The effectiveness of anemophily: Pollination biology of sawgrass, *Cladium jamaicense* Crantz (Cyperaceae)

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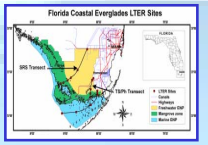

Abstract

Wind pollination, or anemophily, is characteristic of many economically important grain and timber crops (Ackerman 2000, Harder 2000). Basic mating system parameters and genetic consequences of this pollination system, however, have been studied in only a few cases. Sawgrass, the dominant macrophyte in the Florida Everglades, is an herbaceous anemophilous species. I propose to study the effectiveness of wind pollination in sawgrass (*Cladium jamaicense* Crantz) by (1) experimentally determining whether *C. jamaicense* is self-incompatible and (2) quantifying the efficiency of wind pollination *in situ*. These studies are necessary to understand the population biology of sawgrass and will provide data to assist management decisions in the recently legislated $8 billion, 30 year Everglades restoration (U.S. Water Resources Development Act of 2000, Section 601).

Compatibility relations of sawgrass will be determined by examining pollen grain germination and counting seed set in response to controlled self and cross pollinations, with controls for autogamy and pseudogamy. Pollination will be performed in an *ex situ* population of *Cladium jamaicense* at Henington Pond. The effects of temperature and humidity on these parameters will also be analyzed.

The effectiveness of wind pollination will be determined experimentally in natural south Florida sawgrass populations of both dense and sparse stands. Receptive stigmas on target inflorescences will be collected at varying heights and time intervals after stigma expansion, and the number of pollen grains per stigma will be counted. Regression analysis will be used to analyze the effects of length of exposure, where a linear relationship between pollen number per stigma and time of stigma exposure with no effect of flower position is the simplest model. Wind speed, wind direction, humidity, and temperature will be monitored during the experiment to analyze effects of these parameters on pollination efficiency. The results of this study will provide data to understand the reproductive biology, population structure and genetic diversity of sawgrass in the Everglades. It will also provide data to begin to model pollination efficiency in a large, clonal, herbaceous anemophilous species.

Introduction

Abiotic vectors of pollen transport such as wind have received far less attention and credit than their biological counterparts (Crane 1986, Ackerman 2000). Anemophilous plants dominate Poaceae, Cyperaceae, and Juncaceae; many species in these families are also large, dominant herbs with high incidences of cloning (A.J. Richards, 1987). Whether wind pollination allows for large genets to initially form or for large genets to disperse beyond the clone is unknown (A.J. Richards, 1987). In clonal species sexual reproduction may be the only opportunity to maintain genetic diversity. Therefore, mechanisms to prevent self-pollination and/or self-fertilization must exist. Species within Poaceae, Cyperaceae, and Juncaceae are of ecological and economical importance, however relatively few studies have assessed the genetic consequences of anemophily.

Sawgrass, *Cladium jamaicense* (Cyperaceae) is a large, herbaceous, clonal anemophilous species, which covers approximately 65-70% of the Florida Everglades (Loveless 1959). Altered from anthropogenic sources, the Comprehensive Everglades Restoration Plan has been charged with restoring the Everglades to its natural condition (U. S. Water Resources Development Act 2000, Section 601). Models produced include using sawgrass as an indicator of oligotrophic conditions (Sklar *et al*. 2001).

Studies based on sawgrass reproduction have focused mainly on its vegetative propagation. Recent investigations have found *C. jamaicense* to exhibit genetic diversity within populations (Ivey and Richards 2001b). Genotypic variation is found on a m^2 scale (Ivey and Richards 2001a), suggesting that sexual reproduction occurs within populations. The extent and genetic consequence of this mode of propagation has not been studied. I plan to investigate the compatibility relations and efficiency of anemophilous dispersal as an initial study to determine the sexual breeding system of *C. jamaicense*. Understanding of the various modes of propagation, sexual and asexual, may assist in determining replanting practices as part of the restoration plans of the Everglades (Newman *et al.* 1996), as well as providing insight on modeling pollination efficiency of clonal, herbaceous, anemophilous species.

It is unknown how genetic diversity of *C. jamaicense* is maintained. Recent studies (Richards, J.H. personal communication) indicate *C. jamaicense* is usually andromonoeious, with inflorescences bearing branches with spikelets usually containing a male and a hermaphrodite flower. Having a discrete flowering phenology, the male flowers mature first and last approximately one day (figure 1). Two days later, the stigmas of the hermaphroditic flowers mature and last up to one day (figure 2). Then, stamens of the hermaphroditic flower extend and last one day. Pollen release and stigma expansion are synchronous throughout the inflorescence, which suggest that outcrossing may occur. However, it is unknown whether *C. jamaicense* is an obligate outcrosser or is capable of selfing. Therefore, a goal of this study is to determine whether *C. jamaicense* is self-incompatible.

Seed establishment and seedling response in *C. jamaicense* have been studied. However, there is little to no evidence that defines the general characteristics of its pollination biology in the scientific literature. Consequently, another goal of this study is to quantify the temporal dynamics of wind pollination of *C. jamaicense in situ*. In additional to providing further insight to the reproductive phenology of sawgrass, this may also contribute to future studies of other ecological and/or economical anemophilous species in natural settings.

Objectives

1. Determine whether *Cladium jamaicense* is self-incompatible.
2. Analyze the efficiency of pollen capture in natural populations.

Figure 1: Male flowers

Figure 2: Stigmas of hermaphroditic flower

Study Sites

1. Henington Pond (figure 3)
   - A man-made pond located at Florida International University, University Park campus
   - In 1998, a *Cladium jamaicense* population was established around the pond.
2. Shark River Slough (SRS-2), ENP (figure 4)
   - Long hydroperiod marsh with peat soil
   - *C. jamaicense* is the dominant vegetative cover

Figure 3: *C. jamaicense* in Henington Pond

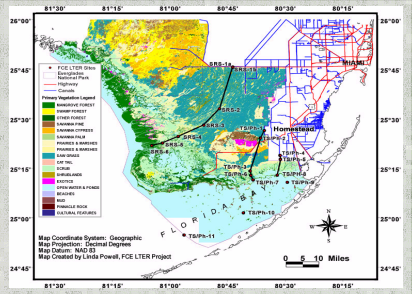

Figure 4: FCE LTER SRS and TS Transects and Vegetative Cover

Methods

Compatibility response of *C. jamaicense*

Plants will be tested *ex situ* at Henington Pond.

Test for autogamy: lateral clusters of flowers off the main inflorescence on 10 plants will be enclosed with aluminum foil.

Self-pollination: pollen will be collected from the lateral clusters of the male flowers of 10 plants. Clusters will be enclosed in foil concluding initial male dehiscence. After stigma maturation, pollen collected from male flowers will be placed on stigmas of flowers from the same plant via hand-pollination.

Cross-pollination: 10 plants' lateral clusters of flowers will be enclosed in foil concluding initial male dehiscence. After stigma maturation, pollen collected from separate populations will be placed on the stigma via hand-pollination.

Open pollination: 10 plants' lateral clusters of flowers will serve to measure natural pollination rates.

- Viability of pollen from 10 individuals will be analyzed using pollen fluorescence after staining with fluorescein diacetate, and will be tested at 0.5, 1, 3, 6, 12, 24, and 48 hours post-anthesis.
- The length of stigma receptivity will be tested by cross-pollinating virgin stigmas at different times following stigma emergence and assessing pollen tube growth. Stigmas from 10 different plants will be pollinated at intervals of 1, 3, 6, 9, 12, and 24 hours post-anthesis. Stigmas will be harvested after 24 hours and examined for pollen tube germination.
- To measure levels of seed set in compatibility experiments, capitula will be randomly marked and collected prior to dispersal. Seed set from autogamous, self-pollinated, cross-pollinated and open pollinated treatments will be counted.

Wind-pollination efficiency

Plants will be tested *in situ* in both dense and sparse sawgrass stands in Shark River Slough, Everglades National Park (ENP).

10 plants that have finished their first male phase but not yet expanded their stigmas will be identified in three 3-m2 plots in sparse and dense sawgrass stands at two different sites off the Shark River Slough LTER transect. Lateral flower cluster at 3 heights will be bagged, then exposed at time 0 on the day they become receptive. Stigmas from these clusters will be fixed in ethanol:acetic acid (3:1) at1, 3, 6, 9, 12, and 24 hours after exposure. Wind speed, wind direction, humidity, and temperature will be measured throughout the exposure period. These stigmas will be mounted on a basic fuchsin mixture, and pollen grains on stigmas counted. We hypothesize that pollen capture increases linearly with time and is independent of stigma position within the inflorescence. The pollen capture data will be used to evaluate this model.

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