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MICRO-ENVIRONMENT CONDITIONS, MYCORRHIZAL SYMBIOSIS, AND SEED GERMINATION IN *CYPRIPEDIUM CANDIDUM*: STRATEGIES FOR CONSERVATION

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Introduction

Cypripedium candidum Muhl. ex Willd. (White Lady's Slipper), a terrestrial northern United States / southern Canada prairie orchid, reproduces both by seed and adventitious buds from older roots and rhizomes. To determine the pollination effects of diverse prairie micro-environments caused by competing foliage, particularly with respect to height and density of surrounding flora, ovary development (pollination success) versus ovary abortion (no pollination) was recorded for every flower in three diverse populations in eastern South Dakota, USA. Dense and medium-dense competing foliage populations correlated with similar orchid seed sets (22%, n=74 and 18%, n=615, respectively), while the more open, exposed orchid population demonstrated a significant increase in success (44%, n=258). In addition, a separate, hand-pollinated population in an open location (n=49) resulted in 100% ovary development. Samples of developing ovaries were histologically examined and more extensive ovule development was observed in ovaries from these open sites. Since micro-environments of tall, dense, competing foliage seem to limit sexual reproduction in *C. candidum* by physically preventing pollinators from accessing the flowers, this orchid also relies on clonal reproduction via its rhizomes. It may also rely on mycorrhizal fungi to receive necessary nutrients and / or enzymes for plant growth to compensate for reduced photosynthates. To locate putative orchid-fungal associations, histological studies of roots and rhizomes were completed. No fungal pelotons were observed in root or rhizome samples harvested in early summer (end of May), but were clearly visible in root cortical cells six to eight weeks later. These

differing levels of mycorrhizal development may be a function of continued or new fungal and / or root growth, or seasonal changes in the soil environment. Further studies of mycorrhizal development throughout the growing season, as well as fungal identification and symbiotic seed germination, are currently underway to help identify these intricate inter-specific associations necessary for the survival and conservation of *C. candidum* in eastern South Dakota.

Description

Cypripedium candidum (White Lady's Slipper) is a perennial terrestrial orchid that is indigenous to 13 states in north-central and north-east United States and southern regions of Ontario and Manitoba, Canada. *Cypripedium candidum* produces solitary stems (10-30 cm), as well as dense clonal clumps, from its short rhizomes and fibrous roots. Each stem produces several strongly ribbed, densely pubescent leaves (5-16 cm x 1.5-6 cm) that are typically held nearly erect, sheathing the stem and overlapping at the base. The uppermost leaf (elliptical, green foliaceous bract) is smaller than the other leaves, standing erect, subtending the flower and sheathing the ovary. The stems are usually terminated by a single flower (occasionally two) characterized by its ivory-white egg-shaped pouch (labellum) for which it was named (from the Latin *Cypris* for "Venus", *pedilon* for "shoe" and *candidum* for "shining white") (Fig. 1). This white shiny or waxy appearing lip petal (1.7-3.3 x 1-1.5 cm) has a rounded opening above with in-rolled edges and may be delicately lined with purple veins toward the bottom and slightly purple-spotted



FIGURE 1. *Cyripedium candidum* (White Lady's Slipper) flower (Wake 2004).

around the pouch opening. The two lateral petals (4-5 cm), which are similar to the sepals, are pale yellow-greenish with lavender veins, pointed, and spirally twisted. The dorsal sepal (2-2.5 cm) is ovate to elliptical and the two lateral sepals are united nearly to the apex. The staminodes, ovate in shape, appear yellow with purple spots and are attached to the style and stigma forming the column. The stigma is borne on the lower side near the base of the column and the pollen is shed in bilateral waxy pollinia. The ovary is inferior, 3-celled, (Fig. 2) and matures into an ellipsoid dehiscent capsule. The seeds are very numerous and minute in size. *Cyripedium candidum* are thought to be pollinated by adrenid and halictid bees (Catling and Knerer 1980).

Habitat

Cyripedium candidum is not a shade tolerant plant and prefers open, wet, rich prairie meadows with alkaline soils and calcareous fens (Galbraith 1996). However, prairie habitats have been transformed by agricultural and urban development, through cropland expansion, extensive grazing, and wetland drainage.



FIGURE 2. *Cyripedium candidum* developing ovary following successful pollination (Wake 2004).

Even in remaining natural areas, the control of wildfires may be endangering the white lady's slipper habitat. In natural ecosystems, wildfires curtail plant succession and prevent development of over-shady conditions that are detrimental to continued *C. candidum* growth and survival (USGS).

Objectives

This project was designed to study the effects of different micro-environments caused by competing foliage, particularly with respect to pollination and ovary / seed growth and development of *C. candidum* on three habitats in eastern South Dakota, USA.

Methods

During the summer of 2003, three different *C. candidum* populations were examined during seed-set and ovary-development stages of the life cycle for sexual reproduction success in relation to diverse surrounding vegetation micro-environments, particularly with respect to height and density of the surrounding flora. By midsummer, flowers were senescing and ovary development in pollinated individuals was evident. The Dense Competing Foliage (DCF) site is a



FIGURE 3. *Cypripedium candidum* senescing / aborting ovary tissue following no pollination (Wake 2004).

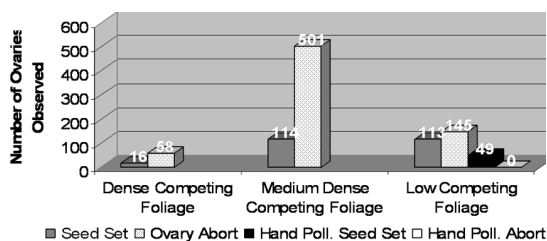


FIGURE 4. Comparisons of *C. candidum* seed set versus ovary abortion in dense competing foliage (DCF), medium-dense competing foliage (MDCF) and low competing foliage (LCF) sites.

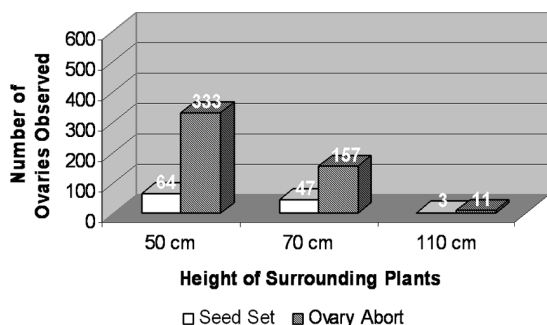


FIGURE 5. Effects of competing foliage heights for seed set in *C. candidum* in medium-dense competing foliage (MDCF) site.

railroad right-of-way very densely populated by brome grass (*Bromus inermis*), a perennial cool-season sod grass with vigorous rhizomes, and the site had not been mowed for many years. The Medium-Dense Competing Foliage (MDCF) site is a federal wildlife production area populated by mixed prairie grasses, forbs and thickets. It had not been grazed for three years, resulting in a range of dense to medium-dense shade / competition for the intermingled orchid population. The Low Competing Foliage (LCF) site is adjacent to a federal waterfowl production area, and, by contrast, was mowed late the previous fall (2002) so the orchids were growing in a much more open and exposed environment.

To compare the pollination success between these different growing conditions, ovary development (successful pollination) (Fig. 2) vs. ovary abortion (no pollination) (Fig. 3) was recorded for every flower located.

Results

The more shaded DCF and MDCF sites demonstrated only 22% ($n=74$) and 18% ($n=615$) seed set, respectively, while at the more open LCF location, successful seed set was 44% ($n=258$) (Fig. 4). In addition, a separate, hand pollinated population at the open LCF site ($n=49$) resulted in 100% ovary / seed development. To further assess the affects of height, as well as density, of surrounding plants in the dense to medium-dense competing foliage (MDFS) site, this area was subdivided by height of surrounding foliage, and ovary development quantified (Fig. 5). In areas averaging 50 cm tall competing foliage, the seed set was 16% ($n=397$); 70 cm tall, it was 23% ($n=204$); and in 110 cm grass, it was 21% ($n=14$). Within this medium-density micro-environment, with a limited number of orchid flowers, differences between surrounding plant height could not be differentiated.

Discussion

Environmental factors, such as temperature, soil type, and light exposure, affect the distribution of *C. candidum*, due to the fact that like many orchids, it is highly specialized in its requirements (Galbraith 1996). Data presented in Fig. 4 demonstrates that a micro-environment of dense, competing foliage can

also affect sexual reproduction in *C. candidum* by physically preventing pollinators from accessing the flowers. Tall, dense surrounding plants would also limit wind-borne seed dispersal, and possibly seed germination. Other habitat degradation threats to consider are trampling of plants and delicate root / rhizomes in the moist soils by over-grazing and all-terrain-vehicle use. Mowing or controlled burns help open the micro-environment to more light for the orchids, but they also remove the thick layer of leaf litter that protects over-wintering rhizomes and next year's fully-formed stem shoots through the harsh northern prairie winters. Even though orchids are able to reproduce vegetatively by underground rhizomes, sexual reproduction provides the critical genetic diversity to insure survival in changing environments. Further study of flower pollination, soil / cultural needs and mycorrhiza-seed germination associations of this native orchid, in relation to land-use management practices, will be useful in protecting this rare natural resource.

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Carol M.F. Wake received her PhD in plant physiology and anatomy and currently teaches botany, plant anatomy, and general biology at South Dakota State University, Brookings, SD, USA. Carol is particularly interested in research encompassing the micro-environmental requirements of *Cypripedium candidum*, an indigenous terrestrial orchid of South Dakota. Several recent projects have been conducted in collaboration with undergraduate botany / plant anatomy students.

PUPULIN - Addenda Orchidaceis Quepoanis