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SEED CAPSULE PRODUCTION IN THE ENDANGERED WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*) IN RELATION TO SPHINX MOTH (LEPIDOPTERA: SPHINGIDAE) ACTIVITY

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ABSTRACT. The endangered western prairie fringed orchid, *Platanthera praeclara* Sheviak and Bowles, is found in remnant tall grass prairie in the northern central plains of North America. The Canadian population of the western prairie fringed orchid produces fewer seed capsules compared to more southern populations in the United States. Pollen vectors of the western prairie fringed orchid include two species of sphinx moths (Sphingidae) in Canada and the orchid can be considered a pollen limited species. The degree to which the presence of sphinx moths may affect pollination success in the western prairie fringed orchid was evaluated using ultraviolet lights to attract sphinx moths and increase nectar feeding activity, thus potentially increasing seed capsule production. Ultraviolet lights were tested at two levels of illuminance. Significantly more individual flowers and plants developed seed capsules in plots with ultraviolet lights than in plots without lights. The number of flowers per plant was unrelated to the number of seed capsules produced per plant. It appears sphinx moth pollinators were equally attracted to small, medium and large sized orchid inflorescences. The degree to which high winds may also decrease the pollinating activity of sphinx moths within the vicinity of orchids is considered. Results indicate that ultraviolet lights may be useful to temporarily manipulate seed capsule production.

Additional key words: western prairie fringed orchid, *Platanthera praeclara*, seed capsules, pollination, sphinx moths, *Sphinx drupiferarum*, *Hyles gallii*.

The endangered western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles) is found in wet sedge meadows in remnant tall grass prairie located in the central plains of North America (Smith 1993; Wolken *et al*. 2001). Loss of habitat is considered the primary cause of its endangered status in Canada and the United States (Davis 1994; U.S. Fish and Wildlife Service 1996), with tall grass prairie being considered one of the most endangered ecosystems in North America (Joyce & Morgan 1989; Samson & Knopf 1994; Hamilton 2005; Whiles & Charlton 2006). When in bloom, these orchids grow 38–85 cm tall and can produce 20 or more flowers, which are arranged on a single racemose spike that opens from the bottom to the top of the inflorescence (Sheviak & Bowles 1986; Pleasants 1993; Pleasants & Moe 1993). The creamy white flowers emit a sweet fragrance that becomes more intense in the late evening during the blooming period of mid June to early July (Sheviak & Bowles 1986). The most striking visual characteristics of the flowers are the large, deeply fringed tri-lobed lower lip and long, slender nectar spur. The only known pollen vectors of *P. praeclara* are several species of sphinx moths (Sphingidae) (Sheviak & Bowles 1986; Cuthrell 1994; Westwood & Borkowsky 2004). Westwood & Borkowsky (2004) described pollination of the Canadian population of the western prairie fringed orchid by two sphinx moths: the wild cherry sphinx, *Sphinx drupiferarum* J.E. Smith and the bedstraw hawkmoth, *Hyles gallii* (Rottenburg) by trapping moths in the act of pollinating individual plants.

The pollen of the orchid is packaged in two pollinaria located on each side of the stigma. Each pollinarium is composed of three structures: the pollinia (pollen masses), the caudicle and a sticky disk called the viscidium (Pleasants & Moe 1993; Johnson & Edwards 2000; Pacini & Hesse 2002). The mechanism of pollen removal from the flower by nectar-seeking sphinx moths involves the adherence of the viscidium to the eye of the sphinx moth. The entire pollinarium is removed from the flower when the moth withdraws its proboscis and moves to another flower where the pollinia may contact the stigma and fertilize the flower (Sheviak & Bowles 1986; Westwood & Borkowsky 2004).

The number of female flowers produced by an individual plant reflects the maximum number of fruits that the plant can produce (Stephenson 1981), but individuals of many plant species produce more flowers than mature fruits (Ågren *et al*. 2008; Spigler & Chang 2008). Flowers and immature fruits may be damaged by environmental phenomena (Inouye 2000; Pilson 2000) or predators (Ågren *et al*. 2008), such that flowers cannot be pollinated or the fruit cannot fully mature. Undamaged flowers often fail to initiate fruit (Stephenson 1981; Heithaus *et al*. 1982) and this disparity between flower and fruit production (as exhibited by the western prairie fringed orchid, which is a self-compatible, facultative out-crosser) is usually attributed to factors that limit fruit production by inhibiting pollination, often by reduced pollinator visitation rates (Roll *et al*. 1997; Parra-Tabla *et al*. 1998; Mattila & Kuitunen 2000; Rathcke 2000; Spigler & Chang 2008). Such plants are said to be pollen limited.

Westwood & Borkowsky (2004) noted a substantially lower level of annual seed capsule production in the Canadian population compared with more southern populations. While the regulation of the level of seed capsule production in the western prairie fringed orchid may be linked to several factors including site quality and herbivory, the abundance of sphinx moth pollinators may also be a factor.

Local abundance of Sphingidae may vary greatly between years (Hodges 1971; Duarte & Schlinwein 2005; Tuttle 2007). Adult sphinx moth surveys indicated that low levels of seed capsule development in the Canadian population of the orchid may be related to a scarcity of pollinators or perhaps environmental factors that diminish pollinator effectiveness (Westwood & Borkowsky 2004). *Sphinx drupiferarum* is uncommon in Manitoba and populations of *Hyles gallii* fluctuate widely on an annual basis with adults being almost absent in some years (Westwood & Borkowsky 2004). Other species of sphinx moths have been identified as pollinators of the orchid in the southern parts of the range (Sheviak & Bowles 1986; Cuthrell 1994; Ralston *et al*. 2008), although most do not occur in Canada. The area surrounding western prairie fringed orchid habitat in Manitoba has become fragmented by agricultural land use ranging from tame pasture to cropland, with insecticide and herbicide usage, and there may be limited habitat available for sphinx moth pollinators. Alternately, environmental factors such as high wind speeds may limit pollinator-orchid contact during the bloom period (Eisikowitch & Galil 1971; Willmott & Búrquez 1996).

We hypothesized that the western prairie fringed orchid population in Canada is pollen limited and that increased visits by sphinx moths would increase seed capsule production. In order to examine the degree to which sphinx moth nectar seeking activity may affect rates of seed capsule production we designed an experiment to artificially attract sphinx moth pollinators to orchid habitat. We hypothesized that ultraviolet lights would attract and hold sphinx moths in the vicinity of orchids compared to areas of orchids without lights, and through increased moth feeding activity there would be a measurable increase in seed capsule production. This increased level of seed capsule production would be an indirect measure of sphinx moth feeding activity. We also examined the effect of individual plant inflorescence size on the number of seed capsules produced, postulating that taller plants with more flowers would be more accessible and attractive to nectar seeking moths. Finally we report on nightly wind speeds in orchid plots and the potential influence on sphinx moth activity.

Study Area and Methods

Study area. The Manitoba Tall Grass Prairie Preserve (hereafter called the Preserve) is located in southeastern Manitoba near the Canada-United States border (49° 05' N, 96° 49' W). The Preserve represents the only known location in Canada where the western prairie fringed orchid occurs (Borkowsky & Jones 1998). The nearest population is located in northwest Minnesota approximately 125 km to the south of the Preserve.

The climate is continental, with an average of 579.1 mm of precipitation annually, a mean summer temperature of 19.6 °C and a mean winter temperature of -18.8 °C (Moore & Fortney 1994). The soil is grey-wooded podzol, having a sandy-loam to clay-loam texture with frequent rock outcrops. The shallow slope of the landscape (1–3%), poor drainage and high water table (within 3m of the surface) generally inhibits agricultural productivity within the Preserve.

The natural vegetation in the Preserve and surrounding area may be grouped into three general communities: aspen woodland, upland prairie and sedge meadow. The areas recognized as aspen woodland are dominated by aspen (*Populus tremuloides* Michx.), interspersed with bur oak (*Quercus macrocarpa* Michx.) and shrubs including saskatoon (*Amelanchier alnifolia* Nutt.), chokecherry (*Prunus virginiana* L.) and hazelnut (*Corylus* spp.). The herbaceous layer is dominated by poison-ivy (*Rhus radicans* L.), meadow rue (*Thalictrum* spp.), goldenrod (*Solidago* spp.), golden alexander (*Zizia aurea* (L.) Koch) and various graminoids. The upland prairie is dominated by big blue stem (*Andropogon gerardi* Vitman) and Indian grass (*Sorghastrum nutans* (L.) Nash) and forbs such as purple prairie clover (*Petalostemum purpureum* (Vent.) Rydb.), wild strawberry (*Fragaria virginiana* Dcne.), goldenrod (*Solidago* spp.) and sunflower (*Helianthus* spp.). Shrubs such as shrubby cinquefoil (*Potentilla fruticosa* L.) and rose (*Rosa* spp.) occur in the upland prairie. The sedge meadow where the orchids are most common is dominated by various sedges (*Carex* spp.)

and rushes (*Juncus* spp.) along with prairie cord grass (*Spartina pectinata* Link), swamp birch (*Betula glandulosa* Michx.) and several species of willows (*Salix* spp.) (Looman & Best 1987; Moore & Fortney 1994).

Field sites. Prior to experimental plot selection, inventory assessments of western prairie fringed orchids from previous growing seasons and general orchid distribution maps for the Preserve were examined to establish potential plot locations (Davis 1994; Borkowsky & Jones 1998). Orchids tend to grow in aggregations and flowering stems become visible in late May (*i.e.*, height of stems approximately 10 cm). The number of flowering stems varies greatly from year to year in the Preserve. In 2001, six plots were selected, each with a minimum of 30 orchid plants that would produce a flowering stem. Plots were separated by a minimum of 500m and were surrounded to some degree by aspen woodland such that they were not visible at 3m above the ground from adjacent plots. Plots were randomly assigned one of two treatments: ultraviolet light or no ultraviolet light (left in a natural state). The three plots assigned to the ultraviolet light treatment were labelled UV-P1, UV-P2, and UV-P3 and the plots without lights NAT-P1, NAT-P2, and NAT-P3. Eight plots (four with ultraviolet lights and four left in a natural state) were used in 2002 as more flowering stems were present.

Sampling methods. In June 2001, the center of each plot was marked with an orange pin flag, and a 60m radius, covering approximately 1.13 ha, was marked with eight additional pin flags to delineate the circumference of the plot. An ultraviolet light covered by a small wooden panel (1m × 1m) was placed in the center of ultraviolet light plots approximately one meter above the ground. The ultraviolet light and its power source were located underneath the panel to prevent water damage to the electrical components. The ultraviolet light used in this study consisted of a single 8 watt florescent bulb assembly from a Ward's® All Weather Insect Bucket Trap which was powered by a 12 volt marine deep cycle battery. A translucent white cloth cover was placed over each light in 2001. In the first year of the study the cover was used to lower the intensity of light emission so as to minimize the visibility of lights from plots without lights. The intensity of the light measured at 0.3m from the cloth covered light was approximately 5.5 ft. candles. The cloth cover was not used in 2002 to test the lights at their maximum intensity (approximately 10.2 ft. candles at 0.3m). In both years, the ultraviolet light was operated on alternate nights between 2000 and 0800 h throughout the flowering period. The ultraviolet lights were operated for 13 nights beginning on 25 June 2001 and nine nights beginning on 6 July 2002. Lights were not placed in natural plots to ensure they resembled normal orchid habitat and sphinx moths did not use them as protective diurnal resting places, which may increase their night nectar foraging activity around orchids. Lights in ultraviolet light plots were examined each morning for the presence of resting sphinx moths.

To estimate the effect of wind on sphinx moth activity the wind speed (km/hr) was recorded during the bloom period on an hourly basis over a 24 hr period (Environment Canada 2008) for each day to determine a mean daily wind speed and also to calculate the mean wind speed for the time period of 2000–0500 h (the period when sphinx moth pollinators are active in the Preserve).

Data analysis. The number of flowers (*i.e.*, inflorescence size), pollinaria available, pollinaria removed, and seed capsules produced were recorded for each plant in 2001 and 2002. Pollinaria removal and seed capsule production have been widely used as proxy measures to gauge sphinx moth feeding activity as direct observation of sphinx moths is difficult due to their nocturnal habit and swift flight (Sheviak & Bowles 1986; Pleasants & Moe 1993; Cuthrell 1994). We calculated the mean number of flowers per plant, and number and percent of pollinaria removed. Number of seed capsules per plant and per flower was calculated for each plot to standardize per capita capsule and flower production. All experimental variables were tested for departure from the normal distribution and transformed where necessary (Zar 1996). Untransformed means are reported in the Results and Tables.

Visual inspection of flowering plant heights and number of flowers per orchid in previous field investigations revealed that plants generally grouped into three broad categories. Smaller plants were well below surrounding vegetation, medium sized plants were approximately level with surrounding sedges, rushes and grasses and larger orchids were often 10 or more cm above the surrounding vegetation. A histogram examination of plant height and number of flowers per plant in 2001 and 2002 confirmed the three broad categories. We postulated that sphinx moths may prefer tall plants with many flowers to maximize ease of nectar collection versus visiting short plants with few flowers partially covered by other herbs and grasses. Three plant size categories were established including small sized plants (1 to 3 flowers), medium sized plants (4 to 10 flowers) and large sized plants (11 or more flowers). The mean number of flowers per plant and standard deviation (7.1 ± 2.8) of all plants was calculated from the pooled 2001 and 2002 data set. The mean and standard deviation were considered to be the medium size category (*i.e.* 4 to 10 flowers per plant).

Plots were used as replicates (and assumed to be independent) for plot type comparisons. Each variable (flowers per plant, percent pollinaria removed, seed capsules per plant and per flower, inflorescence size category) was tested for differences between plot type and the interaction of plot type and inflorescence size using a general linear model ($\alpha = 0.05$). Fisher's least significant difference (LSD) post hoc test was used to separate means when ANOVA was significant for tests between plant inflorescence size categories. An independent t test was used to compare the number of plants in plots by inflorescence size and wind speeds between bloom periods in 2001 and 2002. All statistical analyses were done using SPSS v. 11.0.1 (SPSS Inc. 2001).

Results

The mean number of flowers per plant was 7.1 ± 0.2 and 7.3 ± 0.1 for plots in 2001 and 2002, respectively (Table 1). In 2001 mean percent pollinaria removal was not significantly different between plots with ultraviolet lights (12.9 ± 2.3) and those without lights (10.1 ± 3.6) ($F_{1,4} = 0.42$, $p = 0.550$). In 2002 mean percent pollinaria removal was significantly different between the ultraviolet light plots (7.8 ± 0.5) and plots without lights (6.2 ± 0.2) ($F_{1,6} = 8.94$, $p = 0.024$).

Total seed capsule production in 2001 and 2002 was 11 and 226 capsules, respectively (Table 1). Mean number of seed capsules per plant was not significantly different between the ultraviolet light plots and plots without lights in 2001 ($F_{1,4} = 0.01$, $p = 0.936$) (Table 1). In 2002 number of seed capsules per plant was significantly different between treatments, 0.35 ± 0.02 for the ultraviolet light plots and 0.21 ± 0.02 for plots without lights ($F_{1,6} = 21.46$, $p = 0.004$) (Table 1).

In 2001, mean number of seed capsules per flower (Table 1) was not significantly different between the ultraviolet light plots and plots without lights ($F_{1,4} = 0.76$, $p = 0.431$). In 2002 the difference in seed capsule production per flower was significant ($F_{1,6} = 19.43$, $p = 0.005$), with the number of seed capsules per flower in the ultraviolet light plots almost twice that of plots without lights (0.051 ± 0.004 and 0.028 ± 0.003, respectively) (Table 1).

When plants were placed in inflorescence size categories in 2001, 37.8%, 40.8% and 21.4% of plants fell into the small, medium and large size groups, respectively (Table 2). There was no significant difference in the number plants in the medium and large inflorescence size categories between the plots with ultraviolet lights and without ($t_4 = -0.91$, $p = 0.412$; $t_4 = -1.05$, $p = 0.350$ respectively). There were significantly more small plants in the ultraviolet light plots than plots without lights in 2001 ($t_4 = -4.17$, $p = 0.014$).

In 2002, 29.4%, 49.9% and 20.7% of plants were assigned to the small, medium and large inflorescence groups, respectively (Table 2). There was no significant difference in the number plants in all inflorescence size categories between the plots with ultraviolet lights and without in 2002 (small - $t_6 = -0.78$, $p = 0.465$; medium - $t_6 = -1.13$, $p = 0.299$; large - $t_6 = -1.17$, $p = 0.285$; respectively). The percentage of large inflorescence plants in all plots was similar in 2001 and 2002, although the percentage of smaller plants decreased in 2002 while the number of medium sized plants increased. There was no significant difference in the number of capsules per plant or per flower between plots with ultraviolet lights and those without for all three inflorescence size comparisons in 2001 (Table 3). In 2002, medium sized inflorescences produced more capsules per plant and per flower in ultraviolet light plots than plots without lights (Table 2). When number of seed capsules produced by inflorescence size was pooled over all plots there was a noticeable trend of increasing number of seed capsules with inflorescence size, although the trend was only significant for number of capsules per plant in 2002. There was no significant interaction between the number of seed capsules produced per plant or per flower by inflorescence size and plot type in 2001 ($F_{5,12} = 0.03$, $p = 0.969$; $F_{5,12} = 0.14$, $p = 0.872$, respectively) or 2002 ($F_{5,18} = 0.82$, $p = 0.455$; $F_{5,18} = 1.13$, $p = 0.350$) (Table 3).

The mean daily wind speed over the bloom period was 12.7 ± 1.2 and 12.2 ± 1.1 km/hr in 2001 and 2002, respectively. The mean wind speed over the bloom period from 2000-0500 h was 9.5 ± 0.9 and 9.8 ± 1.1 km/hr in 2001 and 2002, respectively. There was no significant difference in mean daily wind speeds between 2001 and 2002 during the bloom period or the approximate 9 hour period when sphinx moth pollinators are most active (24 hrs: $t_{40} = 0.31$, $p = 0.756$; 9 hr period: $t_{40} = -0.25$, $p = 0.800$).

There was no evidence that adult sphinx moths used the ultraviolet lights for shelter during the day in either year of the study.

Discussion and Conclusion

The mean number of flowers per plant in the present study ranged from 5.4 to 8.5. These values are consistent with the range of 7.0 to 9.4 flowers per plant documented by Pleasants (1993) in Minnesota and North Dakota. However, these values were less than an average of 12.6 flowers per plant reported by Sheviak &

Table 1. Plot summaries for plant, flower and seed capsule variables of flowering western prairie fringed orchids sampled in 2001 and 2002.

Year	Plot[1]	Number of plants	Number of flowers	Number of seed capsules	Seed capsules per plant	Seed capsules per flower	Inflorescence Size[2] Mean ± SE	Inflorescence Size[2] Range	No. Pollinaria Available	No. Pollinaria Removed	% Pollinaria removed
2001	UV-P1	51	320	0	0.000	0.000	6.3 ± 0.5	1 - 16	640	108	16.9
	UV-P2	65	352	6	0.092	0.017	5.4 ± 0.3	2 - 12	704	94	13.3
	UV-P3	68	503	0	0.000	0.000	6.4 ± 0.3	3 - 12	1006	88	8.7
	NAT-P1	99	855	2	0.020	0.002	8.5 ± 0.4	3 - 16	1710	84	4.9
	NAT-P2	75	560	1	0.013	0.001	7.5 ± 0.4	2 - 18	1120	191	17.0
	NAT-P3	29	181	2	0.068	0.110	6.6 ± 0.5	2 - 13	362	31	8.6
Total/Mean		387	2771	11	0.032	0.022	6.9 ± 0.2		5542	596	11.6
2002	UV-P1	87	532	28	0.322	0.053	6.1 ± 0.2	1 - 13	1064	71	6.7
	UV-P2	91	706	32	0.352	0.045	7.8 ± 0.3	1 - 16	1412	123	8.7
	UV-P3	151	1121	50	0.331	0.045	7.4 ± 0.2	1 - 18	2242	160	7.1
	UV-P4	25	159	10	0.400	0.063	6.4 ± 0.4	2 - 11	318	27	8.5
	NAT-P1	150	1068	35	0.233	0.033	7.1 ± 0.2	2 - 20	2136	132	6.2
	NAT-P2	150	1200	33	0.220	0.028	8.0 ± 0.2	2 - 17	2400	141	5.9
	NAT-P3	91	631	12	0.132	0.019	6.9 ± 0.3	1 - 6	1262	75	5.9
	NAT-P4	106	810	26	0.245	0.032	7.6 ± 0.2	3 - 15	1620	111	6.8
Total/Mean		851	6227	226	0.279	0.040	7.3 ± 0.1		12454	840	7.0

[1] UV = Ultraviolet light plot; NAT = Natural plot.

[2] Mean number of flowers per plant.

Table 2. Number of plants per plot based on plant inflorescence size in 2001 and 2002.

2001 Inflorescence size	2001 Plot[1]	2001 Number of plants	2001 Total Plants per plot type/ mean ± SE	2002 Inflorescence size	2002 Plot[1]	2002 Number of plants	2002 Total Plants per plot type/ mean ± SE
Small	UV-P1	29		Small	UV-P1	39	
< 4 flowers	UV-P2	36		< 4 flowers	UV-P2	22	
	UV-P3	29	94/31.3±2.3		UV-P3	38	
	NAT-P1	16			UV-P4	10	109/27.2±6.9
	NAT-P2	22			NAT-P1	56	
	NAT-P3	14	52/17.3±2.4		NAT-P2	31	
					NAT-P3	28	
					NAT-P4	25	140/35.0±7.0
Medium	UV-P1	13		Medium	UV-P1	37	
4-10 flowers	UV-P2	23		4-10 flowers	UV-P2	48	
	UV-P3	27	63/21.0±4.1		UV-P3	80	
	NAT-P1	48			UV-P4	13	178/44.5±13.9
	NAT-P2	36			NAT-P1	63	
	NAT-P3	11	95/31.6±10.9		NAT-P2	74	
					NAT-P3	51	
					NAT-P4	57	245/61.2±4.9
Large	UV-P1	9		Large	UV-P1	11	
> 10 flowers	UV-P2	6		> 10 flowers	UV-P2	21	
	UV-P3	12	27/9.0±1.7		UV-P3	33	
	NAT-P1	35			UV-P4	2	67/16.7±6.6
	NAT-P2	17			NAT-P1	31	
	NAT-P3	4	56/18.6±8.9		NAT-P2	45	
					NAT-P3	12	
					NAT-P4	24	109/28.0±6.9

[1] UV = Ultraviolet light plot; NAT = Natural plot.

Table 3. Effect of inflorescence size on seed capsule production in the western prairie fringed orchid in 2001 and 2002.

2001				2002			
Inflorescence size	Plot[1]	Seed capsules per plant	See capsules per flower	Inflorescence size	Plot	Seed capsules per plant	Seed capsules per flower
Small	UV n = 3	0.018 ± 0.008	0.005 ± 0.001	Small	UV n = 4	0.162 ± 0.082	0.037 ± 0.018
< 4 flowers	NAT n = 3	0.000 ± 0.00	0.000 ± 0.00	< 4 flowers	NAT n = 4	0.013 ± 0.005	0.000 ± 0.000
	$F_{1,4}$ P	1.01, 0.373	1.00, 0.375		$F_{1,6}$ P	1.55, 0.260	1.60, 0.252
Medium	UV n = 3	0.029 ± 0.013	0.004 ± 0.004	Medium	UV n = 4	0.403 ± 0.054	0.056 ± 0.008
4-10 flowers	NAT n = 3	0.037 ± 0.027	0.005 ± 0.003	4-10 flowers	NAT n = 4	0.130 ± 0.003	0.017 ± 0.001
	$F_{1,4}$ P	0.43, 0.845	0.014, 0.912		$F_{1,6}$ P	24.62, 0.003	21.56, 0.004
Large	UV n = 3	0.111 ± 0.011	0.010 ± 0.009	Large	UV n = 4	0.506 ± 0.172	0.044 ± 0.015
> 10 flowers	NAT n = 3	0.112 ± 0.069	0.113 ± 0.007	> 10 flowers	NAT n = 4	0.453 ± 0.088	0.039 ± 0.007
	$F_{1,4}$ P	0.001, 0.990	0.003, 0.962		$F_{1,6}$ P	0.07, 0.794	0.112, 0.749
Inflorescense	Small n = 6	0.009 ± 0.009	0.002 ± 0.002	Inflorescense	Small n = 8	0.109 ± 0.044a[2]	0.025 ± 0.010
Size	Medium (n = 6)	0.033 ± 0.017	0.004 ± 0.002	Size	Medium (n = 8)	0.267 ± 0.057b	0.036 ± 0.008
	Large (n = 6)	0.111 ± 0.058	0.011 ± 0.005		Large (n = 8)	0.479 ± 0.090c	0.042 ± 0.008
	$F_{2,15}$ P	2.25, 0.140	1.22, 0.322		$F_{2,15}$ P	7.72, 0.003	0.931, 0.410

[1] UV = Ultraviolet light plot; NAT = Natural plot.

[2] Means in columns followed by different letters are significantly different (Fishers LSD, $p < 0.05$)

Bowles (1986), who examined orchids from locations across the range of *P. praeclara* in the United States, including states at the southern extent of the orchid's range (Iowa, Nebraska and Kansas). The longer and warmer growing season in the southern part of the orchid's range may produce on average larger plants with more flowers.

To be an effective pollinating agent, a sphinx moth must remove at least one of the pollinaria from an orchid flower and then subsequently visit an unpollinated flower. Increased feeding activity by sphinx moths should presumably lead to an increased number of pollinaria removed. In 2001, the difference in the percent pollinaria removed between the two plot types was not significant, while in 2002 a significantly higher percentage of pollinaria were removed in ultraviolet light plots versus plots without lights, which corresponded to a difference in seed capsule production between plot types. In 2001 and 2002, levels of pollinaria removal in our study in both plot types were considerably lower than levels recorded under natural conditions in North Dakota (33%) (Pleasants & Moe 1993). Sphinx moth pollinator populations may be lower in our study area.

In the present study the overall mean percent pollinaria removed was higher in 2001 (11.6%) than 2002 (7.0%). Pleasants (1993) found a similar difference between study years with overall site averages of 33% and 8% for 1991 and 1992, respectively. Sphinx moth populations may fluctuate from year to year, and between year differences in pollinaria removal may be a result of their fluctuating local abundance (Westwood & Borkowsky 2004). Although the rate of pollinaria removal in 2001 in all plots combined was higher than in 2002, the number of seed capsules produced per plant and per flower in 2002 was more than double that recorded in 2001. While pollinaria removal and subsequent seed capsule production were significantly higher in ultraviolet light plots versus plots without lights in 2002, using only pollinaria removal as an indicator of overall sphinx moth activity needs to be further investigated. During the current study it was incidentally observed that occasionally orchid pollinaria were attached to the ends of orchid petals (although never on the orchid stigmatic surface) and other surrounding vegetation, particularly the leaves of tall grasses such as big blue stem and Indian grass. Cuthrell (1994) suggested that wind may cause accidental pollinaria removal. During windy periods, the inflorescence may contact stems and leaves of surrounding vegetation, especially grasses that equal or exceed the height of the orchid. The combined action of vegetation becoming entangled with the orchid flowers and wind movement could cause pollinaria to adhere to adjacent vegetation (Cuthrell 1994). Thus seed capsule production should be used as the best indicator of pollination success.

In our study wind speeds over the bloom period were very similar in 2001 and 2002. If wind was a major factor in causing a higher percent of pollinaria to be removed in 2001 it is not reflected by wind speed measurement. It is also unlikely that sphinx moths were responsible for the higher pollinaria removal rate in 2001 as there was not a corresponding higher percentage of seed capsules produced. Although seed capsule production is the most accurate measure of sphinx moth activity, orchids have to be carefully monitored as pods take several months to fully develop and orchids may be susceptible to herbivory by a variety of mammals.

Wind can also affect a pollinator's ability to travel between plants. Eisikowitch & Galil (1971) observed a correlation between wind speed and levels of pollination and seed production in an Israeli amaryllis, *Pancratium maritimum* L. Sphinx moth flower visits were common when wind speeds were below 2 m/s, resulting in the highest levels of pollination and seed set (Eisikowitch & Galil 1971). Pollination did not occur when wind speeds were greater than 3 m/s as the sphinx moths did not travel between flowers; wind speeds between 2 and 3 m/s reduced the flight activities of the sphinx moth pollinators and resulted in lower levels of pollination and seed set (Eisikowitch & Galil 1971). Sphinx moth visitations to the flowers of *Merremia palmeri* (S. Wats.) Hallier ended when winds were gusty or became moderately strong (Willmott & Búrquez 1996). We found wind speeds ranged from approximately 2.6 to 2.7 m/s (9.5 to 9.8 km/hr) during the nocturnal pollination period for sphinx moths. These wind speeds are probably close to the upper limit for sphinx moth pollinating activity in the Preserve. High winds during the short bloom period of *P. praeclara* may be a significant factor in reducing seed capsule production and may help explain the large variation in the annual level of seed capsule production. In the Preserve, orchids grow in exposed open areas of prairie and sphinx moths may prefer sheltered areas to seek nectar on windy nights. Future research should examine the effects of wind on both sphinx moth activity and the loss of pollinaria to surrounding vegetation.

In 2002, the increased seed capsule production in the plots with ultraviolet lights may have been due, in part, to removal of the cover sheet to maximize the attractiveness of the plots, although we could not test this effect directly by actually observing moths. The collecting distance of light traps is estimated to be less than 10m (Frank 1988; Southwood & Henderson, 2000) thus there was little chance that moths in one plot could have been attracted by a light from another plot. We hypothesize that moths were probably not attracted from a significant distance (greater than 10m) to plots with ultraviolet lights, but that once attracted by the odor of the orchids and/or visual cues they may have remained in the vicinity of the light and continued to nectar feed in the plot. It appears that the maximum intensity of the light was required to attract moths. It is known that sphinx moths attracted to lights may remain quiescent in the vicinity of lights until daylight (Hodges 1971; Pittaway 1993; Duarte & Schlinwein 2005; Tuttle 2007), and as lights were activated once every 48 hours in our study it is reasonable to expect that moths attracted to the ultraviolet lights may have remained in the plot or the immediate vicinity up to several days.

Less than 7% of flowers produced seed capsules in our study. Seed capsule production rates four to six times greater have been recorded for *P. praeclara* in Minnesota and North Dakota (Pleasants 1993; Pleasants & Moe 1993). Seed capsule production rates in the current study were well below the 49.3% average (range 13.6 to 79.0%) for 11 other North American nectariferous orchids (Neiland & Wilcock 1998).

In 2002 orchids in the medium sized inflorescence category (4–10 flowers) had more seed capsules per plant in the plots with ultraviolet lights than plots without lights. There was also a trend for more seed capsules per plant and per flower to be produced as the size of the inflorescence increased in both years when all plots were pooled, but it was not significant. We could not demonstrate that sphinx moth pollinators had a consistent preference for small, medium or large sized inflorescences. Under natural pollination conditions, Pleasants & Moe (1993) found that seed capsule production was not correlated to the number of flowers in the inflorescence; however, they did not use size categories as we did in the current study.

There are few reported studies that test ultraviolet light as a means of attracting beneficial insects such as pollinators (Nabli *et al*. 1999). Regulations governing the endangered status of the orchid in Manitoba prevent any direct manipulation of large numbers of plants, including removal of flower parts to investigate pollination rates and seed capsule production through the use of techniques such as hand pollination. As the removal of seed capsules from western prairie fringed orchids is not permitted, the temporary use of ultraviolet lights in patches of orchids may attract sphinx moths and inherently increase levels of seed capsule production above natural levels so that capsules can be removed for other research purposes. Successful seed capsule formation in *P. praeclara* is entirely dependent on sphinx moth nectar feeding activity, and in our study, application of ultraviolet lights in prairie habitat significantly increased seed capsule production in *P. praeclara*.

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