Weather conditions and sphinx moth activity in relation to pollination of the endangered Western Prairie Fringed Orchid (*Platanthera praeclara*) in Manitoba

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Abstract – Weather conditions can influence flight activities of sphinx moths (Lepidoptera: Sphingidae), including floral visitation. The rare Western prairie fringed orchid, *Platanthera praeclara,* is exclusively pollinated by several species of nectar-feeding sphinx moths in Manitoba. The orchid is classified as endangered and threatened in Canada and the United States, respectively. We examined several meteorological factors which may influence sphinx moth visitation to *P. praeclara* in Manitoba. Sphinx moth visitation rates, as indicated by orchid seed capsule production and removal of pollinaria, varied between sites but there was no apparent relationship between sphinx moth activity and weather conditions during the flowering period of the Western prairie fringed orchid. We suggest that removal of pollinaria from orchids may be caused by other factors in addition to sphinx moth pollination, such as mechanical disturbance by wind, and that removal rates of pollinaria during the flowering period may not always be an accurate indicator of sphinx moth pollination rates. It is recommended that seed capsule production be used to estimate sphinx pollinator activity and to determine annual rates of orchid reproduction rather than estimates of pollinaria removal during the flowering period.

Introduction

The activity of adult Lepidoptera may be constrained by meteorological conditions, particularly temperature and wind speed (Eisikowitch and Galil 1971; Hardwick 1972; Cruden *et al.* 1976; McGeachie 1989; Holyoak *et al.* 1997; Butler *et al.* 1999; Bailey and Horn 2007). Adult Lepidoptera are active within a range of ambient temperatures, above or below which activity ceases (Daly *et al.* 1978). For nectar-seeking sphinx

moths, there is a positive relationship between ambient temperature within this range and the number of floral visits (Cruden 1973; Cruden *et al.*1976; del Rio and Búrquez 1986). In moderate to high winds, moth activity decreases dramatically (Douwes 1968; Eisikowitch and Galil 1971; Willmott and Búrquez 1996). This may be due to the presumably high energetic costs associated with flight in such conditions or because it is too difficult for a moth to maintain proper positioning in front of the flower while hovering during nectar feeding (Eisikowitch and Galil 1971).

The Western prairie fringed orchid (WPFO) (*Platanthera praeclara* Sheviak and Bowles) (Orchidaceae) is a perennial herb (United States Fish and Wildlife Service 1989). The WPFO is found in tall grass prairie habitats in the midwestern United States (Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, and Oklahoma), (United States Fish and Wildlife Service 2006), and southern Manitoba in Canada (Catling and Brownell 1987) preferring mesic to wet calcareous prairie swales (Sheviak and Bowles 1986; Smith 1993; Wolken *et al.* 2001). It is classified as endangered and threatened in Canada and the United States, respectively (Collicutt 1993; United States Fish and Wildlife Service 1989) and has been placed on the world list of endangered species (NatureServe 2006). The largest population, and only known Canadian population of this orchid occurs in and adjacent to the 2200 ha Tall Grass Prairie Preserve (TGPP) in southeastern Manitoba (Westwood and Borkowsky 2004). The rarity of the WPFO is often attributed to the conversion of its habitat to agricultural use (Sheviak and Bowles 1986; United States Fish and Wildlife Service 1989; Smith 1993; Hof *et al.* 1999).

In Manitoba, two nectar-feeding sphinx moth (Sphingidae) species, *Sphinx drupiferarum* J.E. Smith and *Hyles gallii* (Rottenburg), have been confirmed as pollinators of the WPFO (Westwood and Borkowsky 2004; Borkowsky *et al.* 2011). One other non-native sphinx moth species, *Hyles euphorbiae* L., has been confirmed as a pollinator of WPFO in North Dakota (Fox *et al.* 2013). This species has been observed in the vicinity of the TGPP (Borkowsky pers. comm. 2014, Westwood unpublished data) but has not been recorded as a pollinator of the WPFO in Manitoba.

A WPFO flower contains a pollinarium which is composed of three structures: the pollinium (the pollen mass), the caudicle (stalk), and the viscidium (which is adapted to cement itself to the head or eye of a pollinator) (Sheviak and Bowles 1986; Pleasants and Moe 1993; Johnson and Edwards 2000; Pacini and Hesse 2002). Each WFPO flower has two pollinaria (Sheviak and Bowles 1986). The mechanism of pollinarium removal from a WPFO flower by sphinx moths involves the adherence of the viscidium to the eye of the sphinx moth during the process of nectar removal from the flower (Sheviak and Bowles 1986; Cuthrell 1994; Westwood and Borkowsky 2004). The entire pollinarium is removed from the flower when the moth leaves (Sheviak and Bowles 1986; Westwood and Borkowsky 2004). The pollinium is located in a position protruding from the head of the moth such that when the moth visits the next orchid flower, the pollen mass touches the stigma and pollen is transferred. The number of pollinaria removed from orchid flowers has been used as a measure of pollinator visitation by a number of investigators (Montalvo and Ackerman 1987; Maad 2000; Murren 2002; Maad and Alexandersson 2004). The number of seed capsules produced by the orchids has also been used as a proxy measure of sphinx moth visitation (Cole and Firmage 1984; Patt *et al.*1989; Maad 2000; Lipow *et al.* 2002).

Platanthera praeclara reproduces only by seed (Bowles 1983; Sieg and King 1995; Hof *et al*. 1999), thus pollination is a critical step in its life history. Artificial self and cross-pollination resulted in similar levels of fruit production (Pleasants and Moe 1993) but the positioning of the pollinaria relative to the stigma makes it unlikely that self-pollination would occur commonly in nature (Fox *et al.* 2013). The WPFO is pollen limited, that is, a flowering WPFO fails to produce as many fruits as it has flowers because it lacks the pollen with which to fertilize ovules and stimulate fruit production (Janzen *et al.* 1980; Willson 1983; Calvo 1990; Bertness and Shumway 1992; Westwood and Borkowsky 2004; Darrault and Schlindwein 2005; Borkowsky 2006).

The objective of this study was to determine if meteorological conditions (temperature, relative humidity and wind speed) affected sphinx moth activity in the TGPP and influenced pollinia removal and seed capsule production in the WPFO during the bloom period.

Materials and Methods

Field sampling for this study was carried out in 2006 in the Tall Grass Prairie Preserve (TGPP) in southeastern Manitoba (49°09'N, 96°40'W). The climate is boreal continental with mean temperatures of -17.1°C and 19.8°C for January and July, respectively (Environment Canada 2004). The area receives an average of 382.4 mm of precipitation (86.7% of the annual precipitation) during the months of April through September (Environment Canada 2004). Drainage in the area is poor with soil composed of lacustrine parent material, sandy loam to clay loam upper horizons and a thin organic surface layer (Canada Soil Inventory 1989).

Four undisturbed areas within the TGPP with high densities of the WPFO (orchid beds) were selected to act as sites (sites one, two, three, and four) (Figure 1). Sites were separated by at least 800 m. The approximate radii of the sites were: site 1 – 80 m; site 2 – 40 m; site 3 – 50 m; site 4 – 35 m. As direct observation of the crepuscular and nocturnal pollinators of the WPFO is difficult due to their swift flight and the poor light conditions during their periods of activity (Sheviak and Bowles 1986; Pleasants and Moe 1993; Fox *et al*. 2013), two proxy measures were used to determine visitation rates: removal of pollinaria and seed capsule production.

During the study, we placed a flagged stake beside each flowering orchid at each site and assigned it a unique number. A census of the buds, flowers, and senesced flowers was conducted between 1800 and 2200 hrs five times during the bloom period (only four times for site 4). The number of pollinaria in the flowers on each orchid was counted during each census. The number of pollinaria in the flowers on each orchid was counted again between 800 and 1100 hrs on the morning following each census. The dates of the evening censuses were 27 and 28 June and 4, 5, and 6 July. The seed capsules produced by each orchid at each site were counted on 26 July.

A measure of general sphinx moth activity was also determined. Light trapping was conducted in the TGPP during 10 nights, five nights prior to (13/14, 15/16, 20/21, 21/22, and 22/23 June) and five nights during (27/28, and 28/29 June and 4/5, 5/6, and 6/7

July) the WPFO bloom period in 2006 (approximately 24 June through 8 July). A large white sheet was hung on two opposing sides of a small building at the TGPP field station. An eight watt fluorescent ultraviolet light, with four reflective baffles, from a Ward's All-Weather Insect Trap™ was suspended at a height of 1.5 m in front of each sheet. The lights were operated and monitored for two to four hours between 2200 and 0200 hrs on trapping nights. Sphinx moths which alighted on or near the sheet were caught with containers, which were placed in a cooler until the next morning. The moths were then identified to species, checked for WPFO pollinaria, and marked on the hind wing using a black felt-tipped marker. After identification, the moths were released. For all trapping nights during the WPFO bloom period, the lights were left on until 0430 hrs, at which time any sphinx moths on or near the sheet were captured and identified as described above. There were no WPFO within 500 m of the lights and light traps were not visible from WPFO research sites.

Hourly temperature, wind speed, and relative humidity data for the trapping nights were obtained from a weather station located adjacent to the TGPP field station.

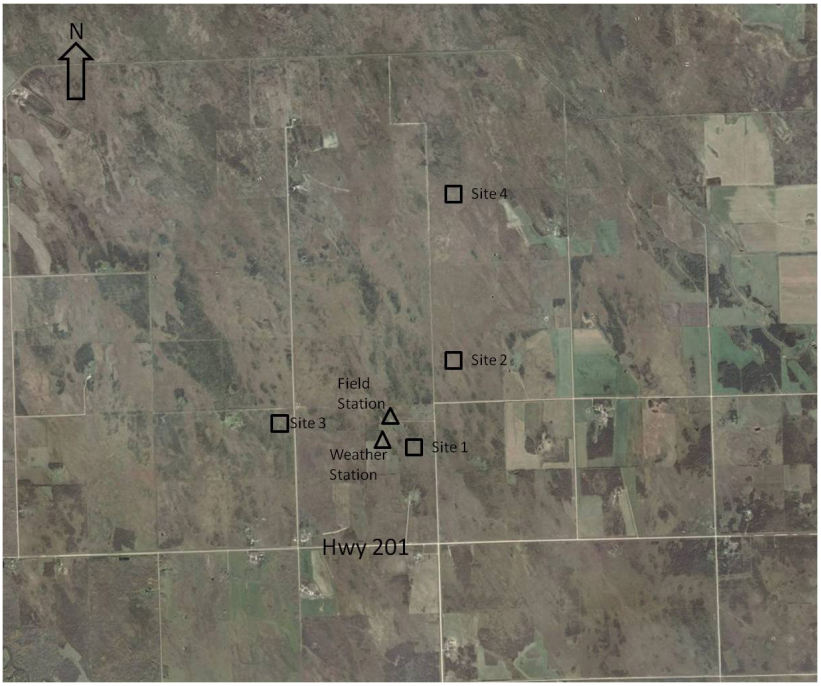

Figure 1. Layout of four orchid sites in relation to the field station (location of moth collection) in the Tall Grass Prairie Preserve in Manitoba (a four-square is one section – 1.6 x 1.6 km)

Statistical analyses

The plant and weather data were first tested for normality by graphing residuals from a general linear model estimate against the estimated values, and assessing the distribution pattern in the scattergram. Data were appropriately transformed (*i.e.,* log(X+1) or square-root-transformed) if heterogeneity of residuals was noted. Untransformed means are reported in the results and tables.

The mean number of flowers per plant per site was derived by dividing the number of flowers by the number of plants, using only the plants which were still intact at the time of the seed capsule survey. Capsules per flower for each plant were also calculated and between site differences examined using the Kruskal-Wallis ANOVA test (the data did not satisfy parametric assumptions after transformation). The percentage of pollinaria removed from the flowers (not including buds or senesced flowers) of each plant during each trapping night was calculated. The Kruskal-Wallis ANOVA test was used to examine the differences in percentage pollinaria removed between sites and between nights. Pearson correlation was used to examine the relationship between number of pollinaria removed and seed capsule production on an individual plant basis.

The mean hourly temperature, wind speed, and relative humidity were calculated during the periods that light traps were operated and observed, and also for the nights associated with pollinaria counts. Tukey's HSD test was used for comparison when differences between means were determined by ANOVA. Between evening and between night differences in these variables were examined using ANOVA during the flowering period. Pair-wise relationships between the weather variables, the number of moths caught and the pollinaria removal rate were analyzed using Pearson correlation over the flight period. Given that weather data from a single weather station were used for all sites, the assessment of pollinaria removal rate x weather variables utilized pooled pollinaria removal counts from all sites per night. A significance level of $\alpha = 0.05$ was used for analyses. All analyses were carried out using SPSS Version 19 (SPSS 2010).

Results

Mean inflorescence size ranged from 4.0 flowers per plant (site 4) to 7.2 flowers per plant (site 3) (Table 1). The number of capsules produced per flower did not vary significantly between sites (Table 1). There was no significant correlation between the number of flowers per plant and seed capsule production or seed capsules per flower and number of plants.

The percentage of available pollinaria removed did not differ significantly between sites (Table 2) or nights (Table 3). The highest single night removal rate (11.1%) and the highest overall removal rate occurred at site 3 (Tables 2 and 3). Pollinarium removal rate tended to increase over the bloom period (Table 3). Pollinarium removal was not correlated with the rate of seed capsule production ($r = -0.072$, $p = 0.928$).

Table 1. Comparison of seed capsule production by the Western prairie fringed orchid (mean ± SE) between four sites in the Tall Grass Prairie Preserve, Manitoba, 2006.

Site	No. Orchids	No. Flowers	Flowers Per Plant	No. Seed Capsules	Capules Per Flower
1	22	122	5.5	22	0.14 ± 0.07
2	39	247	6.3	13	0.05 ± 0.03
3	25	181	7.2	25	0.12 ± 0.06
4	11	44	4.0	14	0.37 ± 0.14
					X^2=6.332
					df=3
					p=0.097

Table 2. Comparison of percentages of Western prairie fringed orchi pollinaria removed from flowers (mean ± SE) between four sites in the Tall Grass Prairie Preserve, Manitoba, 2006.

Site	Night					Mean
	JN 27/28	JN 28/29	JL 4/5	JL 5/6	JL 6/7	
1	0.0	0.8	0.6	1.6	2.8	1.1 ± 0.5
2	0.0	2.7	1.0	0.4	1.1	1.0 ± 0.5
3	---	0.5	2.4	0.7	11.1	3.7 ± 2.5
4	0.0	0.0	1.1	3.0	3.6	1.5 ± 0.7
						$F_{3,15}$=1.018
						p = 0.412

Table 3. Comparison of percentages of Western praire fringed orchid pollinaria removed from flowers (mean ± SE) between five nights in the Tall Grass Prairie Preserve, Manitoba, 2006.

Night	Site				Mean
	1	2	3	4	
JN 27/28	0.0	0.0	---	0.0	0.0 ± 0.0
JN 28/29	0.8	2.7	0.5	0.0	1.0 ± 0.6
JL 4/5	0.6	1.0	2.4	1.1	1.3 ± 0.4
JL 5/6	1.6	0.4	0.7	3.0	1.4 ± 0.6
JL 6/7	2.8	1.1	11.1	3.6	4.6 ± 2.2
					$F_{4,14}$ = 2.362
					p = 0.103

All three weather variables – temperature, relative humidity, and wind speed – varied between trapping nights (Table 4) and between trapping nights during the WPFO bloom period (Table 5). The number of sphinx moths caught per night ranged from two to 17, with a mean of 6.7 (Table 4). Of the 67 sphinx moths caught over the ten nights, no specimens of *S. drupiferarum, H. gallii*, or any other likely pollinator were collected (Table 4). The most abundant and consistently caught sphinx moth was *Smerinthus jamaicensis* (Drury) (Table 4). None of the moths carried WPFO pollinaria or were re-captured on subsequent nights.

The number of moths caught per night was not significantly related to any of the weather variables (with temperature: $r = 0.339$, $p = 0.338$; with relative humidity: $r = -0.001$, $p = 0.997$; with wind speed: $r = 0.088$, $p = 0.808$) over ten nights. For the five trapping nights during the WPFO bloom period, the number of moths caught was not significantly correlated with per cent pollinaria removed ($r = -0.354$, $p = 0.138$). Per cent pollinaria removed was significantly positively correlated with temperature ($r = 0.577$, $p = 0.010$) and wind speed ($r = 0.620$, $p = 0.005$). However, the per cent pollinaria removed had a significant negative relationship with relative humidity ($r = -0.607$, $p = 0.006$). Temperature was positively associated with wind speed ($r = 0.794$, $p = 0.006$) and negatively correlated with relative humidity ($r = -0.705$, $p = 0.023$). Wind speed and relative humidity were negatively correlated ($r = -0.635$, $p = 0.049$).

Discussion

The rate of seed capsule production per flower varied (though not significantly) between sites. While not significant, the percentages of pollinaria removed from flowers varied during the bloom period both within and across sites. While a site effect was not detected in this study, Borkowsky (2006) found considerable differences in WPFO seed capsule production rates between sites in Manitoba. Pleasants and Moe (1993) showed that moth visitation rates to the WPFO increase during the bloom period, with the pollinaria removal rate roughly corresponding with the number of open flowers. Spatial variation in visitation rates has also been recorded for *Platanthera blephariglottis* (Willdenow) Lindley (Cole and Firmage 1984), *P. ciliaris* (L.) Lindley (Smith and Snow 1976), *P. metabifolia* F. Maekawa (Inoue 1986) and *P. okuboi* Makino (Inoue 1985). For these studies, several reasons for the variation were suggested, including differences in flower size (and therefore pollinator attraction) between populations (lnoue 1986), differences in the plant community between populations (Smith and Snow 1976; Inoue 1985), and fluctuating pollinator abundance between years (Cole and Finnage 1984). In the current study, perhaps the sample size was insufficient to show significant differences in pollinaria removal between sites or dates.

The number of seed capsules produced was not correlated with the rate of pollinaria removal. That no relationship was found between these two measures may be due to the different time scales of the measurements – seed capsules per flower were measured after the entire blooming period, while overall rates of pollinaria removal were averages of five nights during the bloom period. If the pollinaria removal rate for the entire bloom period had been calculated using only the plants present when seed capsules were counted, a different relationship between the two measures may

Table 4. Mean weather conditions and sphinx moth catches for ten evenings (2200 – 200 hrs) at the Tallgrass Prairie Preserve, Manitoba, 2006.

	Weather Conditions			Moth Catch			
Evening (no. hours)	Temperature (°C)	Relative Humidity	Windspeed (m/sec)	*Smerinthus jamaicensis*	*Smerinthus cerisyi*	*Pachysphinx modesta*	*Paonias myops*
JN 13/14 (5)	13.8 ± 0.9	95.7 ± 3.4	0.87 ± 0.14	2	0	0	0
JN 15/16 (5)	20.7 ± 0.2	91.6 ± 0.6	2.71 ± 0.21	13	3	0	0
JN 20/21 (4)	16.2 ± 1.0	82.6 ± 4.0	1.72 ± 0.21	5	1	1	0
JN 21/22 (4)	11.6 ± 0.8	91.1 ± 1.2	1.63 ± 0.53	2	0	0	0
JN 22/23 (4)	13.0 ± 1.1	97.7 ± 2.3	0.36 ± 0.10	1	0	1	0
JN 27/28 (4)[1]	11.3 ± 1.2	96.8 ± 3.1	0.20 ± 0.05	7	0	0	0
JN 28/29 (4)	15.8 ± 0.7	82.7 ± 2.1	0.65 ± 0.06	11	1	4	1
JL 4/5 (4)	13.2 ± 1.2	90.4 ± 5.7	0.34 ± 0.05	5	1	0	0
JL 5/6 (4)	17.2 ± 0.8	86.8 ± 1.7	0.83 ± 0.06	6	0	0	0
JL 6/7 (3)	21.5 ± 0.8	63.2 ± 2.7	2.94 ± 0.14	1	0	1	0

[1]June 27/28 to July 5/6 - denotes beginning of flowering period.

have been observed. Alternatively, pollinarium removal rates may not be a reliable measure of pollinator visitation (see below).

Table 5. Comparison of meteorological variables (mean ± SE) among five nights (2200 to 430 hrs) during the Western prairie fringed orchid bloom period at the Tallgrass Prairie Preserve, Manitoba, 2006.

Night	Temperature (°C)[1]	Relative Humidity	Windspeed (m/sec)
JN 27/28	9.2 ± 0.8a	98.6 ± 1.4d	0.19 ± 0.03a
JN 28/29	14.1 ± 0.6bc	84.5 ± 1.4b	0.79 ± 0.06bc
JL 4/5	11.5 ± 0.8ab	94.7 ± 2.7cd	0.50 ± 0.05ab
JL 5/6	15.9 ± 0.5c	89.1 ± 1.4bc	1.05 ± 0.09c
JL 6/7	20.9 ± 0.4d	66.4 ± 1.7a	3.34 ± 0.28d
$F_{4,40}$	45.817	47.756	84.104
p	<0.001	<0.001	<0.001

[1] Means followed by different letters are significantly different

The proportion of flowers producing seed capsules in the present study ranged from 5% to 37% (mean: 17%). These are considerably higher than most previously recorded rates for the WPFO in Manitoba, which ranged from 0.18% to 3.21% (Borkowsky 2006). Borkowsky (1997) recorded a seed capsule production rate of 22% for the WPFO in Manitoba, but this was based on a small sample size, so results must be interpreted with caution. Approximately 30% of flowers in a North Dakota WPFO population produced seed capsules (Pleasants and Moe 1993).

Neither of the known pollinator species of the WPFO in Manitoba was caught at the light traps in this study. There are several possible explanations for their absence. Both *H. gallii* and *S. drupiferarum* are uncommon in Manitoba in some years (Hodges 1971; Westwood and Borkowsky 2004; R. Westwood unpublished data), thus 2006 may have been a year in which populations of both species were low and as such, catching individual moths should have been a rare occurrence. Alternatively, the abundance of these species may not have been low in general, but only in the particular area where the lights were stationed. Potential reasons for low local abundance may include lack of food sources (no known alternate food sources of the adult moths, including *P. praeclara*, were located within 300 m of the lights) or high predator populations in the area.

At least 15 sphinx moth species have been collected in the vicinity of the TGPP during the WPFO bloom period (Westwood and Borkowsky 2004), though only four of these species were collected in this study. Of these four species, none have a proboscis of sufficient length to access the nectar in WPFO flowers (Westwood and Borkowsky 2004) and are thus unlikely to act as pollinators of the WPFO.

The activity of sphinx moths, including their pollination activity, is likely constrained by meteorological factors such as temperature (del Rio and Búrquez 1986; Butler *et al*. 1999; see Willmott and Búrquez 1996) and wind speed (Eisikowitch and Galil

1971; Willmott and Búrquez 1996). There was no significant relationship between the number of sphinx moths caught and three meteorological variables – temperature, relative humidity, and wind speed in our study. There are at least two explanations as to why the expected correlation between weather conditions, particularly temperature and wind speed, and moth abundance was not found. Traps were operated for only 10 nights. Many studies which specifically examine the relationship between light trap catches and weather conditions generally have much larger sample sizes over many more nights (Butler *et al*. 1999). A second potential reason the temperature and wind speed did not correlate with moth abundance in the present study involves the range of conditions in which sphinx moths are active. If the conditions during trapping periods were well within the thresholds of moth activity, a strong linear correlation between moth abundance and weather conditions may not be found (Danthanarayana 1976; Willmott and Búrquez 1996; see Cruden *et al*. 1976; del Rio and Búrquez 1986). Mean temperature during the trapping periods ranged from 11.3°C to 21.5°C and wind speed ranged from 0.20 m/sec to 2.94 m/sec. These values are within the range of conditions in which moths, including sphinx moths, may be active (Eisikowitch and Galil 1971; Cruden *et al*. 1976; Danthanarayana 1976; del Rio and Búrquez 1986; Butler *et al*. 1999). Consistent with previous research (Danthanarayana 1976; Willmott and Búrquez 1996), relative humidity did not have a noticeable effect on sphinx moth abundance, though the problem of a small sample size noted above may apply here as well.

Pleasants and Moe (1993) found 33% pollinaria removal from WPFO flowers in North Dakota. Borkowsky (2006) reported mean percentages of pollinaria removed ranging from 6% to l0% for the WPFO in Manitoba. The percentages of pollinarium removed in our study are more consistent with the findings of Borkowsky (2006) than with Pleasants and Moe (1993). However, the above studies measured removal rates over the entire bloom period rather than daily as in the present study, thus results are not directly comparable.

Percentage pollinaria removal was significantly correlated with all three weather variables (positively with temperature and wind speed, negatively with relative humidity), though the correlation coefficients did not suggest particularly strong relationships. The highest rates of pollinaria removal in the present study occurred when the wind speed was 2.94 m/sec. The upper wind speed threshold for sphinx moth activity is about three metres per second (Eisikowitch and Galil 1971; Danthanarayana 1976). The number of sphinx moths caught at lights, a more direct indicator of sphinx activity than pollinaria removal rates, did not correlate with the weather variables, including wind speed. These observations suggest that the removal of many pollinaria in this study may not have been the result of sphinx moth activity. The relationship between pollinaria removal rates and the weather variables may not have been mediated through sphinx moths but rather through some other feature(s) of the orchid's environment. Other workers have noted that surrounding vegetation, particularly tall grasses, contact the viscidia of WPFO flowers, which then adhere to the grass and are removed from the flower; such events are likely related to grasses swaying in the wind (Cuthrell 1994; Borkowsky 2006; R. Westwood unpublished data). This phenomenon was also noted in the course of the present study. The positive correlation between pollinaria removal and wind speed may be due to increased removal of pollinaria by

surrounding vegetation during windy periods rather than increased moth activity. This suggestion is consistent with the lack of correlation between wind speed and the number of sphinx moths caught at the lights. As in other studies (Cuthrell 1994; Borkowsky 2006; see Fox *et al*. 2013), our study corroborates the finding that the removal of pollinaria from WPFO flowers may not always be an accurate measure of pollinator visitation.

The rate of pollinator visitation to the WPFO in Manitoba, as measured by seed capsule production, did not statistically vary by site. Pleasants and Moe (1993) found a significant, but low ($r = 0.26$) positive correlation between inflorescence size (*i.e*., flowers per plant) and seed capsule production in a North Dakota WPFO population. In the present study, such a trend was not evident between sites. The size of a plant population (*i.e*., number of plants) may be positively related to rates of fruit production (Ågren 1996; Morgan 1999; Waites and Ågren 2004), though this does not appear to be the case for the WPFO (Pleasants and Moe 1993; the present study). There was no significant relationship between seed capsules per flower and the number of plants in the present study.

Four sites may not be a sufficient sample size to demonstrate relationships between pollinator visitation and the variables examined. Although the WPFO population at the TGPP is the largest remaining population in North America, in most years there are not many sites in the TGPP with adequate numbers of orchids for studies such as this. Even if more sites were available and had been used, the resources necessary to sample many additional sites sufficiently were not available for this study.

Temporal variation of pollinator visitation rate also warrants further study, particularly in regards to wind speed. Strong winds during the WPFO bloom period may not only reduce the pollinating activity of sphinx moths (Eisikowitch and Galil 1971; Willmott and Búrquez 1996), but also remove a considerable number of pollinaria from WPFO. The latter phenomenon may increase the incidence of nectar theft experienced by WPFO flowers as they may not have pollinaria to attach to visiting moths which can still access the nectar reward. Nectar theft may lead to lower pollination rates and reproductive implications for the orchid. Long term studies at the TGPP following orchid seed capsule production, meteorological conditions, and sphinx moth abundance may yield important insights into the factors influencing temporal variation in sphinx moth visitation to WPFO flowers in Manitoba.

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