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# Spatial Relationships of *Frankia* and *Myrica cerifera* on a Virginia, USA Barrier Island

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## Abstract

The spatial distribution of *Frankia* and the host plant, *Myrica cerifera*, were examined across Hog Island, a barrier island off the coast of Virginia, USA. *Frankia* distribution was assessed using surface-sterilized *M. cerifera* seeds sown on soil cores collected at 50 m intervals along a 1150 m cross-island transect, where soils varied in age from less than one year to approximately 130 years. Nodulation and the expression of nitrogenase occurred in *M. cerifera* seedlings grown on cores obtained from beneath established *M. cerifera* thickets (6 sites) and on cores obtained at three of fifteen other sites where the host shrub was absent. The latter finding indicates that the distribution of the actinomycete in these soils is not completely dependent on that of *M. cerifera*. Seedlings developed on cores from nine of the twelve remaining sites, but, did not nodulate. This may be attributable to either the absence of *Frankia* or to an inhibition of nodulation. *M. cerifera* seeds did not germinate on soil cores from the remaining three sites, all of which had high salinity ($>$ 500 mol $m^{-3}$ Cl-ions). Seed germination experiments indicated that *M. cerifera* is relatively intolerant of salinity.

The results show that the distribution of *Frankia* in the relatively young soils of a barrier island currently exceeds that of the host and suggests that *Myrica cerifera* has the potential to colonize sites where it does not currently occur. The extent of any additional colonization of these nitrogen poor soils by *M. cerifera* appears to be determined, however, not only by the distribution and abundance of *Frankia*, but also by edaphic factors, including salinity and water availability.

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## 1. Introduction

Actinorhizal plants are frequently pioneers, colonizing early successional or recently disturbed sites with nitrogen poor soils (Baker and Schwintzer, 1990; Dawson, 1990). Their successful establishment on such soils is due to the formation of a nitrogen-fixing association with the actinomycete, *Frankia*, a root-nodule symbiont. In nitrogen limiting environments, such as those created by volcanic activity, glacial retreat, or sand deposition in coastal areas, the successful establishment of an actinorhizal plant is contingent upon the presence of *Frankia* in the soils. Although indirect evidence indicates that *Frankia* may occur in soils that have never supported the host plant, and in soils that have been devoid of the host plant for many years (Baker and Schwintzer, 1990), there is a paucity of information regarding the small-scale distribution of *Frankia* in recently formed, sandy coastal soils.

The actinorhizal shrub, *Myrica cerifera*, is often the dominant woody species on barrier islands along the coast of the southeastern United States (Duncan and Duncan, 1987). This is the case on the barrier islands along the Eastern Shore of Virginia, USA (Dueser et al., 1976). The distribution of *M. cerifera* is not uniform, but rather is restricted to patches across these islands. The objective of the present study was to determine the extent to which the non-uniform colonization by *M. cerifera* was attributable to edaphic factors affecting seedling establishment, versus the distribution of *Frankia* and/or the ability to develop the symbiotic association.

## 2. Materials and Methods

### *Study site*

The small-scale distributions of *Frankia* and *Myrica cerifera* L. (Myricaceae) were studied on the northern end of Hog Island (37° 40′ N, 75° 40′ W), located approximately 8 km out from the Eastern Shore peninsula of Virginia, USA. The island is transitional, with shifting zones of accretion and erosion (Hayden et al., 1991). The study site was a transect located on the accreting, northern end of the island. Soils progressed in age across the island transect, from one year at the ocean front beach to approximately 130 years at the edge of the bay side marsh on the western edge of the island (Hayden et al., 1991).

The vegetation of Hog Island is conspicuously patchy with distinct zonation and sharp transition between patches (Dueser et al., 1976). Grass covered dunes, dominated by *Ammophila breviligulata, Panicum amarum* and *Spartina patens*, alternate with swales containing *Myrica cerifera* thickets that become progressively larger from the ocean side to the bay side of the island, ending with a thicket of relatively tall shrubs (5–6 m) just before the tidal salt marsh. A large interior salt flat is intermittently flooded and several similar salt flats are located among the dunes. The climate is relatively mild and marine dominated with a minimum annual temperature from −6 to −10°C.

*Soil core collection*

To study the distribution of *Frankia* across Hog Island, soil cores were collected in September of 1989 at 50 m intervals along a 1150 m surveyed transect that was oriented east-west. Sampling was initiated at the shoreline on the ocean side and terminated at the edge of the bay side marsh on the west side of the island. Elevations along the transect varied from 2.0 m below to 1.9 m above the high water line. The transect intersected two inland marshes and three *M. cerifera* thickets. Soil cores were not collected at sites that were located in the permanently inundated portions of the two interior marshes. Logistical problems associated with the island field work, especially transport to and from the island, necessitated limiting sample size to 5 cores per sampling point along the transect (105 cores total).

At each sampled point, the presence or absence of *M. cerifera* within a radius of 15 m was noted and four soil cores were collected aseptically. For each soil core, a metal cylinder (30 mm diameter, 35 cm deep) was sterilized by extensive flaming and then inserted into the sandy soil to a depth of 20 cm. The extracted core was transferred to a sterile glass tube (35 mm diameter, 30 cm length) which was flamed, replugged and, after cooling, sealed with parafilm and packed for transport back to the laboratory. A fifth soil core was collected at each site to determine total N and P using standard colorimetric analyses after Kjeldahl digestion (Allen et al., 1986). The five soil cores collected at each site were removed from an area of approximately 0.5 $m^2$.

*Nodulation test for* Frankia *presence*

Seeds of *M. cerifera* were collected from Hog Island in May after natural cold stratification and were kept at 4°C. Prior to use, the waxy outer coating was removed from the stratified seeds and the seed coats were scarified by rubbing against a wire screen. The seeds were then surface sterilized for 20 min in 30%

$H_2O_2$ and rinsed with sterile water. Approximately one week after the soil cores were collected, five seeds were placed on the soil surface in three of the cores from each site and the tube openings were covered with pre-sterilized cotton sandwiched between layers of gauze. The tubes were placed in a glasshouse with a midday photon flux density of approximately 1600 $\mu$mol $m^{-2}$ $s^{-1}$ and a maximum air temperature between 20 and 30°C. Sterile deionized water was added to the tubes as needed, using a sterile syringe. Possible procedural contamination with *Frankia* was checked by autoclaving (120°C for 90 min) the fourth tube from each site prior to adding the seeds. However, percent seed germination was only monitored for the non-autoclaved soil samples.

Seed germination and seedling development were monitored weekly. About four months after germination, when the seedlings reached 8 cm in height, all the soil cores, including the controls, were removed along with the plants. The entire root system was carefully washed free of soil, blotted and examined for nodules prior to placement in a 30 ml glass vial for the acetylene reduction assay of nitrogenase activity (Stewart et al., 1967). The vials were sealed with a rubber stopper and incubated in a 25°C water bath to obtain and maintain an optimum temperature for nitrogenase activity (Bond and Wheeler, 1980). After temperature equilibration, the vials were opened and flushed with air for 3 minutes and resealed. Acetylene was then added to 10% v/v in air and the samples were incubated for 1 hr. A 250 $\mu$l gas sample was withdrawn from each vial for analysis and injected into a Varian model 3300 gas chromatograph equipped with a Poropak N (80–100 mesh) column (2 m×0.002 m) and a flame ionization detector. The oven temperature was 25°C and nitrogen was the carrier gas. After removal of the plants, 10 to 12 $cm^3$ soil samples from each core were also incubated with acetylene to check for any non-symbiotic nitrogen fixation that could affect the results of the plant assays. These samples also provided a check for any endogenously produced ethylene in the soil.

After the seedlings were harvested, the soil cores from the glasshouse experiment were analyzed for total Cl-ions using a chloride electrode (Orion model 9617B) and a 1:5 ratio (w/w) of soil to distilled water with 5 m $NaNO_3$ (2 ml per 100 ml of sample) added as an ionic equalizer. The Cl-ion concentration was expressed as moles per $m^3$ of soil water and, to maintain consistency in concentration, the soil cores were kept near volumetric field capacity throughout the experiment. Field capacity ranged from 12 to 18%, depending on sample location along the transect.

### Myrica *germination in response to salinity*

The effect of salinity on the germination of *M. cerifera* seeds was determined over a period of 35 days. Prior to salt treatment, the waxy coating was removed and seeds were scarified as described above. Five salt solutions (25, 50, 100, 200 and 500 mol Cl-ions per $m^3$ of water) were prepared using sodium chloride in deionized water. For each salinity treatment and a deionized water control, 50 seeds were placed into each of 10 Petri dishes (9 cm diameter) containing three disks of filter paper. The disks were saturated with 7 ml of the appropriate solution and then the dishes were weighed. The seeds were maintained in a growth chamber at 1,000 $\mu$mol $m^{-2}$ $s^{-1}$ photon flux density, using a 14 hr day length and 25/20°C regime, and examined daily. To ensure that relatively constant sodium chloride concentrations were maintained throughout the experiment, the dishes were weighed every third day to determine the degree of water evaporation. Deionized water was added to return them to the original weight.

Except where noted, Student's t-test (Zar, 1984) was used throughout the study to statistically compare means at an alpha level of 0.05.

## 3. Results

The distribution of *Frankia* in soils across Hog Island, as indicated by *Myrica cerifera* seedling nodulation and nitrogenase activity, corresponded quite closely to the natural distribution of *Myrica* thickets on the island (Table 1). However, at 100, 450 and 550 m from the ocean, the soil core-*Myrica* seedling assay revealed that *Frankia* was present despite the absence of naturally occurring *M. cerifera* at those sites. There was complete agreement between the occurrence or absence of nodulation and the presence or absence of nitrogenase activity via the acetylene reduction assay. Further, none of the seedlings in the control (i.e. autoclaved) soil cores became nodulated or exhibited nitrogenase activity and the acetylene reduction assays of the soils to detect non-symbiotic nitrogen fixation were negative for all cores.

*M. cerifera* seed germination on the soil cores ranged from zero at three sites (0, 350 and 1150 m) to 60.0% at the site 1050 m from the ocean, near the bay side of the island (Table 1). At the remaining sites, germination varied from 6.7 to 53.3%. Although samples size was small, germination on soils beneath *Myrica* thickets (40.0±6.4% (SE)) was significantly greater than germination on soils without naturally occurring *Myrica*, (22.2±4.1%) (Table 1).

Total soil Cl-ions ranged from 0.1 mol $m^{-3}$ at 200, 350 and 700 m to over 500 mol $m^{-3}$ at the 0, 300 and 1150 m sites. These values corresponded to the

Table 1. The occurrence of *Frankia*, germination of *Myrica cerifera* seeds, and Cl-ion concentration in soil samples (n=3) from the Hog Island transect. *Frankia* occurrence was monitored by nodule formation and nitrogenase activity. * denotes locations of *M. cerifera* thickets along the island transect and "NG" indicates no seedling growth in the soils.

| Distance from ocean (m) | *Frankia* occurrence (# of samples) | *Myrica cerifera* seed germination (%) | Total soil Cl-ions ( mol $m^{-3}$) |
|---|---|---|---|
| 0 | NG | 0 | 510±42 |
| 50 | 0 | 33.3 | 0.4±0.1 |
| 100 | 1 | 26.7 | 1.0±0.2 |
| 150 | 0 | 13.3 | 0.7±0.2 |
| 200 | 0 | 46.7 | 0.1±0.1 |
| 250 | 0 | 26.7 | 0.3±0.1 |
| 300 | NG | 0 | 601±121 |
| 350 | 0 | 46.7 | 0.1±0.1 |
| 400 | – | – | – |
| 450 | 2 | 33.3 | 1.4±0.4 |
| 500 | 0 | 26.7 | 31.5±16.6 |
| 550 | 2 | 13.3 | 25.3±12.7 |
| 600 * | 1 | 20.0 | 0.5±0.3 |
| 650 | 0 | 6.7 | 0.3±0.1 |
| 700 * | 1 | 26.7 | 0.1±0.1 |
| 750 | 0 | 33.3 | 30.5±24.5 |
| 800 | – | – | – |
| 850 | – | – | – |
| 900 | 0 | 26.7 | 36.8±27.2 |
| 950 * | 2 | 46.7 | 6.0±4.1 |
| 1000 * | 2 | 33.3 | 0.2±0.1 |
| 1050 * | 1 | 60.0 | 0.6±0.2 |
| 1100 * | 3 | 53.3 | 6.2±2.3 |
| 1150 * | NG | 0 | 502±203 |

tidal influence on the ocean and bay sides and an overwash zone (Table 1). The average chloride level at the *Myrica* thicket sites was 2.3±1.2 mol $m^{-3}$, ranging from 0.1 to 6.2 mol $m^{-3}$, and differed significantly from the average for the test of the sampled locations, 124.4±60.2 mol $m^{-3}$, despite high variability (Table 1). Chloride levels for soils from the three additional sites where *Frankia* nodulated seedlings in the core assay ranged from 1.0 to 25.3 mol $m^{-3}$ of Cl-ions.

Analyses revealed that the N levels for the relatively young soils from the ocean side to mid-island were around 400 $\mu$g $g^{-1}$ and that N levels increased to over 2,000 $\mu$g for the oldest soils at the edge of the bay side marsh (Fig. 1). The average soil N level of 791±195 $\mu$g $g^{-1}$ beneath *Myrica* thickets was significantly higher than the 321±14 $\mu$g $g^{-1}$ for the non-*Myrica* sites (excluding the high nitrogen site at the edge of the bay side marsh). Soil phosphorus

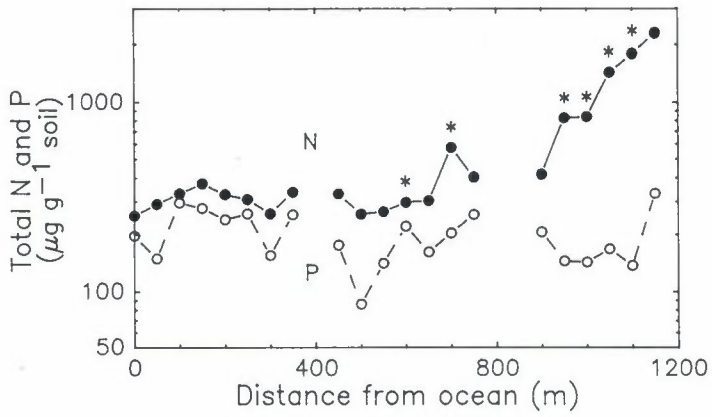


Figure 1. Total nitrogen (N) and phosphorus (P) per gram of dry soil along the Hog Island transect. * identifies sample sites with *Myrica cerifera*. Sites lacking data points were under water.

averaged 200±14 $\mu$g $g^{-1}$ and showed no pattern across the island. It did not differ when soils from non-*Myrica* sites were compared with those under the *Myrica* thickets, nor did it differ in comparison between soils in which *Frankia* was present with those in which it was absent or failed to nodulate *Myrica* (Fig. 1). The ratio of soil N to P varied from a low of 1.1 in the core obtained at 100 m from the ocean to a maximum of 11.7 in the core collected at 1100 m (Fig. 1).

Laboratory experiments investigating the effect of chloride ions on the germination of *M. cerifera* seeds demonstrated that maximum germination (68.0±1.7%) occurred in the absence of salt (Fig. 2). Increases in Cl-ions from 25 to 200 mol $m^{-3}$ resulted in corresponding decreases in germination to only 4.8±0.9% at 200 mol $m^{-3}$. This decrease represented a statistically significant linear relationship based on a least squares regression analysis (Zar, 1984) of Cl-ion concentration versus percent germination with a coefficient of determination ($r^2$) of 0.98. No seeds germinated at 500 mol $m^{-3}$ of Cl-ions. However, at the conclusion of the experiment, the seeds subjected to this treatment were rinsed with deionized water. Most of these seeds subsequently germinated, indicating that the lack of germination was primarily due to elemental or osmotic inhibition and not seed mortality.

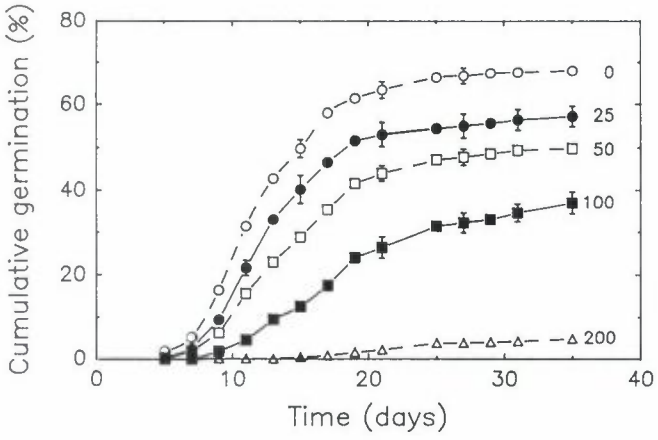


Figure 2. Cumulative germination values for seeds of *Myrica cerifera* subjected to chloride ion concentrations of 0, 25, 50, 100 and 200 mol $m^{-3}$. Values are means ± one standard error (n=10).

## 4. Discussion

Soil core sampling along the Hog Island transect revealed that *Frankia* was present at each of the six sampling sites beneath *Myrica cerifera*. Although the nature of the seedling assay did not exclude the possibility that *Frankia* was present in additional cores, but failed to nodulate *Myrica*, the assay did unequivocally demonstrate that the actinomycete was present at three sites where the host shrub was absent. The latter finding indicates that the distribution of the actinomycete exceeds that of the host, *M. cerifera*, on the barrier island. This is especially significant when considering the remoteness of the island and the relatively young soils which range in age from less than one to about 130 years.

The occurrence of *Frankia* without the host plant nearby has been demonstrated for *M. gale* in bogs (Bermudez de Castro et al., 1976; Rodriguez-Barrueco, 1968), as well as for more developed forest soils associated with several species of *Alnus* (Rodriguez-Barrueco, 1968; Benecke, 1969; Huss-Danell and Frej, 1986) and *Ceanothus velutinus* (Wollum et al., 1968). Moreover, Smolander and Sundman (1987) found *Frankia* in soils that were devoid of host plants for many years. The previous studies, along with the present barrier island work, support the hypothesis that *Frankia* may grow saprophytically in soils, independent of the host plants (Baker and Schwintzer, 1990). However, the possibility that a *Frankia* strain present in the island soils is a spore producer has not been excluded.

Low organic matter content and young soils are both unfavorable for maintaining *Frankia* populations (Becking, 1970; Houwers and Akkermans, 1981). In the present study, although *M. cerifera* seedlings were successfully established from seed in soils from nine of the twelve other sites on the island transect, there was no nodulation or nitrogenase activity. These results indicate either an absence of *Frankia* in the soils, an inhibition of infection, or both. Soil nitrogen levels were not sufficient to adversely affect nodule formation on the *M. cerifera* seedlings. The highest soil nitrogen levels were beneath *Myrica* thickets and all soil cores from these sites yielded nodulated seedlings. While most of the non-nodulated cores came from the accreting ocean side of the island where organic matter content was less than 1% (D.R. Yong, unpublished data), one at 100 m did nodulate. This may reflect the extreme spatial heterogeneity of organic matter in young dunes. Some soils adjacent to clumps of dune grasses may contain relatively high amounts of root detritus, while others may be virtually devoid of organic matter or *Frankia*.

The laboratory seed germination data, as well as the comparison of the total soil chloride data for seed germination on the soil cores and total chlorides for soils beneath island thickets, all indicated that *M. cerifera* is relatively intolerant of salinity. This conclusion is further supported by laboratory experiments with *M. cerifera*, which showed decreased photosynthesis, stomatal conductance and nitrogenase activity at 50 mol $m^{-3}$ NaCl (Sande and Young, in press). Dawson and Gibson (1987) have demonstrated considerable tolerance of specific *Frankia* strains to sodium chloride (over 200 mol $m^{-3}$) and the *Frankia* populations on the island also may be salt tolerant. However, environmental salinity, especially soil chloride levels, appears to be a principal factor influencing the distribution of *M. cerifera* and may also interfere with the infection process. *Myrica* seed germination did not occur on soil cores or in petri dishes when Cl-ion levels were at or above 500 mol $m^{-3}$ and the seedling assay for *Frankia* was consistently negative above 300 mol $m^{-3}$.

As one moves across the island transect from young, accreting soils to older soils, soil nitrogen increases, especially beneath *M. cerifera*. The elevated soil nitrogen levels associated with *M. cerifera* are consistent with the work of Vitousek et al. (1987) and Vitousek and Walker (1989) with *M. faya* in volcanic soils of Hawaii, USA, where *M. faya* alters successional processes via nitrogen enrichment of the soil. Similarly, the success of *M. pensylvanica* as an early successional plant of dunes and impoverished coastal soils of the northern United States is due in part to the nitrogen-fixing capacity of the nodule association (Morris et al., 1974). Clearly, the *Myrica cerifera-Frankia* symbiosis must impact upon many ecological processes in barrier island and coastal environments.

The distribution of *Frankia* currently exceeds that of *M. cerifera* on Hog Island, as evidenced by the soil core assay. All three sites where *Frankia* presence was confirmed in the absence of the host plant can be characterized as swale environments with more than adequate available soil moisture. If other factors are not limiting, *M. cerifera* may gradually colonize these areas. Drought tolerance studies of *M. cerifera* (Young, 1992) have indicated that the shrub requires nearly saturated soils and that the stomates begin to close at moderate plant water potentials ($< 1.0$ MPa). This finding is consistent with the hypothesis that the distribution and future expansion of *M. cerifera* on Hog Island may well be restricted to swales, as it will not tolerate the relatively xeric conditions of the well-drained dune soils.

In summary, the distributions of the host plant, *Myrica cerifera*, and of the root-symbiont, *Frankia*, are patchy on islands along the coast of Virginia, USA. The successful establishment of *M. cerifera* is apparently dependent on the presence of *Frankia* in the soils in order to facilitate symbiotic nitrogen fixation, but the distribution of *Frankia* is independent of the host plant in these sandy soils. In addition, the spatial distribution of both *Myrica cerifera* and *Frankia* and the establishment of the symbiosis appear to be influenced by edaphic factors, especially soil salinity and water availability. Future experiments will focus on identifying whether non-nodulated seedlings were due to inhibition by edaphic factors, the absence of *Frankia*, or both.

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