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Bonnie Warner Alexander

Donald Kirby

Mario Biondini

Edward Dekeyser

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BONNIE WARNER ALEXANDER, DONALD KIRBY[1], MARIO BIONDINI, AND EDWARD DEKEYSER

Department of Science, Valley City State University, Valley City, ND 58072, USA (BWA; deceased)
School of Natural Resource Sciences, North Dakota State University, Fargo, ND 58108, USA (DK, MB, ED)

ABSTRACT A population of threatened western prairie fringed orchid (*Platanthera praeclara*) was selected in 2004 on the Sheyenne National Grassland in southeastern North Dakota to study seed production and maturation for future use in population viability modeling. We randomly collected 30 seed capsules from the population under a permit from the U.S. Fish and Wildlife Service to: 1) identify capsule parameters that might be correlated with seed number and viability, 2) estimate an appropriate sample size to obtain accurate seed production estimates, 3) quantify seed production and viability per seed capsule, and 4) document temporal patterns in seed embryo development. We found that the number of seeds per capsule was weakly correlated with capsule weight ($R^2 = 0.23$, $P = 0.04$), while the proportion of viable seeds within a capsule was weakly correlated with capsule length ($R^2 = 0.20$, $P = 0.01$) and capsule circumference ($R^2 = 0.17$, $P = 0.04$). However, seed production and embryo viability varied extensively in our study to the extent that capsule measurements were not reliable indicators of fecundity or fertility. Our study provides guidance for the sample size required to make statistical inferences regarding seed production and seed viability in western prairie fringed orchid populations. Our data also suggest that orchid seeds undergo maturation up to the time of capsule dehiscence based on increases we observed from August to September in seed weights and proportion of large embryos. Our observations reinforce the importance of moratoriums on grazing and mowing in some areas of orchid habitat until after mid-September.

KEY WORDS North Dakota, orchid, *Platanthera praeclara,* seed production, seed viability, threatened species

The U.S. Fish and Wildlife Service (1989) listed the western prairie fringed orchid (*Platanthera praeclara*) as a threatened species in 1989. The species has declined in numbers and range extent primarily due to conversion of prairie to cropland (Bowles 1983). Western prairie fringed orchids grow in or on the edges of wetlands and in mesic tallgrass prairie. Local extinctions and recolonizations have been observed on a regular basis in populations of western prairie fringed orchids in the Sheyenne National Grassland and are characteristic of some metapopulations (Husband and Barrett 1996, Hof et al. 1999, Sieg et al. 2003*a*, *b*, *c*). The current range of the western prairie fringed orchid extends through Kansas, Missouri, Iowa, Nebraska, Minnesota, eastern North Dakota and into southeastern Manitoba. The plant is no longer found in South Dakota and Oklahoma, and populations have been reduced in Iowa, Kansas, Missouri, and eastern Nebraska (Alexander 2006).

Our study was conducted on the Sheyenne National Grassland, managed by the U.S. Forest Service, in southeastern North Dakota. The orchid population in the Sheyenne National Grassland has been monitored for decades, yet there remains a lack of scientific knowledge of many aspects of its biology, ecology, and phenology, in addition to impacts of grazing management actions.

Armstrong et al. (1997) established the need to collect data on seed production and phenological events in the life history of the orchid. They reported that the western prairie fringed orchid reproduces 99% through seeds although the orchid can also reproduce vegetatively. Bowles (1983) speculated that vegetative reproduction can sustain a population temporarily but growth from seeds is critical for the survival of this species. Reproduction from seeds may be especially critical in the Sheyenne National Grassland where topography, local hydrology, and sandy soils exaggerate effects of flooding and drought. Regular flooding and drought cycles result in annual shifting orchid habitat locations (Hof et al. 1999). An accepted conservation theory is that if seed production were to drop below some minimum critical number, the orchid population might decline (Sieg et al. 2003*a*). However, no such minimum critical number has been established for the western prairie fringed orchid. Some of the most significant threats to orchid survival and reproduction are disruption of habitat, land-use practices that prevent growth and reproduction, and hydrological changes that permanently alter orchid habitat (Armstrong et al. 1997).

Orchid seed capsules begin to form after the flowers are pollinated in mid- to late- July in the Sheyenne National Grassland. Although no standard for classifying capsules has been developed, capsules can be roughly categorized as plump, inflated, partially inflated, twisted, and atrophied (Sieg et al. 2003*c*, Erickson et al. 2006). Atrophied capsules have been found to have no seeds while twisted and partially inflated capsules contained about half as many

[1]Corresponding author email address: donald.kirby@ndsu.edu

seeds as plump capsules (Sieg et al. 1998, Erickson 2003). The seed of the western prairie fringed orchid, like that of most orchids, contains no endosperm and holds tiny embryos with little other tissue (Sharma 2002). Collection of data on proportion of seeds containing viable embryos is critical to the ongoing management of the orchid.

Seed capsules remain green to the end of the growing season, often beyond when the plant has atrophied. In the Sheyenne National Grassland, green capsules usually develop in early August and turn yellow in mid- to late-August before maturation in mid-September. Mature seeds are released through slits in the ripe capsule once the plant has become dormant and the capsule has dried. Seed release usually occurs in mid-September in the Sheyenne National Grassland (Wolken 1995, Sieg et al. 1998). Current livestock grazing management strategies are based on the assumption that viable seeds are not present in green and yellow seed capsules. However, researchers and commercial orchid growers wishing to germinate seeds in culture have collected orchid seeds from closed green and yellow capsules (Böhm 1996, Zettler et al. 2001, Sharma 2002, Royal Botanical Garden Kew 2003). If a majority of viable seeds are produced prior to mid- September, this could have implications for the timing of grazing and mowing strategies.

Thus, our research objectives were to identify capsule parameters that might be correlated with seed number and viability, estimate an appropriate sample size to obtain accurate seed production estimates, quantify seed production and viability per seed capsule, and document temporal patterns in seed embryo development.

METHODS

We conducted our study on the Sheyenne National Grassland (SNG; 46° 28' N latitude, 97° 16' W longitude) approximately 80 km southwest of Fargo, North Dakota, USA. Long-term growing season (April–October) precipitation for the study area averaged 44.8 cm; 38.7 and 50.9 cm were recorded in 2003 and 2004, respectively. We obtained a permit (TE091284-0) from the U.S. Fish and Wildlife Service in 2003 to collect western prairie fringed orchid seeds on the SNG. In 2004, we counted all flowering orchids having inflated capsules and showing no sign of seed predation or damage in a roadside ditch approximately 5 km north of McLeod, North Dakota. We harvested seed capsules from ten randomly selected orchids every 10 days between 21 August and 9 September 2004. On each collection date, we divided orchid inflorescences into thirds by visual estimation and subsequently counted and classified all capsules as plump, inflated, twisted, or atrophied (Fig. 1). We randomly harvested seed capsules from each level of inflorescences to account for possible maturity differences; we collected a minimum of 3 capsules from the bottom one-third, three from the middle one-third, and three from the top one-third of the inflorescence. Due to limited orchid numbers, we collected capsules from plants two or more times.

We weighted and measured circumference, diameter, and length of fresh capsules. We removed and stored seeds in petri dishes until processed. During processing, we cleaned seeds of debris and subsequently weighed, counted, and examined them for presence of embryos using a dissecting microscope at 30X. We placed a grid on the bottom of a petri dish to facilitate examination and accurate counting; we used mechanical counters to increase accuracy. Depending on the number of seeds present, we examined 500–5,000 seeds from each capsule for the presence of embryos. We recorded numbers of seeds containing embryos and classified the embryos as: 1) None, 2) Small (occupying approximately half the testa space, may not be capable of germination), and Large (occupying more than half the testa space, considered capable of germination) (Margaret From, Henry Doorly Zoo, personal communication; Fig. 2). To estimate percentages of small and large embryos, we measured 100 embryos under a Nikon compound light microscope at 40X (Nikon Instruments Inc, Melville, NY, USA). Additionally, we measured and subsequently recorded length and width of the testa and embryos.

We analyzed the data using SPSS for Windows (2001). We used the Shapiro-Wilk normality statistic to test for normality and the Bartlett test for homogeneity of variance. We applied arcsine square root transformations due to non-normally distributed data. We estimated capsule volume using a prolate spheroid equation $4/3\pi ab^2$ (where a = length and b = width) and the number of samples (capsules) needed for an accuracy of ± 10% or ± 20% and a precision of 95% following procedures described by Bonham (1989). We used simple linear regression (Weisberg 1980) to analyze effects of capsule length, diameter, circumference, volume, and weight (predictor variables) on seed number per capsule and percent viable seeds per capsule (response variables).

RESULTS

Capsules and Seeds

Ten capsules each were collected on 21 August, 31 August, and 9 September, respectively. Fresh capsule weight exhibited a wide range of values (0.005 g to 0.40 g) and was weakly correlated with seed number ($R^2 = 0.23$, $P = 0.04$). Capsule length and circumference were also weakly correlated with the percent of potentially viable embryos in the capsules ($R^2 = 0.20$, $P = 0.01$; $R^2 = 0.17$, $P = 0.04$; respectively). Capsule diameter and volume were not correlated with seed number or the number of seeds containing potentially viable embryos.

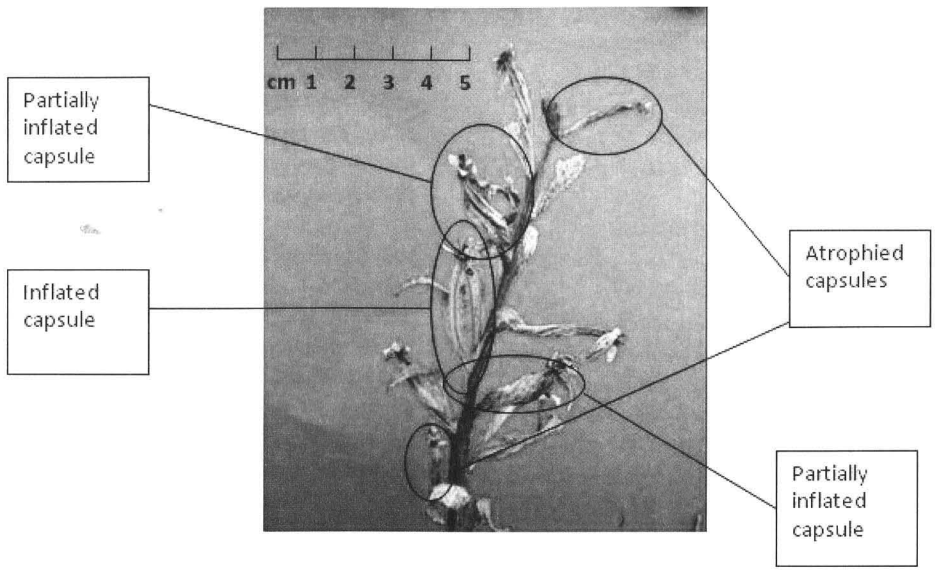

Figure 1. Example of inflated, partially inflated, and atrophied capsules on a western prairie fringed orchid (*Platanthera praeclara*).

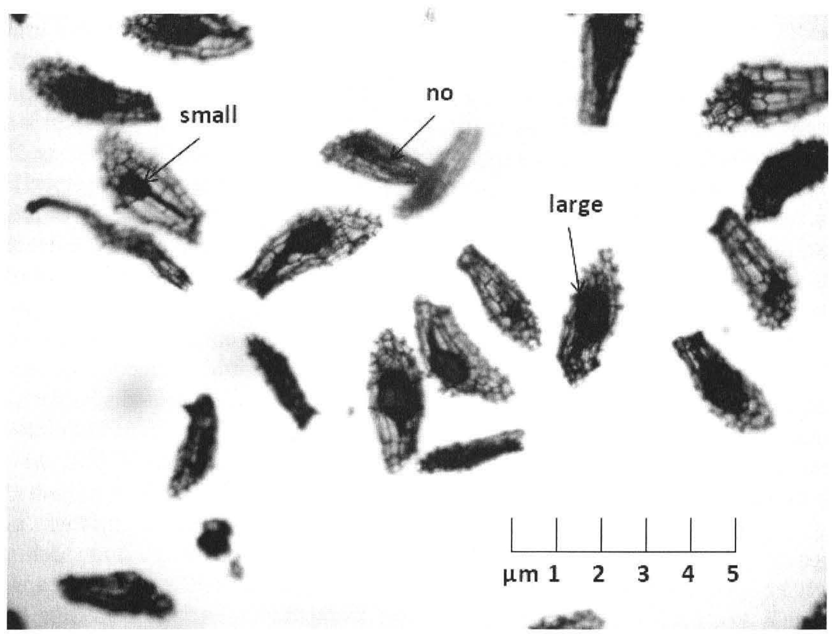

Figure 2. Photograph illustrating western prairie fringed orchid (*Platanthera praeclara*) seeds containing no embryos, small embryos, and large embryos.

Embryos

All embryos measured were ovoid and hyaline, a distinction that is easy to make under the microscope. Small and large embryo average length and width were 1.5 × 0.9 and 1.9 × 1.1 μm respectively. All structures smaller than 0.9 μm by 0.5 μm observed inside the testa appeared as dark linear structures, not ovoid or hyaline, and therefore were not considered viable.

Seed Viability

We estimated a mean of 8,681 seeds per capsule of which 80% or nearly 7,000 were embryonated seeds. However, if only the seeds containing large embryos were considered viable (39%), an average capsule contained approximately 3,000 fewer viable seeds than if all embryos were considered viable. Seeds containing no embryos averaged 20% of the mean seeds per capsule.

Date of Capsule Collection

The proportion of large embryos per capsule nearly doubled (27% to 50%) between August and September, but the differences were only marginally significant ($P \leq 0.09$). Seed number increased minimally as the season progressed, with the percentage of large embryos in capsules increasing and the percentage of small embryos decreasing.

Capsule Position

Mean seed numbers were not different ($P > 0.05$) among capsule positions. In addition, the percentage of seeds that contained potentially viable embryos revealed no difference among tier levels ($P > 0.05$). However, lower proportions of embryonated seeds were found in 69% and 89% of top-tier and bottom-tier capsules, respectively.

Estimated Sample Size

Sample size necessary for estimating viable seed number from fresh capsules with an accuracy of ± 10% or ± 20% and a precision of 95% decreased from August to September (127 versus 118 or 32 versus 29, respectively). Sampling for the potential viability rate based on September embryo status would require 84 or 21 capsules with an accuracy of ± 10% or ±20%, respectively.

DISCUSSION

Robust statistical analyses on the reproduction of this orchid are difficult due to wide annual variations in seed numbers, capsule measurements, percentage of viable embryos, and necessary constraints on the collection and handling of the reproductive parts of this threatened plant. Those difficulties aside, this study generated some useful parameters for estimating seed number, examining embryo viability, and categorizing and classifying capsules.

It is important in managing a federally threatened plant to have research-based sampling techniques for estimating seed production and embryo viability rates. Ideally, these sampling techniques can be used in the field with minimal disturbance to the orchid. In monitoring and research studies on the orchid, capsule diameter often has been used as an indicator of seed number and embryo viability. Our results yielded no significant correlation between capsule diameter and seed numbers. Both seed production and embryo viability varied extensively in our study to the extent that capsule measurements were not reliable indicators of fecundity or fertility. We found that fresh capsule weight was the most important predictor and should be used for estimating seed numbers in harvested capsules.

Seed number per capsule and embryo viability estimates for the western prairie fringed orchid varies widely in the literature. Our seed numbers per capsule ranged from 1,938 to 17,028, and embryo viability, based on embryo status, ranged from 11% to 100%. Mean seed number per capsule (9,825) in our study was lower than that of Richardson et al. (1997; $\bar{x}$= 21,618) and approximated that of Erickson et al. (2006; $\bar{x}$= 15,000), but mean viability in our study was higher (80%) than in these previous studies. Whether these differences resulted from differences in the method of seed counting and viability determination or from variation in growing conditions during the sampling years is unknown.

Since seed production and embryo viability estimates are used in models to project the survival of threatened plant species such as the western prairie fringed orchid, it is important that sampling techniques are consistent and that accurate data are collected and utilized. Future research efforts could use our sample size estimations to collect capsules, count seeds, determine embryo viability, and devise a formula to use for estimating seed production and embryo viability for any particular year. By accepting an accuracy of ± 20%, only 29 capsules collected in September would be required for estimating seed number, and 21 capsules would be needed for embryo viability rates. These sampling levels are desirable and should not impact the long-term population of the orchid.

Most western prairie fringed orchid studies conducted in the SNG (Wolken 1995, Sieg et al. 1998) reported that seeds are not considered mature until capsules are totally atrophied (brown) and dehiscent in mid-September. However, calendar dates of capsule dehiscence are missing from the literature. Capsules observed in this study were beginning to open on 9 September 2004. Collecting or disturbing capsules when they are still green, prior to mid-September in the SNG, may prevent seeds from fully maturing (From and Read 1997), and may result in poor embryo formation and germination rates.

The western prairie fringed orchid is dependant on seed to maintain populations. Reproduction from seed may be especially critical for orchids in the SNG where topography, local hydrology, and sandy soils exaggerate effects of flooding and drought, and result in annual shifting of orchid habitat. Our study suggests that seed maturation occurs in the last few weeks prior to capsule dehiscence. Increase in seed weight and percentage of large embryos (27% to 50%) as time progressed indicate that a seed maturation process is ongoing during late summer. The late season seed coat hardening process (Arditti 1992) could result in better germination, aid in seed survival *in situ* by prolonging the imbibition of water until conditions are optimal for germination, and/or afford the seed long-term protection from contamination by fungi and bacteria, thus allowing orchid seeds to remain viable in the seed bank for longer periods of time. Long-term survival of western prairie fringed orchid seeds would be an important survival strategy considering the extreme climatic conditions that occur in the orchid's habitat (Umbanhower 1991, Sieg and King 1995, Running 1996, Hof et al. 1999).

MANAGEMENT IMPLICATIONS

To optimize mature seed production, we suggest deferment of mowing and grazing until after mid-September to allow more orchid embryos to reach full size and for seeds to complete the hardening process. More research is needed to test for spatial and temporal variation in seed numbers and differences in embryo viability, as well as fertility and fecundity in the orchid. If managers or researchers employ our techniques to estimate seed number and embryo viability they should record detailed information on date of collection, predation on capsules, and employ equal collection of capsules from all positions within the inflorescence to account for possible maturity effects. We recommend closely observing seed capsules starting in early September in order to harvest prior to opening and seed dispersal. Additionally, further research evaluating longevity of western prairie fringed orchid seeds in the environment may be warranted.

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