6-2010

# In Situ Development of Western Prairie Fringed Orchid Seeds, Protocorms, and Seedlings in Grazed and Non-Grazed Prairie Habitat

Bonnie Warner Alexander

Donald Kirby

Mario Biondini

Edward Dekeyser

# *In Situ* Development of Western Prairie Fringed Orchid Seeds, Protocorms, and Seedlings in Grazed and Non-Grazed Prairie Habita

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** In 1989, the U.S. Fish and Wildlife Service listed the western prairie fringed orchid (*Platanthera praeclara*) as threatened. Although this orchid has been monitored for years, there is little scientific documentation of its biology, ecology, and phenology, nor the impacts of management activities on its populations. Our objectives were to document seed germination and seedling production rates after one year *in situ*, and compare seed germination in grazed and non-grazed prairie habitat in the Sheyenne National Grassland (SNG) in southeastern North Dakota. Of 18,717 planted seeds, we recovered 1,561 swollen embryos, 94 protocorms, and 51 seedlings. We documented no difference in germination rate between seeds planted in grazed versus non-grazed prairie. However, our results suggested that 15 new flowering orchids may be produced from each flowering orchid that survives the growing season on the SNG. Thus, our findings confirm successful production of western prairie fringed orchid seedlings after one year in situ. Further research is needed to evaluate potential impacts of livestock grazing on other stages of the orchid life cycle, particularly protocorm and seedling survival rates.

**KEY WORDS** germination, grazing, North Dakota, orchid, *Platanthera praeclara,* seedling survival, threatened species

The U.S. Fish and Wildlife Service (1989) listed the western prairie fringed orchid (*Platanthera praeclara*) as a threatened species. The range of the orchid, once more extensive (Bowles 1983), currently 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). Major threats to the orchid include habitat disruption, land-use practices that interfere with orchid growth and reproduction, and hydrologic changes.

This study was conducted on the Sheyenne National Grassland in southeastern North Dakota (Ransom and Richland counties) where a population of blooming orchids fluctuates between several thousand and 15,000 plants (Alexander 2006). The orchid grows near tallgrass prairie plant communities at various positions from wetland bottoms (swale) to side slopes (hummocks) of remnant Pleistocene sandy beach ridges and sand dune fields (Bowles 1983). The orchid population shifts up and down these wetland slopes and into the swales between the beach ridges and dune troughs in response to changes in moisture levels (Sieg and King 1995, Wolken 1995, Hof et al. 1999).

The western prairie fringed orchid population in the Sheyenne National Grassland has been monitored for over two decades, but there remains a lack of scientific documentation of many aspects of its biology, ecology, and phenology; as well as impacts of management actions (Bjugstad-Porter 1993, Rasmussen 1995). It is assumed from available evidence that western prairie fringed orchids reproduce almost entirely (99%) through seeds (Alexander 2006). The orchid also can reproduce vegetatively by producing a new perennating bud and primary tuber during the growing season. This perennating bud may develop into a new shoot and root system which may develop into a new plant the next growing season. It is thought that vegetative reproduction can sustain a population temporarily, but growth from seeds is essential for continuing the population (Bowles 1983). The necessity to reproduce from seed may be especially true in the Sheyenne National Grassland where hummock-swale (dune and trough) topography, local hydrology, and sandy soils exaggerate effects of flooding and drought (Hof et al. 1999, Sieg and Wolken 1999, Wolken et al. 2001).

A model developed by Sieg et al. (1998) projected a 30% reduction in orchid population growth rates in grazed pastures compared to non-grazed pastures. The USDA Forest Service (2001) suggested protecting either 30% or 50% of the "core" allotments (the area containing most of the orchid populations) in the Sheyenne National Grassland to increase population growth rates of the orchid. A subsequent model developed by Sieg et al. (2003*a*, *b*, *c*) corroborated enhanced population growth rates associated with protecting 30% of core allotments from grazing during the orchid growing season. The authors suggested that cattle grazing could enhance orchid reproduction by creating regeneration niches for the orchid but that this hypothesis had not been tested. Much of the literature emphasizes that effects of grazing can only be ascertained by long-term,

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

replicated direct experimental studies (Sieg and King 1995). Thus, our objectives were to 1) compare the impact of cattle grazing and exclusion on western prairie fringed orchid seed germination, 2) document the phenology of western prairie fringed orchid seed germination after one year *in situ*, and 3) calculate germination and seedling production rates of western prairie fringed orchid after one year *in situ*.

## METHODS

Four orchid habitat areas were selected for this study, including 2 areas in twice-over grazed pastures (South Venlo: 46° 26.728' N; 97° 24.628'W and East Venlo: 46° 26.660' N; 97° 23.246'W) and 2 in cattle exclosures (Penberthy: 46° 28.570'N; 97° 23.788' W and Bjugstad: 46° 26.862' N; 97° 22.622'W). Cattle exclosures had been in place and non-grazed for at least 15 years. Average long-term growing season (April–October) precipitation for the study area was 44.8 cm; precipitation during 2003 and 2004 was 38.7 and 50.9 cm, respectively.

During September 2003, we collected orchid capsules ($n$ = 30) from orchids growing on private property. Care was taken to gather capsules from top, bottom, and middle of the seed head. We weighed, measured, and stored capsules in glass vials in the laboratory until early October 2003 when seeds were removed from each capsule, emptied into a crucible, cleaned of debris, and thoroughly mixed. We examined at least 100 seeds from each capsule using a compound microscope to estimate percentage of seeds within each capsule containing viable embryos. We considered seeds viable if they contained an embryo occupying approximately 50% of the seed's internal space (Margaret From, Henry Doorly Zoo, NE, USA, personal communication).

We constructed seed germination packets ($n$ = 120) using the method devised by Rasmussen and Whigham (1993). For seed packet placement, we divided a 90 m transect into 30, 3-m segments established in orchid habitat within each study area. We randomly buried a single seed packet within each of the 3-m segments. We placed packets 1-cm deep into the ground in mid-October 2003. We wrapped a piece of florist wire around one side of the slide jacket and secured it to a pole barn spike, metal washer, and cattle tag located 1 m east of the packet. Importantly, metal ions are extremely toxic to plants (especially seedlings), so the 1-m distance between the spikes and packet was deemed necessary to avoid toxic effects.

We retrieved seed packets during October 2004 and cleaned them of debris. We scraped and pressed seed pack contents onto agar plates and subsequently examined contents under a dissecting microscope. We evaluated and recorded developmental stages leading to a seedling with a true leaf using the following criteria [adapted from Arditti (1992) and Sharma (2002)]: Stage 0 = no germination; Stage 1 = obviously swollen embryo (double in size), testa rupturing or swollen embryo outside of the testa; Stage 2 = protocorm elongating, a few rhizoids to an enlarged protocorm with many rhizoids; and Stage 3 = tip of first leaf forming to a developed seedling with ≥2 leaves, root initial apparent.

We transformed data using arcsine transformations due to non-normality and/or non-homogeneity of variance. We used the Shapiro-Wilk normality statistic to test for normality and the Bartlett test for homogeneity (Zar 1999). We analyzed differences between grazed and non-grazed treatments for all seed germination stages using a one way analysis of variance (ANOVA; SPSS 2001); results were considered significant at $P < 0.05$.

## RESULTS

We examined 18,717 seeds from the buried packets at the end of one year *in situ*. Of the seeds examined, 5,427 (29%) did not contain viable embryos. A total of 1,706 seeds (9%) had imbibed water and developed enough to double their size (Stage 1) and rupture seed coats (Fig. 1). Additionally, 94 seeds (0.5%) developed to the protocorm stage (Stage 2; Fig. 1) and 51 seeds (0.4%) developed into seedlings (Stage 3; Fig. 2).

Of 13,290 viable seeds, 12.8% demonstrated some germination response, 11.7% of the embryos doubled in size, 0.7% developed to the protocorm stage, and 0.4% developed to some seedling stage from one to two leaves. We documented no differences ($P > 0.05$) in germination rates for all germination stages between grazed and non-grazed plots. Seeds that were considered viable and developed any type of germination response averaged 12.4% and 13.5% for the grazed and non-grazed treatments, while the number of swollen seeds averaged 11.4% and 12.4% for the same treatments. Both grazed and non-grazed treatments averaged 0.7% of seed developing to the protocorm stage with 0.4% developing to the seedling stage.

We estimated a 0.004 probability of seedling production in the first year after planting and a 0.007 probability of protocorm production. Using previous estimates of seedling survival rates (25%), seeds ($\bar{X}$ = 9,825) per capsule, and capsules ($\bar{X}$ = 11; Armstrong et al. 1997, Alexander 2006), approximately 12 adult orchid plants would be produced ≥3 years after seed production and 132 new orchid plants could be produced from each surviving flowering orchid each year.

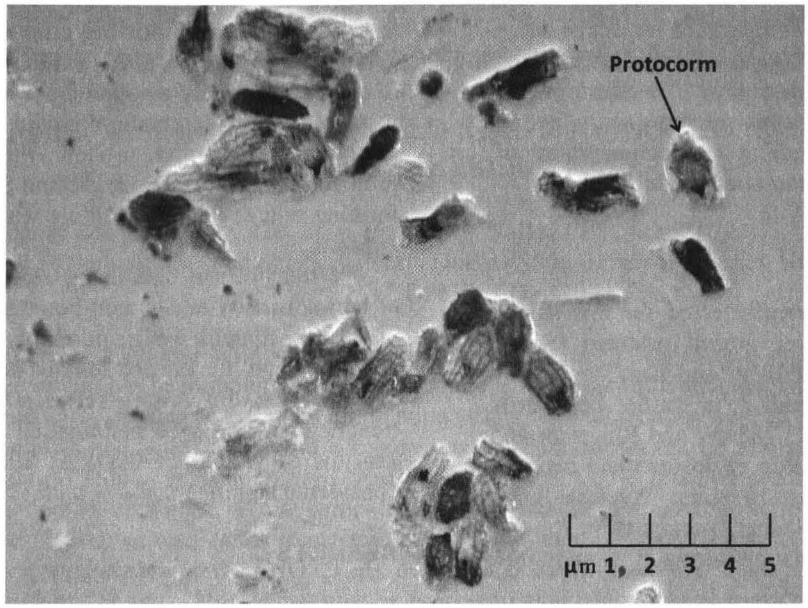


Figure 1. Germinating western prairie fringed orchid seeds recovered from seed packets after one year *in situ*.

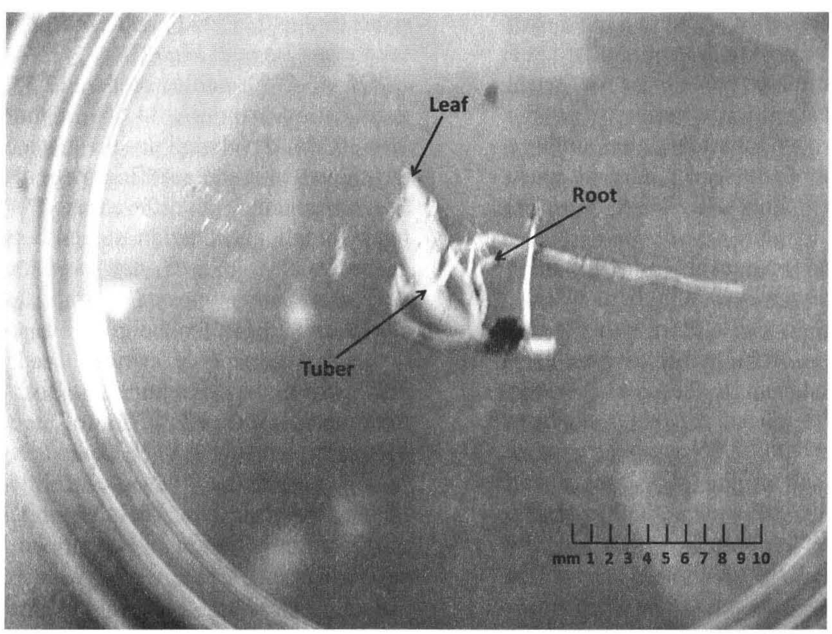


Figure 2. Western prairie fringed orchid seedling recovered from a seed packet after one year *in situ*.

## DISCUSSION

Armstrong et al. (1997) recovered approximately 100 protocorms from seed packets which resulted in about a 0.0056 probability of protocorm production from seed. They estimated that only 25% of seedlings survive to form an adult plant, an average of 25% of newly produced plants flower, and that 75% become vegetative. Our data documented a 0.007 probability of protocorm production which is similar to Armstrong et al. (1997). However, many protocorms do not survive to form adult plants in culture (Rasmussen 1995). Data regarding the survival rate of protocorms *in situ* are not available but would contribute to an understanding of the reproductive potential and recruitment of this orchid.

Although we only estimated a 0.004 probability of orchid seedling production in year one from seeds planted *in situ*, seeds not germinating the first year may germinate in subsequent years which could increase flowering orchid production from a seed lot over our estimate. Stoutamire (1974) observed seeds continuing to germinate for 12 months in culture. No published data are available on seeds and protocorms that continue development *in situ* to produce seedlings in subsequent years. A study of the longevity of orchid seeds *in situ*, as well as their continuing development in subsequent years, may yield valuable information to help establish the long-term reproductive potential of this plant and its ability to resist catastrophic habitat changes.

To our knowledge, phenology of western prairie fringed orchid growth from seedling to flowering *in situ* has not previously been documented. Thus, our findings provide the first published account of orchid development from seed to seedling within the first 12 months *in situ*. Our findings, that no large seedlings were recovered in October, suggests that above-ground growth in the first year is unlikely, and above-ground vegetative growth (and possibly flowering) is probable and may occur in the second year after seed production.

Our analyses compared the germination response of artificially planted orchid seeds in grazed and non-grazed areas. Further, our findings of no observable trend or differences between germination rates in grazed versus non-grazed areas suggest that the microclimate beneath the soil in grazed and non-grazed orchid habitat does not vary in ways that impact orchid seed germination to the 1 to 2 leaf seedling stage during the first 12 months after seed placement in the soil.

## MANAGEMENT IMPLICATIONS

Cattle presence in orchid habitat could impact survival of orchid protocorms and seedlings directly such as being grazed or trampled, or indirectly through subsequent soil erosion and soil moisture depletion. Further research is necessary to better understand how grazing cattle can directly and indirectly impact western prairie fringed orchids during early stages of its lifecycle. Since seed germination is only a small part of the lifecycle of this orchid, a study of seedling establishment and growth to flowering between grazed and non-grazed (or disturbed versus undisturbed) areas across dry and wet climatic cycles is a critical need to further assess the impact of grazing on the orchid population in the Sheyenne National Grassland.

## ACKOWLEDGMENTS

We thank Darla Lenz and the USDA Forest Service, Dakota Prairie Grasslands for their financial and technical support of this research project.

## LITERATURE CITED

Alexander, B. 2006. An analysis of seed production, viability, germination *in situ*, and grazing impacts on the western prairie fringed orchid (*Platanthera praeclara*, Sheviak and Bowles). Dissertation, North Dakota State University, Fargo, USA.

Arditti, J. 1992. Fundamentals of Orchid Biology. John Wiley and Sons. New York, New York, USA.

Armstrong, D., D. Fritz, P. Miller, and O. Beyers. 1997. Population and habitat viability assessment for the western prairie fringed orchid at Eugene Mahoney State Park. Final Report Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA.

Bjugstad-Porter, R. 1993. The western prairie fringed orchid (*Platanthera praeclara*): Its response to burning and associated mycorrhizal fungi. Thesis, University of Wyoming, Laramie, USA.

Bowles, M. 1983. The tallgrass prairie orchids *Platanthera leucophaea* (Nutt.) Lindl. and *Cypripedium candidum* Muhl. Ex. Willd.: Some aspects of their status, biology, and ecology and implications toward management. Natural Areas Journal 3:14–37.

Hof, J., C. H. Sieg, and M. Bevers. 1999. Spatial and temporal optimization in habitat placement for a threatened plant: the case of the western prairie fringed orchid. Ecological Modelling 115:61–75.

Rasmussen, H. N. 1995. Terrestrial Orchids: From Seed to Mycotrophic Plant. Cambridge University Press, Cambridge, England.

Rasmussen, H. N., and D. F. Whigham. 1993. Seed ecology of dust seeds *in situ*: a new study technique and its applications in terrestrial orchids. American Journal of Botany 80:1374–1378.

Sharma, J. 2002. Mycobionts, germination and conservation genetics of federally threatened *Platanthera praeclara* (Orchidaceae). Dissertation, University of Missouri, Columbia, USA.

Sieg, C. H., and R. M. King. 1995. Influence of environmental factors and preliminary demographic analysis of a threatened orchid, *Platanthera praeclara*. American Midland Naturalist 134:307–323.

Sieg, C. H., R. M. King, P. S. Miller, and V. J. Nicholas. 1998. Fruit set of the threatened orchid, *Platanthera praeclara*: Implications for population growth and management. Report from the U.S. Department of Agriculture, Forest Service Rocky

Mountain Research Station, Rapid City, South Dakota, USA.

Sieg, C. H., R. M. King, and F. Van Dyke. 2003*a*. Exercise 12. Creating a stage-based deterministic PVA model – the western prairie fringed orchid. Pages 91–99 *in* F. Van Dyke and contributors. A Workbook of Practical Exercises in Conservation Biology. McGraw Hill, New York, New York, USA.

Sieg, C. H., R. M. King, and F. Van Dyke. 2003*b*. Exercise 14. Using stochastic models to incorporate spatial and temporal variability. Pages 109–113 *in* F. Van Dyke and contributors. A Workbook of Practical Exercises in Conservation Biology. McGraw Hill, New York, New York, USA.

Sieg, C. H., R. M. King, and F. Van Dyke. 2003*c*. Exercise 15. Conservation and management issues and applications in population viability analysis. Pages 115–122 *in* F. Van Dyke and contributors. A Workbook of Practical Exercises in Conservation Biology. McGraw Hill, New York, New York, USA.

Sieg, C. H., and P. M. Wolken. 1999. Dynamics of a threatened orchid in flooded wetlands. North American Prairie Conference 16:193–201.

SPSS. 2001. Statistical Procedures for Social Science. SPSS, Inc., Chicago, Illinois, USA.

Stoutamire, W. P. 1974. Terrestrial Orchid Seedlings. Pages 101–128 *in* The Orchids, Scientific Studies. Wiley-Interscience, New York, New York, USA.

United States Department of Agriculture Forest Service. 2001. Land and resource management plan for the Nebraska National Forest and associated units. U. S. Department of Agriculture, Forest Service, Rocky Mountain Region. Denver, Colorado, USA.

United States Fish and Wildlife Service. 1989. Endangered and threatened wildlife and plants: determination of threatened status for *Platanthera leucophaea* (eastern prairie fringed orchid) and *Platanthera praeclara* (western prairie fringed orchid). Federal Register 54:39857–39862.

Wolken, P. M. 1995. Habitat and life history of the western prairie fringed orchid (*Platanthera praeclara*). Thesis, University of Wyoming, Laramie, USA.

Wolken, P. M., C. H. Sieg, and S. E. Williams. 2001 Quantifying suitable habitat of the threatene western prairie fringed orchid. Journal of Rang Management 54:611–616.

Zar, J. H. 1999. Biostatistical Analysis. Prentice Hall Upper Saddle River, New Jersey, USA.

*Submitted 6 March 2009. Accepted 6 March 2010. Associate Editor was David M. Mushet.*