Interdependence of *Myrica cerifera* Seedlings and the Nodule Forming Actinomycete, *Frankia,* in a Coastal Environment

Annamarie E. Wijnholds and Donald R. Young

Department of Biology
Virginia Commonwealth University
Richmond, VA 23284, USA

ABSTRACT

WIJNHOLDS, A.E. and YOUNG, D.R., 2000. Interdependence of *Myrica cerifera* seedlings and the nodule forming actinomycete, *Frankia,* in a coastal environment. *Journal of Coastal Research,* 16(1), 139–144. Royal Palm Beach (Florida), ISSN 0749-0208.

The shrub, *Myrica cerifera,* and the nitrogen-fixing bacterium, *Frankia,* form a symbiotic relationship that is important for seedling establishment on nutrient poor coastal soils. To determine potential interdependence, the spatial distribution of host plant seedlings was examined in relation to the root symbiont on a Virginia barrier island. Spatial patterns for both species were related to microtopography and soil chlorides. *Myrica cerifera* seedlings were more common in the swale region than on the dune crests or slopes. A bioassay quantified the extent of *Frankia* distribution within a recently formed swale where the shrub seedlings were invading. *Frankia* occurred throughout the swale, but was absent within 20 m of the high tide line. *Frankia* was found in areas devoid of the host plant; *M. cerifera* did not occur at ~43% of the locations where *Frankia* was present. In contrast, the host plant seedlings were limited to areas that contained *Frankia.* Soil chlorides where *M. cerifera* seedlings occurred were lower than locations devoid of the host plant. Similarly, soil chlorides where *Frankia* was present were lower when compared to areas without the root symbiont. Although influenced by microtopography and soil chlorides, the successful establishment and small-scale distribution of *M. cerifera* seedlings in coastal soils depends on the presence of *Frankia.*

ADDITIONAL INDEX WORDS: *Colonization, edaphic factors, establishment, nitrogen fixation, symbiosis, shrub thicket, succession.*

INTRODUCTION

Over the relatively flat topography of barrier islands, a slight change in microtopography may produce significant ecological change leading to discrete boundaries among species (EHRENFELD, 1990). Distribution patterns of plants in coastal environments are often related to the harsh environmental conditions, especially salinity and the resultant interaction with microtography (BARBOUR *et al.,* 1985). Further, coastal systems are usually nutrient deficient, especially in soil nitrogen (ART *et al.,* 1974), so that most species are nutrient limited. Biotic interactions, both positive and negative, also may contribute to community structure and the distribution patterns of species in harsh environments (BERTNESS and CALLAWAY, 1994). For example, soil nitrogen deficiencies may be mitigated through symbiotic relationships with soil bacteria that fix nitrogen. Due to the dominating physical processes in coastal environments, the importance of biotic interactions to the distribution of species has received little attention.

Myrica cerifera L. (Myricaceae) is a dominant woody species on many Atlantic Coast barrier islands (YOUNG *et al.,* 1995). The expansion of *M. cerifera* thickets closely follows soil accretion patterns on barrier islands with regressive shorelines (SHAO *et al.,* 1998). The success of this evergreen shrub may

be related to nodule formation with the actinomycete, *Frankia,* and the resultant fixation of nitrogen (TORREY and CALLAHAM, 1978; TJEPKEMA *et al.,* 1981; SHIPTON and BURGGRAAF, 1983). *Myrica* species improve soil nitrogen levels (MORRIS *et al.,* 1974; YOUNG *et al.,* 1992; 1995) and facilitate the establishment of other species (VITOUSEK and WALKER, 1989; LEVY, 1990).

The distribution pattern of mature *M. cerifera* shrubs across a barrier island landscape was limited to areas where the fresh water table was close to the soil surface, such as low-elevation swales (YOUNG *et al.,* 1992). In comparison to other coastal shrub species, *M. cerifera* is sensitive to salinity (YOUNG *et al.,* 1994). Increased levels of salinity also affect the growth of *Frankia in vitro* and in the field (GIRGIS *et al.,* 1993; MIETTINEN, 1993). At high levels, soil salinity may impair physiological processes of the host shrub and interfere with the infection process by *Frankia* (YOUNG *et al.,* 1992). Factors contributing to the potentially interdependent distribution patterns of *Myrica* species and *Frankia* have yet to be determined, especially relative to seedling establishment in coastal areas. In other environments, *Frankia* can live independent (*e.g.* saprophytically) of the host plant (SCHWINTZER, 1990; ZITZER and DAWSON, 1992; PASCHKE and DAWSON, 1992a; 1992b; HUSS-DANELL, 1997) and has been identified in alluvial soil sediments after transport by freshwater (HUSS-DANELL *et al.,* 1997). In comparison, seedlings of *Myr-*

ica species may be limited to soils containing *Frankia,* especially in low nitrogen soils.

Seedling establishment patterns ultimately influence observed distribution patterns of mature shrubs. To examine the importance of the symbiotic association to the establishment patterns of the host plant and the root endophyte in coastal environments, the distributional relationship between seedlings of the actinorhizal shrub, *Myrica cerifera,* and the nitrogen-fixing actinomycete, *Frankia* were evaluated. We hypothesized that the distribution of *M. cerifera* seedlings is associated with the presence of *Frankia,* but the distribution of *Frankia* extends beyond the distribution of host plant seedlings in coastal environments. Further, *M. cerifera* seedlings and *Frankia* distribution patterns are related to microtopography and soil salinity in coastal environments.

METHODS

The study was conducted on the north end of Hog Island (37°40′N, 75°40′W), a barrier island, ~8 km east of the Eastern Shore peninsula of Virginia, USA. The study site was a portion of recently (<15 yr) developed swale. The highly dynamic nature of the swale makes it ideal to determine factors that influence the distribution pattern of shrub seedlings. The swale is an area of transition, ranging from new soils, populated by pioneer species, to older soils with established shrub thickets. The field study involved intensive local sampling in a study plot as well as sampling along a series of transects.

Study Plot

The study plot (100 m × 100 m) included a swale between newly forming dunes along the beach and the primary dune ridge. The eastern perimeter of the plot was parallel to the beach, approximately 20 m from the high tide line. The western perimeter of the plot was on the slope of the first dune ridge, approximately 20–30 m from the leading edge of established *M. cerifera* thickets. The plot had variable microtopography and vegetation that included small dunes, and relatively flat, grassy areas with encroaching *M. cerifera*. To determine the distribution of *M. cerifera* seedlings within the newly formed swale, sampling locations were delineated with a 10 m interval grid (n=121) throughout the study plot. At each location in June, 1996, the number of *M. cerifera* seedlings was counted within a 1 m radius.

The distribution of the root symbiont, *Frankia,* was determined via bioassay (HUSS-DANELL, 1997), using the field method developed for coastal soils (YOUNG *et al.,* 1992). At each location within the plot, five soil samples were collected in sterile, capped 50 ml plastic vials in June, 1996. Samples were collected within a 1 m radius sampling area at regular intervals along the circumference of the site. Samples were capped, returned to the laboratory and refrigerated (4° C) prior to the bioassay (~1 wk). In addition, *M. cerifera* seeds were collected in mid-April, 1996 on Hog Island and stored at 4° C. Seeds were scarified and wax removed by rubbing them against a metal screen. They were surface sterilized by soaking in 30% hydrogen peroxide for 15 min, then rinsed three times with sterile, deionized water. The sterile seeds were

placed on top of sterile perlite in a clear plastic container, and watered with deionized water. They were then placed in an environmental chamber (model E15, Conviron, Pembina, N.D.; 25/20° C, 16 hr photoperiod) until germination.

After the appearance of two or more sets of leaves, the nonnodulated seedlings were transplanted into each of the soil samples. Controls (n=15) consisting of seedlings planted in sterile soil (autoclaved at 230° C, 90 min) were placed randomly among the other samples in order to detect any cross-contamination of *Frankia*. Newly planted seedlings were watered with deionized water as needed, and maintained in the environmental chamber. Plants that died (<5%) were replaced with sterile seedlings, up to three times. After 10 wk from the time of transplantation, seedlings were harvested at weekly intervals according to size and date planted. The plants were removed from the vials, and the roots were gently shaken over fine wire mesh. Roots were examined for developing nodules under a dissecting scope. Presence of nodules indicated the presence of *Frankia* in the soil sample; however, absence of nodules may be attributable to the absence or low density of *Frankia* or to an inhibition of nodulation (YOUNG *et al.,* 1992). Thus, the bioassay is a conservative estimate of the distribution of *Frankia*.

To determine if *M. cerifera* is dependent on the presence of *Frankia,* 100 *M. cerifera* seedlings in the study plot and the surrounding swale were uprooted and examined for nodules in June, 1996. These data supplemented additional seedling and shrub excavations (>500) over the past eight years from numerous locations on Hog Island and other Virginia barrier islands (YOUNG, unpublished data).

To determine the association of microtopography with *M. cerifera* and *Frankia* distribution, each location within the plot (n=121) was visually assessed and characterized as either dune crest, dune slope, or swale. Further, to determine if the host shrub or *Frankia* distribution was associated with edaphic factors, one soil sample from each location (n=121) was collected and analyzed for pH and chlorides. The samples were oven-dried at 80° C for 72 hr. Subsamples (40 g) were diluted with 100 ml of deionized water, and the pH measured with a pH electrode (Orion). Total soil chlorides for each of the water-extracted samples were determined with a chloride electrode (Orion) (YOUNG *et al.,* 1994). Data were statistically evaluated with X^2 and Kolmogorov-Smirnov goodness of fit tests (ZAR, 1984), which identified potential associations between the presence of *M. cerifera* and *Frankia* relative to microtopography (dune crest, dune slope, and swale). Similarly, variations in soil chlorides and pH values were related to microtopography. One-way single factor ANOVA determined significance among mean soil chloride and pH values for the microtopographic regions.

Transects

The transect baseline covered ~500 m along the high-tide line. To examine *M. cerifera* seedling distribution relative to soil age and microtopography, five transects were established perpendicular to the beach and extending from the baseline to *M. cerifera* thickets. Sampling locations were established at intervals of 25 m along each transect. At each location, the
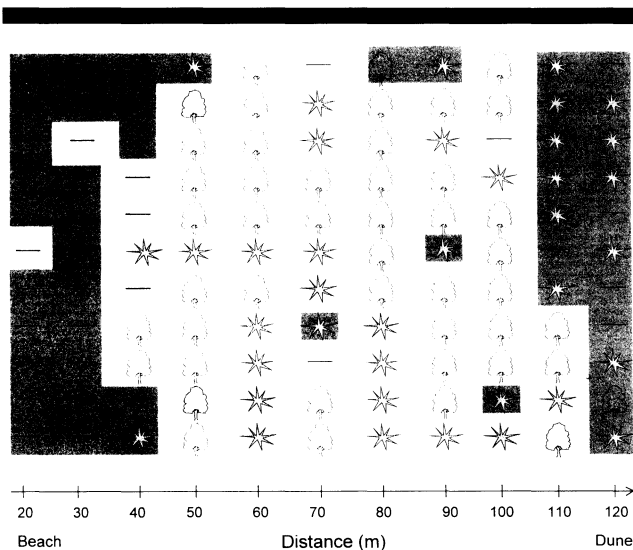
Figure 1. The distribution of locations with *M. cerifera* (shrub), with *Frankia* (star), and with no *Frankia* or *M. cerifera* (———) in relation to microtopography; dune crest (□), dune slope (□), and swale (□) in the study plot on Hog Island.

Figure 2. The occurrence of locations without *M. cerifera* or *Frankia* (□), with *Frankia* but no *M. cerifera* (▨), and with *M. cerifera* (■) in relation to microtopographic regions in the plot on Hog Island.

number of *M. cerifera* seedlings within a 2.5 m radius was quantified. Microtopography was assessed using the previous categories, with the addition of thicket and beach categories. Three soil samples from each sampling location were collected and tested for pH and chlorides, using the previously mentioned techniques. Data were statistically evaluated with X^2 and Kolmogorov-Smirnov goodness of fit tests, which identified potential associations between the presence of *M. cerifera* seedlings relative to microtopography. Similarly, variations in soil chlorides and pH values were related to microtopography. One-way single factor ANOVA determined significance among mean soil chloride and pH values for the microtopographic regions.

RESULTS

Study Plot

There were 4.6 ± 0.5 *M. cerifera* seedlings per sampling location (*i.e.* 1.5 ± 0.2 seedlings/m²) within the study plot and the number of seedlings ranged from 3 to 8 per sampling location at 61% of the locations. Neither *M. cerifera* nor *Frankia* was detected within 20 m of the beach (Figure 1). The presence of *M. cerifera* and *Frankia* in the plot was related to microtopography. The shrub seedlings were found predominantly in the protected swale regions; only two locations with seedlings occurred in the dune crest region (Figure 1). The shrub seedlings were not distributed proportionally among the microtopographic regions ($X^2 = 96$, p < 0.05) of the plot (Figure 2). *Frankia* was found in all the locations that contained *M. cerifera*, but it also occurred in locations with no shrub seedlings (Figure 1). Similar to the host plant, *Frankia* also was not distributed proportionally among the microtopographic regions and was most abundant in the swale, less on the dune crest, and least on the dune slope (X^2
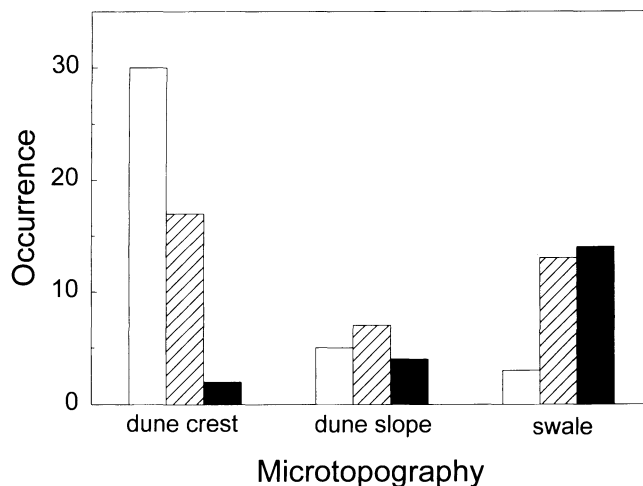
= 38, p < 0.05) (Figure 2). However, relative to *M. cerifera*, *Frankia* was more frequent on dune crest and dune slope regions. All nodulated bioassay seedlings had only one *Frankia* nodule. All excavated shrub seedlings were nodulated; >90% had 1 nodule/seedling. Similarly, all of the previously observed *M. cerifera* seedlings and shrubs (1 to 10 nodules/plant) were nodulated.

Soil chlorides ranged from 0–24 μg Cl⁻/g soil, and 42% of the locations occurred within the range 4.5–7.0 μg Cl⁻/g soil. Chloride levels differed significantly among microtopographic regions of the plot (ANOVA, F = 25.4, p < 0.001). Chloride levels in the swale were higher than in dune crest and dune slope areas (Table 1). Locations in the plot were not distributed proportionally among chloride levels (K-S test, d_{max} = 51, p < 0.05). The frequency distribution of chlorides in locations with *M. cerifera* differed significantly from the chloride distribution of all locations in the plot (K-S test, d_{max} = 17, p < 0.05) (Figure 3). However, the distribution of chlorides for locations with *Frankia* only was not significantly different (p > 0.05) from the chloride distribution of all locations (K-S test, d_{max} = 3.72) (Figure 3). There was no significant difference in pH levels among microtopographic regions (ANOVA, F = 0.64, Table 1). Soil pH averaged 5.8 units. The frequency distribution of pH of *M. cerifera* locations and of *Frankia* locations did not differ significantly from

Table 1. *Soil chlorides (μg Cl /g soil), soil pH, and percentage of locations in the plot among microtopographic regions (n = 121). Values are means ± standard errors; * denotes statistical significance (p < 0.05) as compared to other regions.*

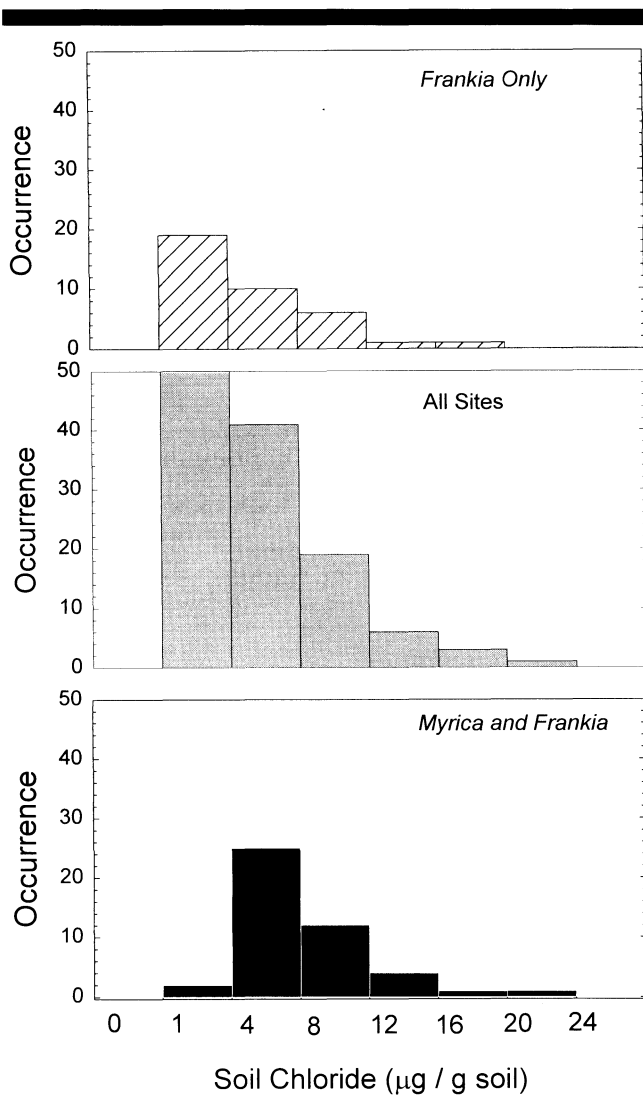| Micro-topography	Chlorides	pH	% of locations
Dune crest	3.3 ± 0.2	5.9 ± 0.1	40.5
Dune slope	5.4 ± 0.9	5.8 ± 0.1	13.2
Swale	9.3 ± 0.7*	5.8 ± 0.1	46.3

Figure 3. The occurrence of locations with *Frankia*, all locations, and locations with *M. cerifera* in relation to soil chloride levels (μg Cl$^-$/g soil) in the plot on Hog Island.

Microtopography

Figure 4. The occurrence of all locations ($\square$) and locations with *M. cerifera* ($\blacksquare$), in relation to microtopographic regions in the transect on Hog Island.

the distribution of pH of all the locations (K-S test, d_{max} = 1.39, d_{max} = 7.4, respectively).

Transects

Myrica cerifera seedlings were not distributed proportionally among microtopographic regions along the transects (X^2 = 30, p < 0.05). Locations with host shrub seedlings were most frequent in the swale and thicket regions, with only two locations found on the dune crest (Figure 4). No seedlings occurred in the beach or dune slope regions. Similarly, shrub seedling density significantly differed with microtopography (ANOVA, F = 11.4, p < 0.01). Seedling density was greatest in the swale (Table 2). Soil chloride levels ranged from 1 to >1,000 μg Cl$^-$/g soil, and 53% of the locations along the transects occurred within the range of 1–10 μg Cl$^-$/g soil. Chlorides differed significantly among the microtopographic re-
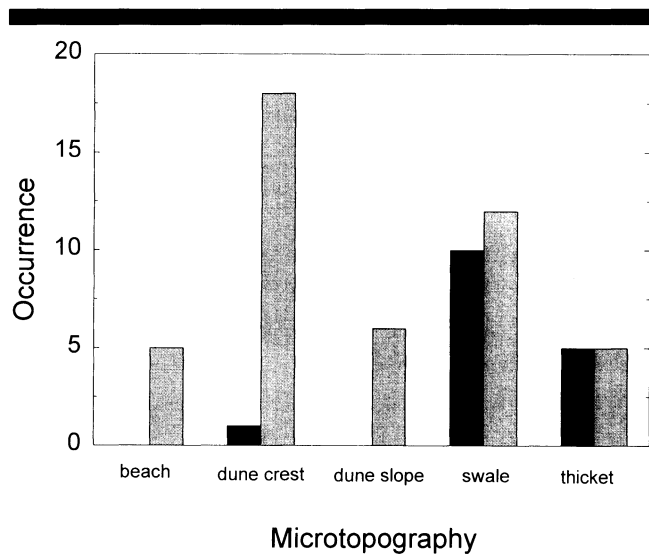
gions along the transects (ANOVA, F = 30.2, p < 0.001). Soil chlorides in the beach region were significantly higher than in dune crest, dune slope, swale or thicket regions (Table 2). Thus, locations along the transects were not distributed proportionally among chloride levels (Kolmogorov-Smirnov test, d_{max} = 14.5, p < 0.05) (Figure 5). The distribution of chlorides for locations with *M. cerifera* did not differ significantly from the distribution of chlorides of all the locations in the transect (K-S test, d_{max} = 2.66). Soil pH differed significantly among the microtopographic regions along the transects (ANOVA, F = 7.56, p < 0.001). Soil pH was significantly lower in the thicket than on the beach, dune crest, dune slope, and swale (Table 2). The frequency distribution of pH of *M. cerifera* seedling locations did not differ significantly from the frequency distribution of soil pH of all locations (K-S test, d_{max} = 3.54).

DISCUSSION

Myrica cerifera seedlings only occurred in areas where *Frankia* was present within the study plot on Hog Island, but *Frankia* was also found in areas devoid of the host plant. The

Table 2. *Myrica cerifera* seedling density (#/m^2), soil chlorides (μg Cl / g soil), soil pH, and percentage of locations along the transects for the microtopographic regions (n = 46). Values are means ± standard errors; * denotes statistical significance (p < 0.05) as compared to other regions.

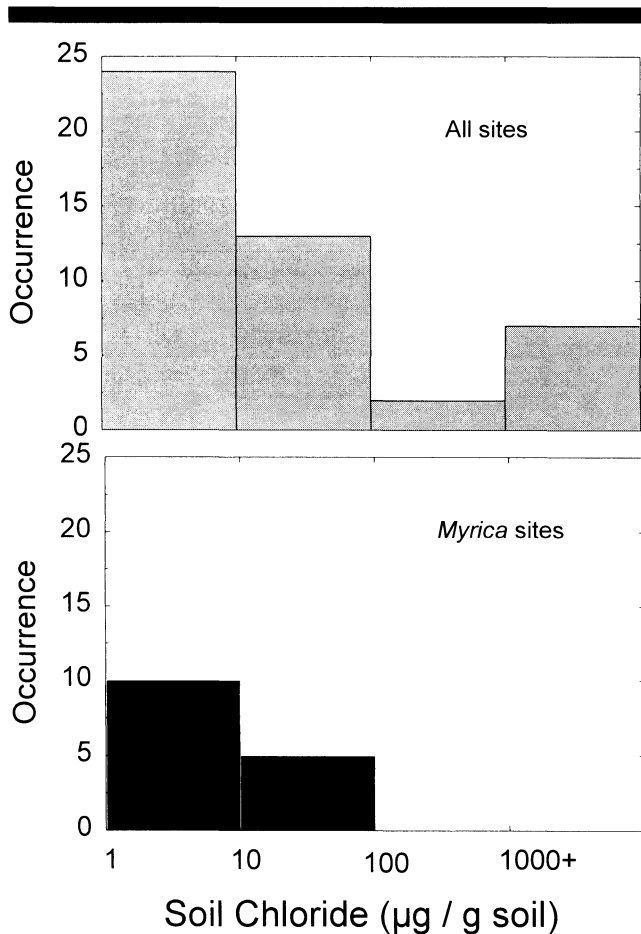| Micro-topography	Seedling density	Chlorides	pH	% of locations
Beach	—	5955 ± 1548*	7.9 ± 0.2	10.9
Dune crest	0.2 ± 0.1*	38 ± 11	7.5 ± 0.1	41.3
Dune slope	—	39 ± 17	7.9 ± 0.2	10.9
Swale	2.1 ± 0.2*	17 ± 2	7.7 ± 0.1	26.1
Thicket	1.0 ± 0.1*	5 ± 1	6.2 ± 0.2*	10.9

Figure 5. The occurrence of all locations and locations with *M. cerifera* in the transect in relation to soil chloride levels (μg Cl⁻/g soil) on Hog Island.

shrub seedlings were most abundant in the swale areas with low salinity. The distribution of *Frankia* was similar, but *Frankia* was less constrained by microtopography. *Myrica cerifera* is relatively salt intolerant (YOUNG, 1992; SANDE and YOUNG, 1992; CARTER and YOUNG, 1993). YOUNG, SANDE, and PETERS (1992) concluded that soil moisture and water retention limits mature *M. cerifera* shrubs to swale regions. This evergreen shrub is relatively intolerant of the xeric and high salinity conditions of the open beach (YOUNG *et al.,* 1992). Thus, the low-lying swale is a suitable environment for *Myrica* seedling establishment.

Locations that did not contain the host plant or root symbiont occurred in the dune crest region closest to the beach. We expected *M. cerifera* to be absent from these areas because of intolerance to high salinity (YOUNG *et al.,* 1994). Although chlorides were significantly different among microtopographic regions within the study plot, the chloride range was unusually narrow relative to other coastal studies. The soil chlorides in the plot ranged from 0–24 μg Cl⁻/g soil, whereas YOUNG, ERICKSON, and SEMONES (1994) detected soil chloride ranges of 1–500 μg Cl⁻/g soil for soils beneath *M. cerifera* on Hog Island. As expected, there was greater

variability in soil chlorides along the transects. The summer of 1996 had unusually high precipitation, and many locations in the swale had standing water. In chronically wet soils, chlorides are less concentrated and evaporation is low; therefore, chlorides do not accumulate (BARBOUR *et al.,* 1985). For both the plot and transects, the chloride distribution at locations with *M. cerifera* differed significantly from the distribution of chlorides at all locations, indicating a relationship between the spatial pattern of the shrub seedlings and soil chloride levels. YOUNG, ERICKSON, and SEMONES (1994) determined that salinity is a major factor contributing to the small-scale distribution patterns of the shrubs, *Myrica cerifera, Baccharis halimifolia,* and *Iva frutescens*; *M. cerifera* was the most sensitive to salinity.

The *Frankia* bioassay was a conservative estimate of the chloride limits of *Frankia.* Although the presence of nodules provides indirect evidence of *Frankia* (TORREY and CALLAHAM, 1978), unnodulated seedlings do not necessarily indicate the absence of *Frankia. Frankia* may be present in too low a density to cause nodulation. Further, high salinity, low soil moisture, or an unidentified edaphic factor may inhibit the growth of *M. cerifera* or the formation of the symbiotic association (HUSS-DANELL, 1997). PASCHKE and DAWSON (1992a) determined that soil quality can limit infectivity of *Frankia,* regulating the soil populations of the root symbiont.

The success of *M. cerifera* is due in part to the symbiotic relationship with *Frankia*; however, the nature of the relationship appears inequitable. Mature shrubs were found only in the presence of *Frankia* (YOUNG *et al.,* 1992) and our results indicate that *M. cerifera* seedlings require the root endophyte. All *M. cerifera* seedlings examined were nodulated. Similarly, VITOUSEK and WALKER (1989) found all *M. faya* on a Hawaiian island nodulated, even young seedlings. Yet, *Frankia,* occurred in volcanic soils without *M. faya* (BURLEIGH and DAWSON, 1994). Indeed, *Frankia* has been identified in soils where no known hosts have grown (HUSS-DANELL, 1997). For coastal soils, YOUNG, SANDE, and PETERS (1992) determined that the general distribution of *Frankia* exceeded that of the mature *M. cerifera,* which concurs with the results of our study. SMOLANDER and SUNDMAN (1987) found *Frankia* in acidic soils where the host plant had not occurred. The distribution of *M. cerifera* is associated with the presence of *Frankia,* therefore the root symbiont may directly influence the distribution of the host plant in this coastal environment. Thus, factors limiting *Frankia* will also limit the host plant. These factors include microtopography and soil chlorides.

Prior to the successful establishment of *M. cerifera* and, perhaps, regardless of favorable edaphic factors, *Frankia* must be present in the soil and the symbiotic association formed. Without this mutualistic relationship, the host shrub may not tolerate the low nitrogen conditions typical of newly formed coastal soils. The dispersal of *Frankia* to newly accreted soils becomes critical for the expansion or establishment of *M. cerifera.* Although the mode of dispersal has not been ascertained in coastal environments, transport by freshwater has been demonstrated in river and lake sediments (ARVEBY and HUSS-DANELL, 1988; HUSS-DANELL *et al.,* 1997), as well as by flooding (SMOLANDER and SUNDMAN,

1987; ZITZER and DAWSON, 1992). Freshwater flooding from rain storms may contribute to *Frankia* dispersal in coastal swales, but the potential for transport by seawater remains uncertain. Air-borne dispersal of *Frankia* may occur over short distances (HUSS-DANELL, 1997). This may be important for shrub thicket expansion after the initial establishment of *M. cerifera*.

In harsh environments, such as coastal systems, small-scale distribution patterns are controlled primarily by physical factors. The relationship between *M. cerifera* seedlings and *Frankia* demonstrates the importance of biotic factors when evaluating distribution patterns. Actinorhizal plants typically colonize open, nitrogen poor sites (HUSS-DANELL, 1997), adding nitrogen (MORRIS *et al.*, 1974; YOUNG *et al.*, 1995), and thereby influencing the establishment of other species (VITOUSEK and WALKER, 1989; LEVY, 1990). These characteristics make actinorhizal species ideal for reclamation and soil restoration (HUSS-DANELL, 1997). To ensure success in reclamation efforts, especially in coastal soils, these species should be inoculated with *Frankia* prior to planting.

ACKNOWLEDGEMENTS

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