PROCEEDINGS

OF THE

FIFTEENTH SYMPOSIUM

ON THE

NATURAL HISTORY OF THE BAHAMAS

Edited by
Robert Erdman
and
Randall Morrison

Conference Organizer
Thomas Rothfus

Gerace Research Centre
San Salvador Bahamas
2016

Cover photograph - "Pederson Cleaning Shrimp" courtesy of Bob McNulty

Press: A & A Printing

ISBN 978-0-935909-16-6

REPRODUCTIVE BIOLOGY OF BAY LAVENDER (*HELIOTROPIUM GNAPHALODES* L.; BORAGINACEAE)

Kathryn M. Everson[1,2], Carol L. Landry[3] and Erin Richardson[4]

[1]Department of Evolution, Ecology, and Organismal Biology
The Ohio State University
Columbus, OH 43210
[2]Current address: Department of Biology and Wildlife
University of Alaska Fairbanks
982 N. Koyukuk Dr
Fairbanks, AK 99775
[3]Department of Evolution, Ecology, and Organismal Biology
The Ohio State University
Mansfield, OH 44906
[4]Department of Horticulture and Crop Science
The Ohio State University
Columbus, OH 43210

ABSTRACT

Plants in the beach-foredune community have a unique and important role as dune stabilizers, but are increasingly subjected to disturbance from tropical storms, rising sea levels, and human activity. Understanding the reproductive ecology of dune species is critical to their conservation and to the conservation of coastlines. Here we investigate the reproductive biology of a common Bahamian dune plant, *Heliotropium gnaphalodes* L. (Bay Lavender; Boraginaceae), by examining three reproductive stages: pollination, fruit set, and fruit dispersal. We observed and recorded visits by animal pollinators, compared fruit set in two hand-pollination treatments – self-pollen, and self-pollen augmented with outcross pollen – and confirmed that mature fruits can float. Bay Lavender was visited by many generalist pollinators, and preliminary dispersal data suggests that seeds may be water dispersed. Differences in fruit set number between plants that were pollinated with self-pollen and plants that were pollinated with both self and outcross pollen were not significant, indicating that Bay Lavender may be self- compatible. A seedling germination study comparing cross types will confirm this. Self-compatibility would allow Bay Lavender to colonize new dune areas from a single propagule, thus making it a candidate species for use in beach-dune stabilization projects. This study provides evidence that Bay Lavender may be an important species for beach and dune maintenance and restoration, and provides a framework by which the reproductive biology of other native dune plants may be evaluated.

INTRODUCTION

In the Caribbean, tropical storms and hurricanes cause high levels of disturbance to island flora most years between the months of September and November (Gerace et al. 1998). Several studies have shown that hurricanes are capable of stripping plants of their leaves, adding additional stressors such as excess water and salt, and diminishing pollinator populations, all of which can negatively affect plant reproduction (Rathcke 2000, Pascarella 1998). This disturbance is compounded by the detrimental effects that can be caused by human activity, such as those that have occurred on San Salvador Island. When the beach resort Club Med was built on San Salvador, non-native ornamental plant species were introduced which have since posed a serious risk to floral biodiversity, and local extinction of native plants is now a potential reality (Eshbaugh 1996). As a low-island system, San Salvador is also subject to ramp disturbances such as sea level rise (Ross et al. 2009), which makes coastline plants such as *Heliotropium gnaphalodes* L. (Bay Lavender; Boraginaceae) a subject of conservation concern.

Plants in the beach-foredune community play an important role in island ecosystems as dune builders and stabilizers. The deep root systems of dune plants bind sand to anchor the dune, and allow new dunes to form in front of them by the process of accretion (Ranwell 1972). These dunes serve as "natural coastline protection systems," by storing fresh water and acting as natural sea walls against storms (Sealey 2001). Without the stabilizing effects of dune plants, beaches and dunes erode rapidly (Martinez et al. 2001), especially in locations like East Beach on San Salvador Island, where strong winds and waves can dramatically change the landscape in a relatively short amount of time (e.g. Figure 1).

A beach-foredune plant's ability to colonize new areas is influenced by its breeding system, or whether or not it is self-compatible. Under certain conditions, self-compatibility is beneficial. It provides reproductive assurance where pollinators are unpredictable, and it can allow for opportunist range expansions, as a single propagule is sufficient to start a sexually reproducing population (Baker 1955). However, many plants have mechanisms to reduce selfing because outcrossing provides the important benefits of reducing inbreeding depression and promoting genetic variability (Whitehouse 1950). Reproductive strategies vary between plant species based on the costs and benefits for a given environment, and can be empirically determined by a pollination study.

Knowledge of the pollinators and mating system of Bay Lavender is vital for the development of effective conservation and management strategies (Kearns et al. 1998). If the re-introduction of this species to a beach or island was required due to recent local extinctions, or if Bahamian businesses wished to propagate the plant for use as an ornamental, then it would be necessary to know whether the species is self-compatible, the relative success of selfed versus outcrossed progeny, and which pollinators are necessary for its reproductive success.

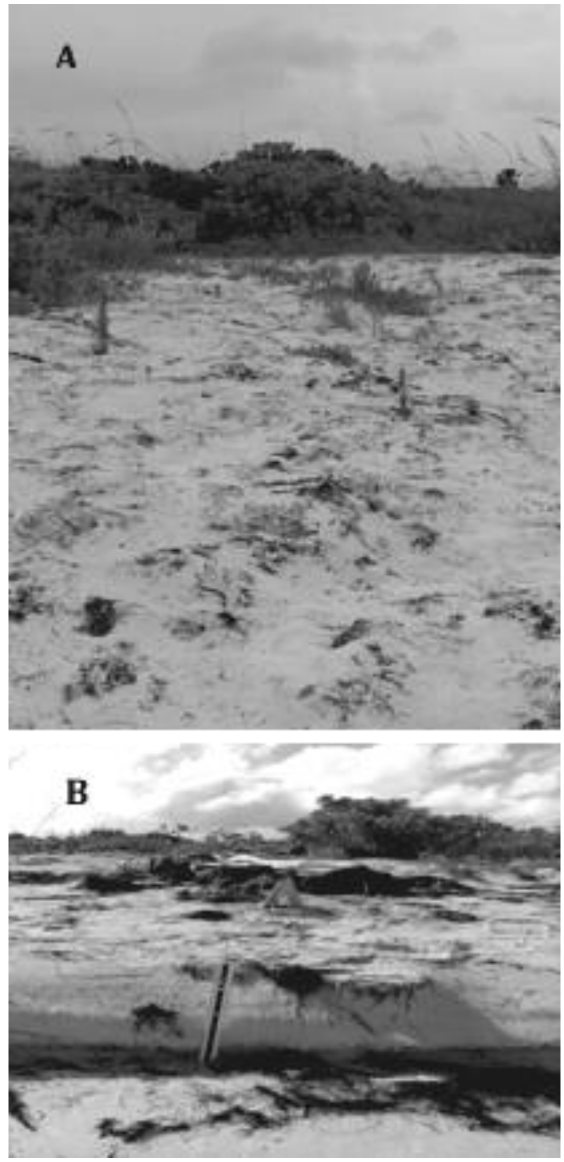

Figure 1. Dramatic landscape changes on the dunes of East Beach between (A) June 2010 and (B) December 2010. Photos by C. Landry.

The propensity for autonomous self-pollination of Bay Lavender was hypothesized in a previous study (Kass et al. 2011), but hand-pollination and seedling germination experiments were not performed, so self-compatibility could not be concluded. To determine the mating system of Bay Lavender, we compared the fruit set of selfed flowers to those of flowers treated with self and outcross pollen. In this study, we specifically addressed the following questions: (1) What is the mating system of Bay Lavender, and (2) What are its pollinators?

METHODS

The Species and Populations

Bay Lavender is found in the beach-foredune community on San Salvador Island in The Bahamas (Landry et al. 2013). Its range extends throughout the Caribbean (Gillis 1976). Bay Lavender is a stout shrub, 1-1.5 m high, often forming dense clumps. The branches and leaves are covered in fine, straight hairs, giving them a silky appearance. Small, fragrant, white flowers are arranged in tight, elongated, curved clusters (Kass 2009; Figure 2). Each cluster consists of 8-16 flowers and many flowers open on one plant at a time (Correll and Correll 1982). The corolla is made up of five white petals forming a petal tube that may have a pink-tinged throat and a green interior base. The stamens of Bay Lavender are attached to the corolla tube at the point where the petals separate into lobes. The pistil has a 4-celled ovary and a very short style, capped with a triangular-shaped stigma. The top of the pistil is located below the attachment of the stamens, thus self-pollination is possible (Kass 2009).

Figure 2. Bay Lavender inflorescence showing size and arrangement of flowers. Photo by K. Everson.

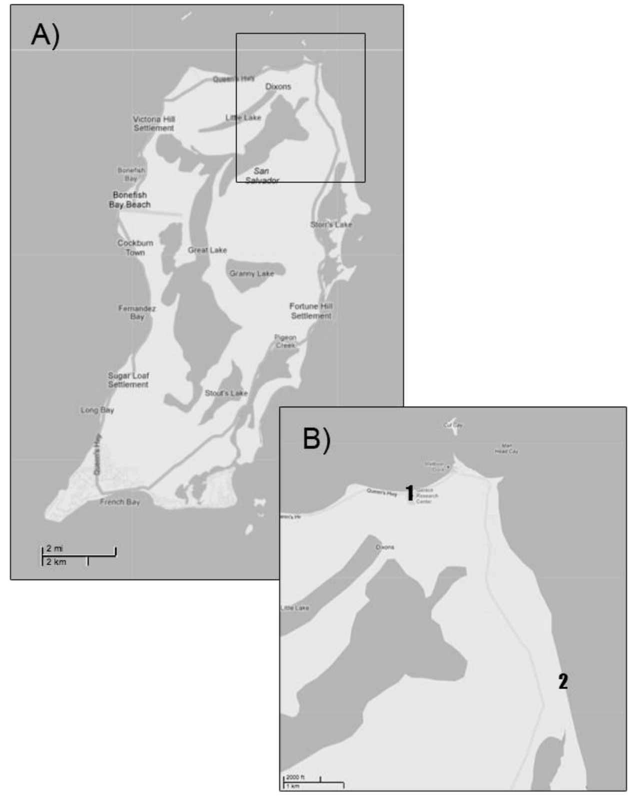

Figure 3. Map of San Salvador Island, Bahamas. (A) Entire island, (B) northeast corner of island, with study locations. 1 = Grahams Harbour site, 2 = East Beach site. Map revised from maps.google .com.

Breeding and Mating System

Hand-pollinations were performed on Bay Lavender on March 23 and 24, 2011. Our two study populations were 5 km apart (Figure 3), located at Graham's Harbor on the north side of the island, and at East Beach on the east side of the island. Graham's Harbor is relatively sheltered from heavy wind and waves, so dune formation and landscape changes are less active than at the East Beach location. Plants were 10-20 meters from the surf. Ten plants were tagged and numbered at each site, and several unopened inflorescences were bagged on each plant. After one day the bags were removed and open flowers were hand-pollinated using one of two treatments, either self-cross or self-cross with an out-cross pollen supplement. An outcross only treatment was not possible because stamens could not be removed without damaging the flower.

Because the petal tubes were too slender to transfer pollen using traditional methods, all crosses were performed using a tool of the authors' design, which functioned as a thin cotton swab to access both the pistil and stamens. Self-crosses, testing the potential for autonomous self-fertilization, were performed by brushing the cotton swab across the anthers of an open and receptive individual, then dabbing the pollen-logged cotton on the stigma of the same flower. The outcross supplement treatment was performed in a similar manner, but pollen from a different individual was collected on the cotton before pollinating the maternal flower. After hand-pollination, a bag was placed over the inflorescences to prevent insect visitation and fruit dispersal.

On June 12 and 13, 2011, the treated inflorescences were relocated and fruit set was recorded. Mature fruits were collected on July 30 and 31, 2011. Two-way ANOVA was used to test for differences in fruit set (SPSS Statistics v19.0.0). Percent fruit set data were arcsine transformed to normalize distributions before statistical analysis. Pollinator species were observed and/or collected on Bay Lavender flowers at each site. The number and species of the pollinators was noted, as well as the approximate size of the plant and number of flowers each insect visited.

To investigate the hypothesis that Bay Lavender seeds are water-dispersed, it was first necessary to determine whether or not the seeds could float in salt water. This was accomplished by placing 50 seeds in a saltwater live well and recording the percentage of floating seeds each day for fourteen days.

RESULTS

Infructescences formed in both pollen treatments at both locations. Fruit set did not differ between site locations ($p = 0.46005$, f-ratio = 0.5601, df = 1) and there was not an interaction between pollination treatments and site locations ($p = 0.6085$, f-ratio = 0.26796, df = 1), so data from the two locations could be combined. Fruit set did not differ significantly between the two pollination treatments (Figure 4; $p = 0.89738$, f- ratio = 0.01692, df = 1).

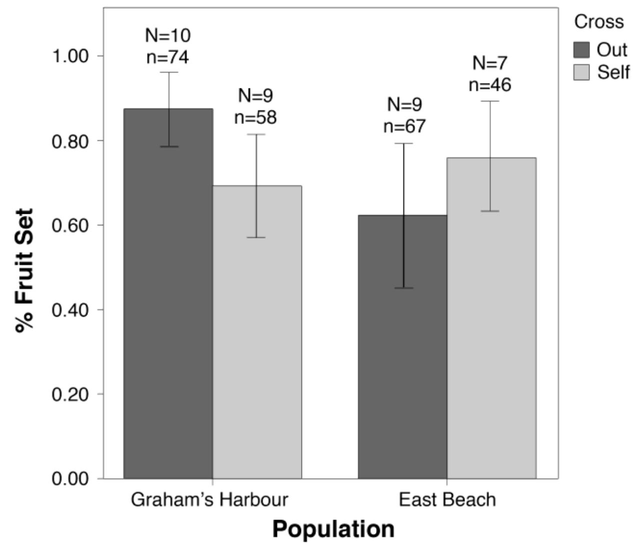

Figure 4. Percent fruit set per inflorescence, with SE bars. Out = outcross pollen added treatment, Self = self-pollen treatment, N = number of plants, n = number of flowers. No significant differences were detected across or within treatments and locations.

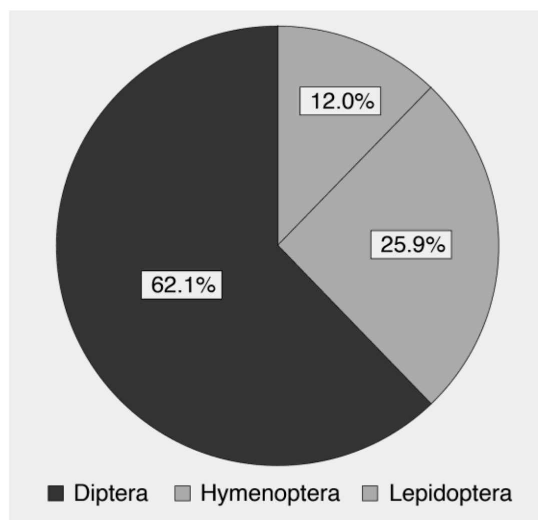

Figure 5. The relative number of pollinator visits to Bay Lavender observed and/or collected. Gray tones represent orders of insect. N = 58.

Bay Lavender was visited by a variety of insects (Table 1). The orders Diptera, Hymenoptera, and Lepidoptera were represented. Dipterans were the most frequent visitors, followed by Hymenopterans then Lepidopterans (Figure 5). Two Dipterans in particular, *Ligyra* *cereberus* (F.) and *Copestylum eugenia* (Will.), were the most common species observed visiting Bay Lavender.

In the test of the seeds' abilities to float in salt water, 98% of the seeds were still floating in the live well after two weeks, suggesting that salt water is a potential dispersal medium for this plant.

DISCUSSION

The fruit set data gathered in this study suggest that Bay Lavender is self-compatible (Figure 4). However, it is currently still unknown whether the seeds produced are viable. Inbreeding depression can appear at any of several life history stages, including reduced germination in selfed progeny (Whitehouse 1950). It would be necessary to conduct an F1 germination study to determine whether offspring of selfed individuals are less likely to germinate than those of outcrossed individuals.

If Bay Lavender is self-compatible, as the preliminary data suggests, it would be afforded a significant advantage. Unlike an obligate outcrosser, which passes on one haploid copy of its genome through its own seeds and one copy each time it fertilizes another plant, a selfing plant would pass on two copies of its genome in every selfed seed (Barrett 1991). Additionally, previous studies have shown that high levels of selfing are beneficial under certain conditions, such as pollinator failure or population bottlenecks. In these cases, increased selfing can actually reduce the level of inbreeding depression by rapid purging of deleterious alleles (Schemske and Lande 1985). These highly selfing populations perform well as long as environmental conditions remain stable, but genetic homogeneity leaves them vulnerable when new elements such as disease, herbivores, and climate change enter the system.

If Bay Lavender does not suffer from inbreeding depression, then it would aid in the understanding of its ecological role. Selfing plants are typically better pioneers (Baker 1955), so the ability to self makes Bay Lavender better capable of colonizing new dune areas and starting new populations. The ability to pioneer is an especially advantageous characteristic on San Salvador Island, where storm and human disturbance periodically wipes out local plant and insect populations, leaving open areas free from competition and resource limitation (Eshbaugh and Wilson 1996).

Occasional outcrossing, however, may still be important for the reproductive success of Bay Lavender. Most self-compatible plants employ an intermediate mating system, with a mix of outcrossing and selfing strategies (Goodwillie et al. 2005). Several hypotheses have been proposed to explain the maintenance of mixed mating strategies in natural systems, ranging from the prevention of deleterious allele accumulation via inbreeding depression (Lenormand and Otto 2000, Johansen-Morris and Latta 2006), to reproductive success in times of pollen limitation (Vallejo-Marin & Uyenoyama 2004). A mixed mating system would be advantageous for Bay Lavender because self-pollination would be beneficial when pollinators were scarce following hurricanes, but outcrossing would increase genetic variation.

This study's documentation of pollinators is also important in understanding Bay Lavender's role in the beach-foredune community. Although it does not seem to have bird or beetle pollinators, it is regularly visited by a large variety of lepidopterans, hymenopterans and dipterans. Most of the visitors are generalists, so Bay Lavender may provide pollinator resources when other plant species are not flowering, having a facilitative effect on other plants in the community. As hurricanes have been found to greatly diminish population sizes of invertebrates in island communities (Willig and Camilo 1991), Bay Lavender's presence on the island would be quite beneficial to a recovering insect population that had been disturbed by a tropical storm.

Plants in the beach-foredune community must have unique adaptive traits, like the ability to tolerate saltwater spray, storm waves, and alternate periods of sand burial and erosion (Crawford 1989). Decomposing organic matter may provide extra root anchorage for these difficult conditions, and increases moisture retention and nutrient availability (Provoost 2004). On the dunes on San Salvador, the macroalga *Sargassum fluitans* (Børgesen) and seagrass *Thallasia testudinum*

(Banks ex König) are brought in with waves and make up the majority of decomposing organic matter, along with dead invertebrates, fecal matter and other organic detritus (Landry unpubl. data). The presence of this detritus may have a positive impact on Bay Lavender seedling success, but additional work is needed to test this hypothesis.

Ongoing and Future Work

Other members of the Boraginaceae are water-dispersed, which further supports this hypothesis (Quilichini and Debussche 2000, Skarpaas and Stabbetorp 2003, Mackey 1999). The ability of Bay Lavender nutlets to float in salt water for an extended period of time would allow for water dispersal, if seeds remain viable. A comparison of germination rates in seeds that were immersed in saltwater for different periods of time would test this hypothesis.

Further studies of insect floral foraging bouts would also be relevant to the mating system question of the relative proportions of selfed versus outcrossed flowers. If insects tend to forage on the same plant for a long period of time, traveling from flower to flower, high rates of self-fertilization are likely. If, however, pollinators spend short periods of time on each plant, traveling between patches of Bay Lavender when they forage, then outcrossing is more likely occurring (Snow et al. 1996).

ACKNOWLEDGMENTS

We thank the Gerace Research Centre and its staff for their support, especially Dr. Thomas Rothfus. We also thank Dr. Nancy Elliott for assistance and expertise with insect collections and identifications. We also thank the people of San Salvador for sharing their island and for their continued support of our research. This research was funded in part by a grant from The Ohio State University – Mansfield.

REFERENCES

Baker, H.G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution* 9(3): 347-49.

Barrett, S.C.H. & J.R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants—implication for conservation. *Genetics and Conservation of Rare Plants*. New York, NY: Oxford University Press. pp 3-30.

Correll, D.S. & H.B. Correll. 1982. *Flora of the Bahama Archipelago*. Forestbourgh, NY: Lubrecht and Cramer.

Crawford, R.M.M. 1989. *Studies in plant survival.* Blackwell: Oxford.

Eshbaugh, W.H. & T.K. Wilson. 1996. On the need to conserve Bahamian floral diversity. *Proceedings of the Sixth Symposium on the Natural History of the Bahamas*: 77-82.

Gerace, D.T., G.K. Ostrander & G.W. Smith. 1998. San Salvador, Bahamas. *Caribbean Coral Reef, Seagrass and Mangrove Sites:* 229-46.

Gillis, W.T. 1976. Flora and vegetation of the Cay Sal. *Bahamas Naturalist* 2(1): 36-41.

Goodwillie, C., S. Kalisz, & C.G. Eckert 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* 36: 47-79.

Johansen-Morris, A.D. & R.G. Latta. 2006. Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution* 60: 1585-95.

Kass, L.B. 2009. *An Illustrated Guide to Common Plants of San Salvador Island, Bahamas,* 3rd edition, A.J. Kowalski, illustrator. Gerace Research Centre, San Salvador Island.

Kass, L.B., N.B. Elliott, & C.L. Landry. 2011. The natural history of Bahamian dune plants: Importance in coastal conservation. Pp. 35-45. In: J. Baxter and E. Cole, (Eds.). *Proceedings of the 13th Symposium on the Natural History of the Bahamas*. Gerace Research Centre, San Salvador Island, The Bahamas.

Kearns, C.A., D.W. Inouye, & N.M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29:83-112.

Landry, C. L., N. B. Elliott, A. Finkle, and L. B. Kass. 2013. Pollination networks- what's the buzz? A preliminary study of coastal community pollination dynamics on San Salvador Island, The Bahamas. Pp. 95-112. In: C. Tepper and R. Shaklee, (Eds). *Proceedings of the 14th Symposium on the Natural History of The Bahamas*. Gerace Research Centre, San Salvador Island, The Bahamas.

Lenormand, T. & S.P. Otto. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* 156(1): 423-38.

Mackey, S.S.D. 1999. A life history study and geometric population growth model of a rare borage, *Cryptantha crinita* Greene. M.S. Thesis. Chico, CA. California State University.

Martinez, M.L., G. Vazquez, & S.S. Colon. 2001. Spatial and temporal variability during primary succession on tropical coastal sand dunes. *Journal of Vegetative Science* 12:361-372.

Pascarella, J.B. 1998. Resiliency and response to hurricane disturbance in a tropical shrub, *Ardisia escallonioides* (Myrsinaceae), in south Florida. *American Journal of Botany* 85: 1207-15.

Provoost, S., C. Ampe, D. Boute, E. Cosyns, & M. Hoffman. 2004. Ecology, management and monitoring of grey dunes in flanders. *Journal of Coastal Conservation* 10(1): 33-42.

Quilichini, A. & M. Debussche. 2000. Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). *Acta Oecologica* 21: 303-13.

Ranwell, D.S. 1972. *Ecology of Salt Marshes and Sand Dunes*. London: Chapman and Hall.

Rathcke, B. 2000. Hurricane causes resource and pollination limitation of fruit set in a bird-pollinated shrub. *Ecology* 81(7): 1951-58.

Ross, M.S., J.J. O'Brien, R.G. Ford, K. Zhang, & A. Morkill. 2009. Disturbance and the rising tide: the challenge of biodiversity management on low-island ecosystems. *Frontiers in Ecology and the Environment* 7(9): 471-78.

Schemske, D.W. & R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39: 41-52.

Sealey, N.E. 2001. Coastal Erosion in the Bahamas. *Bahamas Journal of Science* 9(1): 15-21.

Skarpaas, O. & O.E. Stabbetorp. 2001. Diaspore ecology of *Mertensia maritima*: effects of physical treatments and their relative timing on dispersal and germination. *Oikos* 95: 374-82.

Snow, A.A., T.P. Spira, R. Simpson, & R.A. Klips. 1996. The ecology of geitonogamous pollination. Lloyd, D.G. & S.C.H. Barrett, (eds.). *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York. 191–216.

Vallejo-Marin, M. & M.K. Uyenoyama. 2004. On the evolutionary costs of self-incompatibility: incomplete reproductive compensation due to pollen limitation. *Evolution* 58: 1924-35.

Whitehouse, H.L.K. 1950. Multiple-allelomorph incompatibility of pollen and style in the evolution of the angiosperms. *Annals of Botany* 54: 199-216.

Willig, M.R. & G.R. Camilo. 1991. The effect of hurricane Hugo on six invertebrate species in the Luquillo experimental forest of Puerto Rico. *Biotropica* 23: 455-61.hurricane Hugo on six invertebrate species in the Luquillo experimental forest of Puerto Rico. *Biotropica* 23: 455-61.

Table 1. Visitors to Bay Lavender on San Salvador Island, Bahamas. EB = East Beach site, GH = Grahams Harbor site.

Order	Family	Genus/Species	EB	GH
Diptera	Bombyliidae	*Ligyra cereberus* (F.)	X	X
		Chrysanthrax maculipennis Scarbrough and Davidson		X
		Copestylum eugenia (Will.)		X
Hymenoptera	Anthophoridae	*Xylocopa cubaecola* Lucas		X
	Scoliidae	*Campsomeris trifasciata trifasciata* (Fabr.)	X	
	Sphecidae	*Stictia signata* (L.)	X	
	Vespidae	*Polistes bahamensis picturatus* Beq. and Salt	X	
	Undetermined	Undetermined wasp species	X	
Lepidoptera	Hesperiidae	*Hylephila phyleus* Drury		X
	Lycaenidae	*Strymon acis armouri* Clench		X
	Pieridae	*Ascia monuste eubotea* Latreille	X	
	Undetermined	Undetermined butterfly species	X	