ERDC/CERL TR-00-42

Construction Engineering Research Laboratory

US Army Corps of Engineers®
Engineer Research and Development Center

Reproductive Ecology of the Sandhills Pyxie Moss (*Pyxidanthera barbulata* var. *brevifolia*) in North Carolina

Ann-Marie Shapiro, Jeff Zimpfer, and Moni Bates

December 2000

ERDC/CERL TR-00-42

US Army Corps of Engineers®
Engineer Research and Development Center

Reproductive Ecology of the Sandhills Pyxie Moss (*Pyxidanthera barbulata* var. *brevifolia*) in North Carolina

Ann-Marie Shapiro, Jeff Zimpfer, and Moni Bates

December 2000

Construction Engineering Research Laboratory

Foreword

This study was conducted for U.S. Army Corps of Engineers under Military Interdepartmental Purchase Request W31RY081386852/PO, work unit PT8, "Managing At-Risk Species in the Southeastern Longleaf Pine Ecosystem," and the Conservation Assistance Program (CAP) under MIPR0A48R00024, work unit RF0, "Pyxie Moss Habitat Characterization." The technical monitor was Alison Dalsimer, ODUSD(ES) EQ-LP.

The work was performed by the Ecological Processes Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Principal Investigator was Ann-Marie Shapiro. Jeff Zimpfer is a student research assistant with the Department of Forestry, University of Illinois at Urbana-Champaign. Moni Bates, a botanist and plant ecologist, is a contractor from Summerfield, NC. The technical editor was Linda L. Wheatley, Information Technology Laboratory. Stephen Hodapp is Chief, CN-N, and Dr. John T. Bandy is Chief, CN. The associated Technical Director was Dr. William D. Severinghaus, CVT. The Acting Director of CERL is William D. Goran.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Director of ERDC is Dr. James R. Houston and the Commander is COL James S. Weller.

Contents

List of Tables and Figures

Tables

Figures

1 Introduction

Background

Sandhills pyxie moss (*Pyxidanthera barbulata* var. *brevifolia*) is a creeping evergreen sub-shrub that grows in dense mats resembling moss on the floor of sparsely wooded, xeric sandhill communities. The plant blooms during February and March, displaying a dense mat of white flowers. Each flower is 5-6 mm across, with an upright, bell-shaped corolla (Russo et al. 1993). Although the species produces seed, there are no documented cases of individuals of pyxie moss becoming established through sexual reproduction.

Sandhills pyxie moss is a Federal Species of Concern (FSC), and is listed as Endangered in the state of North Carolina. Currently, legal land-use restrictions due to the presence of this species are not imposed on the Army. Army conservation policy states, however, that installations will not jeopardize the continued existence nor take actions that will lead to Federal listing of the species. Since sandhills pyxie moss is widespread across Fort Bragg, the species is important to future land-use flexibility on the installation. The Army and Fort Bragg have an opportunity to contribute ecological information about this little-studied species and identify any measures needed for long-term conservation.

Objectives

The objective of our research was to assist Fort Bragg to continue its conservation of the sandhills pyxie moss and prevent its Federal listing under the Endangered Species Act, with the ultimate goal of supporting the military mission of the U.S. Army.

Approach

Three basic research questions were pursued related to the reproductive biology of the sandhills pyxie moss. This research (1) described the breeding system of the sandhills pyxie moss, (2) assessed the existence and mechanisms of successful pollination in natural populations, and (3) tested the ability of the species to

germinate under experimental conditions. The implications for management of pyxie moss and its habitat are discussed where relevant.

Mode of Technology Transfer

Technology transfer of this research will be through publication of a journal article in the year 2001.

2 Materials and Methods

Fort Bragg, North Carolina

Fort Bragg is within the Coastal Plain physiographic province, in the Sandhills Region of North Carolina. The fort occupies portions of Cumberland, Harnett, Hoke, and Moore counties within a total of 58,054 hectares.

Fort Bragg is dominated by longleaf pine/wiregrass ecological communities that form a distinctive two-layer environment. Longleaf pine is the dominant canopy species, while wiregrass dominates the herbaceous layer. Pyxie moss is found within two such plant communities on Fort Bragg: xeric sandhills scrub and pine/scrub oak sandhills.

Assessment of Breeding System

To determine the breeding system of the Sandhills pyxie moss, researchers measured capsule production under each of the following breeding systems: autogamy (self-fertilization within a single flower), geitonogamy (self-fertilization between two or more flowers), and xenogamy (cross fertilization between different plants). Experiments were conducted in March 1999.

Potential study sites were selected by avoiding pyxie moss populations likely to experience mechanized or frequent military training events. The study was conducted within two large populations in the southwest corner of Fort Bragg: North Carolina Natural Heritage Program Element Occurrence (EO) 58 sub C, located next to firebreak 15, and EO 58 sub A, located next to firebreak 17, both near King Road. Treatments were assigned in a blocked random design (three flowers on a given individual pyxie moss were each assigned a different treatment). At both sites, 12 clumps of plants scattered throughout the populations were selected. All cages for all treatments consisted of fine-mesh mosquito netting and twist ties.

To test for autogamy, individual flowers were caged prior to anthesis and left undisturbed throughout the flowering season to determine if they could produce seed without the aid of pollinators. In the geitonogamy treatment, individual

flowers were caged before anthesis. Once their stigmas exerted and became receptive, the cages were removed and pollen was manually transferred with a dissecting needle from another flower to the receptive stigmas. The cages were then replaced to wait for capsule production. For the geitonogamy treatment, pollen was collected from a different flower on the same inflorescence within 6 cm of the experimental flower. Experimental flowers were hand-pollinated with pollen from another plant at least 3 m away to test for xenogamy. Since pollen is applied to the point of stigma saturation, this treatment also ensures that pollen availability is not limited, and thus it is also referred to as the enhanced outcrossing pollination treatment.

The experimental design included two control treatments. One control tested for the effect of pollinator excluder cages on capsule production. This control is necessary to determine that the absence of capsule production in the autogamy and geitonogamy treatments is due to the exclusion of pollinators, and not from the cages interfering with capsule production. Flowers were again manually pollinated, and a cage was placed over each flower for the duration of the flowering season. Collected pollen came from flowers in clumps at least 3 m away from the control flower.

The second control included flowers that were left uncovered for natural pollination. A comparison of capsule production in the open, naturally pollinated control and the enhanced outcrossing treatment helped determine if pollinator availability limited capsule production.

The breeding system treatments were placed on flowers between 24 February and 22 March 1999. Every treatment was performed on each experimental plant clump. Final data was collected on 12 May 1999 after capsule formation was completed in the field. Only 3 flowers out of 120 were lost during the experiment: one autogamy-treated flower and two caged control flowers. Capsule production between different breeding system treatments was compared with chi-square tests.

Identification of Pollinators

Pollinators were sought on 10 sites with 3 different burn histories — those last burned in 1996, 1997, and 1998. The incorporation of three different burn histories allowed the return patterns to be ascertained for potential pyxie moss pollinators following fire. Three populations in sites 57C and B were burned in 1996. The three populations in 58A, C, and D burned in 1997. Due to reduced and delayed flowering in the 1998 burn sites, four sites were selected: 93D, 93C, 16,

and 33A. A total of 206 pollinator observation sessions were conducted across all sites: 70 were observed in 1996 burn sites; 88 in 1997 burned areas; and 48 in those burned in 1998. Pollinator observations from 1998 are fewer as a result of reduced flowering in those sites. Each observation session lasted 10 minutes, for a total of 2,060 minutes. Sessions were conducted between 25 February and 8 April 1999. The following information was recorded during each observation session:

- site number and year of last burn
- date and time
- temperature (not recorded for every observation)
- presence of flower and plant visitors (by species)
- presence of pollinators (by species)

The presence of pollinator species was recorded instead of species frequency because of the difficulty of field identification and lack of multiple visits by individual species. Only ant pollinators were frequently noted in multiple numbers during an observation. Since the showy mass of white pyxie moss flowers could potentially attract nocturnal pollinators, evening observations were conducted. Thirty-eight 10-minute observations were made at each site between the hours of 1900 and 2242.

Insects observed visiting or probing pyxie moss plants were captured for identification. Each collected insect was viewed under a dissecting microscope to determine the presence or absence of sandhills pyxie moss pollen and then identified by Ken Ahlstrom at the North Carolina Department of Agriculture in Raleigh.

Plant visitors, flower visitors, and confirmed pollinators were identified and recorded. Simple linear regression analyses were performed to test whether different groups of pollinators varied through time or with increasing temperatures. Difference among different pollinator groups across sites with different burn histories was tested with chi-square analyses.

Seed Germination Experiments

Seed collected at Fort Bragg, both from the seed bank and from reproductive plants, was used in manipulative experiments in greenhouse facilities at the University of Illinois at Urbana-Champaign (UIUC) to examine factors related to seed viability and germination.

As much open-pollinated seed as possible was collected from pyxie moss plants on Fort Bragg during the third week of May 1999. This seed was sent to the UIUC greenhouse, where the germination experiments were conducted. A total of 1,540 seeds was divided into four treatments. One-half of the seeds were soaked in 21 °C water for 15 minutes, while the other half were soaked in 65 °C water for 15 minutes. Half of the seeds from each water treatment were then placed into a 4 °C cooler for 6 weeks, followed by growth under fluorescent growth lights for 12 weeks. The other half of the seeds from each water treatment were placed directly under fluorescent growth lights for 12 weeks. A total of 385 seeds underwent each of the four treatment combinations. Seed bank excavations were taken in July 1999 from three sites: 017G, 057M, and 033E. Within each site, the seed banks surrounding three robust, reproductively active pyxie moss individuals without close (within 2 m) neighbors were sampled using a bulb planter. Cylindrical cores were taken at the immediate edge of each plant, and at 50-cm and 100-cm distances from the edge, along a transect line, for a total of 3 excavations per plant and 27 samples total. Each excavation removed a sample of soil that was approximately 10 cm in diameter and 10 cm deep. Samples were transported to the UIUC greenhouse facility.

In the greenhouse, each sample was split in half and assigned to one of two treatments. Each was mixed thoroughly, moistened, and placed into clear 20 x 20 x 10 cm plastic boxes. The bottoms of the boxes were lined with four layers of institutional paper towels to retain moisture. The soil samples were spread out evenly over the bottom of the boxes. The depth of the soil in each box was approximately 1 cm to maximize exposure to light. Half of the boxes were placed into a 4 °C cooler for 6 weeks and then placed in the greenhouse at 23 °C with supplemental growth lights for 12 weeks. The other half of the samples was placed immediately into the greenhouse at 23 °C with supplemental growth lights for 12 weeks ("no cooler" treatment). While in the greenhouse, the photoperiod was extended to 16 hours by using 1,000-watt Sylvania LU lamps.

3 Results

Assessment of Breeding System

Overall, sandhills pyxie moss produces few capsules in response to pollination, regardless of pollen source (Table 1). Sandhills pyxie moss is not self-compatible. Only the controls and xenogamy treatments produced capsules in the breeding system study (Table 1). None of the autogamy or geitonogamy treatments produced capsules, indicating that pyxie moss relies on pollinators for successful fertilization. The caged control flowers produced six capsules, indicating the cages did not interfere with capsule production.

Preliminary data were also found to suggest that pollinator availability does not limit capsule production in the study populations. Although overall capsule production was extremely low in both treatments, capsule production in the xenogamy treatment (enhanced outcrossed flowers) was not significantly different from natural, open-pollination control treatment (chi-square=0.76; $p>0.05$). Additional data will be collected in 2001 to further examine this initial conclusion.

Table 1. Capsule production by treatment.

Treatment	Number of Flowers Capsules Present	Number of Flowers Capsules Absent	Total Treated Flowers
Autogamy (Self-fertilization)	0	23	23
Geitonogamy (Fertilization from different flower on same plant)	0	24	24
Bag Effect (Control)	6	16	22
Xenogamy (Enhanced Outcrossed)	2	22	24
Natural Open Pollination (Control)	4	20	24

Identification of Pollinators

Plant visitors are those insects that were observed on the plants' foliage but did not probe the flowers. Six insect species in this category were observed. Plant visitors included flies, ants, and one species of katydid. Two species were small flies (Diptera): Phoridae *Megaselia* sp. and Mycetophilidae *Boletina* sp. The former was observed on a corolla with its wings folded back; the latter was observed on a flower capsule. Three ant species were identified — *Formica* sp., *Aphaenogaster* sp., and *Crematogaster* sp. There was one possible flower probe by *Formica* sp.; however, no pollen was observed on its body.

The katydid, Orthopera: Tettigoniidae *Atlanticus* sp. was observed eating the anthers and petals of pyxie moss, and carrying spent corollas. However, no pollen was found on katydids during field observations or on collected specimens.

Insects observed probing flowers but not transferring pollen were considered to be flower visitors. Fifteen insect species were identified as flower visitors on pyxie moss (Table 2). Most species were small and entered the corolla without touching the anthers. On numerous occasions, *Hylemya platura* (seed corn maggot) were seen probing flowers, but pollen was absent from all specimens.

Thirteen species of insects were observed visiting pyxie moss flowers and carrying pollen grains on their bodies. Pyxie moss pollinators include five Diptera species (flies), two species of Hymenoptera (Formicidae – ants), and six Hymenoptera species other than Formicidae (bees/wasps). One yellow butterfly was observed nectaring on pyxie moss; however, capture was unsuccessful and this species is unconfirmed as a pollinator. A hand lens was used to observe ant pollinators triggering the anther beaks, which released pollen onto their bodies (as noted by Beal 1878). Depending on their body orientation, they collected pollen on either their ventral or dorsal surface. If their dorsal surface triggered the anther, then their ventral surface was in position to contact the stigma. Pollen grains were also attached to their heads and legs. *Emphoropsis laboriosa laboriosa* individuals were noted with pollen on their proboscises, heads, ventral and dorsal surfaces, and legs, but mostly on their ventral surface. Frequently, it was necessary to capture several individuals of one species before pollen grains were observed, confirming the species as a pollinator. Multiple captures were not possible for every species observed on the pyxie moss plants, so documented flower visitors were considered to be potential pollinators.

Table 2. List of flower visitors, plant visitors, and pollinators.

Species Name	Behavioral Category
Diptera: Syrphidae *Toxomerus marginata* (Say)	Flower visitor
Diptera: Syrphidae *Toxomerus graminatus* (Say)	Flower visitor
Diptera: Empidae *Hilara baculifer* (Melander)	Flower visitor
Diptera: Calliphoridae *Calliphora vomitoria* (Linnaeus)	Pollinator
Diptera: Calliphoridae *Phaenicia* sp.	Pollinator
Diptera: Phoridae *Megaselia* sp.	Plant visitor
Diptera: Phoridae *Dohrniphora* sp.	Flower visitor
Diptera: Tachinidae *Gonia* sp.	Pollinator
Diptera: Tachinidae *Jurinia* sp.	Pollinator
Diptera: Tachinidae *Catharosia nebulosa* (Coquillett)	Pollinator
Diptera: Mycetophilidae *Boletina* sp.	Plant visitor
Diptera: Milichidae *Leptometopa latipes* (Meigen)	Flower visitor
Diptera: Anthomyiidae *Hylemya platura* (Meigen) Seed corn maggot	Flower visitor
Orthoptera: Tettigoniidae *Atlanticus* sp.	Plant visitor - suspected this insect of eating anthers
Hymenoptera: Formicidae *Formica* sp.	Plant visitor *
Hymenoptera: Formicidae *Monomorium minutum* (Buckley)	Flower visitor
Hymenoptera: Formicidae *Tapinoma* sp.	Flower visitor
Hymenoptera: Formicidae *Linepithema humilis* (Mayr) Argentine ant	Pollinator
Hymenoptera: Formicidae *Aphaenogaster* sp.	Plant visitor *
Hymenoptera: Formicidae *Crematogaster* sp.	Plant visitor
Hymenoptera: Formicidae *Prenolepis imparis* (Say)	Pollinator
Hymenoptera: Eupelmidae *Calosota metallica* (Gahau)	Flower visitor
Hymenoptera: Scoliidae *Campsomeris (Dielis) plumipes fossulana* (Fabricius)	Pollinator
Hymenoptera: Halictidae *Halictus* sp.	Pollinator

Species Name	Behavioral Category
Hymenoptera: Halictidae *Augochlorella aurata* (Smith)	Pollinator
Hymenoptera: Halictidae *Sphecodes* sp.	Flower visitor
Hymenoptera: Andrenidae *Andrena* sp.	Flower visitor
Hymenoptera: Anthophoridae *Emphoropsis laboriosa laboriosa* (Fabricuis)	Pollinator
Hymenoptera: Apidae *Apis mellifera* (Linnaeus)	Pollinator
Hymenoptera: Colletidae *Colletes*sp.	Pollinator
Hymenoptera: Chalcididae *Conura* sp.	Flower visitor
Lepidoptera: Lycainidae *Incisalia augustus* (Kirby) Brown elfin	Flower visitor
Lepidoptera: Gelechidae *Aristotelia roseosuffusella* (Clemens)	Flower visitor
Hemiptera: Reduviidae *Zelus (Zelus) cervicalis* (Stal)	Plant visitor
* Only one specimen seen and collected.	

The Pollination Ecology of Sandhills Pyxie Moss

Insect size appears important for successful triggering of the anther beaks and release of pollen. In general, medium to large-sized insects trigger the anthers while probing flowers. Small ants like *Tapinoma* sp. and *Monomoruim minutum* could crawl between the anthers without triggering the beaks. A small fly, *Leptometopa latipes*, was witnessed probing its head and body into corollas; however, no pollen was seen on its body. *Calosota metallica*, a tiny parasitic wasp, crawled into the corollas by entering between the anthers but avoided picking up pollen.

Most observed pollinators are 1 cm or more in length. All of the Diptera (fly) pollinators, *Calliphora vomitoria, Jurinia* sp., *Gonia* sp., and *Phaenicia* sp., are over 1 cm in length (only *Catharosia nebulosa* is not). Pollen was observed on the proboscis of *Phaenicia* sp. and *Jurnica* sp. Pollen was noted on the proboscis and ventral surface of *Gonia* sp. The smallest Diptera (fly) pollinator, *Catharosia nebulosa*, acquired pollen on its head and dorsal and ventral surfaces.

Of the six flying Hymenoptera pollinators, five are 1 cm or more in length. These include Scoliidae *Campsomeris (Dielis) plumipes fossulana*, Anthophoridae

Emphoropsis laboriosa laboriosa, Halicitdae *Augochlorella aurata*, Apidae *Apis mellifera*, and Colletidae *Colletes* sp. The smallest one is Halictidae *Halictus* sp. *Campsomeris (Dielis) plumipes fossulana*, a large wasp, accumulated pollen on both its ventral and dorsal surfaces. *Augochlorella aurata* collected pollen on the ventral surface of its head. *Apis mellifera* was observed with very few pollen grains on its body. *Colletes* sp. acquired pollen on its head and ventral surface while probing pyxie moss flowers.

Evening observations were performed because the showy mass of white flowers could potentially attract nocturnal pollinators. Pollinator species were observed visiting the flowers in 63 percent (24/38) of the nocturnal sessions. They were absent in 37 percent (14/38) of the sessions. Ant species were the only nocturnal pollinators. No pollinating flies or bees were observed during the night, even when temperatures were above 32 °C.

The presence of Hymenoptera (bee/wasp) pollinator species varied throughout the flowering season. A significant relationship exists between the presence of bee pollinators and date (Figure 1; F-ratio = 5.65; p = 0.02). This relationship is echoed by a similar response to temperature (Figure 2; F-ratio = 9.61; p = 0.002). Bee and wasp species were first recorded on 18 March when the temperature was 32 °C. No bee or wasp pollinators were present at temperatures below 32 °C. Therefore, bees and wasps served as pollinators from peak flowering to the end of the flowering period.

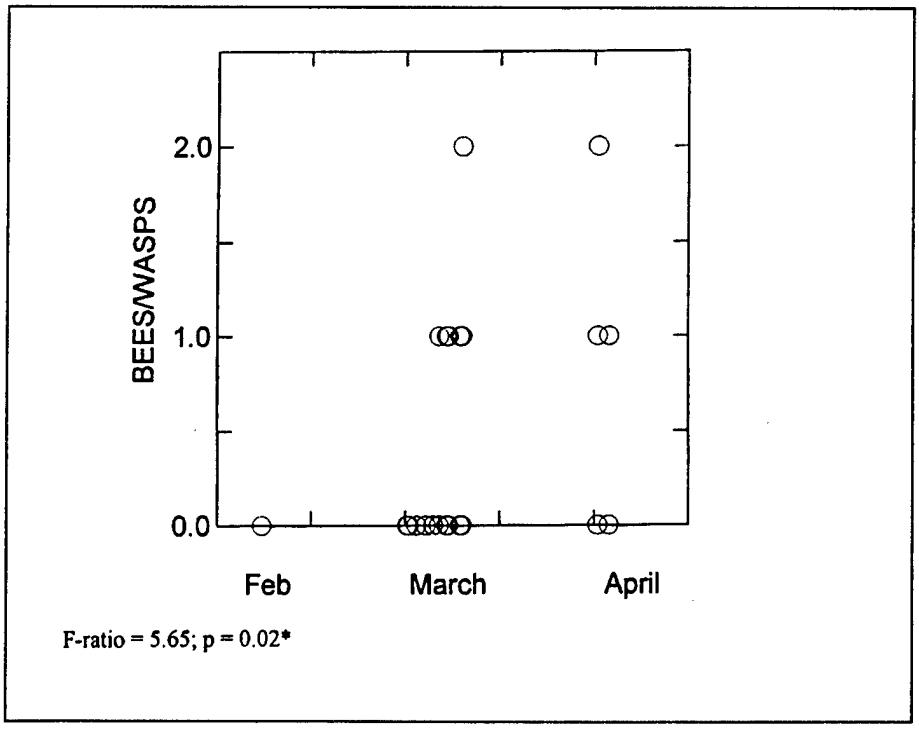

Figure 1. Bee/wasp pollinator occurrence related to date.

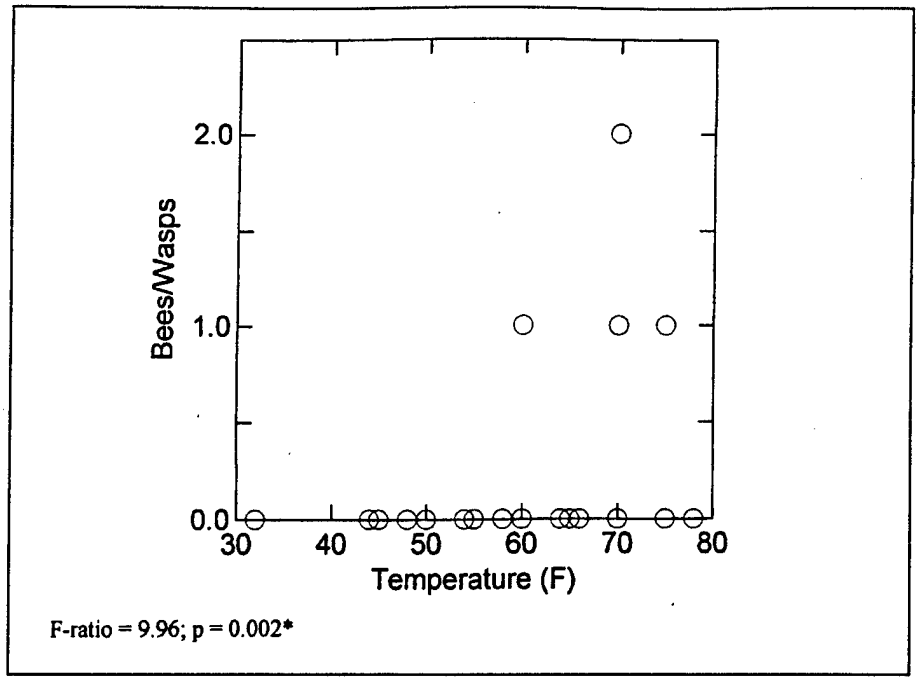

Figure 2. Bee/wasp pollinator occurrence related to temperature.

Diptera (fly) pollinators first appeared at flowers on 11 March, 2 weeks later than ant pollinators. No significant relationship exists between the presence of fly pollinators and date (F-ratio = 1.32; p = 0.25) nor temperature (F-ratio = 1.37; p = 0.24). Fly pollinators were active during the day, especially during the peak flowering period.

Ant pollinators occurred throughout the study periods from 24 February to 8 April. No significant relationship exists between the presence of ant pollinators and date (F-ratio = 0.00; p = 0.99) nor temperature (F-ratio = 0.18; p = 0.67). Only ant pollinators were active throughout the entire flowering period. They were present early in the season, during the day and at night, and when temperatures were low. The other pollinator species were absent during these periods of cooler temperatures.

Because different pollinator insects were present throughout the flowering period, no relationship exits between all pollinators combined and date (F-ratio = 2.51; p = 0.12) and temperature (F-ratio = 2.45; p = 0.12).

The three groups of pollinators did not show strong responses to fire history across the 10 study sites. In all cases, ant pollinators were more common than fly and bee/wasp pollinators regardless of burn history. The percent of observations where ant pollinators were present ranged from 53 percent in areas burned 3 years prior, 48 percent in those burned 2 years prior, and 69 percent in those

burned 1 year prior. In contrast, the percent of observations where fly pollinators were present ranged from 4 to 7 percent. Bees and wasps were present 9 to 15 percent of the time among the three burn years (Table 3).

The three pollinator groups were examined separately for response to fire history. The variation in presence of fly and bee/wasp pollinators among sites is very small and shows no response to burn histories (fly test statistic = 1.52; Table 4, and bee/wasp test statistic = 2.89; Table 5). The response to fire history by ant pollinators is borderline significant at the 0.05 level (test statistic = 5.65). The highest presence of ant pollinators occurred in sites burned 1 year (69 percent) and lowest in sites burned 2 years prior to study (48 percent; Table 6).

Table 3. Percentage and number of fly, ant, and bee/wasp pollinator species present during pollinator observation sessions in sites burned 1, 2, and 3 years prior to study.

Time Since Last Burn (# observations)	Chi-square test statistic and significance for rows	% of Sessions Fly Pollinators Present (recorded #)	% of Sessions Ant Pollinators Present (recorded #)	% of Sessions Bee/Wasp Pollinators Present (recorded #)
3 Years (N = 70)	187.3+	4 (3/70)	53 (37/70)	9 (6/70)
2 Years (N = 88)	55.0+	7 (6/88)	48 (42/88)	9 (8/88)
1 Year (N = 48)	58.5+	2 (1/48)	69 (33/48)	15 (7/48)
+ At the 0.01 level, the chi-square test statistic must be greater than 9.210 to be significant				

Table 4. Presence/absence of fly pollinators in sites burned 1, 2, and 3 years prior to study.

Time Since Last Burn (Sample Size)	# of Times that Fly Pollinators were Present	# of Times that Fly Pollinators were Absent
3 Years (N = 70)	3	67
2 Years (N = 88)	6	82
1 Year (N = 48)	1	47
At the 0.05 level, the chi-square test statistic must be greater than 5.991 to be significant Test Statistic = 1.52 (not significant)		

Table 5. Presence/absence of bee/wasp pollinators in sites burned 1, 2, and 3 years prior to study.

Time Since Last Burn (Sample Size)	# of Times that Bee/Wasp Pollinators were Present	# of Times that Bee/Wasp Pollinators were Absent
3 Years (N = 70)	6	64
2 Years (N = 88)	8	80
1 Year (N = 48)	7	41
At the 0.05 level, the chi-square test statistic must be greater than 5.991 to be significant Test Statistic = 2.89 (not significant)		

Table 6. Presence/absence of ant pollinators in sites burned 1, 2, and 3 years prior to study.

Time Since Last Burn (Sample Size)	# of Times that Ant Pollinators were Present	# of Times that Ant Pollinators were Absent
3 Years (N = 70)	37	33
2 Years (N = 88)	42	46
1 Year (N = 48)	33	15
At the 0.05 level, the chi-square test statistic must be greater than 5.991 to be significant Test Statistic = 5.65 (borderline significant)		

Seed Germination Experiments

None of the seeds collected from open-pollinated plants on Fort Bragg germinated under the experimental treatments during this study.

Seed bank samples that were placed directly into the greenhouse did demonstrate the presence of a modest seed bank. A total of 10 seedlings germinated from 9 soil samples taken at the edge of pyxie moss plants in the 3 sites. A total of 15 seedlings germinated from 9 soil samples taken 50 cm from the edge of pyxie moss plants in the 3 sites. Only one seedling emerged from soil collected 100 cm from pyxie moss plants (Table 7). Differences appear to exist in the viability of the seed bank among the three sites sampled, with 17G appearing to contain the most viable seed bank.

A similar germination pattern occurred with seed bank samples stored in a cooler before being placed in the greenhouse, again demonstrating a modest seed bank. A total of 17 seedlings germinated from 9 soil samples taken at the edge of pyxie moss plants in the three sites. A total of seven seedlings germinated from nine soil samples taken 50 cm from the edge of pyxie moss plants in the three sites. No seedlings emerged from soil collected 100 cm from pyxie moss plants (Table 8). Seeds collected in site 33E appeared to germinate at a higher rate following the cooler treatment, but this was not the case for sites 17G and 57M.

Table 7. Number of seeds germinated from pyxie moss seed bank no cooler treatments.

EO (site)	Distance from plant	# total seeds
017 G	edge	5
	50 cm	13
	100 cm	1
057 M	edge	1
	50 cm	2
	100 cm	0
033 E	edge	4
	50 cm	0
	100 cm	0

Table 8. Number of seeds germinated from pyxie moss seed bank with cooler treatments.

EO (site)	Distance from plant	# total seeds
017 G	edge	5
	50 cm	6
	100 cm	0
057 M	edge	0
	50 cm	0
	100 cm	0
033 E	edge	12
	50 cm	1
	100 cm	0

4 Discussion

This study indicates that sandhills pyxie moss is an obligate outcrosser that requires insect pollinators for successful fertilization, but that current pollinator activity results in capsule production equal to enhanced outcrossed treatments in the two populations examined. Of concern is that all capsule production was extremely low. However, at this time, no evidence shows that management of Fort Bragg natural resources, including an aggressive prescribed burning program, is placing important pyxie moss pollinators at risk or is contributing directly to low observed capsule production.

A total of 13 insect species were identified as pollinators. These insects belong to three groups: Diptera (five fly species); Hymenoptera: Formicidae (two ant species); and Hymenoptera (five bee and one wasp species). The presence of ant pollinators was significantly greater than flies and bees/wasps for all sites, regardless of time-since-last burned. Ant pollinators occurred from the beginning of the flowering period to the end, both day and night. Since ants were not limited by temperature, they served as effective pollinators during nocturnal and early flowering periods when temperatures were cool. Temperature appears to restrict the activities of bee and wasp pollinators during the flowering season of pyxie moss; these species serve to enhance daytime pollination from peak flowering (mid-March) to the end of the flowering season (first of April). It is concluded that two ant species: *Prenolipis imparis* (Say) and *Linepithema humilis* (Mayr) function as the major pollinators of sandhills pyxie moss.

Fire is a dominant process across the Fort Bragg landscape and could affect sexual reproduction in plants found in the understory of natural plant communities. This study showed that the presence of sandhills pyxie moss pollinators was not affected by "time since last burn" over the range of fire histories studied here (ranging from 3 years to less than 1 year since last burn).

However, as time was spent in other subpopulations on Fort Bragg, low levels of flowering effort were observed in some sites. One site that burned during the previous dormant season did not flower in 1999. Only 7 percent of all pyxie moss clumps located at another site, last burned in 1996, showed evidence of either flowers or capsules when we surveyed in June. It is possible that by the third year following a burn, the ground cover is thick enough to reduce pyxie moss

flowering due to shade. Such factors may or may not combine with other factors to limit sexual reproduction of pyxie moss on Fort Bragg but, at the very least, they have the potential to reduce the number of years a given individual of pyxie moss would reproduce sexually, at least in some sites. These observations suggest that longer-term study to specifically examine effects of fire management may be worthwhile.

The lack of germination from any open-pollinated fruits may be due to the timing of seed collection. All of our experimental seeds were collected from the very last individual fruits available in the field in early May 1999. They may have resulted from the last few blooms, which may suffer from decreased viability due to physiological stresses or the reduction in available pollinators. Or perhaps these capsules had formed earlier, but failed to dihisce, and also contained inviable seed. To better determine whether this is a typical result, large quantities of seed should be collected throughout the entire fruiting period of the pyxie moss, from sites with a variety of recent fire histories. Nonetheless, it is interesting that none of the seeds germinated, since a small seed bank appears to exist for this species.

A viable seed bank does exist near clumps of pyxie moss, but dispersal appears limited. It appears that a cool period is not necessary to trigger seed bank germination. Perhaps banked seed germinates throughout the year whenever environmental conditions are favorable. If this is the case, burning during any season of the year is no more likely to damage newly emerging seedlings of pyxie moss. It could be that microbial degradation of the seed coat is necessary, or perhaps phenolics or other substances that inhibit germination must be leached before germination can occur.

All seedlings from the successful seed bank germination trials turned brown and died within weeks of germinating, for undetermined reasons. Some of the seedlings died while they were still growing in the sandy soil excavated from the three study sites. Others were moved into moist peat with antifungal and antibacterial properties, in an attempt to prevent their deaths, but they also perished. Researchers were able to extend their longevity through the application of organic fish-based fertilizer. There is a chance that seeds in natural settings experience similar problems, dying off during a critical early life stage unless nutrient conditions are optimal, thus limiting sexual reproduction in this species.

The single primary root of transplanted seedlings was at least five times the length of the shoots. This observation is consistent with field observations for this study in which pyxie moss root systems also appeared to be greater in mass than the aboveground shoots and penetrated deeply into the soil. This may

explain why these slow-growing and low-growing plants can persist in areas of frequent fire.

The large number of insects observed pollinating the pyxie moss during the whole flowering period is consistent with the breeding study result showing no significant difference between the enhanced outcrossed treatment and naturally open-pollinated control. However, general low capsule production and lack of germination of open-pollinated seeds indicate that sexual reproduction is limited in this species. Additionally, a lack of viable seeds in the seed bank beyond 50 cm from pyxie moss clumps indicates that the plant has limited dispersal ability or that insect or other seed dispersal agents are no longer available to the plant.

References

Beal, W.J., "Insects needed to fertilize *Utriculata* and *Pyxidanthera*," *American Naturalist*, vol 12 (1878), pp 552–554.

Russo, M.J., B.A. Sorrie, B. Van Eerden, and P.E. Hippensteel, *Rare and Endangered Plant Survey and Natural Area Inventory for Fort Bragg and Camp Mackall Reservations, North Carolina* (The Nature Conservancy, Carrboro, North Carolina, and the North Carolina Natural Heritage Program, DPR, NC, 1993).

CERL Distribution

Chief of Engineers
ATTN: CEHEC-IM-LH (2)
ATTN: HECSA Mailroom (2)
ATTN: CECC-R

ODUSD(ES) EQ-LP 22202 (3)

Engineer Research and Development Center (Libraries)
ATTN: ERDC, Vicksburg, MS
ATTN: Cold Regions Research, Hanover, NH
ATTN: Topographic Engineering Center, Alexandria, VA

Defense Tech Info Center 22304
ATTN: DTIC-O

12
12/00

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
12-2000	Final	

4. TITLE AND SUBTITLE
Reproductive Ecology of the Sandhills Pyxie Moss (Pyxidanthera *barbulata* var. *brevifolia)* in North Carolina

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ann-Marie Shapiro, Jeff Zimpfer, and Moni Bates

5d. PROJECT NUMBER
MIPRs

5e. TASK NUMBER
W31RY081386852/PO;
MIPR0A48R00024

5f. WORK UNIT NUMBER
PT8 and RF0

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Construction Engineering Research Laboratory (CERL)
P.O. Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/CERL TR-00-42

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of the Deputy Undersecretary of
Defense (Environmental Security)
1225 Old Jefferson Davis Highway
Suite 1500
Arlington, VA 22202

10. SPONSOR/MONITOR'S ACRONYM(S)
ODUSD(ES)EQ-LP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

14. ABSTRACT

Sandhills pyxie moss (*Pyxidanthera barbulata* var. *brevifolia*) is a creeping, evergreen subshrub of the sparsely wooded sandhills pine-dominated plant communities on Fort Bragg, North Carolina. This research described the breeding system of the sandhills pyxie moss, examined the pollination biology of the species, and assessed germination of the species. The breeding system was evaluated using experimental manipulations of pollen transfer on plants in their natural environment. The results indicate that sandhills pyxie moss is not self-compatible, but reproduction does not appear to be limited by pollinator availability. The existence of pollinators was studied through intensive observation, collection, and identification of insect visitors in the natural environment. The results indicate that bees, wasps, ants, and other insects visit and pollinate the sandhills pyxie moss, but ants appear to be the most important, effective pollinator in these study populations. Germination was tested by planting seed under greenhouse conditions. Sparse germination was observed from seed bank samples, but the viability of seedlings was very low. These results shed some light on the reproductive biology of sandhills pyxie moss, but do not establish evidence of successful sexual reproduction.

15. SUBJECT TERMS
Sandhills Pyxie Moss, Ft. Bragg, NC, *Pyxidanthera barbulata* var. *brevifolia,* endangered species, Federal Species of Concern (FSC), natural resource management, ecosystem management

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Ann-Marie Shapiro
Unclassified	Unclassified	Unclassified	SAR	26	19b. TELEPHONE NUMBER (include area code) (217)352-6511x7416