25 Factors Influencing Establishment Success of *Melaleuca quinquenervia* (Cav.) S.T. Blake in Everglades National Park

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Abstract

Seedling establishment often creates a bottleneck in a plant's life history. The requirements of adult plant growth are often distinct from the requirements for germination and early growth. Grubb (1977) terms these changing requirements "the regeneration niche" and describes differences in requirements for light, moisture, mutualisms, and other factors between processes in a plant's life history. For the major invasive plants in south Florida, there is very little information available on factors affecting seed dispersal, seed germination, and seedling establishment, the regeneration niche, despite the importance of these processes to the spread of invasive plants. We conducted a series of growth chamber, greenhouse, and field experiments to investigate factors affecting seed dispersal, germination, and establishment of *Melaleuca quinquenervia* (Cav.) S.T. Blake. We have particularly focused on the effects of water depth, as one potential management strategy would be to manipulate water levels in the Everglades (Johnson and Fennema 1989; Laroche 1994).

Introduction

Melaleuca quinquenervia (Myrtaceae; melaleuca, paperpark, or cajeput tree) is an Australian tree introduced to southern Florida several times over the last 80 years. In Australia, it occupies flooded riverine sites and seasonally flooded sites behind coastal mangroves and sand dunes. Within Everglades National Park, melaleuca is mostly found invading sawgrass glades under many different hydrological regimes in the eastern Everglades. The species is also invasive in pine flatwoods and cypress dome edges on the west coast of Florida. Melaleuca has persistent serotinous capsules that open after fire, frost, or other damage to a branch (Ewel 1986). The seeds are less than 1 mm in length and are dispersed by wind and water (Woodall 1982). The distance that seeds are dispersed determines the pattern of colonization of melaleuca.

Stands of melaleuca are scattered, and each stand often consists of a single "founder" individual surrounded by rings of progressively younger trees. Trees grow very densely, and little native vegetation occurs under a mature stand. Once established, trees can tolerate a wide range of environmental conditions including flooding, drought, and fire (Ewel 1986). However, there have been very few studies on factors affecting melaleuca's establishment (Woodall 1981).

Specifically, the project addressed the following questions:

How does light affect seed germination rates? Seeds may land in areas with very low light such as under a mature melaleuca stand or within a true hammock, or in high light conditions on exposed ground in a sawgrass glade. We wanted to determine what the optimal light conditions were for melaleuca germination.

How do water and seedling damage affect seed germination and seedling establishment? How seeds and seedlings respond to submersion in water is critical to how the species will perform in a wetland environment such as the Everglades. We designed an experiment to test the effects of inundation of varying lengths of time on melaleuca seeds. When results of the experiment showed that melaleuca seed germinated while submerged in water, we thought it would be important to know if these seedlings could gain a roothold in soil while they were submerged. This would have important consequences in judging the effectiveness of a water dispersal strategy. Further, we tested the relative growth rates of seedlings in flooded versus low water treatments, and the affect of removing plant biomass on growth under the different water treatments.

What requirements do seedlings have for establishment in the field? We designed an outplanting experiment manipulating environmental factors such as light and soil depth in a natural and a restored sawgrass prairie habitat to determine under what conditions melaleuca could establish.

What are natural seedling establishment success rates in the field? Seedlings are rarely sited in the field, but saplings and adult trees are common. We wanted to determine what seedling establishment success was like for very young seedlings. After a fire in the East Everglades Acquisition Area (EEAA) of the park, we began censusing a new cohort of seedlings.

Methods

Light requirements for germination

We collected seeds from trees in the EEAA. We placed 20 seeds of melaleuca on

No. 3 filter paper moistened with distilled water, in each of 10 petri dishes, for each treatment, for a total of 200 seeds per treatment. There were three light treatments under flourescent lighting. For the high light treatment, the petri dishes were completely exposed to the light. In the low light treatments, the petri dishes were covered with 73% shade cloth. The dishes were wrapped with aluminum foil for the dark treatment. We placed all the petri dishes in growth chambers (Percival Model E-30B) set for 15 h of daylight followed by 9 h of darkness. The relative humidity was set at 70% and the temperature varied from 24°C during darkness to 29°C during 6 h of daylight. The high and low light treatments were evaluated weekly for germination, and the dark treatment was evaluated once at the conclusion of the study. Results were analyzed by conducting a one-way ANOVA on the final number of seeds germinated for each treatment at 40 days.

Seed inundation and establishment

The inundation treatments were as follows: 0 days, 1 day, 1 week, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. We placed twenty melaleuca seeds in each of 5 replicate vials containing 10 ml of distilled water for each treatment. In addition, we placed 20 melaleuca seeds on moistened No. 3 filter paper in each of 5 petri dishes as a control (no inundation) treatment. The vial and petri dishes were placed in a growth chamber set at 65-70% relative humidity, 27°C, and a cycle of 12 h of daylight to 12 h of darkness. We monitored the vials weekly for germination, and also noted whether the seed was floating or sinking and if the seed had germinated while submerged. After the specified inundation times for each treatment, we removed the seeds from the vials, placed them on moistened filter paper in petri dishes, and put them back into the growth chamber. We examined the petri dishes weekly for germination. The proportion of seeds germinated in each treatment was compared using Analysis of Variance (SAS 1985).

To look at seedling establishment under water, we compared establishment on two inundated substrates and one moistened substrate. The two inundated substrates were a fine clay loam soil and a sphagnum peat. The moistened substrate was also sphagnum peat. For the inundated substrates, we placed 20 melaleuca seeds in vials containing 20 ml of distilled water. For the moistened substrate, we placed 20 seeds directly onto the substrate. There were 10 replicates for each treatment. The vials were monitored weekly for germination, numbers of floating versus sinking seeds, and whether the sunken seedlings took root in the substrate.

We also did a greenhouse experiment determining the responses of melaleuca to submersion in water and above-ground biomass removal. We used two water depths, high (8-10 cm above the soil surface) and low (6 cm below the soil surface). The biomass treatments were uncut and cut. For the cut treatment, all above-ground biomass was removed at the beginning of the experiment. We potted 24 approximately 2 cm tall seedlings for each treatment in a 1:1 sterile sand to potting soil (Metromix 550) medium. The pots were placed in 1.5 m diameter plastic pools in a split plot design. We measured plant height at the start of the experiment, and at 125 and 160 days.

Seed and seedling outplantings

We planted seeds in a series of grids at the Hole-in-the-Donut (HID) restoration area in the park and at Chekika in the EEAA in June 1994 and at HID again in January 1995. At HID, we also planted seedlings in June 1996. At the HID restoration area, there were 20, 20 m^2 grids, each composed of 25 points. At Chekika, there were two 6 x 21 m grids, each containing 126 points. Grid 1 was located south of the parking lot and grid 4 was on the southern side of the dirt road near the ranger residence.

Within these grids, we selected points for planting using a stratified random design. The sample plots differed in elevation within a 1 m range. The variables manipulated in the experiment were light, soil depth, nutrient availability, and soil disturbance. We limited light by using shade tents, increased soil depth by adding potting soil, increased nutrients by adding fertilizer, and scarified the soil with a gardening claw. Controls included planting with no manipulation and unplanted blank controls. We planted approximately 50 seeds in 20 cm squares at each selected point. We planted 5 seedlings at each of three points in each HID grid.

During late May and early June 1994, we counted seedlings at each seed planting point. We recounted the seedlings at HID at the end of June, and in December 1994, January 1995, March 1995, March 1996, and finally in January 1997. We counted and measured all the outplanted seedlings in October 1996 and January 1997. The water depths at Chekika prevented us from monitoring the seedlings there until January 1997. In January 1997, we removed the grids from Chekika and HID.

Field seedling census

At Chekika, at the northern corner of 168th Street and 237th Avenue, we had previously set up a seed dispersal experiment that was destroyed by fire in April 1996. This experiment had six approximately 6 m tall trees in it, around each of which all melaleuca trees had been cleared for a 160 m radius. Three of the trees survived the fire, and in January 1997, we began to census seedlings that had germinated after the fire around each of these trees. We counted all of the seedlings in a 10 m^2 area around each tree and counted all seedlings in 2 m wide transects radiating due north, south, east, and west from each tree. The transects were run for at least 20 m or until no more seedlings were found at least 10 m from the last seedling found. We mapped 10 groups of seedlings around one tree and 7 groups around another tree, and in each group we marked between 6-11 seedlings. We recensused the number of seedlings in each of these groups in March 1997 and January 1998 to follow seedling survival.

Results

Light requirements for germination

Forty days after initiation of the experiment, the high and low light treatments had significantly higher germination rates (17% and 12%, respectively) than the dark treatment (5%) ($F=7.17$, $p<0.003$) (Fig. 1). We expected the germination rate to decrease as light decreased; however, we were surprised that seeds germinated in the dark treatment. This implies that seeds could potentially germinate under other vegetation, or under periphyton.

Seed inundation and establishment

There were no significant differences among treatments in the percentage of seeds germinated ($F=1.05$; $p=0.42$). The germination rate for the control treatment was approximately 8%, and for the different treatments, the germination rate ranged from 14% after 4 months of submersion to 6% after 2 months of submersion (Fig. 2). There was no clear pattern of germination success with time submerged. For the inundation treatments, all germination occurred by the fifth week of inundation. For the control treatment (no submersion), all germination occurred within 3 weeks. Of all the melaleuca seeds, 73 seeds floated and 727 seeds sank. Germination rate among the "floaters" was as high as 46.6%. Germination rates for the "sinkers" was much lower (6.6%).

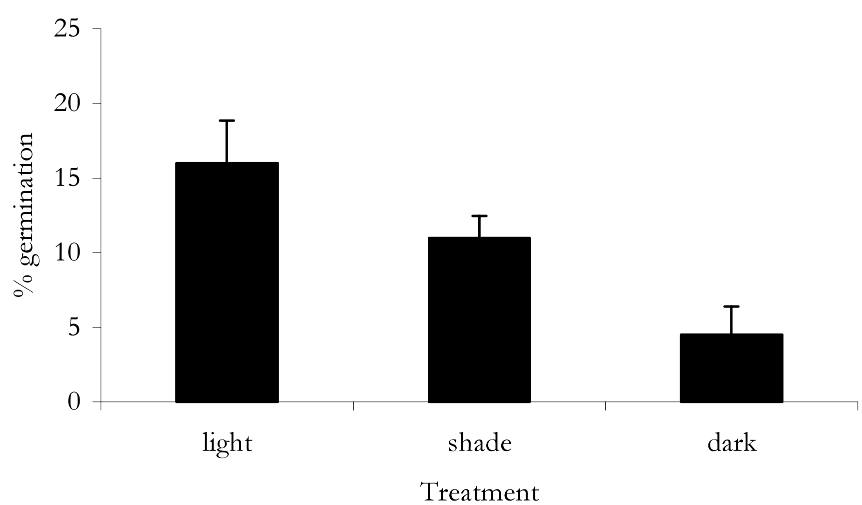

Figure 1. Effect of light on seed germination. We used 20 seeds in each of ten replicates for each treatment. Bars represent one standard error.

In the establishment study, after 2 months of inundation, approximately 45% of the germinated seeds in the submerged clay treatment were able to root in the substrate (Fig. 3). The germination rate for this treatment was 7.5%. For the submerged peat and moist peat treatments, 100% of the germinated seeds were able to root within both substrates. The germination rates for each of these treatments were 5% and 6.5%, respectively.

In the greenhouse study on response of seedlings to submersion and biomass removal, melaleuca grew more slowly when submerged (Fig. 4a), but submersion did not significantly increase mortality (Fig. 4b). Biomass removal decreased growth rate (Fig. 4a) and it increased mortality under low water conditions (Fig. 4b). It did not significantly increase mortality under flooded conditions.

Seed and seedling outplantings

No melaleuca seeds germinated and none of the outplanted seedlings survived under any treatment at either site. Chekika was inundated for a long period of time, and although previously established stands of melaleuca in the area survived, the seeds we planted may have been washed away or may not have survived the inundation once they germinated. We were more surprised by the lack of establishment of melaleuca at HID where water levels were lower.

Field seedling census

In a 10 m^2 area around each tree, seedling density ranged from 0.5-4.7 seedlings/ m^2. Grouping seedling counts for the 4 transects around each tree showed a patchy distribution of seedlings (Fig. 5). Three months after marking the seedling clumps, there was 98% seedling survival. One year later, an average of 58% of the seedlings survived. The seedlings grew very little over the year. This may be due to the high water levels of 1997. Often, the underwater portions of seedlings were covered in periphyton, which may be inhibiting photosynthesis.

Discussion

Our experiments demonstrate that the processes of seed germination and seedling establishment represent a bottleneck in the life-history of *M. quinquenervia.* Seed germination was consistently below 10%, and of those seeds that germinated, only half established. In our field experiment, there was no establishment, although there was natural establishment around trees at Chekika following a fire. Despite low germination and establishment rates, it is important to keep in mind that melaleuca trees each produce millions of seeds. Because of the quantity of seeds produced, the likelihood of some trees establishing is high.

Melaleuca's ability to grow under high water conditions should also be taken into

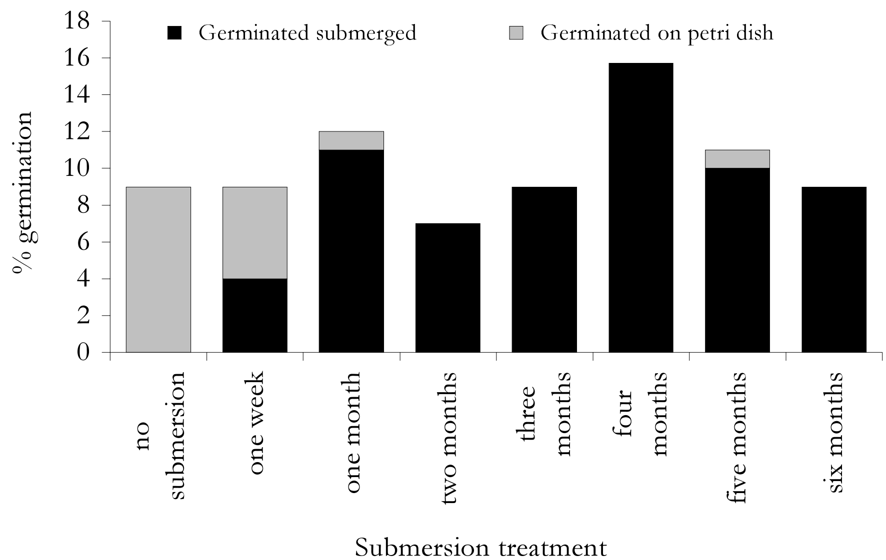

Figure 2. Effect of inundation on seed germination. We used 20 seeds in each on 5 replicates for each treatment and control (no inundation). Seed inundation treatments were 0 days, 1 day, 1 week, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. Bars represent one standard error.

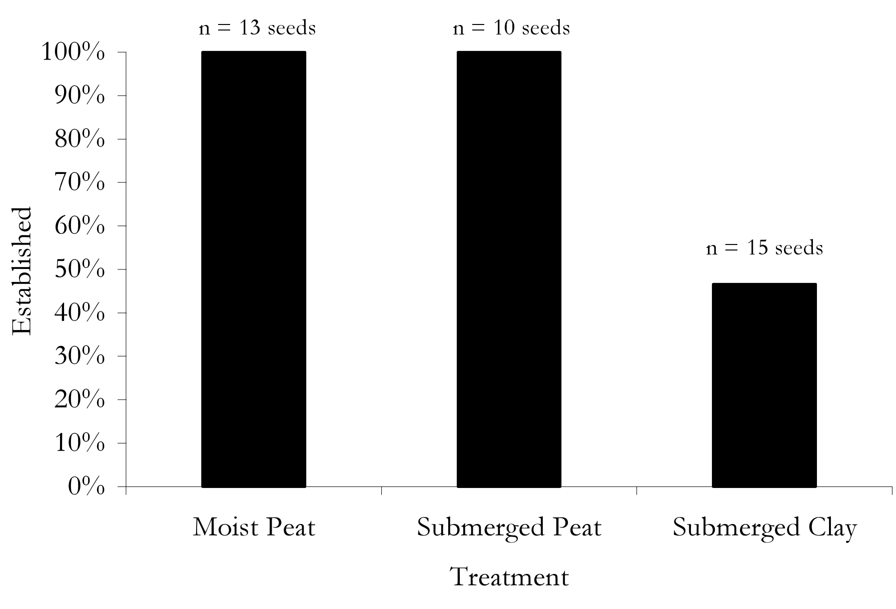

Figure 3. The percent of germinated seeds that established on inundated and moistened substrates. Sample sizes varied due to different germination rates in the different treatments.

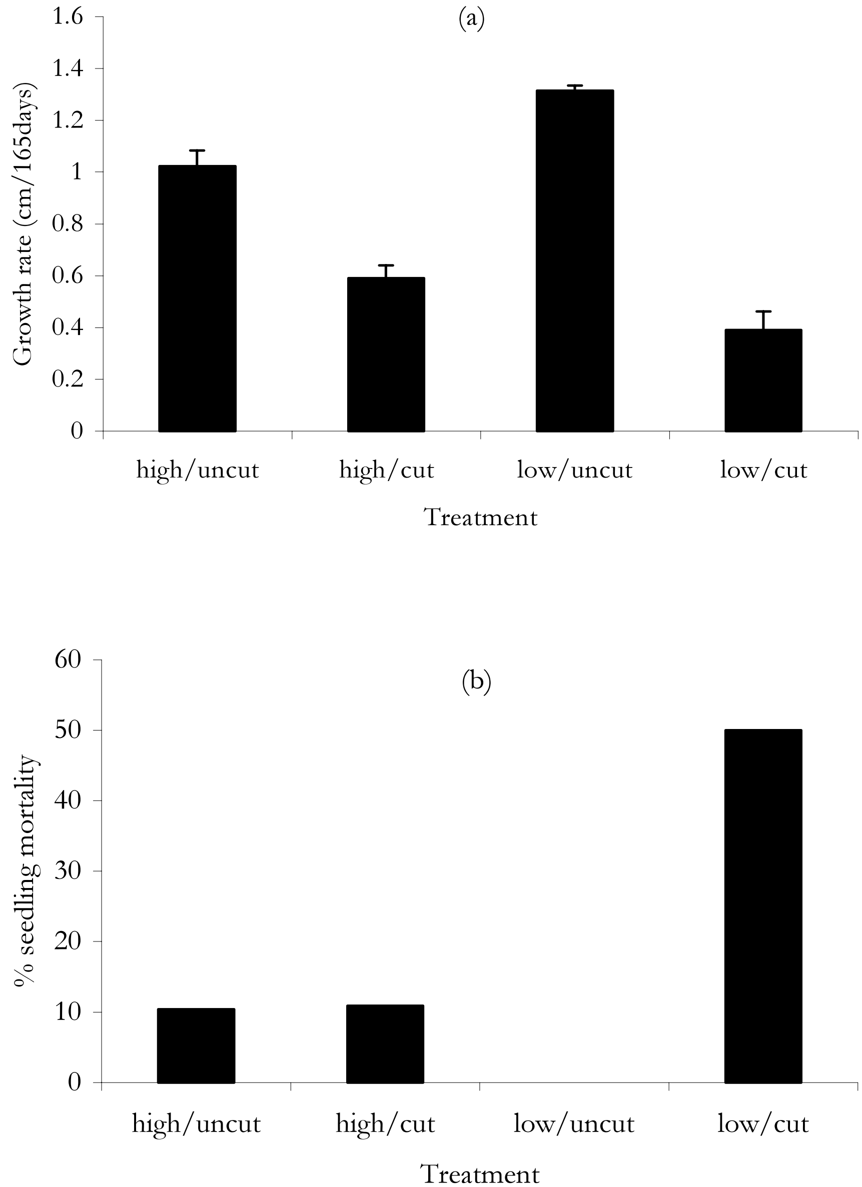

Figure 4. Effect of inundation (high or low) and biomass removal (cut or uncut) on (a) seedling growth rates and (b) mortality in the greenhouse.

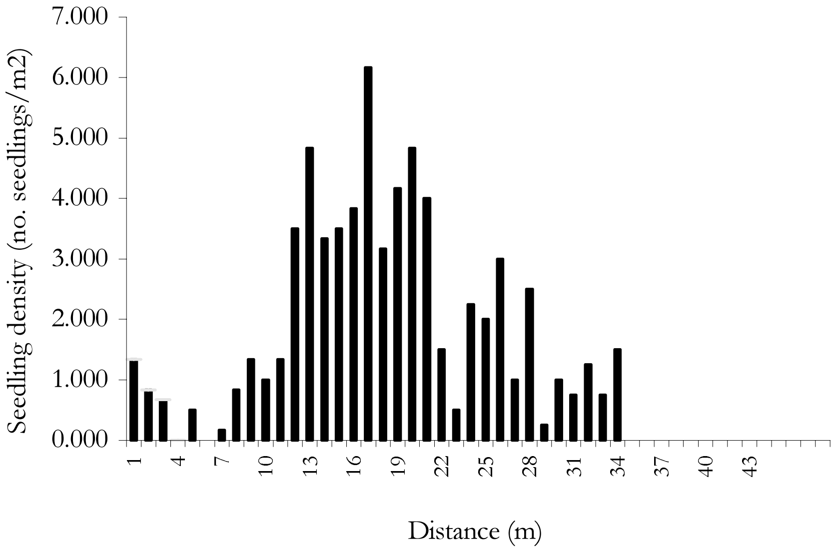

Figure 5. Average distribution of seedlings along transects at the Chekika site. Transects began at the parent trees.

consideration. It is able to germinate and establish underwater in the lab. Seedlings can grow underwater for at least 9 months in the greenhouse (J. Hartman, unpubl. data). Our field seedling census also indicates that seedlings survive for at least a year underwater. Lockhart (1995) found that seedlings grew best under a hydroperiod with 60% wet/dry time, but they still grew under her highest 80% hydroperiod. Seedlings begin to produce heterophyllic leaves after submersion for a few weeks (Lockhart 1995).

Water may also be more important to seed dispersal than previously thought. Most literature focuses on wind as a dispersal mechanism for melaleuca (e.g., Woodall 1982) . However, in a system like the Everglades (or in melaleuca's native wetland habitat), water dispersal is a viable strategy for moving seeds to new, appropriate habitats. The viability of submerged seeds did not differ from that of unsubmerged seeds, and the amount of time submerged, up to 6 months, did not lower the viability of the seeds. Seeds submerged during dispersal can still germinate. The relatively high germination rate of melaleuca seeds that float could be a dispersal strategy for high water conditions. Establishment was high underwater in this experiment as well, increasing our concern that increasing water levels may have little effect on reducing the establishment success of melaleuca.

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