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Seed Longevity of *Melaleuca quinquenervia*: A Burial Experiment in South Florida

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ABSTRACT

Burial and removal techniques with seed bags were used to examine the viability and longevity of *Melaleuca quinquenervia* seeds at four field sites representing different soil types and hydrological conditions in South Florida. Seed viability was determined over different burial durations in the soil through a combination of germination tests and 2,3,5-triphenyl-tetrazolium chloride (TTC) treatments. Control seeds kept dry at 25 C in the laboratory maintained same viability of ca. 15% over the 3-year study. In the field, seed viability decreased with increased burial duration. Most buried seeds lost viability by ca. 1.5 year at seasonally flooded and permanently flooded sites, whereas seeds buried at non-flooded sites survived over a period up to 2 to 2.3 years. Burial depth increased seed viability at the non-flooded sites ($P < 0.0001$), but had little effect on viability at seasonally ($P = 0.3691$) and permanently flooded sites ($P = 0.0735$). Soil types also had significant effect on seed viability. Seeds buried in organic (muck) soils decreased viability significantly faster ($P < 0.0001$) than those in sandy loam soils. Our results suggest that it may be possible to greatly reduce soil seed populations of *Melaleuca* in south Florida within 2 to 3 years after the seed source is eliminated.

Key words: Melaleuca, paper bark tree, seed viability, germination, dormancy.

INTRODUCTION

Melaleuca quinquenervia (Cav.) Blake (melaleuca or paperbark tree) is an aggressive weed tree of Australian origin that has invaded sensitive Everglades ecosystems and surrounding areas in South Florida. The invasive behavior of *M. quinquenervia* seems attributable to its inherently aggressive regeneration strategies and the associated massive seed production, coupled with favorable growing conditions of the South Florida environment (Meskimen 1962, Turner et al. 1998). Melaleuca is a prolific seeder, and a 21-m tall open-grown tree may produce 34 kg of mature capsules that contain up to 100 million seeds (Van et al. unpublished data). A large proportion (ca. 85%) of the seed crop is actually composed of empty testa, but most of the filled seeds are viable and germinate, indicating very little seed dormancy in melaleuca (Rayachhetry et al. 1998). Capsular fruits remain persistent in the canopy for several years and seeds retained within these capsules can remain viable for 10 years (Meskimen 1962), although, germinability declines with age of the capsules (Rayachhetry et al. 1998). Meskimen (1962) determined that some capsules apparently open continuously releasing an almost continuous light rain of seeds onto the ground, which acts to continually replenish the soil seed bank.

Since there is little dormancy in melaleuca seeds and germination usually occurs within ca. 5 days of wetting (Myers 1975, Rayachhetry et al. 1998), field germination and recruitment could happen soon after current seed fall (seed rain) on moist soil. During dry and cool conditions typical of the winter months in south Florida, seeds may fail to germinate immediately. Woodall (1983) found that some seeds remained viable after 10 months in the sandy soils of a well-drained saw-palmetto prairie, whereas others reported survival of melaleuca seeds submersed in water for 6 to 12 months (Meskimen 1962, Myers 1975). Persistent seeds in soil are important sources for the regeneration of plant communities (Murdoch and Ellis 1992), and such persistence entails maintenance of seed viability in the soil. We buried seeds at two depths in the top-soil of the melaleuca forests at four locations in south Florida and assessed the status of the seeds at intervals over a 3-year period or until all viability was lost. The main objective of this study was to determine how long melaleuca seeds remain viable in the soil under different hydrological conditions in south Florida.

MATERIALS AND METHODS

Study sites. The experiment reported herein is part of a larger study investigating the impacts of released biological control agents on populations of *M. quinquenervia* in south Florida. For the seed longevity study, four burial sites inside melaleuca forests were selected to encompass different soil types and hydrological conditions in South Florida as previously described (Van et al. 2000). Three of the sites consist of typical 'glades' characterized by high organic (muck) soils, but each represents a different habitat category of non-flooded (Holiday Park), seasonally flooded (Krome), or permanently flooded (Water Conservation Area). The fourth site (Corkscrew) was also non-flooded, but consists of sandy loam soils. Biomass and other structural characteristics of the melaleuca forests at the study sites, as well as monthly averages of temperature and rainfall for South Florida have been presented elsewhere (Van et al. 2000, 2002).

Seed burial and testing. Burial and removal techniques with seed bags (Lunt 1995) were used to examine seed longevity

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in *M. quinquenervia.* Laboratory and field studies were conducted simultaneously, using seeds collected in 1997 from the middle capsules of freshly cut trees from a concurrent biomass study in Broward County (Van et al. 2000). Seeds obtained from the middle capsule clusters (II, III, and IV) had germination rates (ca. 15%) consistently higher than seeds from immature (Cluster I) or older (Clusters V, VI, and VII) capsules (Rayachhetry et al. 1998).

Seeds were separated, cleaned, and air dried for at least a week, placed in a glass jar, and stored at room temperature (25 C). Before burial, each group of 200 seeds were placed in folded fiber glass (Miracloth) packets (2.5 by 2.0 cm) that were permeable to water. Two of these packets were then inserted in a 10 × 7 cm nylon bag filled with coarse sand and closed by a nylon string. In preliminary studies, direct contact of seed with fiber glass and nylon screen during incubation in petri dishes did not affect germination. In August 1998, thirty bags were buried (Figure 1) at depths of 0.5 cm and 5 cm below the soil surface, inside melaleuca forests in non-flooded and seasonally flooded habitats. These two burial depths are representative of the soil layer where most melaleuca seeds are found. At the permanently flooded sites, where water levels fluctuated from 0.3 to 1.3 m during the course of the study, the seed bags were attached to a floating litter trap (Van et al. 2002), using nylon strings of different lengths to suspend the bags in water at either the surface or the bottom of the water. This was to simulate a falling seed that either floats on surface or sinks down to the bottom of the flooded water. In the laboratory study, the seed bags were stored dry in the dark at room temperature (25 C).

Two nylon bags were harvested after 2, 4, 8, 16, 24, 32, 40, 60, 81, 104, and 114 weeks of burial. Two seed packets from each nylon bag were then opened and placed on moist filter paper in two separate petri dishes. Thus for each depth at a given site, four seed packets (each containing 200 seeds) were removed after a given burial duration and evaluated for seed germination and viability. Similarly, four packets of control seeds stored in the laboratory were also tested for germination and viability along with the buried seeds at each of the designated burial durations. The general procedure for viability testing was that of Rayachhetry et al. (1998). Seeds were allowed to germinate in an incubator at 28 C and 12-hour fluorescent light for 10 days. Germinated seeds were counted and removed. The remaining seeds that did not germinate after 10 days were then immersed in 2 ml of 1% (w/v) of 2,3,5-triphenyl-tetrazolium chloride (TTC), sealed, and then placed back in the incubator at 28 C and in the dark for additional 7 days. Seed viability was calculated as the sum of germinated seed and non-germinated seed stained with TTC. The percentages of viable seeds remaining in the soil at time of recovery were calculated based on the numbers originally buried. Analysis of variance of arcsine transformed data was used to determine whether site, burial depth, and time had significant effects on viability and germination. The data were analyzed as a randomized complete block design using the GLM procedure of the Statistical Analysis System (SAS 1988). The data for each burial site were then fitted to an exponential decay model ($y = a.e^{-bx}$), where y is the proportion of seeds still viable, x is time since burial in weeks, and a and b are the regression parameters.

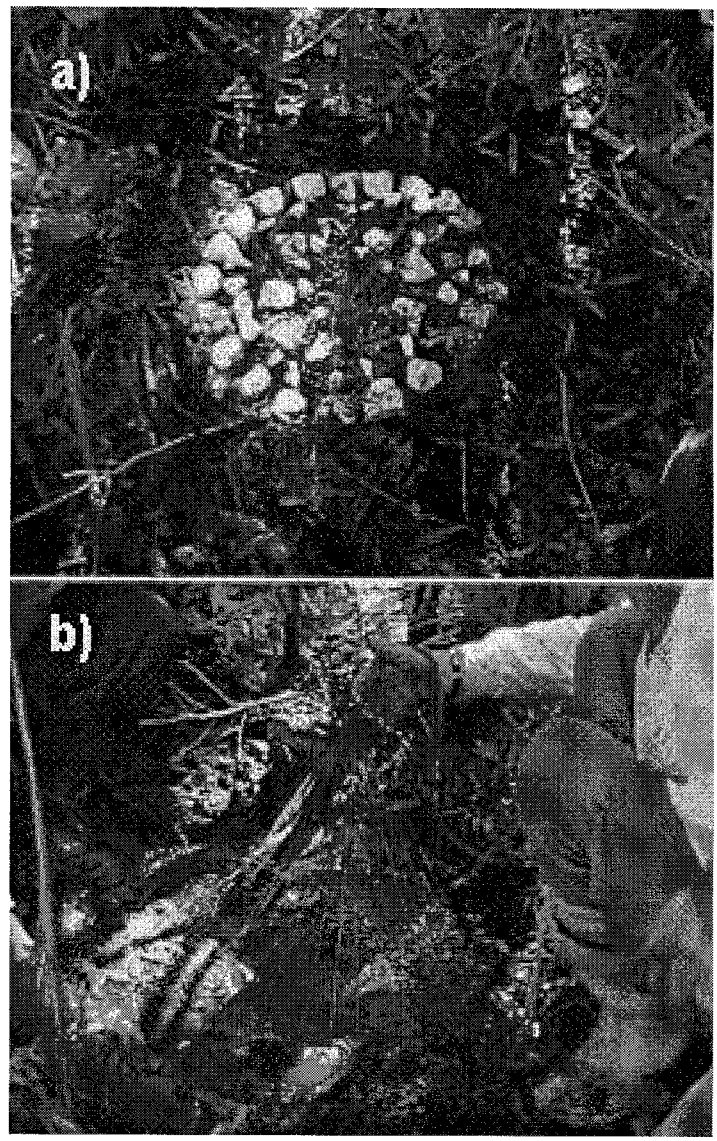

Figure 1. a) Seed bags of *Melaleuca quinquenervia* were buried at four different melaleuca infested sites selected to encompass different hydrological conditions in South Florida. b) A nylon string was tied to each of the seed bags to facilitate their removal after a given period of burial, with minimum disturbance to other nearby bags.

RESULTS AND DISCUSSION

Percent germination and viability of melaleuca seeds over the three-year burial period are presented in Figure 2. Very little difference was observed between rates of seed germination (left) and seed viability (right), indicating low levels of inherent seed dormancy in melaleuca, as reported in earlier studies (Rayachhetry et al. 1998). Both germination (Figure 2a) and viability (Figure 2f) in control seeds (kept dry at 25 C in the laboratory) were about 15% initially, and did not change during the course of the experiment. In fact, continued samplings through 2004 revealed little change in viability of these melaleuca seeds after 7 years of storage in the laboratory (Table 1). Melaleuca maintains both aboveground (canopy) and soil seed banks. The canopy seed bank

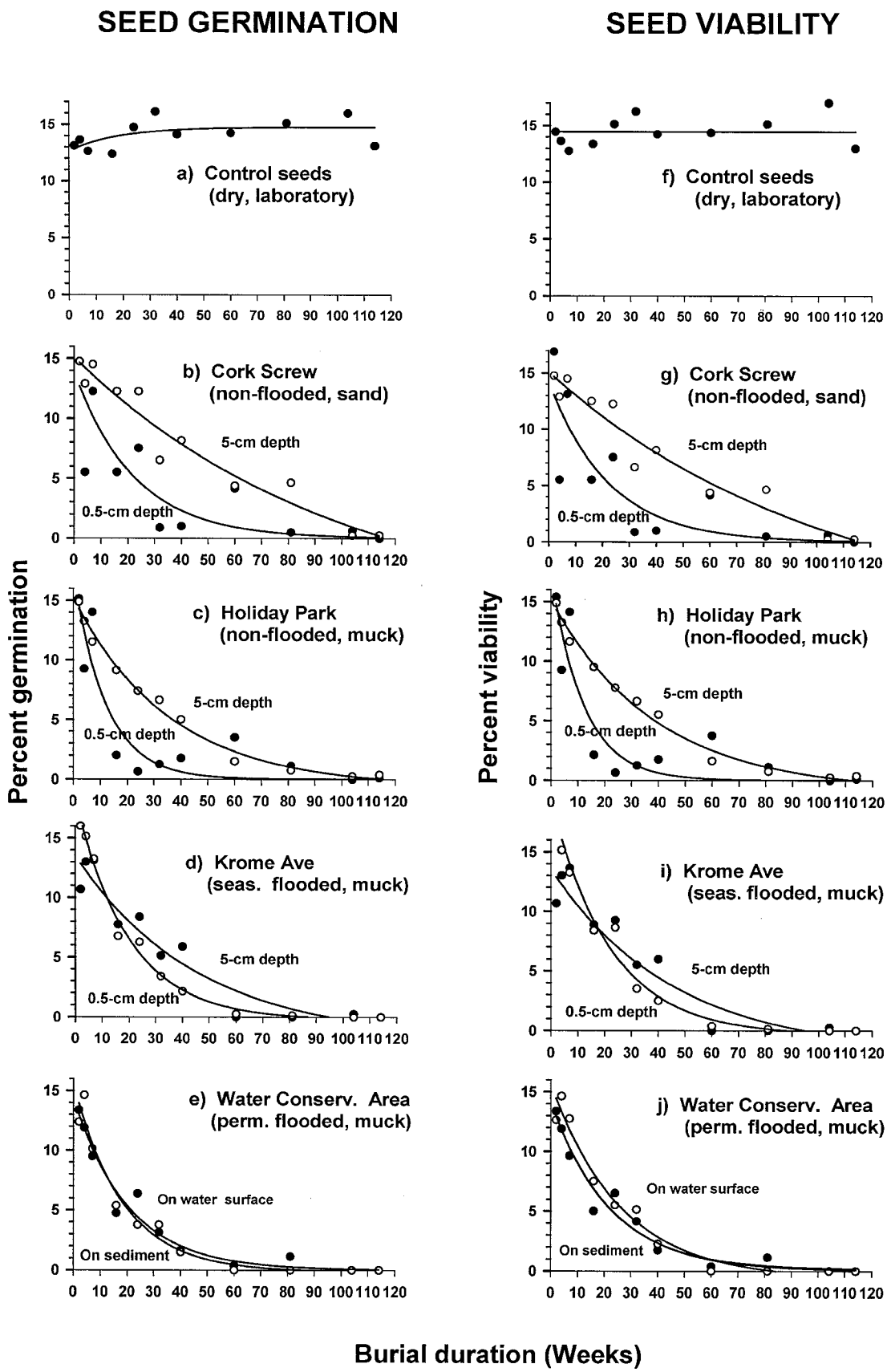

Figure 2. Longevity (germination and viability) of *Melaleuca quinquenervia* seeds buried at 0.5 cm (closed circle) or 5 cm (open circle) at four different locations in South Florida. Data were fitted with negative exponential decay curves.

TABLE 1. PERCENT GERMINATION AND VIABILITY[1] OF *MELALEUCA QUINQUENERVIA* SEEDS OVER TIME. SEEDS WERE COLLECTED IN 1997 AND STORED IN A GLASS JAR AT 25 C.

Year	Germination (%)	Viability (%)
1998	13.83 ± 2.90	14.33 ± 2.88
1999	14.91 ± 3.52	14.97 ± 3.59
2000	14.50 ± 2.12	15.07 ± 2.74
2002	14.50 ± 3.54	15.50 ± 4.95
2003	14.76 ± 4.76	16.20 ± 2.64
2004	13.57 ± 2.06	14.23 ± 0.93

[1]Data are means (± standard deviation) of 6 to 15 petri dishes, each containing 100 seeds.

consists of seeds held in capsules on branches in the canopy. Our data suggest that seeds held within capsules on the canopy are long lived, corroborating an earlier observation that they may remain viable up to 10 years (Meskimen 1962).

In the soil, germination and viability of seeds buried at all studied sites declined exponentially with time over the course of the experiment. Generally, seeds buried at the wet sites lost viability much faster than those at drier sites ($P < 0.0001$). All viability was lost in seeds buried at the permanently flooded Water Conservation Area site (Figure 2j) after about 1.5 year. Seeds at the Krome site (Figure 2i), which was flooded for over a year during the early part of the experiment, also were not viable after 1.5 year. In contrast, a small percentage of seeds at the non-flooded Corkscrew site (Figure 2g), which was sandy and dry, remained viable up to 2.3 years after burial.

It was not possible to determine whether the decline in viability observed here was the result of lethal germination in the seed bags or solely owing to death and decay of the small seeds. Nevertheless, soil type had a significant effect on seed viability. Seeds buried in organic (muck) soils (Figure 2h) lost viability significantly faster ($P < 0.0001$) than those in sandy loam soils (Figure 2g). It is probable that higher organic content may have a large impact on soil biota and decomposition processes. This, in turn, may have either a direct or indirect influence on the longevity of seeds in the soil. The difference in germination rates between seeds stored in the laboratory or buried in soil indicate some sort of induced dormancy that occurred while buried. Induced dormancy of buried seeds in soil have been reported earlier (Taylorson 1970), and probably function as a delay mechanism that increase the chance of burial and persistence in the field.

Several researchers have reported a faster decline in seed viability with shallow rather than deep burial (Gleichsner and Appleby 1989). This rapid decline in seed viability near the soil surface has often been attributed to greater environmental extremes and greater seed germination in the seed bags buried near the surface. This was also the case in our study where seed viability declined more rapidly ($P < 0.0001$) at the shallower (0.5 cm) burial depth at the non-flooded sites. However, depth was not an important factor to seed viability at seasonally flooded ($P = 0.3691$) and permanently flooded ($P = 0.0735$) sites. We did not look at seed viability at greater soil depth, as our previous seed bank study had indicated that distribution of melaleuca seeds was limited to the first 5 cm of the soil surface (Van, unpublished data). On the other hand, we did not leave the seed bags on the soil surface either, mainly because in nature, strong winds could easily cover the small seeds with dust, leaf litter, and soil particles.

Knowledge of seed longevity under different field conditions has implications for weed management. Management of melaleuca using biological control depends on the consistent suppression of seed production and depletion of the seed bank. The aim is to reduce fecundity to such an extent that recruitment becomes seed-limited (Crawley 1990). Our results suggest that it may be possible to severely reduce soil seed populations of melaleuca in 2 to 3 years after the seed source is eliminated. It should be noted, however, that even with a relatively short-lived soil seed bank, annual inputs of seeds from continual seed rain could replenish a seed bank that would otherwise decline rapidly.

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