

## LONG-TERM FLOODING STIMULATES STILT-ROOT PRODUCTION IN *AVICENNIA MARINA*

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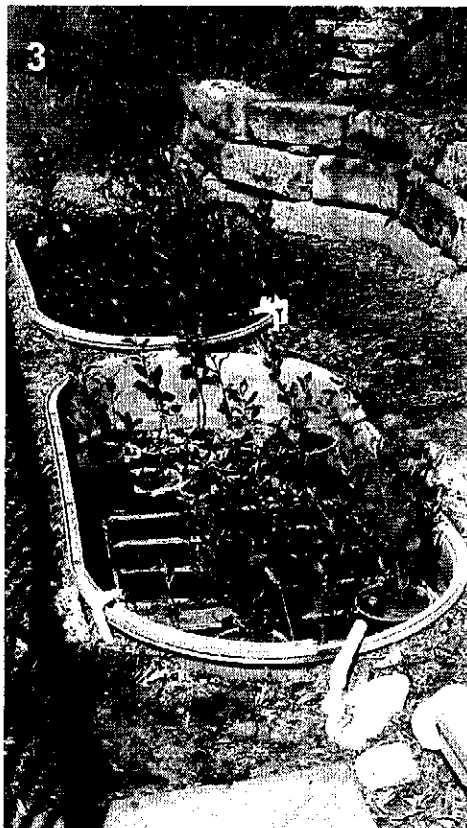
### Abstract

Plants of *Avicennia marina* (Forssk.) Vierh. var. *australasica* (Walp.) Moldenke ex N. C. Duke that had been continuously flooded for about two months produced numerous downward-growing roots well above the soil surface, as they sometimes do after pollution events in the field. These downward-growing roots (stilt roots) were not produced by the non-flooded plants. The stilt roots generally resembled inverted normal pneumatophores, and had many lenticels on their surface. We suggest that a stimulus for stilt root production in this species may be an increase in ethene (ethylene) resulting from restricted ventilation. We suspect that there is a requirement for some availability of oxygen to support the growth of new ventilating root structures, and that this criterion is satisfied near the air-water interface. The number of aerenchymatous organs including all types was the same in both flooded and non-flooded plants, and we speculate that this number may be pre-determined in some way in plants of this size.

**Keywords:** mangrove, pneumatophore, stilt-root, aerenchyma, flooding

### Introduction

In *Avicennia* the major organs of aeration of the root system are the pneumatophores. These are negatively geotropic roots that emerge from the soil, and are covered with lenticels and full of aerenchyma (Haberlandt 1884, Baylis 1950, Chapman 1975, Tomlinson 1986). These organs allow uptake of oxygen to the rest of the root system at low tide (Scholander et al. 1955, Curran 1985, Curran et al. 1986). Some other mangroves, notably species of *Rhizophora*, are largely supported by stilt roots which also function in aeration (Scholander et al. 1955, Chapman 1976). Stilt roots have already been noted in the genus *Avicennia*. In *A. officinalis* and *A. alba*, stilt roots from the main stem seem to be normal features, while in other species of the genus they may be a wound-response (Tomlinson 1986). In *A. marina* their formation often follows damage or environmental change such as contamination by oil (Allaway 1982, Böer 1993). We have also observed them following disturbance to pneumatophore aeration due to a filamentous algal bloom (Figures 1, 2), and we have anecdotal evidence of their occurrence in situations of impeded drainage elsewhere. However the conditions which lead to the formation of these anomalous roots in *A. marina* have not been experimentally investigated.



*Figures 1 and 2.* Aerial adventitious root development (stilt roots) observed on *Avicennia marina* trees which had experienced disturbance to pneumatophore aeration due to filamentous algal bloom first reported about a month before these photographs were taken. Both illustrations also show the field of normal negatively geotrophic pneumatophores surrounding the plants. Bayview, north of Sydney, NSW, 17 November 1986. *Figure 1.* Stilt roots arising about 30 cm above soil level on a sapling. The inset shows a close-up view. *Figure 2.* Base of a tree about 20 cm in diameter showing stilt roots of different age. The uppermost stilt roots were new growth, while the lower stilt roots were obviously much older.

*Figure 3.* The artificially-tidal troughs used in this experiment. The western trough is nearer the camera. The experimental plants are in the central portion of each trough, with the other plants at each end.

*Figure 4.* Stilt roots on long-term flooded plants in the western trough. The small "granular" structures on the surface of the stilt roots are lenticels. Scale is in centimetres.

*Figure 5.* Enlargement of normal pneumatophores of non-flooded plants in the eastern trough, showing numerous rather hypertrophied lenticels. Scales in centimetres.

Here we describe an experimental occurrence of long-term flooding during which stilt roots formed on plants of *Avicennia marina*.

## Materials and Methods

Propagules of *Avicennia marina* (Forssk.) Vierh. var. *australasica* (Walp.) Moldenke ex N. C. Duke, produced in our tidal tanks by plants from Botany Bay, NSW, were planted on sand in pots in November 1997. Twenty of these seedlings selected for similarity were planted out in February 1999 in sand, one at either end of each of 10 polystyrene foam planter boxes of internal dimensions 80.5 cm x 16.5 cm x 14 cm deep with fertiliser tubes. They were watered with tap water and occasionally with seawater, and kept in a glasshouse (minimum temperature 22 °C) until September 2000, when the plants were culled to one per box, again selected for similarity overall. The planter boxes were then moved in October 2000 to an artificial tidal system outdoors, five randomly chosen boxes being put into each of the two tidal baths. New fertiliser tubes, one at each end of each box, were inserted at

this time. There were also 12 (eastern) or 15 (western) older plants of *A. marina* and five *Aegiceras corniculatum* (L.) Blanco in each of the tanks, but these had not been preselected randomly into the baths.

The arrangements for growing the plants and the artificial tidal system (Figure 3) were as described by Curran et al. (1985), except that the tanks in this tidal system were ReIn plastic cattle troughs 2.3 m x 1.0 m x 0.5 m deep (Allaway et al. 2001). The clocks and pumping arrangements were set so that each bath was flooded with about 50% seawater to a depth of about 25 cm above the sand surface for two approximately 6-hour high tides each 25 hours, alternating with two low tides the same length, pumping periods occupying the remaining hour in each cycle. However, at an unknown time (presumably during the Christmas-New Year University closure) one clock failed, with the result that the western trough remained full and the water level in the eastern bath remained below the level of the sand, apart from occasional manual pumpings for inspection purposes, until 21 February 2001 when the

observations described below were made. Thus the plants in the western trough had been continuously flooded for about two months, while those in the eastern trough had remained wet but not flooded. On 21 February 2001 all pneumatophores, and stilt roots originating 6 cm or more above the sand surface, were counted and the plants photographed. Additionally counts were made of downward-growing roots which originated less than 6 cm above the sand surface: these were presumed to be adventitious cable roots as normally found in this species, and were designated 'low stilts' here. The results were analysed by Student's t test (Bailey 1981).

## Results

After about two months' flooding, the tops of the long-term flooded plants were smaller and in poorer condition than in the non-flooded plants (Figure 3). One of the long-term flooded plants had died, and the foliage of all the other long-term flooded plants showed some chlorosis, while the non-flooded plants' foliage looked normal. The living long-term flooded plants had all produced stilt roots (average 4 per plant), but these were absent from the non-flooded plants. Conversely, normal negatively geotropic pneumatophores were more abundant in the non-flooded plants, averaging 8 per plant versus about 3 for the flooded plants, although this difference was not statistically significant (Table 1; Figures 4, 5). Low stilts were present in both groups.

The stilt roots were dull green in colour and resembled inverted pneumatophores, and they had abundant lenticels (Figure 4). The tips of some of the stilt roots had entered the sand. All stilt roots were

completely below the flood level, except on one plant where two stilt roots emerged 2 cm above flood level.

In the *A. marina* plants which had not been randomly selected before the flooding occurred there were no significant differences between treatments in the number of stilt roots, low stilts or normal pneumatophores. Pneumatophores were not produced by *A. corniculatum*, but the flooded plants of this species had unusually abundant lenticels on stem and exposed roots in all but one of the flooded plants, while on the non-flooded ones the lenticels were fewer. All of the flooded plants of both species showed some leaf chlorosis, while the non-flooded plants' leaves were their usual colours.

## Discussion

Stilt roots are here shown to have been induced on *Avicennia marina* saplings by the experimental (although unintended) treatment of long-term flooding. No injurious treatment such as oil or other damage was imposed. The principal effect of the long flooding would have been to reduce the ventilating capacity of the root system severely, leading to a reduction in the oxygen concentration in the internal gas spaces throughout the root system to a very low level, and to its maintenance there (cf. Skelton & Allaway 1996). Exactly what this low level was cannot now be known, but it is likely to have been around  $1 \text{ mol m}^{-3} \text{ O}_2$  or less (cf. Skelton & Allaway 1996).

The production of stilt roots below the level of the water would have had no effect on gas exchange capacity of the root system, except to increase the total oxygen demand by adding respiring tissue. Only if stilt roots occurred

above the water level would they have any ameliorating effect on the oxygen level, by increasing the gas exchange capacity and conducting oxygen from the air. In fact nearly all these stilt roots were completely below the flood level. This gives the enigmatic result that the plants produced what could reasonably be supposed to be structures that function in aeration - the stilt roots - in underwater positions where they could have little or no aerating effect at all. It is clear that in this experimental case of prolonged flooding an actual improvement of the oxygen status is not necessarily a result of their formation. However in situations to be expected in estuaries

even a weather-induced flood is likely to be transitory. In this case the stilt roots would ensure a return to good oxygen exchange with the atmosphere as soon as the flood starts to fall: thus the formation of stilt roots is likely to be beneficial in estuarine situations. The observed stilt-root growth would also be consistent with the creation of a new root system at a level where subsequent pneumatophore formation would be useful. This would be of functional significance in cases where the restriction of ventilation was caused by siltation rather than a rise in water level.

Table 1. Stilt roots, low stilts, and normal pneumatophores on *Avicennia marina* plants after 2 months of flooding or non-flooding.

Plant No	Treatment	Number of stilt roots	Number of low stilts	Normal pneumatophores
1	not flooded	0	1	6
2	not flooded	0	0	12
3	not flooded	0	6	2
4	not flooded	0	3	4
5	not flooded	0	3	16
<b>mean</b>	not flooded	0.0	2.6	8.0
<b>s e m</b>		0.0	1.0	2.6
6	flooded	3	3	1 a
7	flooded	1	5	0
8	flooded	9	1	3
9	flooded	-	-	- b
10	flooded	3	2	7
<b>mean</b>	flooded	4.0	2.8	2.8
<b>s e m</b>		1.7	0.9	1.5
		s	ns	ns

a. also 1 dead pneumatophore. b. dead plant. s. statistically significant difference between treatments,  $p < 0.05$ . ns. not statistically significant,  $p > 0.05$

The stem base of plants of this age and size has been demonstrated to have a ventilating capacity which is similar to that of a large pneumatophore (Curran et al. 1986), with gas spaces continuous with the remainder of the root system. Thus in the flooded plants the oxygen level in the gas spaces within the above-water portion of the stem would have been high, and a distinct oxygen concentration gradient from the stem to the roots would have been evident.

The production of 'low stilts' in all plants is consistent with their development as cable roots, a normal part of the root system, which is completely adventitious in this species. The dull green colour of all stilt roots indicated the presence of chlorenchyma, as in pneumatophores (where carbon dioxide fixation has been reported, Aiga et al. 1995). Presumably the chloroplasts in the chlorenchyma have some capacity to generate oxygen in photosynthetic light reactions and it seems likely that the stilt roots also resemble the pneumatophores in this respect.

Increase in ethene (ethylene) level in the gas spaces could be a stimulus for stilt root formation. Ethene has been postulated as stimulating the formation of lysigenous gas spaces (i.e. spaces formed by the breakdown of cells) in the finest roots of this species (Allaway et al. 2001) but not in larger roots where the gas spaces are schizogenous (i.e. where they form between intact cells). We are unaware of any measurements of ethene levels in mangrove root systems related to other developmental events. However, in many plants, ethene accumulation has been correlated with restricted ventilation and low oxygen tension (see review by Vartapetian and

Jackson 1997) and these are exactly the conditions which would have applied in our flooded mangroves. Ethene has a wide range of effects, including stimulation of adventitious root formation which often occurs in locations where some oxygen is available (see review by Vartapetian and Jackson 1997). In our flooded mangroves the potential exists for ethene, accumulated in areas of restricted gas exchange further down the root, to diffuse to a region of more plentiful oxygen supply near the air-water interface, where stimulation of root growth could occur as stilt-roots. We have observed a somewhat similar phenomenon in partly submerged river red gum seedlings (*Eucalyptus camaldulensis*), where new adventitious roots and side shoots grow just above the cortical swelling and aerenchyma development is stimulated by moderate but long-term flooding.

The production of the normal negatively geotropic pneumatophores was not increased by the long flooding, in contrast to the production of stilt roots. The possible interaction between ethene and oxygen levels presented above as a potential stimulus for formation of ventilating root structures (cf. Vartapetian and Jackson 1997) could explain this, since although a high level of ethene would be expected in the cable roots where pneumatophores originate, oxygen levels would be very low in this region distant from the stem base. Overall this seems to be a testable hypothesis which could reward further study.

In these results, both flooded and non-flooded plants averaged about ten aerenchymatous root tips per plant (the flooded plants  $9.5 \pm 1.8$ ; non-flooded plants  $10.6 \pm 2.3$ , see Table 1). We

have previously suggested for younger saplings that the formation of gas space is pre-programmed rather than adaptive (Hovenden et al. 1995) up to the formation of the first pneumatophores. Could it be that the production of new aerenchymatous root tips (of all kinds, whether downward-growing, low downward-growing or normal pneumatophores) is also pre-programmed for a given size of plant? Exactly how such programming might occur is baffling. These observations highlight how little is known about the process of pneumatophore initiation and development, and of what determines the spacing of ventilating structures.

### Acknowledgments

We thank Mark Dickson and Hamlet Giragossyan for assistance. The work was entirely funded by The University of Sydney. W G Allaway thanks Professor Bengt Söderström and Lund University, Sweden, for facilities while on Study Leave.

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