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AVIAN DISPERSAL OF THE ACTINOMYCETE *FRANKIA* ACROSS A BARRIER ISLAND LANDSCAPE

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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ACROSS A BARRIER ISLAND LANDSCAPE

By Spencer Nathaniel Bissett, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2008

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In the nutrient-poor soils characteristic of coastal environments, symbiotic association with the nitrogen-fixing root endosymbiont *Frankia* is essential to establishment and survival of the woody shrub *Morella cerifera*. Nutrient deficiency quickly becomes severe unless seedlings are infected by *Frankia* soon after germination. However, the means of arrival of *Frankia* prior to shrub establishment has not been determined. Using sterilized lab-grown *M. cerifera* seedlings and fecal samples collected from passerine birds on the Eastern Shore of Virginia, viability of avian dispersal of the bacteria was tested. Although passerine fecal samples did produce nodules on some sterilized *M. cerifera* seedlings, these experimental inoculations did not lead to significantly higher likelihood of nodulation, relative to sterilized reference seedlings. Non-sterilized seedlings displayed greatest percent nodulation; results suggest that passerines contribute to *Frankia* dispersal, but also that the actinomycete is contained on or within viable seeds or fruits of *M. cerifera*, and therefore may be co-dispersed directly from the parent plant.

Introduction

In coastal ecosystems, plants are exposed to a unique set of harsh environmental factors which may act alone or in concert, limiting survival. Chief among these are sea spray, saltwater intrusion, nutrient-poor soils, low freshwater availability, high wind, shifting sand, and storm-related disturbances (Ehrenfeld, 1990; Stalter and Odum, 1993; Hayden et al., 1995; Shao et al., 1996). Proximity to the shore determines the severity of many of these factors, and thus contributes to the development of environmental gradients which affect plant community composition. Increasing distance from the ocean is reflected by a predictable pattern of successional seral stages (Ehrenfeld, 1990; Levy, 1990; Stalter and Odum, 1993). In the context of barrier islands, the physical pressures associated with a coastal landscape are compounded by the transitional nature of the environment. Drivers such as shifting sand and sea level rise, combined with storm-related overwash, erosion, and accretion, contribute to long-term migration of the islands, and all of these factors influence the establishment of successional plant communities between the beach and island interior (Stallins and Parker, 2003; Davis and Fitzgerald, 2004).

On the barrier islands of the Mid-Atlantic United States, recently-accreted soils are dominated by burial-tolerant colonizing grasses such as *Ammophila breviligulata*, which capture blowing sand, build dunes and create habitat for sand-binding dune crest species such as *Spartina patens* (Levy, 1990). Over time, a woody shrub community may establish and thrive in the protected swales behind sand dunes, resulting in generally monospecific, dense-canopied thickets of *Morella cerifera* (L.) Small (Myricaceae) (Crawford and Young, 1998a), formerly *Myrica cerifera* (L.) (Wilbur, 1994). Thus, in the absence of disturbance events, biotic interactions may supersede physical controls and lead to autogenic succession.

Critical to the establishment of early successional communities in coastal environments is the acquisition of limiting nutrients in young, sandy soils (Sundareshwar et al., 2003). In the absence of weathering, much of the basic cation input of barrier island ecosystems may be provided by sea spray and other meteorological inputs (Art et al., 1974), but nitrogen availability remains low in these recently deposited sandy soils (Morris et al., 1974; Ehrenfeld, 1990; Sundareshwar et al., 2003). In these environments, plants that form symbiotic associations with nitrogen-fixing bacteria have a distinct advantage over non-symbiotic plants. On the eastern seaboard of North America, *M. cerifera* and *M. pensylvanica* are the dominant woody species (Stalter and Odum, 1993), owing largely to their association with the root nodule-forming endosymbiont *Frankia*. Additionally, these and other actinorhizal members of the family Myricaceae associated with effectively N_2-fixing *Frankia* may play a vital role in succession by providing an autochthonous source of available organic nitrogen (Morris et al., 1974; Young et al., 1992; Vitousek and Walker, 1989; Shumway, 2000). Vitousek and Walker (1989) calculated nitrogen input from fixation by invading *Morella faya* on Hawaiian volcanic soils to be 18.5 kg ha^{-1} yr^{-1}. Shumway (2000) also found soil nitrogen levels to be higher beneath *M. pensylvanica*, effectively facilitating growth of two herbaceous sand dune species in the shrub thicket understory.

On Mid-Atlantic barrier islands, thickets dominated by *M. cerifera* represent the first woody seral stage of succession, and may similarly increase concentration and availability of soil organic nitrogen; Young et al. (1992) measured soil N levels of 791 ± 195 μg g^{-1} beneath *M. cerifera* thickets, while levels were 321 ± 14 μg g^{-1} outside of thickets. Indeed, while individuals of the non-nitrogen-fixing woody species *Baccharis halimifolia* and *Ilex vomitoria* may establish prior to *M. cerifera* arrival, they exist only at low densities in young soils. However, densities of these shrubby species may become considerable when found alongside *M. cerifera* or *M. pensylvanica*, or in old soils which have previously been occupied by the actinorhizal shrubs (Stalter and Odum, 1993; personal observation).

Infection by *Frankia* occurs at the point of a developing root hair, where the bacteria enter the plant tissue via either the intracellular or intercellular pathway (Wall, 2000). Because the association is essential to survival of *Morella cerifera* in low-nutrient coastal soils, *Frankia* must be present in barrier island soils at the time of seed germination (Young et al., 1992; Wijnholds and Young, 2000). However, means of *Frankia* dispersal to the sandy soils of these islands has not been clearly defined (Wijnholds and Young, 2000). Active dispersal of propagules is limited to hyphal growth, and this cannot account for movement at the landscape scale either across or between islands, or between island and mainland soils (Paschke and Dawson, 1993). If previously established, the actinomycete may persist in an environment via spore production, the timing of which may be related to host cell death (VandenBosch and Torrey, 1985). *Frankia* strains may be spore-positive or spore-negative, and while both phenotypes may be induced in pure culture in the lab, spore-positive nodules are rarely observed in the field (Schwintzer, 1990). Spores may also be referred to as bacteroids or granules (VandenBosch and Torrey, 1985), which Gardner (1976) documented in nodules of *M. cerifera*. Few, if any, spore-positive nodules have been identified in subsequent field observations of *M. cerifera*, though both types are known to occur in root nodules of the related species *Myrica gale* (Myricaceae) in Maine (Schwintzer, 1990). Of note, however, Burleigh and Dawson (1994) indicated that *Frankia* does not produce an above-ground sporophyte, and therefore sporulation cannot be expected to provide an effective means of long-distance dispersal, although the potential for a narrowly-distributed spore positive strain of *Frankia* on mid-Atlantic barrier islands has not been conclusively excluded.

Documented passive dispersal mechanisms include movement via freshwater (Huss-Danell et al., 1997) and animal vectors (Reddell and Spain, 1991; Paschke and Dawson, 1993; Burleigh and Dawson, 1995). Reddell and Spain (1991) successfully inoculated *Casuarina equisetifolia* using casts of earthworms taken from soil mixed with nodule material. Paschke and Dawson (1993) induced nodulation of *Alnus glutinosa* and *Elaeagnus umbellata* seedlings using dilutions of bird nest material, and Burleigh and Dawson (1995) fed *Frankia* spore and hyphal material to parakeets, whose fecal matter successfully inoculated *Casuarina equisetifolia*. If birds are indeed capable of transporting infective *Frankia* propagules, co-dispersal of the actinomycete with fruits may occur, as *M. cerifera* fruits are an important food source for both local and migratory species (Stiles, 1980; Borgmann et al., 2004). Additionally, while *Frankia* strains exhibit varying degrees of host specificity, actinorhizal members of the genera *Myrica* and *Morella*, specifically *Myrica gale*, *Morella pensylvanica*, and *Morella cerifera*, are considered to be promiscuous hosts, forming associations with strains isolated from a wide variety of sources (Torrey, 1990; Huss-Danell, 1997; Clawson and Benson, 1999). Therefore, *Frankia* transported by passerines need not necessarily originate from a similar *M. cerifera* thicket in order to provide a source of infective and effective bacteria.

Frugivorous birds have a major effect on the plant community composition of barrier islands, particularly the members of The Nature Conservancy's Virginia Coast Reserve (VCR) Long-Term Ecological Research (LTER) site. Members of this barrier island chain serve as valuable native bird habitat, and passerine populations also increase during the autumn migratory season (Mabey et al. 1993). On barrier islands, Mabey et al. (1993) documented greater numbers of species, as well as individuals, than on the coastal mainland; *Dendroica coronata coronata*, the yellow-rumped myrtle warbler, is three to five times as abundant on the islands. As reviewed by Ehrenfeld (1990), a large percentage of shrubs and vines on Atlantic coast barrier islands produce fleshy fruits and are bird-dispersed. *Ilex opaca*, *I. vomitoria*, *Smilax spp.*, and *Sassafras albidum*, in addition to *M. cerifera* and *M. pensylvanica*, may be found in mid-successional communities of these islands, and are also species which are primarily dispersed via avian consumption and defecation (Stiles, 1980; Levy, 1990; Stalter and Odum, 1993; Crawford and Young, 1998b). Species exploiting bird-mediated seed dispersal are particularly successful in barrier island environments because such species are capable of rapid recolonization from external sources following disturbance (Spiller et al., 1998). In shrub thickets of the VCR, seedling recruitment of bird-dispersed species in newly-formed gaps advances successional processes as well. Perch sites become available to native and migratory passerine birds, and the resulting seed rain serves as an input of new species (Crawford and Young, 1998b).

Symbiosis with *Frankia* is essential to the establishment of *M. cerifera* shrubs and thickets, so it is necessary to understand how infective propagules of effective *Frankia* are dispersed to soils of barrier islands, prior to host colonization. Because active dispersal is limited to short distances, animal-mediated transport is a likely candidate for introducing the actinomycete to nutrient-poor recently accreted or recently disturbed soils, thus supplying the prerequisite for establishment of *M. cerifera* seedlings. Although several experiments have demonstrated the viability of animal-mediated dispersal of *Frankia* propagules to non-infective soils, the question of whether this is a predominant means of dispersal *in situ* remains unanswered. The presence of non-native *Frankia* in volcanic Hawaiian deposits and the infectivity of barrier island soils previously devoid of the host plant strongly suggest that some means of long-range dispersal is necessary to the establishment of effectively associated actinorhizal plants (Vitousek and Walker, 1989; Young et al., 1992).

Greater understanding of nitrogen-fixing plants may provide insight to improve understanding of the complex nature of global change. As atmospheric carbon dioxide concentration increases, plants with the capacity to use atmospheric dinitrogen may possess an advantage over other species, but nutrient cycling, soil microbial processes, hydrology, land use, and plant community composition will be affected if these plants experience greater success (Soussana and Hartwig, 1995; Temperton et al, 2003; Hungate et al., 2004; Brantley and Young, 2008). Native plant communities may be jeopardized, as actinorhizal trees and shrubs have already proven to be effective colonizers in alien habitats such as disturbed areas, volcanic soils, and grasslands (Vitousek and Walker, 1989; Orr et al., 2005; Baer et al., 2006). Successful management may depend on an understanding of the ecology of these plants. In addition, actinorhizal species may provide benefits in the fields of agriculture and management, as they may supply a means of increasing available soil nitrogen (Morris et al., 1974; Young et al., 1992; Vitousek and Walker, 1989; Shumway, 2000). One major aspect of the ecology of actinorhizal plants is the means of their establishment in new habitats, which is dependent on the prior arrival of *Frankia* propagules. To determine the viability of a potential means of long-range dispersal of the actinomycete, I evaluated avian feces collected from three field sites, using two different methods. My primary hypothesis was that native and migratory passerine birds are capable of dispersing *Frankia* propagules. An accepted bioassay technique was used to evaluate fecal samples for the presence of infective and effective *Frankia* propagules. Additionally, I tested the hypothesis that *Frankia* propagules are associated with fruits or seeds of *Morella cerifera* prior to ingestion and excretion by frugivorous birds.

Materials and Methods

SITE DESCRIPTION

All *Morella cerifera* fruits and a small number of fecal samples were collected on Hog Island, a barrier island included in the Virginia Coast Reserve (VCR) Long-Term Ecological Research (LTER) site off the coast of the Eastern Shore of Virginia (Fig. 1a). Hog Island is located approximately 10 km east of the Delmarva Peninsula, and is owned by The Nature Conservancy. The island is approximately 10 km long, and up to 2.5 km wide at its northern end, where field sampling for this study was conducted (37° 26' N, 75° 39' W). The accreting north end of the island displays distinct patterning of vegetation with increasing distance from the shoreline as, over time, dune-building colonizers such as *Ammophila breviligulata*, *Spartina patens*, *Panicum amarum*, and *Cakile edentula* are competitively excluded in protected swales by increasingly dense thickets of *M. cerifera*.

A second passive fecal collection site was located on Smith Island, a southern island of the VCR LTER (37° 06' N, 75º 54' W) (Fig. 1b). Smith Island is approximately 11.4 km long and up to 1.4 km wide. Primary dune community composition and successional processes at the developing southern end of Smith Island, where samples were collected, are similar to those observed at the northern end of Hog Island. Recently accreted coastline is colonized by many of the same dune-building species, although infrequent patches of *Uniola paniculata* are also present. Shrub thicket composition of Smith Island is more complex, as *M. cerifera* is co-dominant with *M. pensylvanica*. *Ilex vomitoria* may also be found sporadically in the swale shrub community (personal observation).

The majority of fecal samples were collected during routine bird-banding operations at the Coastal Virginia Wildlife Observatory (CVWO), located in Kiptopeke State Park near the southern tip of the peninsula (37° 26’ N, 75º 39’ W) (Fig. 1c). Mist nets (22) at the CVWO are situated to allow exposure to the most dominant local habitat types. The nets are primarily surrounded by maritime forest dominated by *Pinus taeda*, and this area is bordered by old field areas as well as a low-lying area of dune habitat dominated by *M. cerifera* and *M. pensylvanica* (personal observation). Banding at the CVWO has been conducted annually since its establishment in 1963, and daily operations throughout the autumn migratory period are typically carried out between August and November. Mist nets are opened before dawn and closed between 1300 and 1600 h EST, weather permitting (Wilson and Watts, 1997).

SAMPLE COLLECTION

Morella cerifera fruits were collected from haphazardly selected, distinctly individual shrubs on March 14, 2007, and November 30, 2007. Locations varied from the bay side of a well-developed thicket approximately 15 m from the strand to interiors of older, mid-island thickets, and the fruits were maintained at 4°C until sterilization and/or germination, or pulverization for use as an experimental inoculant.

Fecal samples from Hog and Smith island were passively collected using avian fecal collection devices (AFCDs), constructed from rain gauges (127 mm ClearVu®; Taylor USA, Oak Brook, IL), 2.5 m long 2.5 cm x 5.0 cm weather-resistant posts, and plastic-coated copper wire (Fig. 2). Wires were attached to provide favorable perching sites, and to orient birds such that defecation was generally directed into collectors. During the study period, AFCDs at all sites showed evidence of visitation and successful collection. All site locations are labeled in Figure 1. On Hog Island, nine collectors were erected east of colonizing *Morella cerifera* thickets on primary dunes, six were placed between *M. cerifera* thickets on secondary dunes, and three were located in a freshwater marsh on the bay side of Hog Island, near multi-specific thickets composed primarily of *M. cerifera*, *Baccharis halimifolia*, and *Iva frutescens* (Fig. 1a). Seven more AFCDs were positioned on primary dunes of Smith Island, between established and colonizing thickets of *M. cerifera* and *M. pensylvanica* (Fig. 1b). All collectors were placed in close proximity to edges of established *M. cerifera* thickets on both islands, generally upon dune crests to maximize attractiveness and visibility to birds. AFCDs were examined bi-weekly during summer, but less regularly during the peak migratory period, due to logistical difficulties. Rain gauges containing fecal matter or showing other evidence of visitation (seeds, insect parts, feathers, etc.) were rinsed into sealable plastic bags using deionized water. When necessary, a spatula was used to scrape dried material from the sides of collectors. The spatula was sterilized between uses with 50% ethanol. Collectors were returned to position, and samples were sealed and returned to the lab, where they remained either frozen at -10 °C, or refrigerated at 4 °C until used for seedling inoculations.

Collection at the CVWO was the primary source of fecal samples tested. Birds were extracted from nets and processed by qualified staff, held in paper bags until evident defecation, and promptly released. Birds not defecating within 45 min of extraction were released. Fecal matter was removed from paper bags and separated by species. Samples were moistened with deionized water, and stored on ice in sealed plastic bags until returned to the lab, at which point they were kept frozen until seedling inoculation.

Root nodules from healthy, non-clonal *Morella cerifera* seedlings at expanding edges of thickets were used for positive reference seedling inoculations. Live plants were carefully excavated to avoid separation of nodules from roots, returned to the lab, and maintained in an environmental chamber until use. Nodules were removed, pulverized with a mortar and pestle, and mixed with deionized water.

SEEDLING PREPARATION, TREATMENT, AND EXAMINATION

Seedling treatments were: (1) sterilized positive reference, (2) sterilized negative reference, (3) non-sterile reference, (4) sterilized, with experimental inoculation by crushed fruit of *M. cerifera*, (5) sterilized, with experimental inoculation by AFCD fecal material, and (6) sterilized, with experimental inoculation by CVWO fecal material. Fruits for sterilized seedlings were rubbed vigorously against wire mesh to remove the waxy outer coating and scarify seed coats. Following Young et al. (1992), seeds were then surface sterilized for 20 min in 30% H_2O_2, rinsed five times with deionized water, sown in transparent plastic trays filled with one inch of Perlite growth medium, and watered as necessary with deionized water. For non-sterilized reference plants, whole fruits were crushed with a mortar and pestle to break the waxy coating and scarify seeds, but no material was removed before sowing in Perlite. Plants with at least three sets of secondary leaves were considered sufficiently resilient to survive transplant, and were shifted from

Perlite to sand in individual pots, and fertilized once per week with ¼-strength nitrogen-free Hoagland's solution (Hoagland and Arnon, 1950).

After approximately three weeks, or as nitrogen deficiency became apparent, seedlings were uprooted and inoculated with an experimental solution. Roots of seedlings were rinsed, non-destructively examined for presence of nodules, and replanted in new pots with fresh sand after application of experimental inoculation material. Air temperature was maintained between 25 and 30 °C in an environmental chamber (Conviron E15; Controlled Environments Limited, Winnipeg, Manitoba, Canada), with a 15 h photoperiod and daytime photon flux density of 180 μmol m^{-2} s^{-1}. Application of reference and experimental materials was accomplished by dipping root masses of seedlings into one of five suspensions of material. For sterilized positive reference plants, roots were dipped in crushed nodule suspension. Sterilized negative reference plants were transplanted without inoculation, as were non-sterile reference plants. Sterilized experimental plants were dipped in either *M. cerifera* crushed fruit suspension, AFCD suspension, or CVWO fecal suspension. Inoculated seedlings were returned to the environmental chamber, and again watered as necessary with deionized water and fertilized once per week.

Seedlings were grown for up to seven weeks after inoculation before destructive examination. Cork stoppers in drain holes were then removed and sand was washed away, to avoid loss of any present root nodules, and minimize loss of root hairs. Roots were carefully removed, and examined under a dissecting microscope; 40X magnification was sufficient for viewing of individual root hairs. Roots were thoroughly examined, and any potential nodules were removed and preserved for further evaluation in deionized water with ten percent ethanol.

DATA ANALYSIS

Chi-square testing of homogeneity was performed using SAS 9.1 to determine whether actual numbers of nodulated plants differed from expected (Zar, 1999). Observed number nodulated was compared with expected number nodulated for sterilized positive reference plants, non-sterile reference plants, sterile plants with crushed fruit inoculation, sterile plants with AFCD inoculation, and sterile plants with CVWO inoculation. A least squares post-hoc test in SAS 9.1 was used to determine significant differences between groups.

Results

In total, 132 *Morella cerifera* seedlings were grown and analyzed, 51 (39%) of which developed root nodules in response to *Frankia* infection. Visibly apparent signs of nitrogen deficiency, particularly yellowed leaves and comparatively small size, were apparent in many seedlings which were not nodulated. Therefore, because not all uninfected seedlings displayed visible indications of N deficiency, this was not a reliable basis for determination of infection, and root masses of all seedlings were examined thoroughly for nodules.

Nodulated seedlings were present in all groups tested (Figure 3), and a chi-square value of 23.7826 (p=0.0002) for all seedlings indicated significant differences among groups. Post-hoc tests confirmed specific differences between groups, which are displayed in Table 1. Percentages of nodulated seedlings differed significantly from expected values for all groups except positive reference (x^2=1.10, p=0.2949) and crushed fruit (x^2=1.10, p=0.2949) seedlings, showing 50% and 25% nodule development, respectively.

Surface-sterilized negative reference plants (x^2=48.15, $p < 0.0001$) and AFCD feces plants (x^2=0.4.39, $p = 0.0362$) demonstrated significantly less nodulation than expected, at 28% and 19%, respectively. 88% of seedlings which were not surface-sterilized developed nodules, showing significantly greater incidence than expected (x^2=4.95, $p = 0.0260$). 40% of plants inoculated with CVWO fecal material developed nodules, demonstrating significantly more successful infections than expected (x^2=15.43, $p < 0.0001$).

For both islands, AFCDs at primary dune sites showed consistently greater seed and fecal matter content than those erected at other sites, particularly during and after the autumn migratory period. Percentages of successful inoculation by AFCD materials from Hog Island and Smith Island primary dunes were 33% and 25%, respectively. Marsh and secondary dune site AFCDs of Hog Island also exhibited notable fecal matter and seed input, but, as tested, resulted in zero percent nodulation of seedlings.

At the Coastal Virginia Wildlife Observatory, 213 individual birds belonging to 32 species provided fecal materials for experimental inoculation (Fig. 4). Thirty samples, representing 85 individuals of 20 species, were used for the *Frankia* bioassay. Where necessary, individual samples were pooled by species. Positive infection occurred for twelve seedlings exposed to CVWO fecal materials representing 39 birds of ten species (Table 2). Feces of four warbler species (American Redstart, Northern Waterthrush, Pine Warbler, Yellow-rumped Warbler), three sparrow species (Slate-colored Junco, Song Sparrow, Swamp Sparrow), two wren species (House Wren, Winter Wren), and one nuthatch (Red-breasted Nuthatch) resulted in nodulation.

Discussion

Results supported the hypothesis that *Frankia* propagules are transported to barrier island soils by avian consumption and defecation. Fecal samples collected directly from birds were more likely to nodulate sterile *Morella cerifera* seedlings, but feces from field site collectors resulted in successful nodulations as well. While avian transport may not to be the dominant mode of dispersal at small scales, bird dispersal in this environment appears to be viable, and likely provides an important mechanism for initial island colonization by *Frankia*, as well as *M. cerifera*.

Establishment and expansion of woody species are critical stages of successional processes in environments around the world. In coastal environments, as well as others in which soil nutrients are severely limited, species that associate with symbiotic nitrogen-fixing bacteria may advance succession, conditioning soil by providing an autochthonous nitrogen input through root exudates and tissue senescence. Crucial to the establishment of these species in such challenging habitats is the prior presence of the symbiont in the soil; only if infection occurs shortly after germination will the plant survive. While the importance of these species to community succession has been appreciated, the mechanism or mechanisms of the bacteria's arrival in advance of host plants have not been well established. Using a bioassay technique, I tested passerine fecal materials for the presence of infective, effective *Frankia* propagules.

For sterilized positive and negative reference seedlings, I expected nodulation to approach 100% and 0%, respectively. Seedlings inoculated with fecal matter were expected to show significant nodulation percentages. However, I anticipated fewer successful nodulations by seedlings treated with AFCD material than those treated with CVWO samples. In contrast to samples collected directly from birds at the banding station, samples collected in AFCDs were exposed to potentially damaging environmental conditions in the field. Between sterilized seedlings inoculated with crushed fruit and non-sterilized reference seedlings, I anticipated similar nodulation rates. However, quite unexpectedly, non-sterile seedlings demonstrated the highest percent nodulation, significantly higher even than positive reference seedlings. More surprisingly, only 50% of positive reference seedlings developed nodules, despite a manner of treatment which should have ensured more than adequate exposure to *Frankia* propagules. The results demonstrated a far greater nodulation rate in the single group of seedlings which remained unsterilized, suggesting that viable *Frankia* cells persist either on or within some fruits or seeds of *M. cerifera*, but that these propagules were destroyed by the sterilization procedure applied to other groups of seedlings.

As anticipated, fecal material from the Kiptopeke banding station (CVWO) resulted in greater incidence of nodulation than those collected with AFCDs. However, seedlings inoculated with fecal matter collected from AFCDs exhibited the lowest rate of inoculation of all groups, including both negative reference and non-sterilized reference plants. Even this low percentage, however, indicates that viable, infective *Frankia* can exist in passively-collected field samples, particularly when considered alongside seedlings treated with CVWO materials. Other factors may have contributed to the low number of successful inoculations in samples treated with AFCD feces. Because samples collected in AFCDs were subject to destructive environmental conditions such as desiccation, intense sunlight, and saline sea spray, infective material in these samples was expected to be relatively low, compared to fecal matter collected directly from birds. Additionally, because neither continuous monitoring nor immediate collection after each use of all AFCDs was possible, no species identifications were possible using this passive technique, therefore only geographic identities for these samples are available.

As projected, collectors located on primary dune crests of both islands were consistently more successful at attracting birds than those placed at the marsh or secondary dune sites of Hog Island. Samples gathered from these primary crest locations were the only AFCD materials tested which resulted in nodulation. While this may indicate that those birds which are potential vectors of infective *Frankia* prefer near-shore shrub thickets as habitat or foraging grounds, it is also likely due to our selective preference for sites atop dunes. These sites, located close to shrub thickets, were considered more likely to be favored by passerines, and to result in greater visitation by these birds. Similarly, over the study period, successful inoculation by AFCD materials increased for samples from September and November. This increase may be attributable to greater numbers of birds and species present on the VCR islands during the autumn migration, but time between retrievals of collected material also increased during this period. Therefore, although the likelihood of visitation by birds carrying *Frankia* may have increased during this late period, the increase in delay between collection of samples would suggest that the collectors were more likely to contain feces from multiple individuals and species.

Thirty CVWO specimens, representing twenty species, were used for experimental treatments. Species whose feces resulted in successful nodulation were four warblers, three sparrows, two wrens, and one nuthatch. It should be noted that sample sizes for the majority of these species were relatively small, some consisting of only one individual. Species chosen for inclusion as experimental treatments were those which were found to be most conspicuously present at the CVWO as well as on the barrier islands. Yellow-rumped (myrtle) warblers, in particular, are present in great numbers on the Virginia barrier islands, and are voracious consumers of the fruits of *M. cerifera* (Borgmann et al., 2004). Because of their preference for *M. cerifera* fruits, and consequent prevalence in the shrub thickets of Hog Island, these birds were considered a likely potential vector for the co-dispersal of *M. cerifera* fruits and *Frankia* propagules. No observable relationship was found to exist between likelihood of nodulation and either migratory status or dietary category; nearly all bird species tested were migratory and omnivorous, with preference for insects and invertebrates. However, the results of AFCD and CVWO treatments do indicate that passerines are capable of serving as vectors for the movement of *Frankia*.

As members of all groups of both experimental and reference seedlings formed nodules, our evidence suggests that *Frankia* may in fact be dispersed from the adult shrub in tandem with seeds of *M. cerifera*. While the concept of seed transmission of bacteria is not novel, it generally has been applied within the context of disease and plant pathogens (Baker and Smith, 1966; Guo et al., 2001; Samish et al., 2006). There is also evidence that the symbiotic diazotroph *Rhizobia* may exist within stem vascular tissue of some graminoid species (Rosenblueth and Martínez-Romero, 2006; Roesch et al., 2008). Further study is necessary to determine whether the systemic transport of *Frankia* through a woody plant's vasculature to its developing fruits is possible. Future studies will indicate whether *Frankia* is indeed found on or within healthy, intact fruits or seeds of *M. cerifera*.

Both in field conditions and in lab experiments, external factors exist which, either alone or in concert, may act to limit survival of *M. cerifera* or *Frankia*, to delay or prevent aspects of the symbiont's infection process, or to minimize the actual physical manifestation of root hair infection. In addition, potentially significant sources of error existed in the methods of this experiment, including cross-contamination of seedlings and time or temperature-related loss of viable *Frankia* in samples. Some experimental materials, particularly the sand, deionized water, and Perlite used for seedlings, were presumed to be sterile. Of particular concern is the store-bought sand used here, which was naturally sourced. Depending on the actual site from which this sand was removed, *Frankia* propagules may have been present, as in the sandy soil of the VCR islands. Also complicating data analysis were the small sample sizes. Due to experimental limitations (e.g. environmental chamber size, time constraints, seedling mortality), sizes were limited.

While success of *M. cerifera* is dependent upon infection by *Frankia*, the mode or modes of dispersal across the long distances necessary for colonization across a barrier island landscape remain inconclusively determined. Promising evidence for bacterial movement via bird vectors exists, as birds may ingest *Frankia* together with soil particles and soil invertebrates such as earthworms (Reddell and Spain, 1991; Paschke and Dawson, 1993; Burleigh and Dawson, 1995). Considering the importance of birds to plant community composition of these islands, along with the possibility that bacterial endophytes may become established or transported within plant tissues other than root nodules, there is reasonable expectation that co-dispersal of *M. cerifera* seeds and *Frankia* propagules may prove to be an important means of long-distance dispersal and successful colonization by these shrubs. Other actinorhizal plants, particularly those which are bird-dispersed, may employ avian vectors for their respective symbionts as well. Dispersal modes of *Frankia* deserve further attention, as incidence of woody plant expansion and invasion may increase in an environment of elevated atmospheric CO_2, and effective co-dispersal of *Frankia* with seeds is even more critical to plants without the capacity to associate with multiple strains of the bacteria.

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Table 1: Reference and experimental seedling groups, total number seedlings analyzed, and expected and observed nodulation values. Chi-square and *p* values are also listed.

	Total seedlings	Number (percent) nodulated			
		Expected	Observed	x^2	p
+R	8	3 (39%)	4 (50%)	1.10	0.2949
-R	53	20 (39%)	15 (28%)	48.15	< 0.0001
non-sterile	17	7 (39%)	15 (88%)	4.95	0.0260
crushed fruit	8	3 (39%)	2 (25%)	1.10	0.2949
AFCD	16	6 (39%)	3 (19%)	4.39	0.0362
CVWO	30	12 (39%)	12 (40%)	15.43	< 0.0001

Table 2: Results of *Frankia* bioassay of Coastal Virginia Wildlife Observatory fecal material. Samples were pooled by species, and introduced to roots of one or more sterile *Morella cerifera* seedlings.

Bird Species	Samples pooled	Seedlings tested	Seedlings nodulated
American Redstart	9	3	1
American Robin	1	1	0
Black-and-white Warbler	1	1	0
Black-throated Blue Warbler	1	1	0
Common Yellowthroat	2	1	0
Gray-cheeked Thrush	1	1	0
Gray Catbird	7	2	0
House Wren	7	2	1
Northern Mockingbird	1	1	0
Northern Waterthrush	1	1	1
Pine Warbler	1	1	1
Red-breasted Nuthatch	1	1	1
Slate-colored Junco	1	1	1
Song Sparrow	3	1	1
Swamp Sparrow	9	3	2
Veery	1	1	0
Winter Wren	2	2	2
Western Palm Warbler	6	3	0
White-throated Sparrow	2	1	0
Yellow-rumped Warbler	28	2	1

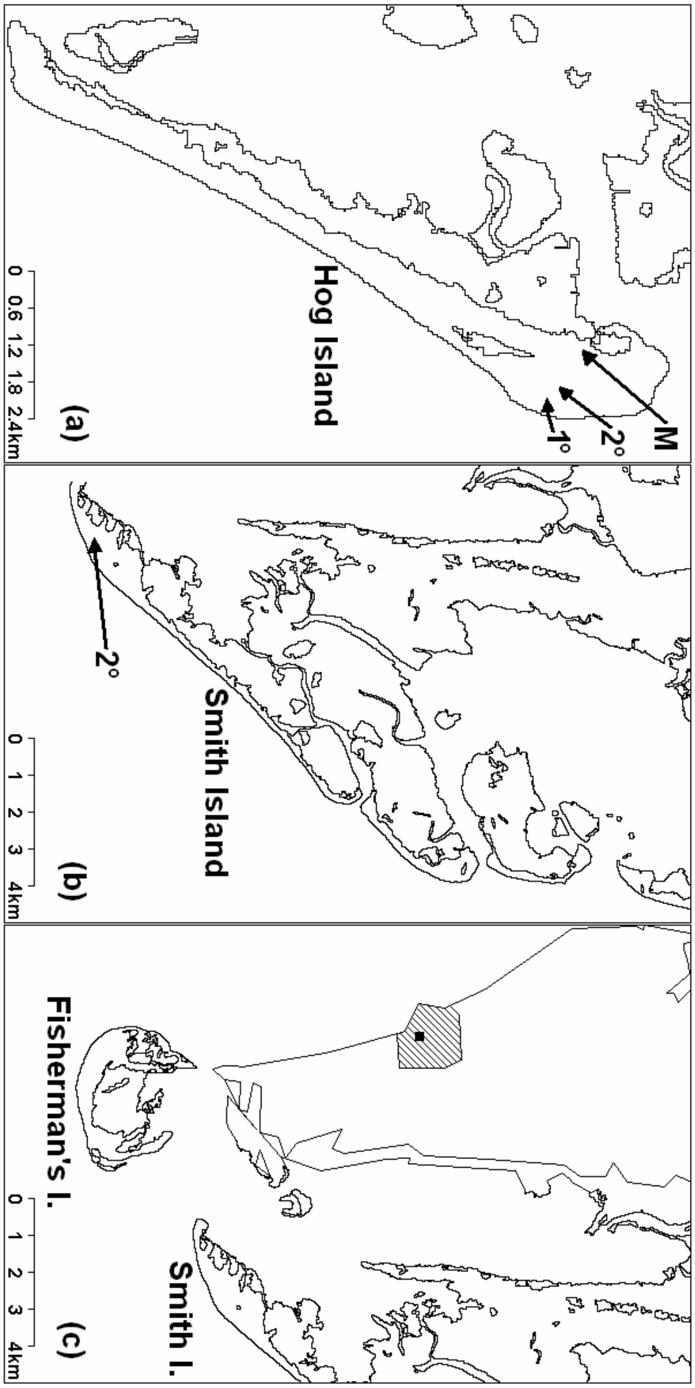
Figure Legends

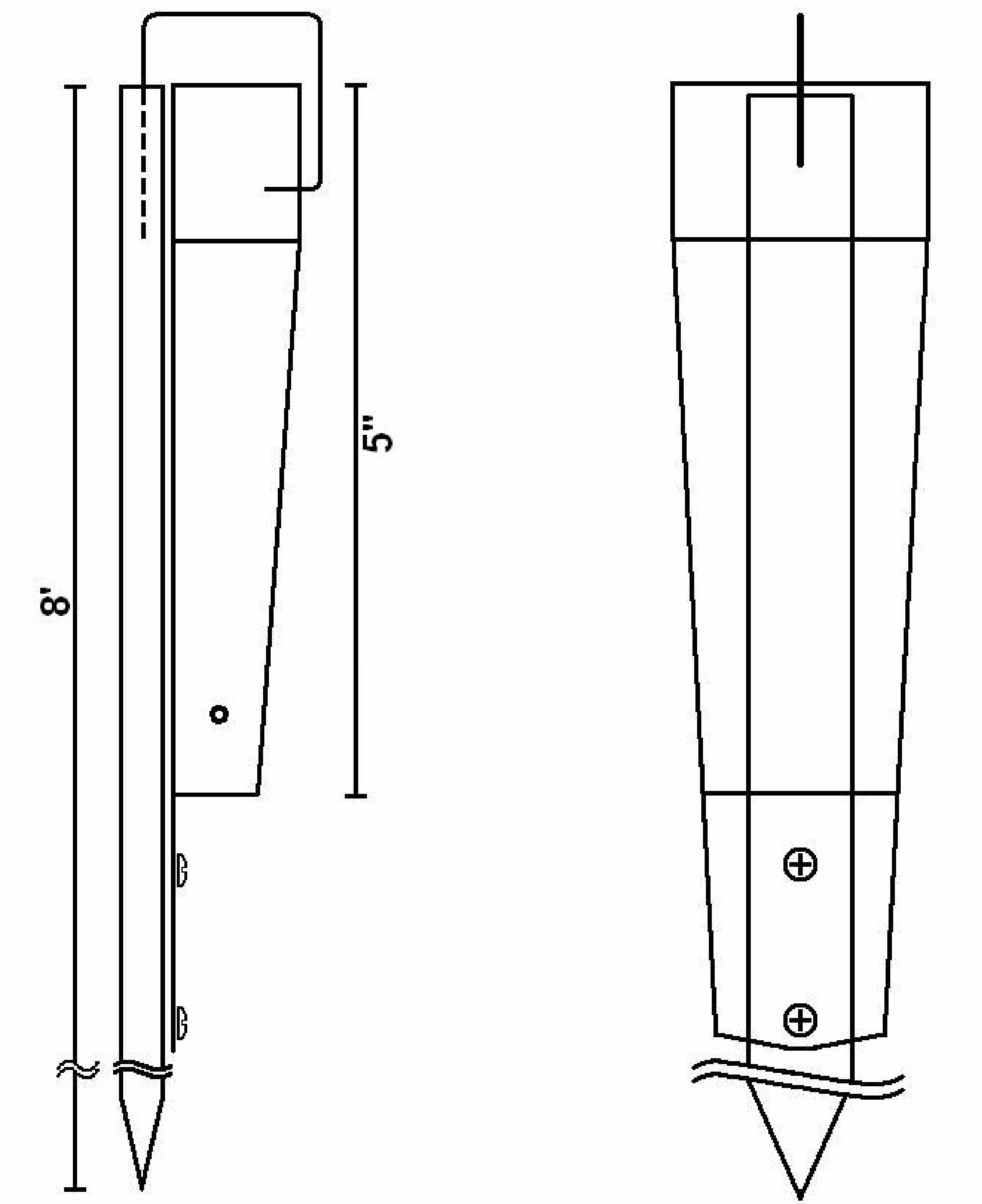
Figure 1. Site locations at the Virginia Coast Reserve LTER site. Hog Island (a) sites are marsh (M), secondary dunes (2°), and primary dunes (1°). Smith Island (b) sites are secondary dunes (2°). On the mainland peninsula, birds were sampled at the Coastal Virginia Wildlife Observatory ■ , within Kiptopeke State Park ▨ (c).

Figure 2. Schematic representation of avian fecal collection devices used for field sampling.

Figure 3. Percent nodulation for all reference and experimental seedling groups. Each column is labeled with number nodulated / total seedlings analyzed for that group.

Figure 4. Total number of fecal samples collected from each species during sampling at the Coastal Virginia Wildlife Observatory. Standard four-letter alpha codes are used.


M
2°
1°
Hog Island
0 0.6 1.2 1.8 2.4km
(a)
2°
Smith Island
0 1 2 3 4km
(b)
Smith I.
Fisherman's I.
0 1 2 3 4km
(c)


8'
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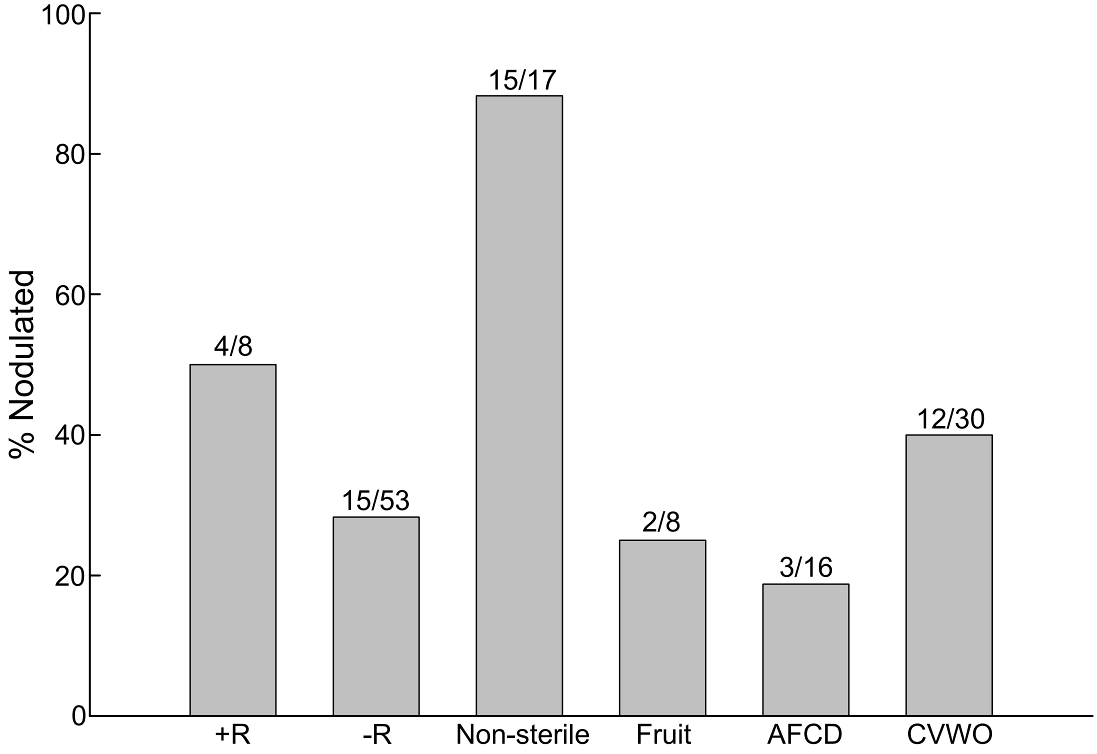
% Nodulated
100
80
60
40
20
0
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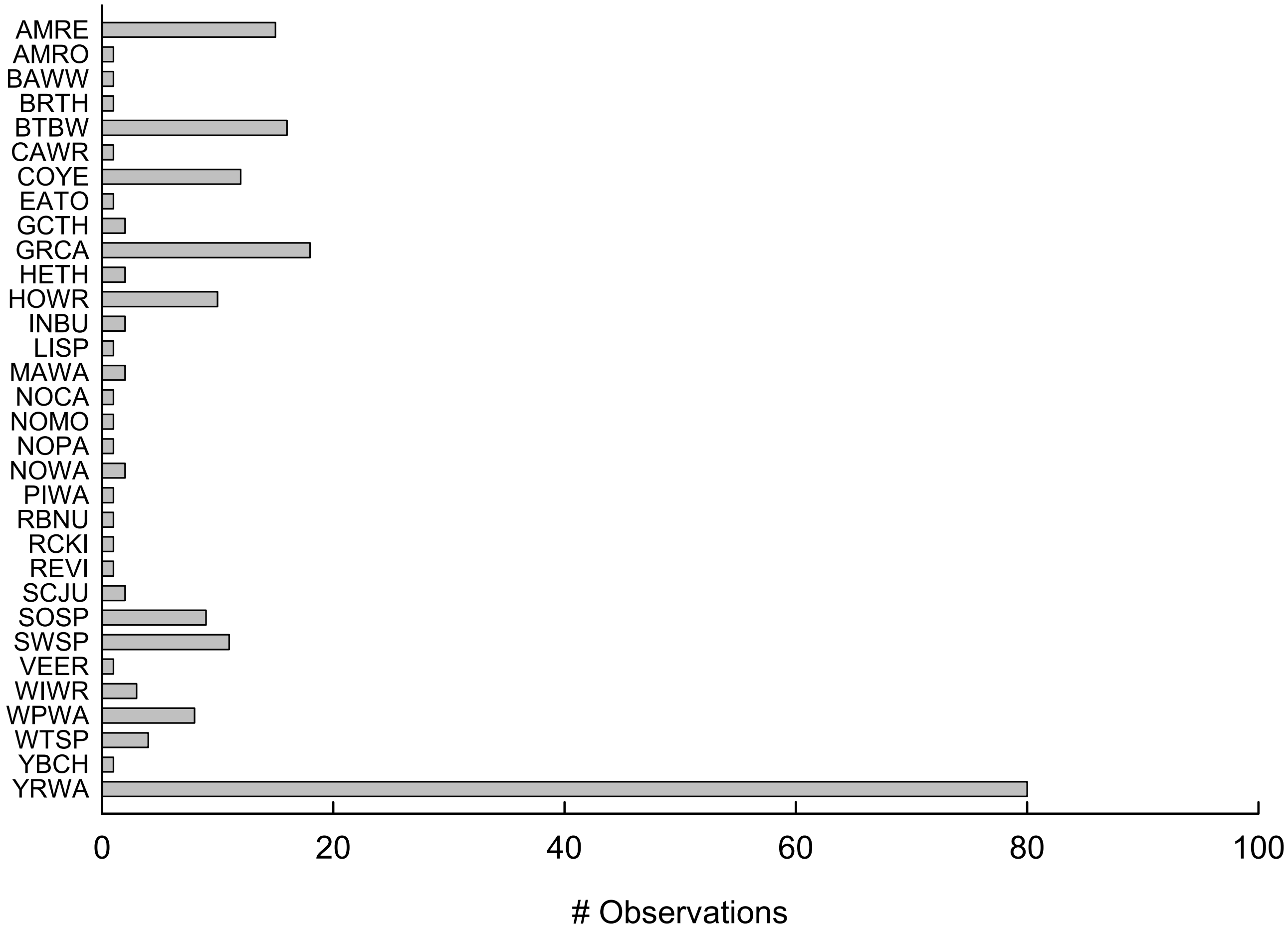
15/53
15/17
2/8
3/16
12/30
+R
-R
Non-sterile
Fruit
AFCD
CVWO


AMRE
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COYE
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GCTH
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NOCA
NOMO
NOPA
NOWA
PIWA
RBNU
RCKI
REVI
SCJU
SOSP
SWSP
VEER
WIWR
WPWA
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0
20
40
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Observations

VITA

Spencer Nathaniel Bissett was born and raised in Rockbridge County, Virginia. His interest in the sciences initially manifested itself as an ongoing search for climbable trees, aquatic invertebrates, and gross things. As a perpetually grass-stained youth, Spencer showed an interest in plants and gardening, and in sixth grade he was enrolled in an extra-curricular horticulture course; here he was introduced to formal plant identification and landscape design. Prior to ninth grade, Spencer participated in Virginia's Field Biology Governor's School, and after graduating with honors from Rockbridge County High School, he attended Wake Forest University and majored in biology. Excited by an introductory ecology class with Dr. William K. Smith, Spencer entered Dr. Smith's upper-level Plant Physiological Ecology course, and also worked as a research assistant and technician in the Smith laboratory, both before and after graduating. Time spent in the lab and field with Dr. Smith and his students led Spencer to pursue a Master's degree in plant physiological ecology, and Dr. Don Young accepted Spencer into his Coastal Plant Ecology Lab at Virginia Commonwealth University. After a summer spent studying pollination ecology of two plant species at Florida's Archbold Biological Station, Spencer entered VCU intending to pursue studies of plant-insect interactions; naturally, this goal led to extensive collection of bird feces. While concluding this M.S. project, Spencer entered the Integrative Life Sciences Ph.D. program at VCU in 2008, and looks forward to research with a bit more emphasis on plant physiological ecology.