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Toward Understanding Lakela's and Savannas Balm, *Dicerandra immaculata*

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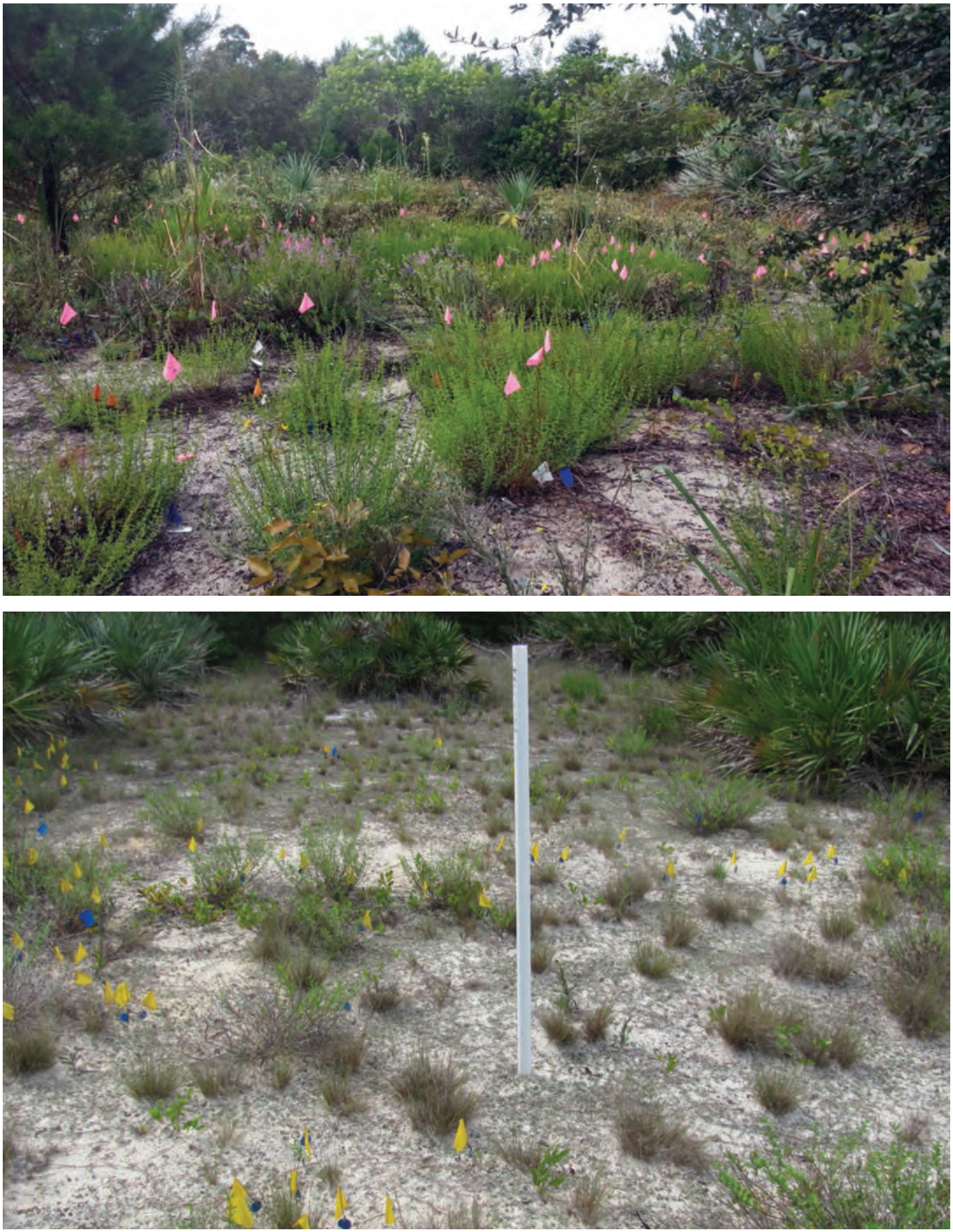
The plant genus *Dicerandra* (Lamiaceae) is the highest-ranked genus of rare southeastern endemic plants in the United States and contains six perennial and five annual species that are restricted to sandhill and scrub habitats primarily in Florida (Estill and Cruzan 2001). Two-thirds of the species have extremely small geographic ranges, populations are likely isolated with little gene flow between them, and their habitats are being destroyed through human development or becoming overgrown due to suppression of natural fires. Some *Dicerandra* species are included on state and federal endangered species lists, but this affords little protection and populations continue to decline.

The Center for Plant Conservation (CPC), a national organization of gardens and other institutions in the United States, strives to preserve germplasm of rare plants and uses the germplasm for research and cultivation. The Rare Plant Conservation Program at Bok Tower Gardens (BTG; Lake Wales, FL), managed by Cheryl Peterson, is a member institution of the CPC and has worked for 31 years to study

Above, top to bottom: Lakela's balm in flower. The petals of both Lakela's and Savannas balm are a rose-lavender color and lack the spots which appear on the petals of other *Dicerandra* species. The most noticeable difference between Lakela's and Savannas balm is leaf size – Savannas balm has much larger leaves.



Above: Lakela's and Savannas balm are most frequently found in scrub habitat where there are gaps in the canopy and at ground level. This is true in both natural sites (top) and a few years after introducing them into new sites (bottom).

Above and right: The critically endangered Lakela's and Savannas balm are propagated at Bok Tower Gardens to augment natural populations and to create new populations to ensure their survival.

and protect remaining populations, restore declining populations, and create new populations of rare plants in Florida. This work has included *Dicerandra* species, such as Lakela's balm (*D. immaculata* var. *immaculata*) and Savannas balm (*D. immaculata* var. *savannarum*).

Lakela's balm is a critically endangered short-lived perennial plant that is found in fragmented scrub habitat on the Atlantic Coastal Ridge. Lakela's balm persists at five of its historical sites in St. Lucie and Indian River Counties, but many of these sites are not protected and have become severely overgrown, so large areas within these sites no longer have any surviving individuals. One of these locations has been augmented with plants propagated at BTG as part of a program to conserve this species. Three populations of Lakela's balm were also introduced at new sites in Florida (St. Lucie and Martin Counties) and are naturalized.

Lakela's balm has multiple inflorescences, each of which is between 15-25 cm long and has overlapping cymes that bear up to five flowers in leaf axils (Kral 1982). Flowering typically begins in early October and concludes in November. Flower petals are a rose-lavender color and lack spots on the petals, unlike other *Dicerandra* species that have spotted petals. The four stamens per flower extend beyond the corolla and the anthers are spurred, which means that insects are likely necessary to prompt the release of pollen (Nelson 1996). The stigma extends farther than the stamens. The stigma and anthers are laterally spread out within the corolla, which is predicted to reduce nectar robbing compared to the flower morphology in other species of *Dicerandra* and deposit pollen on the sides of pollinators instead of the venter (illustrated and discussed in Deyrup and Menges 1997). Our prior work on the breeding system of Lakela's balm (discussed in Richardson et al. 2016) shows that it is a facultative outcrosser, which means that self-pollination is possible, but seed set is highest when pollen comes from another plant. We observed the honey bee *Apis mellifera*, a non-native pollinator, visiting plants more frequently than any other pollinators. Once on a plant, honey bees also visit more flowers within plants than native pollinators, which could lead to higher rates of inbreeding. Native pollinators such as the bumble bee *Bombus impatiens* are attracted more frequently to shorter plants, but when they do visit taller plants the bumble bee tends to stay longer and visit more flowers than on shorter plants. Despite having fewer total and pollinated flowers, shorter plants had a higher output of intact seeds than taller plants, which could be due to differences in efficiency between native and non-native pollinators or other factors.

Broadly speaking, Lakela's balm grows in scrub habitat.

However, not every spot within scrub habitat is suitable for Lakela's balm, and plants are often found in clumps. Therefore, we characterized the microhabitat where plants are found within sites and compared it to microhabitat in random locations and microhabitat where plants were extirpated (discussed in Richardson et al. 2013). This comparison allows us to be able to identify necessary habitat for restoration and also allows us to identify appropriate natural locations for planting greenhouse-grown plants. We commonly find Lakela's balm in areas with fewer woody stems, shorter understory vegetation, lower percent canopy coverage, and lower percent ground cover of detritus than random locations and locations with extirpated Lakela's balm. In addition, bare ground decreased at extirpated locations because other plant species expanded their coverage, water saturation of the soil increased, diversity of shrubs decreased, and composition of the overstory changed compared to locations with Lakela's balm. These results highlight the need for gaps in the canopy and at ground level in order to maintain populations of Lakela's balm. Lakela's balm is able to coexist with some taller woody plants such as pines, but is unable to coexist with many common woody plants such as oaks, scrub hickory, and sabal palms when they become abundant. Oak, hickory, and palms become more abundant on the Atlantic Coastal Ridge through suppression of fire and the resulting habitat succession.

Savannas balm is also a short-lived perennial plant and shares many of the same characteristics as Lakela's balm. It was discovered in St. Lucie County in 1995 at two locations separated by less than 0.3 km (Huck 2001) and bisected by the Florida East Coast Railway. One site was just adjacent to the Florida East Coast Railway and Savannas Preserve State Park (SPSP) boundary and the second was located along a residential road that spanned three private properties within a subdivision near SPSP. These are the only locations known to historically support Savannas balm and likely supported a single contiguous population before construction of the railroad and other anthropogenic disturbances. Approximately 200 individuals were counted on well-drained ridge habitat at these locations in 1995, but continued disturbance and increasing overgrowth has led to their decline, and as few as eight plants have been observed within the natural population. However, beginning in 2005, germplasm has been regularly collected and transported to BTG for propagation and preservation in the CPC National Collection. Plants propagated in the greenhouse were used to establish three populations within SPSP beginning in 2006. Survival, reproduction, and recruitment were monitored yearly through 2011 in the first of these established populations so that we could determine how genotype and environmental factors, including microhabitat, influenced these life history events. Since little was known about the habitat preferences of Savannas balm prior to the establishment of the population, we introduced plants into microhabitats that differed in their amount of sunlight and detritus. Survival and reproduction of introduced Savannas balm and recruitment of new plants was higher in microhabitats in full sun and no leaf litter and lower in partially shaded habitats. Ultimately, Savannas balm seems to thrive in the same microhabitats as Lakela's balm.

Savannas balm is considered a wide-leafed variety of Lakela's balm based on morphological similarity of the two varieties. However, there are differences that set them apart. Savannas balm has floral volatiles that differ from Lakela's balm (unpublished data) and a phylogenetic analysis shows that Savannas balm has divergent nuclear and chloroplast genomes that make it unique in the *Dicerandra* genus (Oliveira et al. 2007). Savannas balm is also generally longer-lived and larger on average than Lakela's balm, is quicker to root during clonal propagation, and its seeds have approximately twice the germination rate (unpublished data). Due to inherited differences in the width of leaves, we speculated that Lakela's and Savannas balm may also vary in their number of chromosome sets (i.e., cytotypic variation).

Cytotypic variation is common within plant species and can influence their morphology, physiology, phenology, and other life history traits, as well as community structure of their associated arthropod pollinators and herbivores. Research published 20 years ago showed that Lakela's balm is tetraploid (four complete sets of chromosomes), but chromosome counts for Savannas balm were not made in that initial study or in the intervening period (Huck and Chambers 1997). We received a grant from the Florida Native Plant Society and partnered with Dr. Lane Rayburn and his graduate student Jeff Bishop at the University of Illinois at Urbana-Champaign to determine whether there was cytotypic variation between Lakela's and Savannas balm. We sent fresh leaves to their lab and they used flow cytometry and direct counts of chromosomes to determine that Lakela's and Savannas balm are tetraploid, so they do not vary in their number of chromosome sets and cytotypic variation cannot explain the differences in morphology between the varieties.

There is still much to learn about Lakela's and Savannas balm in order to inform conservation efforts and prevent their extinction. Germination rates of seeds in the greenhouse are low, and germination rates in the wild are even lower because of seed predators and other environmental factors. We still do not fully understand what influences their spatial distribution within habitats, their recruitment, or their long-term survival. We do not know how honey bees influence pollination compared to native pollinators and whether the former are responsible for the low seed set we observed on some plants. We need to identify whether or not our efforts to augment populations and create new populations is a viable strategy over time (i.e., are the introduced plants genetically diverse, likely to survive,

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and likely to contribute to the next generation), and which land management strategies, such as prescribed fire, best maintain suitable habitat. Lastly, the largest unknown may be how climate change will influence long-term viability of populations because Florida is expected to experience higher temperatures and changed patterns of precipitation.

However, efforts are underway to investigate many of these unknowns. We are currently engaged in research to better understand spatial seedling recruitment, population demography, seed predators and reproductive biology of both balms, and are currently working on modeling to understand how climate change will influence populations of Lakela's balm. In time, each piece of the puzzle will help us better understand what influences survival of these balms and develop strategies for their long-term conservation.

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