The cryptoviviparous fruit is the dispersal unit of the tree (referred to as a propagule after abscission from the tree) and consists of a single embryo encased within a thin pericarp. The fruit is roughly spherical to ovoid (about 3 cm) and flattened, with one rounded side and one flatter side (Tomlinson 1994). The embryo comprises an embryonic axis (radicle, hypocotyl, epicotyl, and plumule) and two large, fleshy cotyledons that contribute the bulk of the propagule. The cotyledons are tightly packed and folded in half, the inner one stacked inside the outer one. Mature fruit fall from the trees in late spring and early summer (Duke 1990; Clarke and Myerscough 1991a) and may be dispersed by water via the buoyant pericarp (Steinke 1986; Clarke and Myerscough 1991b; Clarke 1993). The pericarp splits (i.e., germination occurs) within a few days and establishment generally occurs within 1–3 weeks (Clarke and Myerscough 1991a, 1993). After seedling establishment, the cotyledons unfold, one on either side of the stem. Seedlings grow for several months during the summer and early autumn, after which the cotyledons are withered and fall from the seedling. Seedlings can survive at this height for several years in a "seedling bank" (Burns and Ogden 1985; Clarke and Allaway 1993).

The insects

The larvae of two species of insects have been documented as common herbivores on *A. marina* fruit in mangrove forests: the mangrove fruit fly *Euphranta marina* and the mangrove plume moth *Cenoloba obliteralis* (McAlpine 1965; Hutchings and Recher 1974, 1982; Common 1990; Hockey and De Baar 1991; Clarke 1992; Permkam 1993; Permkam and Hancock 1995).

The mangrove fruit fly *E. marina* was first reported by McAlpine (1965) and derives its specific name from its host plant *A. marina* (Permkam and Hancock 1995). Permkam (1993) has described the life cycle of *E. marina* within the fruit of *A. marina*. Briefly, a female *E. marina* pierces the pericarp and deposits her egg (usually one, but can be more than three) in the fruit while still on the tree. After hatching, the larva bores into and feeds exclusively on the developing cotyledons. The larva develops through three instars and once full grown (7–8 mm in length) it tunnels to the surface of the fruit and makes a round hole in the surface of the cotyledon and the pericarp for emergence (hereafter referred to as an exit hole). The larva then pupates within the cotyledons near the surface of the fruit and emerges as an adult through the exit hole. Thus, the activities of larvae produce a labyrinth of tunnels within and exit holes on the surface of the cotyledons. Under laboratory conditions, larval development within the fruit takes about 4 weeks (of which 1 week is spent as a pupa) and the adult lives about 3 weeks.

The life cycle of the mangrove plume moth *C. obliteralis* is less well known. A female lays her eggs outside the flower cluster or on the surface of the fruit and, after hatching, the larva burrows into the stalk of the flower cluster or directly into the fruit (Clarke 1992). The larva develops by feeding on the floral buds and developing cotyledons, creating irregular galleries in the cotyledons (Common 1990; Clarke 1992). The mature larva leaves these galleries and pupates in white, silky cocoons either within or outside the fruit (Common 1990; Hockey and De Baar 1991). Consequently, the larva might generate an exit hole in the surface of the cotyledon and the pericarp as it leaves the fruit.

Patterns of frugivory

To determine how patterns of herbivory by larval insects on fruit were transferred to the propagule and seedling life history stages, we examined (a) fruit from the trees, (b) propagules 1 and 2 days after abscission from the trees, and (c) the cotyledons of seedlings in three size classes (5–10, 15–20, and 25–30 cm) that had established naturally in the upper part of the mangrove forest. Fruit and propagules were examined for frugivory five times between 7 December 1995 and 2 January 1996. At each sampling time, hundreds of mature fruit were collected from about 25 trees in the upper part of the mangrove forest and then mixed together. To ensure that fruit were mature and roughly the same size, only those that fell after we shook the branches of trees were used (see also Clarke and Myerscough 1991b). We were concerned that if damaged fruit fell from the trees more easily than those that had not been consumed, then this method of collection might bias (i.e., inflate) our estimates of frugivory. Observations of fruit on the trees revealed that this was not the case: our procedure yielded a representative sample of fruit. Immediately after collection, 100 fruit were haphazardly selected and scored for frugivory. The others were put into plastic mesh bags and placed in the mangrove forest where they would not be inundated by the tide. On each of the following 2 days, 100 of these propagules were scored for frugivory. The cotyledons of seedlings in each of the three size classes were examined at each of two times depending on their availability after establishment: 5–10 cm seedlings on 21 December 1995, 5–10 cm and 15–20 cm seedlings on 16 January 1996, 15–20 cm and 25–30 cm seedlings on 17 February 1996, and 25–30 cm seedlings on 15 March 1996. On each day, cotyledons were randomly collected from 50 seedlings in each size class and then scored for frugivory. Therefore, cotyledons were sampled from 100 seedlings in each of the three size classes. Only seedlings with both cotyledons that showed no signs of post-dispersal damage by crabs or other animals were selected.

We scored damage due to frugivory in two ways: externally and internally. By assessing frugivory both externally and internally, we could determine whether the number of exit holes could be used as a proxy for the degree of internal damage caused by the insects. First, we examined the external surfaces and counted the number of exit holes in all fruit, propagules, and cotyledons of seedlings. Exit holes are visible with the naked eye and easily counted. Second, we quantified internal damage for a subset of the fruit from the trees and cotyledons of seedlings in each of the three size classes. Ten fruit and the cotyledons of ten seedlings in each size class with either zero (i.e., no damage), one, two, or three exit holes were examined internally with the naked eye. For each, damage was determined by assessing the condition of the cotyledons and embryonic axis (for fruit only). The larvae were also counted. The embryonic axis was examined externally, broken open and inspected internally, and then classified as either damaged or undamaged. The two cotyledons of the fruit or seedling are folded in half, yielding four distinct "half-cotyledons" that can be assessed for damage. Each of the four half-cotyledons was examined externally and then broken open and inspected internally. The condition of each half-cotyledon was classified according to the severity of the damage: no damage, minor damage, or major damage. Pinhole-sized tunnels within the cotyledons or superficial grazing tracks were classified as minor damage. Large bore holes,

galleries, and pupal cases in the cotyledons or large portions consumed at the surface of the cotyledons were classified as major damage. Therefore, for each pair of cotyledons, this method gave an index of damage that could range from 0 to 4 for minor damage and 0 to 4 for major damage. For example, if there was minor damage to two of the half-cotyledons and major damage to the other two half-cotyledons, then the pair of cotyledons of this fruit or seedling was assigned a score of 2 for minor damage and 2 for major damage. The maximal damage to a pair of cotyledons would occur when all four half-cotyledons had major damage, and this fruit or seedling would receive a score of 0 for minor damage and 4 for major damage.

To identify the larval insects, we incubated fruit, propagules, and the cotyledons of seedlings containing larvae in opaque, plastic bags at room temperature and collected the insects that pupated.

Frugivory and the establishment, growth, and survivorship of seedlings

Two experiments were done to test hypotheses that frugivory by insects negatively affects the (a) establishment, (b) growth, and (c) survivorship of seedlings.

Propagule experiment

In the first experiment, we collected mature fruit from the trees as described previously and separated them into four treatments according to the number of exit holes in their pericarps: zero (i.e., no damage), one, two, or three holes. Five quadrats (50×50 cm) of each treatment were randomly located in an area (10 m wide ×25 m parallel to shore) under the canopy in the upper part of the mangrove forest. Quadrats were separated by at least 3 m and located on flat areas between mounds created by the burrowing activities of crabs (see Warren and Underwood 1986) because the establishment of seedlings has been shown to vary with topography of the forest floor (Minchinton, in press). We first removed the few propagules that had naturally dispersed to the area. Most fruit had fallen from the trees by this time, so the subsequent natural dispersal of propagules into quadrats was not a problem. We then removed the pericarps of the mature fruit so that they would not float away and placed 12 propagules in each quadrat. Within each quadrat, propagules were uniformly arranged on the substratum with their flat sides down. The secondary dispersal of propagules without pericarps is minimal in the upper part of the forest and, therefore, propagules did not intermix among quadrats (Minchinton, in press).

The experiment began on 21 December 1995 and the fates of the propagules and then seedlings were followed every 2–4 weeks until the end of the experiment about 10 months later on 13 October 1996. Percent establishment of seedlings in each quadrat was estimated as the number of propagules out of 12 that established. Seedlings were considered to have established when roots had anchored and the cotyledons were lifted off the substratum. Percent mortality of seedlings in each quadrat was estimated as the proportion of established seedlings that died by the end of the experiment. The growth of seedlings was estimated by their heights and number of leaves at the end of the experiment.

Seedling experiment

In the second experiment, we monitored the growth and early survivorship of naturally established seedlings with cotyledons that had either been consumed or not consumed by larval insects. At each of two sites (about 10 m wide×20 m parallel to the shore and separated by about 200 m) under the canopy in the upper part of the mangrove forest, 30 seedlings were randomly selected that had either no exit holes (i.e., they were undamaged) or one or more exit holes (i.e., the propagules from which the seedlings arose had

experienced frugivory). We also included a third treatment where we removed the cotyledons from 30 randomly selected seedlings. This treatment was included to determine the importance of cotyledonary reserves to the early growth and survivorship of seedlings. It also simulated the case of seedlings that establish from propagules with extreme amounts of damage and then lose their cotyledons soon after establishment. The initial heights of seedlings were similar among treatments (site 1: no exit holes=7.6± 0.28 cm, exit holes=7.9±0.26 cm, removed=8.2±0.27 cm; site 2: no exit holes=8.1±0.29 cm, exit holes=7.9±0.32 cm, removed= 7.8±0.26 cm; mean±SE, n=30 for each), and all seedlings had only their first pair of true leaves. Seedlings exhibiting post-dispersal damage by crabs or other animals were not selected.

Seedlings were individually labelled with flagging tape on 18 January 1996 and then monitored every 2–3 weeks until the end of the experiment on 28 May 1996. The experiment was ended at this time because the cotyledons had withered and fallen from all the seedlings. Percent mortality of seedlings at each site was estimated as the proportion of seedlings that died by the end of the experiment. The growth of seedlings was estimated by their heights and number of leaves at the end of the experiment. The cotyledons of seedlings were monitored to determine whether damage affected how quickly they withered and were shed.

Results

The insects

The fruit, abscised propagules, and cotyledons of seedlings of *A. marina* contained numerous larval insects that had inflicted considerable damage. We found two types of larvae within the cotyledons, a larger one that resembled that of the mangrove fruit fly *E. marina* (see Permkam 1993) and a smaller one that we assumed to be that of the mangrove plume moth *C. obliteralis*. Adults of both *E. marina* and *C. obliteralis* were reared from the fruit, propagules, and the cotyledons of seedlings, but unfortunately we could not link the two larval types to the specific adults. We observed both types of larvae feeding on the cotyledons. Feeding activities produced bore holes and galleries in and grazing tracks on the surface of the cotyledons. Although both insects were common, larvae and adults of *E. marina* appeared to be more abundant than those of *C. obliteralis*. Many pupal cases of *E. marina* were observed in chambers within the cotyledons of fruit, abscised propagules, and seedlings, and these occupied considerable space and thus resulted in substantial
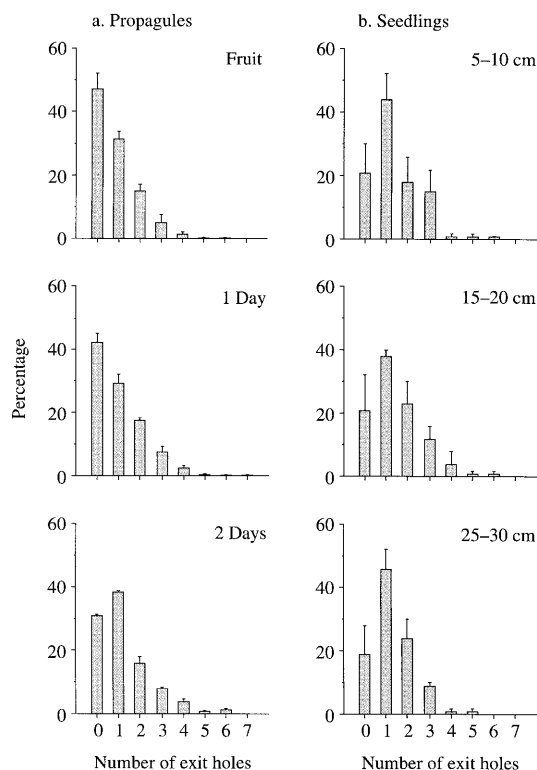
Fig. 1 Mean (±SE) percentage of propagules (fruit and propagules 1 and 2 days after abscission from the trees; n=100 at each of five sampling times) (**a**) and cotyledons of seedlings in each of three size classes (5–10, 15–20, or 25–30 cm; n=50 at each of two sampling times) (**b**) with zero (i.e., no damage) to seven exit holes

Table 1 Mean (±SE) damage (number with internal damage, amount of minor and major damage) and number of larvae for ten fruit and cotyledons of ten seedlings in each of three size classes with different numbers of exit holes (zero, one, two, or three)

	Number of exit holes	Number with internal damage	Minor damage		Major damage		Number of larvae	
			Mean	SE	Mean	SE	Mean	SE
Fruit	Zero	1	0.1	0.10	0.1	0.10	0.0	0.00
	One	10	0.8	0.20	1.6	0.22	0.8	0.20
	Two	10	0.5	0.22	2.3	0.15	1.2	0.25
	Three	10	0.3	0.15	3.1	0.28	1.5	0.34
Seedlings (5–10 cm)	Zero	0	0.0	0.00	0.0	0.00	0.0	0.00
	One	10	0.7	0.33	1.7	0.30	0.8	0.51
	Two	10	0.8	0.25	2.6	0.22	1.3	0.50
	Three	10	0.4	0.22	3.2	0.20	1.5	0.34
Seedlings (15–20 cm)	Zero	0	0.0	0.00	0.0	0.00	0.0	0.00
	One	10	0.4	0.16	1.8	0.29	0.7	0.42
	Two	10	0.6	0.16	2.1	0.31	0.4	0.22
	Three	10	0.5	0.22	3.2	0.20	0.8	0.51
Seedlings (25–30 cm)	Zero	0	0.0	0.00	0.0	0.00	0.0	0.00
	One	10	0.3	0.15	1.2	0.29	0.0	0.00
	Two	10	0.5	0.22	2.2	0.20	0.0	0.00
	Three	10	0.6	0.22	2.9	0.23	0.3	0.15

Table 2 Analyses of variance of minor damage, major damage, and numbers of larvae for cotyledons at different stages of early life history (fruit, seedlings in three size classes: 50–100, 150–200, 250–300 cm) with different numbers of exit holes (zero, one, two, or three) (see Table 1 for data) (***$P<0.001$; NS not significant)

Source	df	Minor damage		Major damage		Number of larvae	
		MS	F	MS	F	MS	F
Stage (S)	3	0.12	0.34 NS	0.63	1.37 NS	6.07	7.16***
Exit holes (H)	3	2.74	7.66***	68.42	147.93***	7.41	8.73***
S×H	9	0.28	0.78 NS	0.25	0.54 NS	0.93	1.10 NS
Residual	144	0.36		0.46		0.85	

damage. We found only a few of the silky cocoons of *C. obliteralis*, suggesting that many of the larvae must leave the propagule to pupate. Cocoons were located between the folded halves of the cotyledons in fruit and even on one seedling. Consequently, pupation by this species does not appear to cause significant internal damage. Interestingly, we also reared several species of hymenopterans from the fruit that were suspected to be *Opius* sp. and *Eriborus* sp., which are parasites of *E. marina* and *C. obliteralis*, respectively (Hutchings and Recher 1974).

Patterns of frugivory

More than half of the cotyledons of fruit, abscised propagules, and seedlings had been infested by larval insects (Fig. 1). For both undamaged fruit and those with exit holes, there was on average one exit hole per fruit, and fruit with at least one exit hole had on average two exit holes per fruit. Frequency histograms of the number of exit holes generally declined exponentially, and few cotyledons had more than three exit holes (Fig. 1). The percentage of cotyledons with exit holes increased with life history stage. About 53% of the cotyledons of fruit had exit holes, and this increased to 69% for propagules that had abscised from the trees 2 days previously, and to 80% for established seedlings (Fig. 1). The percentage of fruit with exit holes increased with the time since they had abscised from the trees (Fig. 1a; *G*-test, *G*=7.0, *P*<0.05). Moreover, the percentage of cotyledons of established seedlings with exit holes was greater than that for fruit and abscised propagules (Fig. 1; *G*-test, *G*=9.7, *P*<0.05). The percentage of cotyledons of seedlings with exit holes was, however, independent of seedling height (Fig. 1b; *G*-test, *G*=0.2, *P*>0.90).

The degree of major damage was positively and significantly related to the number of exit holes in the cotyledons of fruit or seedlings [Tables 1, 2; Student-Newman-Keuls multiple-comparison tests after significant analysis of variance (SNK), *P*<0.05]. Larval densities showed a similar trend, and both densities of larvae and minor damage were significantly greater within cotyledons with exit holes than within those without exit holes (Tables 1, 2; SNK, *P*<0.05). Therefore, these results demonstrate that the number of exit holes in the fruit can be reliably used as a proxy for the degree of frugivory by these larval insects. Interestingly, one prop-
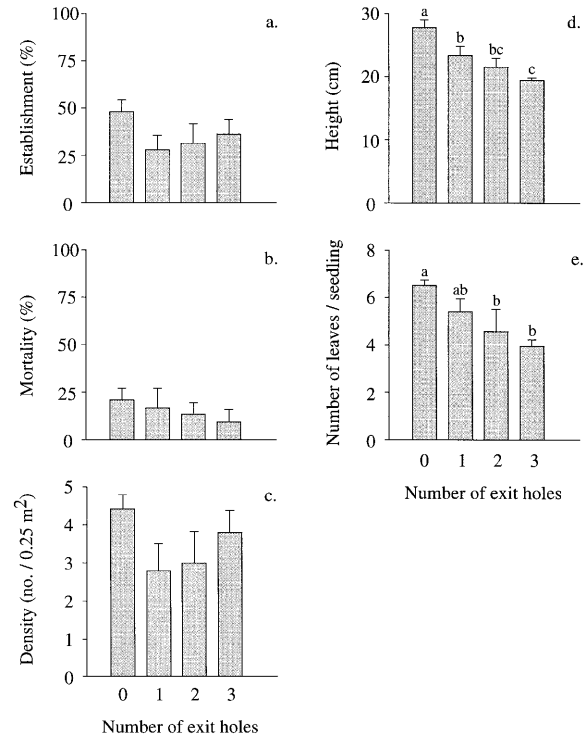
Fig. 2 Mean (±SE) percent establishment (**a**), percent mortality (**b**), density (**c**), height (**d**), and number of leaves per seedling (**e**) of seedlings from propagules with either zero, one, two or three exit holes. *Letters above bars* indicate the results of Student-Newman-Keuls multiple-comparison tests after significant analysis of variance. For each graph, bars with different letters are significantly different at *P*<0.05

agule without an exit hole was internally damaged, probably because the larvae had died or not yet emerged (Table 1). Importantly, not one propagule had a damaged embryonic axis, suggesting that frugivory does not affect propagule viability.

The greatest number of larvae found within a single fruit was six. For both undamaged fruit and those with exit holes, there were on average 0.9 larvae per fruit, and fruit with at least one exit hole had on average 1.2 larvae per fruit. Larval densities within the cotyledons decreased from the fruit through the seedling stages of the life history, even though all stages exhibited similar amounts of internal damage (Tables 1, 2). Larvae were significantly more abundant in the cotyledons of fruit and
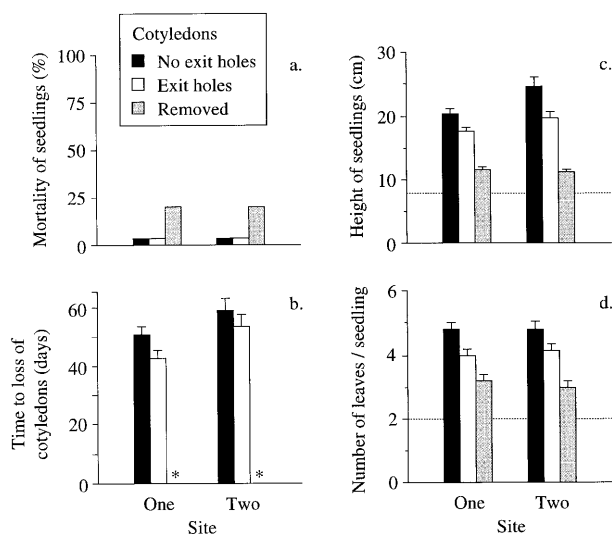
Table 3 Analyses of variance of the time to loss of cotyledons, heights of seedlings, and number of leaves per seedling for seedlings with different levels of damage to their cotyledons (no exit holes, one or more exit holes, removed) at each of two sites (see Fig. 3 for data) (*$P<0.05$, **$P<0.01$; NS not significant)

Source	Cotyledons			Height			Leaves		
	df	MS	F	df	MS	F	df	MS	F
Site (S)	1	2,655.4	7.9**	1	172.6	7.4**	1	0.004	0.003 NS
Damage (D)	1	1,324.7[a]	4.0*	2	1,625.9	21.8*	2	39.972	131.227**
S×D	1	68.3	0.2 NS	2	74.6	3.2*	2	0.305	0.263 NS
Residual	112	334.5		158	23.3		158	1.159	

[a] To increase the power of the test, the estimate of mean squares used in the denominator of the *F*-ratio is a pooled estimate from the mean squares of the S×D interaction and the residual, and then the effect of D was tested with 1 and 113 degrees of freedom (see Winer et al. 1991 for pooling procedures)
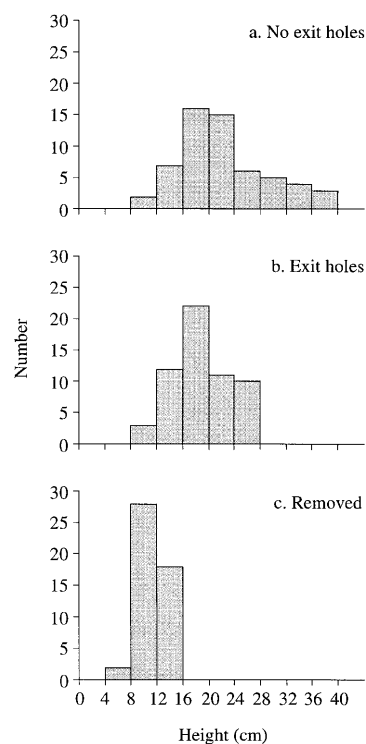

Fig. 3 Percent mortality (**a**) and mean (±SE) time to loss of cotyledons (**b**), height (**c**), and number of leaves per seedling (**d**) of seedlings with different levels of damage to their cotyledons (no exit holes, one or more exit holes, removed) at each of two sites. The *asterisk* indicates that this treatment is not relevant because cotyledons had been removed at the beginning of the experiment. *Dashed lines* indicate the average heights and number of leaves of seedlings at the start of the experiment

Fig. 4 Height-frequency histograms of numbers of seedlings with different levels of damage to their cotyledons: no exit holes (*n*=58) (**a**), one or more exit holes (*n*=58) (**b**), removed (*n*=48) (**c**). Data for the two sites are combined

seedlings that were 50–100 cm tall than in those of seedlings 250–300 cm tall (Tables 1, 2; SNK, $P<0.05$).

Frugivory and the establishment, growth, and survivorship of seedlings

Propagule experiment

Seedlings established in greatest numbers from propagules without exit holes, but there was no significant difference in percent establishment among treatments [Fig. 2a; one-factor analysis of variance (ANOVA), $F_{3,16}=1.3$, $P>0.30$]. Fewer than 25% of the established seedlings died, and mortality was independent of damage to propagules (Fig. 2b; one-factor ANOVA, $F_{3,16}=0.4$, $P>0.70$). Consequently, densities of seedlings 10 months after the start of the experiment were not affected by the damage caused by the larval insects (Fig. 2c; one-factor ANOVA, $F_{3,16}=1.3$, $P>0.30$). In contrast, there was a negative relationship between the degree of frugivory and the growth of mangrove seedlings (Fig. 2d, e). Seedlings of undamaged propagules were significantly taller (by about 30% on average) than those of damaged propagules and produced significantly more leaves than seedlings from propagules with two or three exit holes (Fig. 2d, e; one-factor ANOVA, height: $F_{3,16}=9.4$, $P<0.001$; leaves: $F_{3,16}=3.6$, $P<0.05$).

Seedling experiment

Few seedlings died during the experiment and, as in the propagule experiment, there was no influence of frugivo-

ry on the survival of seedlings (Fig. 3a). In contrast, removing the cotyledons of seedlings resulted in a more than fivefold increase in their mortality (Fig. 3a). Cotyledons with no exit holes remained attached to seedlings significantly longer (by about 5–10 days) than those that had been consumed by insect larvae, and cotyledons were lost earlier at site 1 than at site 2 (Fig. 3b, Table 3). As in the propagule experiment, seedlings with undamaged cotyledons grew significantly taller (by about 20% on average) and produced significantly more leaves than those with damaged cotyledons (Fig. 3c, d, Table 3; SNK, $P<0.05$). Seedlings that had their cotyledons removed grew very little and thus were significantly shorter and had significantly fewer leaves than seedlings with cotyledons (Fig. 3c, d, Table 3; SNK, $P<0.05$). The significant interaction between site and cotyledon damage for seedling height occurred because seedlings with no exit holes in their cotyledons were significantly taller at site 2 than at site 1, whereas those with exit holes or no cotyledons were similar in height at both sites (Fig. 3c, Table 3). Frequency histograms of seedling heights revealed that seedlings whose cotyledons had been removed or damaged by larvae did not grow sufficiently to be represented in the taller size classes (Fig. 4).

Discussion

Herbivory by insects on the fruit of *A. marina* did not affect the establishment of seedlings because larvae did not feed on the embryonic axis. In contrast, seedling growth was significantly and negatively related to the degree of frugivory. Larval insects appear to reduce the size of seedlings by consuming the cotyledons which provide the energy reserves for initial growth. The early mortality of seedlings was minimal and not influenced by differences in size due to frugivory. Whether the largest seedlings derived from undamaged propagules have a competitive advantage (e.g., increased light capture) that enhances their future growth and survivorship awaits longer-term observations. Evidence from this and other studies (see discussion below) indicates that frugivory by insects plays a role in the early life history of mangroves.

Patterns of frugivory

There was a high incidence of herbivory by insects on the fruit of *A. marina* at Woolooware Bay. Fruit hosted numerous larvae of multiple taxa (dipterans, lepidopterans, hymenopterans), indicating that the presence of one larva did not deter others from entering or adults from laying eggs into the same fruit (Permkam and Hancock 1995). A single mangrove propagule of *A. marina* thus harbors a small community of insects from which numerous adults can emerge. Similarly, propagules of tropical mangroves often have multiple bore holes and diverse assemblages of insects (Robertson et al. 1990; Farnsworth and Ellison 1997).

In this study, 53% of mature fruit had been infested by insects, and such levels of frugivory are comparable to those recorded for *A. marina* in the Sydney region (43%: Clarke 1992) and other temperate and tropical mangrove forests (59–65%: Robertson et al. 1990; 10–62%: Clarke 1992; 10–90%: Farnsworth and Ellison 1997). The larvae of two common insects in temperate mangroves inflicted the damage: the mangrove fruit fly *E. marina* and the mangrove plume moth *C. obliteralis* (McAlpine 1965; Hutchings and Recher 1974, 1982; Common 1990; Hockey and De Baar 1991; Clarke 1992; Permkam 1993; Permkam and Hancock 1995). Moth larvae are ubiquitous herbivores of propagules of *A. marina*, and flies and beetles are also important consumers of mangrove propagules (Onuf et al. 1977; Rabinowitz 1977; Murphy 1990; Robertson et al. 1990; Feller 1995; Farnsworth and Ellison 1997).

The degree of internal damage to fruit was positively related to the densities of larvae they contained and, consequently, to the numbers of exit holes in the cotyledons. Determining this relationship allowed us to perform experiments relating the severity of frugivory to the early life history of *A. marina*. Insect larvae only preyed upon the cotyledons of fruit, and both *E. marina* and *C. obliteralis* appear to cause damage. The few studies that have examined herbivorous insects of mangrove propagules have found that some moth larvae burrow between and feed on the surfaces of cotyledons (Robertson et al. 1990; Farnsworth and Ellison 1997), whereas others create galleries in the cotyledons (Common 1990; Murphy 1990; Clarke 1992). Moth larvae appear to leave the galleries within the cotyledons and pupate between the cotyledons within or outside the fruit (Common 1990; Hockey and De Baar 1991). We found a few cocoons between the cotyledons of fruit and seedlings and these did not appear to damage the propagules. In contrast, larvae of *E. marina* are known to pierce the surface of the cotyledons, and consume the cotyledons from within as they develop and create chambers for their pupal cases (Permkam 1993). Therefore, we speculate that larvae of *E. marina* do more damage than those of *C. obliteralis* to the cotyledons. Nevertheless, more information on the natural history and relative abundance of these and other herbivorous insects is needed before we can assign observed damage to particular species and determine their relative importance to the population dynamics of *A. marina*.

There was a significant trend for the proportion of cotyledons with exit holes to increase with life history stage: fruit on the trees had the lowest percentage of damaged propagules, propagules abscised from the trees had an intermediate level, and seedlings had the highest level. Because *E. marina* and *C. obliteralis* enter the fruit while it is on the tree (Common 1990; Permkam 1993), this suggests that larvae are developing within the cotyledons over time and an increasing number emerge from the time the fruit is on the tree until after the seedlings become established. This idea is supported by the result that the numbers of larvae within the cotyledons

decreased significantly from the fruit to the seedling stage, with the tallest seedlings with the oldest cotyledons containing almost no larvae. Larvae of *E. marina* take about a month to develop (Permkam 1993) and those of *C. obliteralis* may take just as long (Hockey and De Baar 1991). Therefore, as observed for weevils attacking propagules of the mangrove *Avicennia alba* in the tropics (Murphy 1990), these larvae are likely consuming the cotyledons and growing, pupating, and emerging while the propagule disperses in the water and as the seedling establishes and grows (Permkam 1993). Consequently, even though larvae enter the fruit while it is on the tree, frugivory by these insects is occurring from the pre-dispersal stage while the fruit is on the tree, during and following dispersal, and even after establishment of the seedling. Consequently, these herbivores not only affect the production of fruit (see Clarke 1992), but they also have the potential to influence the dispersal of propagules, and the establishment, growth, and survivorship of seedlings (see discussion below).

Frugivory and the establishment, growth,
and survivorship of seedlings

The establishment of seedlings was unaffected by the herbivory of insect larvae on fruit of *A. marina*. Our study only included propagules with up to three exit holes, so we do not know whether propagules with four or more exit holes are similarly viable. Nevertheless, the embryonic axis of propagules with up to seven exit holes was not damaged, and thus it seems reasonable to assume that these severely damaged propagules would also be viable. In studies using propagules collected from the ground and planted in pots in a shadehouse, Robertson et al. (1990) also found that frugivory by insects did not influence the viability of propagules of *A. marina* in the tropics (although they did find that insects reduced the viability of other mangrove species; see also Onuf et al. 1977). Therefore, frugivory by insects appears to have little influence on initial patterns of seedling recruitment of *A. marina*.

The primary influence of herbivory by larval insects on the cotyledons of fruit was to reduce seedling growth. Frugivory reduced the heights of seedlings by about 20–30%, a result comparable to that for the effects of herbivorous insects on seedling growth of *A. marina* in the tropics (Robertson et al. 1990). Moreover, the growth of seedlings was related to the degree of damage to their cotyledons (see also Robertson et al. 1990 for the mangrove *Xylocarpus granatum*). Seedlings with undamaged cotyledons were about double the size of those whose cotyledons we removed, indicating that initial growth depends on cotyledonary reserves. As insect larvae consume the cotyledons of fruit, they deplete the energy reserves available for the initial growth of seedlings after establishment. The result that cotyledons infested by larvae withered and fell from seedlings sooner than undamaged cotyledons indicates that reserves had been exhausted sooner. Damage to the cotyledons of seedlings by crabs and other animals was negligible (about 5% of cotyledons showed signs of attack) and not influenced by the presence of insects, so this cannot account for the earlier fall of the cotyledons containing larvae (unpublished data; see Smith 1987b). Therefore, frugivory reduces cotyledonary reserves required for growth, producing shorter seedlings with fewer leaves.

The effect of frugivory on seedling growth was that the larger size classes of seedlings were never achieved, not that there were simply more short and fewer tall seedlings. This initial period of growth before a seedling loses its cotyledons is a critical time because a seedling typically remains at this height in a "seedling bank" (Burns and Ogden 1985) until other factors, such as the creation of a light gap or sediment disturbance, catalyze the transition from seedling to sapling (see Clarke and Allaway 1993). The largest seedlings derived from undamaged propagules would therefore be expected to have a future competitive advantage over the smaller seedlings whose cotyledons had been preyed upon by larval insects. For example, if a gap in the canopy were to occur, then the taller seedlings with more leaves would have a greater ability to capture light and grow than would the smaller seedlings. Consequently, the larger seedlings might have an increased probability of becoming saplings and eventually trees.

Longer-term monitoring is necessary to determine conclusively whether the largest seedlings obtain a future competitive advantage. Our experiments do show that the extremely short seedlings produced when we removed their cotyledons suffered increased mortality in only 4 months. Seedlings derived from propagules that have been heavily consumed and thus have few cotyledonary reserves (e.g., those with more than three exit holes) might fare similarly to those with experimentally removed cotyledons. Indeed, Robertson et al. (1990) observed that propagules of the mangrove *X. granatum* with one bore hole usually survived, whereas those with three or more bore holes had greater mortality. Similarly, Smith (1987b) observed that the removal of cotyledons from seedlings of *A. marina* by crabs in the tropics increased mortality of these seedlings over time. Therefore, it seems reasonable to predict that the shorter seedlings derived from propagules consumed by larval insects might experience increased mortality in the future. Alternatively, because seedlings can live for many years in a seedling bank (Burns and Ogden 1985), these initial differences in size may not confer a future survival advantage. Current evidence suggests that larval insects and post-dispersal herbivores such as crabs which displace or severely damage the integrity of the cotyledons can potentially limit the recruitment of mangrove seedlings.

The nature of the interactions between insects and mangroves is not well understood (see Hutchings and Saegner 1987; Tomlinson 1994). Here we have documented the negative consequences of frugivory by larval insects on the growth of mangrove seedlings. Interesting-

ly, the moths and flies that inflict the damage are also potential pollinators of *A. marina* (Hutchings and Saegner 1987; Clarke and Myerscough 1991a; Tomlinson 1994). Indeed, *E. marina* derives its specific name from its host, *A. marina*, but how tightly these two species are coupled is not known. More detailed and longer-term studies are needed to determine the net influence of these insects on the population dynamics of *A. marina*.

An important aspect of this study was that the viability of propagules was tested in the field following quantitative assessment of frugivory (see also Robertson et al. 1990). Often, the consequences of herbivory (e.g., the viability of propagules) are assumed by simply examining the severity of damage to the propagule (e.g., Smith et al. 1989; Clarke 1992). This may inflate or deflate the assessment of the actual importance of damage to propagules in structuring plant communities (see Andersen 1988). For example, a common criterion used in studies of herbivory of mangrove propagules is to classify a propagule as not viable if >50% of its tissue has been consumed. In this study, propagules with substantial portions of their cotyledons consumed by larval insects established successfully because these herbivores did not consume the embryonic axis of the propagule (although the long-term consequences of this herbivory were not assessed). Based on observations and results found here, propagules of *A. marina* with greater than 50% of their cotyledons consumed possibly can establish successfully. Testing the viability of propagules subjected to herbivory is critical before assuming that herbivory is an important limit to recruitment (Robertson et al. 1990). Further, linking actual insect damage to the viability of propagules and growth of seedlings is important for forestry and restoration efforts. For example, when restoring mangrove forests by reseeding areas with propagules or seedlings, should time be spent sorting through the propagules to exclude those damaged by insects? Our results demonstrate that damage by insects can decrease the size of seedlings in the short-term, but to answer this question we need to know the longer-term consequences of frugivory on the growth and survivorship of seedlings.

The relative importance of frugivory in determining patterns of recruitment and growth of mangrove seedlings will depend on the other factors (e.g., post-dispersal herbivory, competition, physico-chemical conditions) that also influence their early life history (e.g., Robertson 1991; Smith 1992; Clarke and Allaway 1993; Clarke and Myerscough 1993; Minchinton, in press). For example, the severe post-dispersal herbivory by crabs on mangrove propagules in some areas of the tropics probably outweighs the impacts of herbivorous insects (see Robertson 1991; Smith 1992). In temperate mangroves of the Sydney region, post-dispersal herbivory by crabs on propagules of *A. marina* is relatively less intense than in the tropics (Clarke and Allaway 1993; Minchinton, in press). Therefore, frugivory by insects should play a more significant role in the post-dispersal life history of mangroves in these temperate forests.

Here we have shown that herbivory experienced by fruit on the trees before dispersal negatively affects the early life history of seedlings of *A. marina*. Therefore, predicting the patterns of recruitment and growth of seedlings of mangroves and other plants not only requires knowledge of the proximate factors in the seedling environment, but also of those that affect the plant at earlier stages of its life history. The influence of herbivores that consume propagules on the population dynamics of plants in terrestrial habitats is well-documented (Crawley 1992; Louda and Potvin 1995), and there is every reason to believe that this factor may be equally important in structuring populations of marine plants. Herbivory by insects on mangrove propagules is a common and global phenomenon (Robertson et al. 1990; Farnsworth and Ellison 1997). Studies thus far suggest that it is an important yet still underappreciated factor affecting the demography and population dynamics of mangrove trees.

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