ENHANCING POLLINATION OF THE ENDANGERED WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*) BY SPHINX MOTHS (LEPIDOPTERA: SPHINGIDAE) IN TALL GRASS PRAIRIE IN SOUTHEASTERN MANITOBA AND AN EXAMINATION OF ORCHID NECTAR PRODUCTION

by

Christie L. Borkowsky

A Thesis submitted to the Faculty of Graduate Studies

The University of Manitoba

In Partial Fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Entomology

University of Manitoba

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FACULTY OF GRADUATE STUDIES

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This is dedicated to the memory of my grandfather, Frank Schalla, for his keen interest in wildlife and countless stories about his adventures hunting, fishing and wildlife watching. His enthusiasm for nature captured my curiosity and developed my interests in wildlife that has guided my educational and career choices.

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ABSTRACT

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Enhancing pollination of the endangered western prairie fringed orchid (*Platanthera praeclara*) by sphinx moths (Lepidoptera: Sphingidae) in tall grass prairie in southeastern Manitoba and an examination of orchid nectar production.

Major professor: A. R. Westwood

The endangered western prairie fringed orchid, *Platanthera praeclara* (Sheviak and Bowles), is found in remnant tall grass prairie in southeastern Manitoba, and has a lower level of seed capsule development in comparison to more southern populations in the United States. Pollination of *P. praeclara* is limited to few select species of sphinx moths, Sphingidae, Lepidoptera, a group that is less abundant in comparison to other lepidopteran families. This study tested the hypothesis that the presence of ultraviolet light sources near orchids would attract more sphinx moths into orchid habitat and increase feeding activity of sphinx moth pollinators, thus increasing seed capsule production. The second part of this study measured orchid nectar quality and quantity during the bloom period and the possible link to pollination success. A significantly larger number of individual flowers and plants developed seed capsules in the ultraviolet light treatment plots (5.13 ± 0.42 % of available flowers; 35.12 ± 1.74 % of total plants) than the control plots (2.78 ± 0.42 % of available flowers; 21.76 ± 2.58 % of total plants). The intensity of the ultraviolet light treatment may have also had an effect on pollination success. Ultraviolet lights influenced seed capsule production by attracting sphinx moths into experimental plots. Results also indicated that ultraviolet light may be useful to manipulate seed capsule production for other research purposes. Nectar quality and quantity varied considerably during the study. The mean sugar concentration over the

sampling season was 23.9 ± 0.2 %; however, values ranged from a low of 13 % to high of 34 %. Nectar sugar concentration decreased by approximately 6 % as the flowering period progressed over the season.

CHAPTER 1

INTRODUCTION

The endangered western prairie fringed orchid, *Platanthera praeclara* Sheviak and Bowles, is found in remnant tall grass prairie in southeastern Manitoba. It also occurs in the United States including North Dakota, South Dakota, Minnesota, Nebraska and Iowa (Sheviak and Bowles 1986; Bray and Wilson 1992; U.S. Fish and Wildlife Service 1996). It is protected under the Manitoba Endangered Species Act and listed as an endangered species under the federal Species at Risk Act (Manitoba Conservation – Species at Risk 2006; Environment Canada – Species at Risk 2006). When in flower, these orchids will grow 38-85 cm tall (Sheviak and Bowles 1986). The inflorescence of creamy white flowers makes this species highly visible during its flowering period from late-June to mid-July in Manitoba. The flowers give off a sweet fragrance that becomes more intense in the late evening. The most striking visual characteristics of the flowers are the large, deeply fringed, tri-lobed lower petal and long, slender nectar spur (Sheviak and Bowles 1986). These floral characters limit pollination to a few select species of sphinx moths, Sphingidae, Lepidoptera (Cuthrell 1994, Westwood and Borkowsky 2004, Ralston *et al.* 2006).

Platanthera praeclara only occurs in wet sedge meadows within remnant tall grass prairie habitat in central North America, and loss of habitat is considered the leading cause for its' endangered status in Canada and the United States (Davis 1995, U.S. Fish and Wildlife Service 1996). Tall grass prairie is considered the most endangered ecosystem in North America (Samson and Knopf 1994, Hamilton 2005, Whiles and Charlton 2006) and in Canada the prairie is one of the most endangered

natural regions (World Wildlife Fund 1989) with less than 0.5% of the original habitat remaining in Manitoba (Joyce and Morgan 1989). Westwood and Borkowsky (2004) have described the pollination process for *P. praeclara* in Canada. Westwood and Borkowsky (2004) also noted that there is a low level of seed capsule development in the Canadian population in comparison to more southern populations of *P. praeclara.*

This low level of seed capsule development may be related to the scarcity of pollinators (Westwood and Borkowsky 2004). Sphingidae are generally less abundant than many other lepidopteran families (Covell 1984, Duarte and Schlindwein 2005). The area surrounding *P. praeclara* habitat in Manitoba has become fragmented by agricultural land use ranging from tame pasture development to conversion to cropland, with considerable insecticide and herbicide usage. Westwood and Borkowsky (2004) suggested that nocturnal pollinators may be drawn to the light sources prevalent in the previously dark countryside, such as farm lights and intersection lights at highway junctions and away from the less inhabited areas that support populations of *P. praeclara.* Other factors that may influence pollinator visitation may include lack of larval host plants or competition from alternate nectar sources.

To test the hypothesis that low production of seed capsules in *P. praeclara* in Manitoba is dependent on sphinx moth density an experiment was designed to attract sphinx moth pollinators into orchid habitat to increase pollination success. This study tested the hypothesis that the presence of ultraviolet light sources near orchids will increase feeding activity of sphinx moth pollinators, thus increasing seed capsule production. Westwood and Borkowsky (2004) also noted that there exists no published data on nectar production in the western prairie fringed orchid or possible links between

nectar quality and quantity, moth attraction and subsequent pollination success. There is evidence that the time frame available for pollination may be relatively short as sphinx moth pollinators are nearing the end of their flight period when the orchid is reaching the height of the flowering period (Westwood and Borkowsky 2004). This study also examined the role that orchid nectar quantity or quality may play in attracting sphinx moths during the important overlap period of orchid flowering and moth flight.

CHAPTER 2

LITERATURE REVIEW

Distribution of the Orchidaceae. The Orchidaceae is regarded as the second largest family of flowering plants; however, the estimated number of species remains inconsistent. Early estimates for the number of species ranged from 17000 (Willis 1973 in Dressler 1981) to over 24000 species (Hawkes 1961). Nilsson (1992) considered the possibility of 25000 species, while Dressler (1993) suggested that there are approximately 19500 species worldwide. More recently, estimates suggest 800 genera and 22000 to 35000 species (Romero-González *et al.* 2003). Dressler (1981) lists 153 known species representing 26 genera of orchids native to North America, however, Romero-González *et al.* (2003) includes 208 orchid species in 70 genera. Within the province of Manitoba, there are 36 known species of orchids from 14 genera (Ames *et al.* 2005). Three species, *Spiranthes magnicamporum* Sheviak, *Platanthera praeclara* Sheviak and Bowles, and *Cypripedium candidum* Muhl. Ex Willd. have been designated as endangered under the Manitoba Endangered Species Act (Manitoba Conservation – Species at Risk 2006).

Tropical regions possess thousands of orchid species compared to the hundreds of species found in temperate zones. Orchid abundance and diversity are dependent primarily on rainfall, with regions receiving annual rainfall of approximately 2.5 m or more having the greatest abundance and diversity (Dressler 1981). Orchids have evolved to fit particular niches within a habitat, growing in the ground (terrestrials), on rocks or cliffs (lithophytes) and perched upon trees or shrubs (epiphytes) (Dressler 1981). A few species are semi-aquatic and two genera of Australian orchids, *Cryptanthemis* and

Rhizanthella, are considered subterranean as only the flowers reach the surface of the ground (Hawkes 1961).

Orchid morphology relevant to pollination. Orchids are perennially flowering, herbaceous plants bearing a single seed-leaf during germination, thus belonging to the monocotyledon group of plants (Hawkes 1961). The orchid flower is composed of structures similar to those of other families of plants such as sepals, petals, anthers containing pollen, stigma, and ovary; however, through processes of reducing, enlarging, and/or fusing of these basic structures, orchids have evolved into a multitude of forms (Hawkes 1961, Dressler 1981, 1993). Nevertheless, a few generalizations may be made regarding the flowers of the Orchidaceae. Orchid flowers are bilaterally symmetrical and three fused carples form an inferior ovary (van der Pijl and Dodson 1966). In many orchid flowers, both the sepals and petals occur in sets of three with the sepals being of similar shape while the petals are not. Typically, one of the three petals, most often the median petal, is modified to form the labellum (often referred to as the lip), which may act as a landing platform for the pollinator (Hawkes 1961, van der Pijl and Dodson 1966). These structures are identified for an orchid and non-orchid flower in Figure 1. Dressler (1981) identified and described three morphological features common to all orchids. Firstly, stamens are shifted to one side of the flower, secondly the stamens and pistol have united to form a compound structure called a column, and lastly the seeds are small and very abundant. Orchid seeds are often microscopic or dust-like as they lack an endosperm and are produced by the thousands (Bowles 1983, Light and MacConaill 2002, Lehnebach and Riveros 2003). The remaining characteristics, when present, reflect

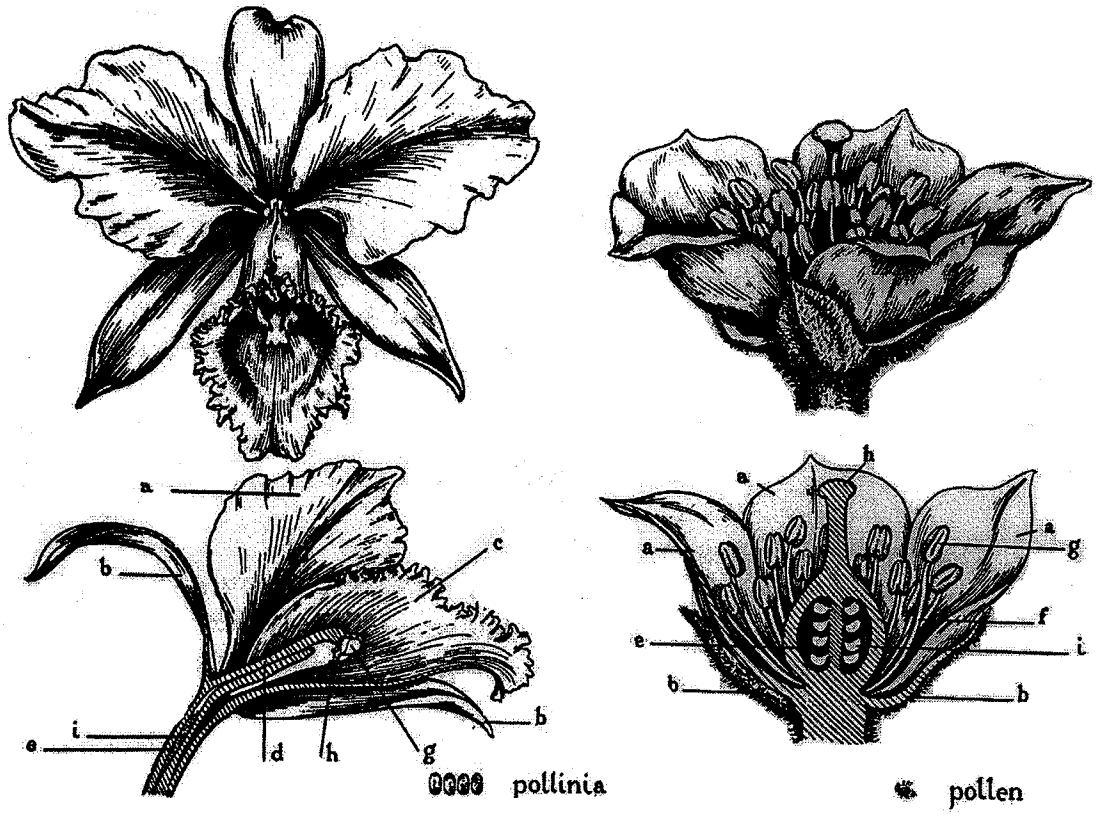

Figure 1. Comparison of a generalized orchid flower (left) with an uncomplicated flower (right). A, Petal; B, Sepal; C, Petal transformed into the labellum; D, Column; E, Ovary; F, Filament of a stamen; G, Anther; H, Stigma; I, Ovule. (from van der Pijl and Dodson 1966.)

modifications of the basic floral design found in most plants (Dressler 1981). The labellum is positioned opposite the column and the pollen is usually bound together in a few, large masses called pollinia (Dressler 1981). During orchid pollination the rostellum (the larger median lobe of the stigma) is involved with pollen transfer; providing an adhesive substance as in the *Vanilla* spp. or forming the viscidium structure of the pollinaria as in the *Platanthera* spp. In both instances, the rostellum secures the pollen to the pollinator and is a functionally important structure (Dressler 1981) (Figure 2). Finally, during their development, the flowers of most orchids twist in a process termed resupination (Dressler 1981). In early development, the bud is held such that, relative to the stem, the labellum is proximal while the stamen is distal. As the bud develops and matures, the ovary twists, such that when the flower opens the stamen becomes proximal to the stem and the labellum is positioned distally (Dressler 1981).

Among monandrous orchids (diandrous species, such as lady'slippers, have two anthers) the pollinarium is a specialized pollen bearing structure that becomes attached to the pollinator (Luer 1975, Johnson and Edwards 2000). The pollinarium consists of the pollinia (pollen masses) that are attached to a stipe, of varying length, the caudicles and the viscidium (Dressler 1993). The caudicles are highly variable among orchid species. They may be minute discs located between the stipe and the pollinia (Darwin 1904) or up to 20 mm in length as in *Cynorkis uniflora* Lindl. (Nilsson *et al.* 1992). The caudicles are important to the orientation of the pollinia after the pollinarium is attached to the pollinator (Darwin 1904, Johnson and Edwards 2000). The pollinarium has evolved via fusion of the stigma's median lobe and stamen(s) (Dressler 1983). The size and shape of

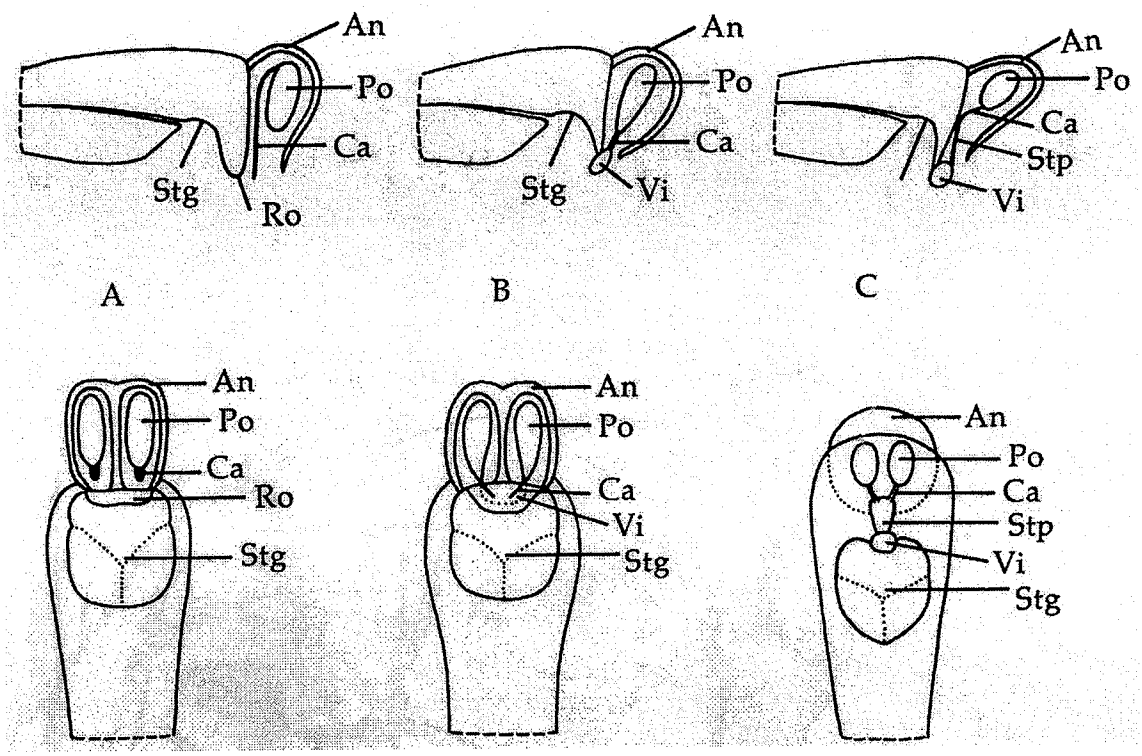

Figure 2. The relative position of the reproductive structures for three arrangements. Longitudinal sections above, ventral view below. A, Column with rostellum, but no viscidium; B, Column with viscidium; C, Column with viscidium and stipe. An, Anther; Ca, Caudicle; Po, Pollinium; Stg, Stigma; Stp, Stipe; Vi, Viscidium. (modified from Dressler 1981).

the pollinarium is unique among orchid species (Dressler 1981); pollinators can be associated with a particular orchid species through the identification of the pollinaria they carry (Ackerman 1983). As many orchids are autogamous or self-pollinating (van der Pijl and Dodson 1966), it would follow that this would be the most reliable method to guarantee pollination of a flower; however, several mechanisms will reduce the likelihood of this occurring. Firstly, the position of the pollinarium and stigma reduce the possibility of self-pollination. Relative to the stigma, the pollinaria are distally located at the end of the column within a protective pouch (Figure 3) (Dressler 1981). This spatial separation of the reproductive structures, i.e. pollinaria and stigma, is referred to as herkogamy. Webb and Lloyd (1986) suggested that herkogamy evolved to reduce self-pollination of a flower and self-pollination within a plant producing multiple flowers. The pollinarium is pulled from a protective pouch when the pollinator draws away from the flower and does not pass near the stigma. Secondly, the orientation of the pollinarium, immediately after removal from the pouch, does not align with the stigma and the pollinarium must undergo specific movements to do so. This movement, illustrated in Figure 4, is caused by differential drying of the stipe and/or caudicles, to change the orientation of the pollinia by as much as 90 degrees within a period of a few seconds or up to a few minutes (Darwin 1904, Dressler 1981, Johnson and Edwards 2000). Luyt and Johnson (2001) observed that after several minutes, movement of the pollinarium from *Mystacidium venosum* Harv. ex Rolfe flowers was completed and only then could contact be made with a specific notch in the stigma of subsequent flowers visited by the pollinator. Another important set of structures related to pollination are the flower nectaries. Nectaries are the nectar producing structures or glands in plants (Fægri

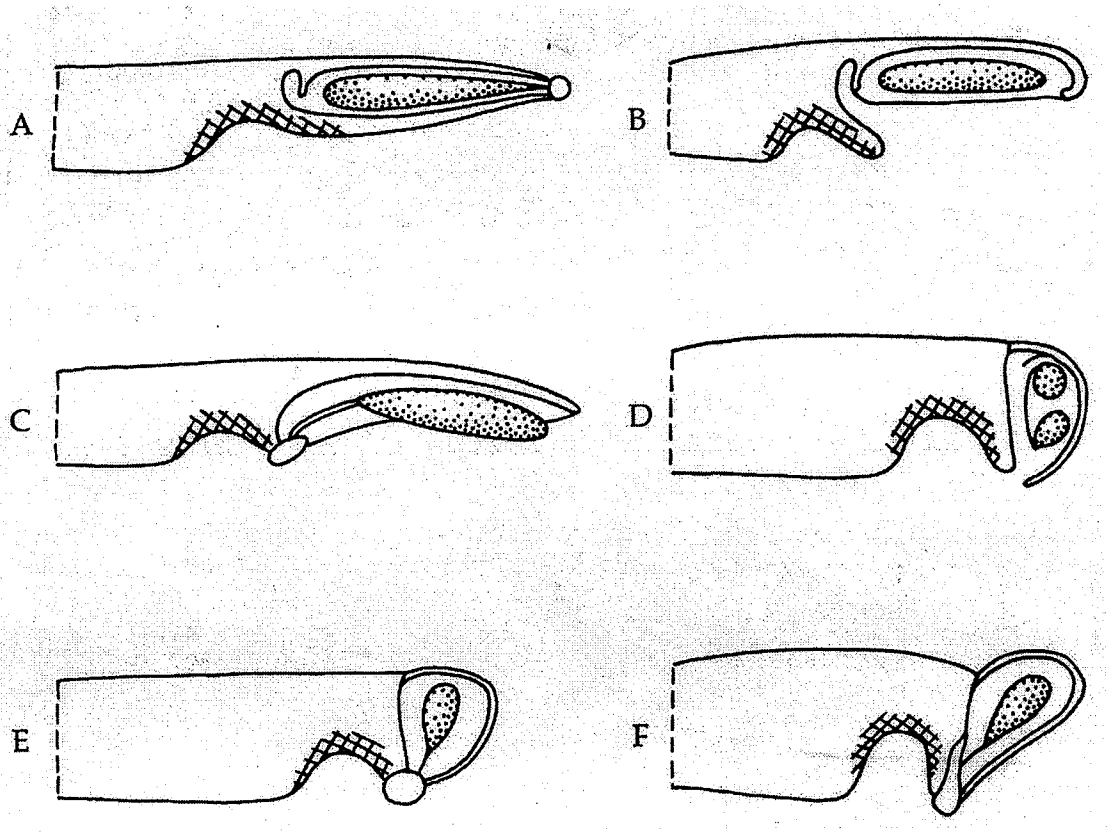

Figure 3. Diagrams showing relationships of pollinarium to stigma. Pollen, stippled. Stigma, cross-hatched. A, Spiranthoideae, with anther dorsal and rostellum subequal to pollinarium; B, Neottieae, pollinarium terminal, projecting beyond rostellum; C, Orchideae, with basal viscidia; D, Epidendroideae, with incumbent pollinarium; E, Vandoideae or advanced Epidendroideae, with viscidium, F, Vandoideae, with viscidium and stipe. (from Dressler 1981).

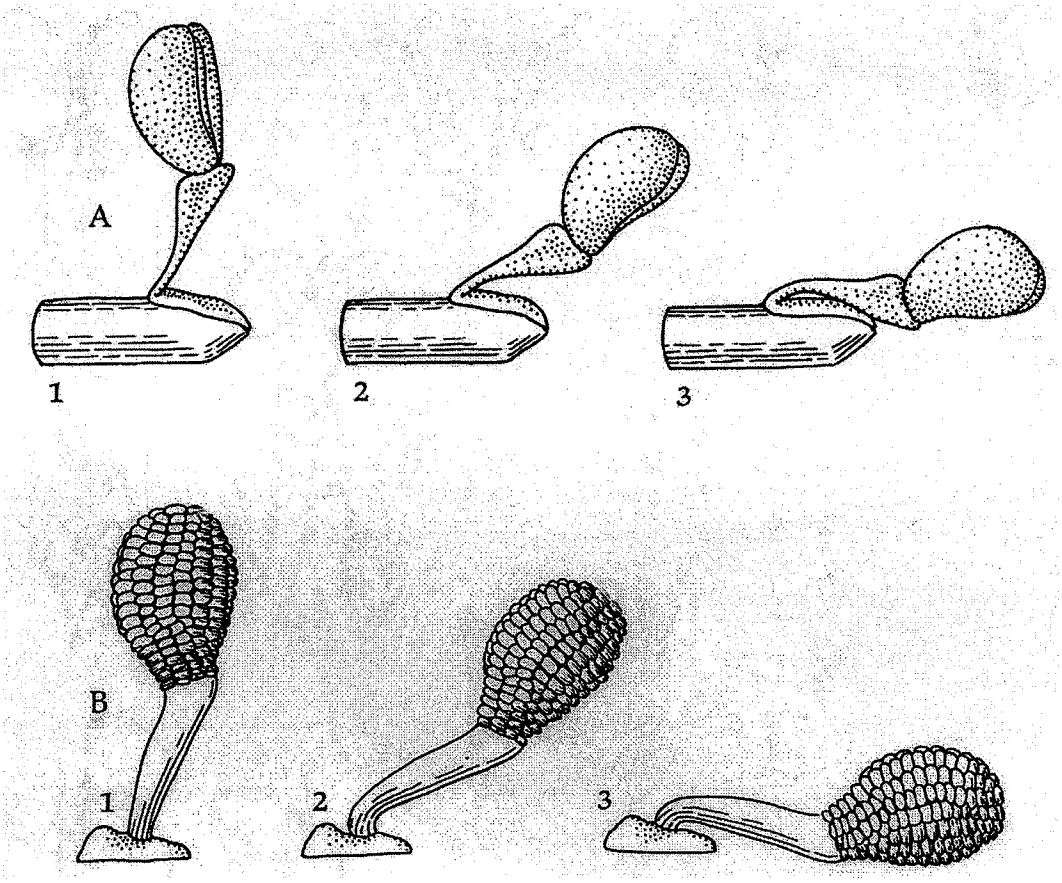

Figure 4. Movement in pollinaria. A, *Himantoglossum* spp.; B, *Rossioglossum* spp. (from Dressler 1981).

and van der Pijl1979). Many orchids produce a ready supply of nectar; however, pollinator access varies among the many taxa. To acquire nectar from *Cattleya*, *Epidendrum* and *Sobralia* species, pollinators must pierce the wall of the nectary before the nectar may be consumed; whereas in other orchids, such as the *Platanthera*, *Habenaria* and *Brassavola* species, nectar is stored in elongate spurs that hang beneath the flower (van der Pijl and Dodson 1966). The nectar spur may be formed by a fold in the labellum tissues or via fusion of the column and labellum tissues (Figure 5). Several orchid genera, such as the *Angraecum*, have greatly extended spurs that measure more than 10 cm in length (Nilsson *et al.* 1985).

Flower pollination in orchids. Pollination is the process in which pollen grains are transferred to the stigma, which is followed by fertilization of the ovules and development of seeds (Proctor *et al.* 1996). Plants rely on a variety of abiotic and biotic methods to achieve pollination of their flowers. For many plant species, abiotic reproductive methods, including wind pollination (anemophily) and water pollination (hydrophily), are prevailing approaches for successful pollination (Fægri and van der Pijl 1979). In both processes, plants produce a substantial amount of pollen that is released into the environment, with the prospect that a few grains will reach the stigma of another flower of the same species. This non-specific, non-directional transfer may seem wasteful (Fægri and van der Pijl 1979); however, in instances with highly gregarious plants, such as the grasses of temperate grasslands, anemophily is more efficient at pollen transfer than biotic counterparts (Proctor *et al.* 1996). Unlike grasses and other gregarious

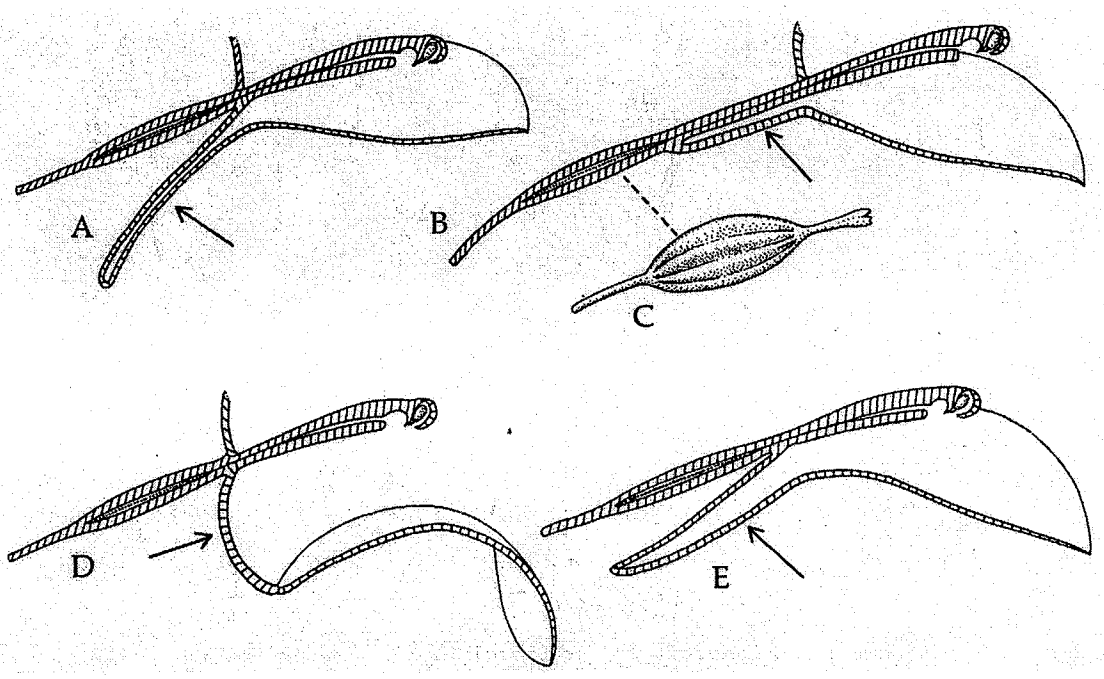

Figure 5. Formation and variation of the nectaries. A and B, Spur formed by the labellum; C, Beaked fruit (the beak representing the cuniculus of the flower; D and E, Spur formed by the column and labellum. (from Dressler 1981).

species, many orchids often occur at low densities and rely almost exclusively on biotic pollination methods to complete their reproductive cycle (van der Pijl and Dodson 1966, Wyatt 1983, Patt *et al.* 1989, Cuthrell 1994, Light and MacConaill 2002, Lehnebach and Riveros 2003).

The common factor characterizing biotic pollination is the organism that transports the pollen to the stigma - the pollination agent, the pollen vector, or the pollinator (van der Pijl and Dodson 1966, Fægri and van der Pijl 1979). To be recognized as a pollinator, and to rule out indiscriminate flower visitors, the organism must make regular visits to the flowers during its lifetime and effectively deposit the pollen on the stigma (Fægri and van der Pijl 1979). Wyatt (1983) identified various forms of biotic pollination as related to the pollinator taxon: sphingophily (hawkmoth pollination), phalaenophily (small moth pollination), psycophily (butterfly pollination), melittophily (bee pollination), myophily (syrphid and bee fly pollination), sapromyophily (carrion and dung fly pollination), cantharophily (beetle pollination), orinthophily (bird pollination) and chiropterophily (bat pollination). Most abiotic pollination systems do not occur in the Orchidaceae (i.e. anemophily and hydrophily) nor have there been observations of chiropterophily (van der Pijl and Dodson 1966); however, the remaining previously listed biotic systems have been observed in orchids (Patt *et al.* 1989, Rodríguez-Robles *et al.* 1992, Voss and Riefner 1983, Larson 1992, Johnson 1994, Cuthrell 1994, Light and MacConaill 2002, Lehnebach and Riveros 2003, Singer and Koehler 2003, Westwood and Borkowsky 2004).

Each of the pollination syndromes utilized by the Orchidaceae consist of a set of floral characters, reflective of the attractiveness of the flower from the perspective of the

pollinator (van der Pijl and Dodson 1966, Fægri and van der Pijl 1979). These characters are easily recognizable and include features such as floral odours, the colour and shape of flowers, abundance of nectar as well as the presence or absence of landing platforms and nectar guides (van der Pijl and Dodson 1966, Dressler 1981). Wyatt's (1983) list of syndromes included a distinction between sphingophily, and phalaenophily; however, other authors such as van der Pijl and Dodson (1966), Fægri and van der Pijl (1979), and Dressler (1981) have not separated the two pollination types. Since the floral characteristics are nearly identical according to Wyatt's (1983) description, no distinction was made in this review and phalaenophily refers to pollination by all moths.

Most orchids, except those utilizing an orinthophilous system, produce an odour to alert pollinators of their presence, and many odours are identified as sweet or, at the very least, agreeable to the olfactory receptors of most humans (van der Pijl and Dodson 1966, Dressler 1981). Floral odours are of little benefit to orinthophilous orchids as most birds have a poor sense of smell (van der Pijl and Dodson 1966, Pettingill, Jr. 1985). In contrast, flowers visited by birds are more brilliantly coloured (*i. e.* vivid shades of red, yellow, and cerise) than those pollinated by insects and only orinthophilous and psycophilous flowers are pure red in colour (Grant and Grant 1968, Dressler 1981). Rodrígues-Robles *et al.* (1992) identified the pollinator of *Comparettia falcata* Poepp. and Endl., which has odourless and red coloured flowers, as *Chlorostilbon maugaeus* (Audebert and Vieillot), a hummingbird endemic to Puerto Rico. A similarly red flowered orchid, *Disa ferruginea* (Thunb.) Sw. is pollinated by a butterfly *Meneris tulbaghia* (L.) as it mimics a nectar-producing iris *Tritoniopsis triticea* (Burm. f.) P. Goldblatt (Johnson 1994). Orchids that employ an orinthophily or psycophily

syndrome, or even a phalenophily method, have a tubular floral shape that is markedly different from the cupped flowers of myophilous and sapromyophilous species (Wyatt 1983). *Platanthera praeclara* and *Mystacidium venosum* are classic examples of phalenophilous flowers as both species have white flowers, emit a sweet scented odour during the night and produce a ready supply of nectar stored in a nectar spur (Sheviak and Bowles 1986, Luyt and Johnson 2001).

Melittophilous flowers in the Orchidaceae do not show conformity towards a particular floral shape and various forms are observed (van der Pijl and Dodson 1966). Landing platforms are present in many orchids, the exception being those pollinated by moths and birds. These platforms may aid in the attraction of pollinators by providing a place to rest or increasing the visibility of the flower. When landing platforms are not developed, it is common to find horizontal or hanging flowers as in the phalenophilous and orinthophilous flowers. Orchids using myophily and sapromyophily syndromes are more variable in the development of landing platforms and flower position (van der Pijl and Dodson 1966, Dressler 1981).

Pollen and nectar are common attractants for many plant genera; however, orchid pollen is not consumed by Hymenoptera, therefore nectar becomes the primary reward with most orchids producing an ample supply (Fægri and van der Pijl 1979, Dressler 1981). Orchids that do not produce nectar must rely on other means such as deception and mimicry to attract pollinators (Nilsson 1992, Johnson and Nilsson 1999). Some sapromyophilous species rely on odours that mimic the smell of decaying animal tissues to attract pollinators, e.g. *Bulbophylumm* spp. (van der Pijl and Dodson 1966). For species that have tubular shaped flowers, the nectar supply may be concealed deep within

the flower through the development of an elongated nectar spur formed by the folding of a portion of the sepal and/or petal (Dressler 1981). Nectar spurs are evident among several genera of orchids including, *Platanthera*, *Angraecum*, and *Comparettia*, and their pollinators' must possess a proboscis long enough to withdraw nectar (Bowles 1983, Dressler 1993, Rodrígues-Robles *et al.* 1992). Concealing the food reward in a restricted location reduces the possibility that a non-pollinator could remove nectar without assisting in the transfer of pollen (Fægri and van der Pijl 1979). Furthermore, the relative position of the nectar spur opening and the reproductive structures (e.g. pollinaria or modified stamens) is important in assuring that pollination is successful (Dressler 1981, Nilsson 1983). To retrieve nectar, the insect must correctly align itself with the flower as it inserts its proboscis; which will increase the likelihood that the pollinator will make contact with the pollinarium to remove it from the flower or strike it against the stigmatic surface.

Many plant genera may be limited to a single group of insects for pollination (Proctor *et al.* 1996). Among the orchids, 12 species of *Stanhopea* and 11 species of *Catasetum*, exhibit strict melittophily with pollinators belonging to either the moth genera *Euglossa* or *Eulaema*. However, a few orchid genera have developed a variety of pollination syndromes, as is the case of the *Disa* complex in southern Africa. Johnson *et al.* (1998) reviewed 27 species in the *Disa* complex and found that both psycophily and melittophily had evolved twice, while phalenophily and myophily occurred three and four times, respectively, among unrelated clades of the *Disa* complex. At the species level, Tremblay (1992) found that 67% of the 456 orchids examined relied on a single

pollinator species whereas 14% relied on two pollinator species and 17% utilized three or more pollinator species.

Western prairie fringed orchid. *Platanthera praeclara* is found in small areas of remnant tall grass prairie in southeastern Manitoba. Manitoba is the only known location in Canada where these orchids are found. The population fluctuates widely from 2000 flowering plants to more than 20000 flowering plants annually (Borkowsky and Jones 1998). In the United States there are small isolated populations of the orchid (each of only several hundred or less plants) in North Dakota, South Dakota, Minnesota, Nebraska and Iowa (Sheviak and Bowles 1986; Bray and Wilson 1992; U.S. Fish and Wildlife Service 1996).

Extensive agricultural activities throughout southern Manitoba and the central United States have radically changed the landscape including areas once characterized as tall grass prairie. In Manitoba, the extent of this once vast area of prairie grasses, forbs, shrubs and accompanying wildlife has been reduced to a few small locations, with the majority concentrated in the vicinity of Vita and Tolstoi, in the Rural Municipality of Stuartburn (Joyce and Morgan 1989). This Preserve was established through the cooperation of a number of partners including the Manitoba Naturalists Society, World Wildlife Fund, Wildlife Habitat Canada, Manitoba Conservation and Manitoba Habitat Heritage Corporation with the Nature Conservancy of Canada and Environment Canada joining the partnership shortly thereafter. In the United States less than 4% of the 60 million ha original tall grass prairie exists (Samson and Knopf 1994). The western prairie fringed orchid is confined to isolated patches within these last remnant tall grass

prairie areas, and before being discovered in the Tolstoi-Vita area (now Manitoba Tall Grass Prairie Preserve) it was not known to exist in Canada. The orchid has been placed on Canada's list of endangered plants and its endangered status is recognized on a worldwide basis (Collicutt 1993; Davis 1995). The government of Manitoba listed the species, as endangered under the Manitoba Endangered Species Act on April 19, 1996 (Manitoba Conservation – Endangered Species Act).

The western prairie fringed orchid was first documented in Manitoba in 1987 (Catling and Brownell 1987). Previously collected specimens were identified as the prairie white fringed orchid [(*Platanthera leucophaea*)(Nutt.) Lind.] (Johnson 1985). A distinction was made between the eastern and western plants of the prairie white fringed orchid when Sheviak and Bowles (1986) demonstrated that in addition to geographical displacement, the plants also possess different pollination mechanisms and floral characters. These differences are consistent and suggest that hybridization is unlikely. The discovery of this perennial orchid and remnant parcels of tall grass prairie habitat lead to the formation of the Manitoba Tall Grass Prairie Preserve. The entire orchid population exists within an area of approximately 12 000 ha, including agricultural lands, such as pastures that may support orchids and cultivated cropland and upland prairie that do not. In Manitoba, the orchid is associated with sedge meadows that are dominated by various sedge species (*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 and Best 1987, Moore and Fortney 1994). Since 1992, the Preserve and surrounding area has been surveyed for flowering western prairie fringed orchids. These annual surveys have lead to the discovery of 61 quarter sections

with flowering orchids, of which 17 quarter sections are located within the Preserve (Borkowsky 1996). It is estimated that 63% of the flowering population in Manitoba occurs within the Preserve. The population found in Manitoba is the largest in North America (Davis 1994).

To date most research on the biology of the western prairie fringed orchid has occurred in the most southern parts of its range in the United States (Pleasants and Moe 1993; Sieg and King 1995; Hof *et al.* 1999, Sharma *et al.* 2003). Little is known about the biology of the orchid specific to the Manitoba population. In Manitoba, several organizations including The Manitoba Museum, local universities and other non-profit groups have investigated certain life cycle aspects of the orchid, but the results of most of these studies have not been published in the general literature to date. It has become evident however, that the population of western prairie fringed orchids in Manitoba may have low seed capsule production compared to populations in the United States (Sheviak and Bowles 1986, Westwood and Borkowsky 2004). The role that varied seed capsule production between northern and southern populations may play in maintaining healthy populations in the few remaining areas where the orchid is found, is unknown. Although higher levels of seed production may ensure adequate reproduction over the long term and help maintain core orchid numbers in the southern populations, the significance of lower seed capsule production in the northern population is unknown.

When in flower, western prairie fringed orchids will grow 38-85 cm in height (Sheviak and Bowles 1986). The inflorescence of creamy white flowers makes this species highly visible during its flowering period of mid-June to mid-July (Figure 6). The number of flowers contained within the inflorescence is highly variable. Sheviak

Figure 6. Western prairie fringed orchid inflorescence and close up of flowers. A, inflorescence in early bloom; B, plant in full bloom; C, front view of individual flower; D, side view with complete nectar spur. Ns, Nectar spur; Nso, Nectar spur opening; Pol, Pollinarium; Stg, Stigma.

and Bowles (1986) reported a mean of 12.6 flowers per plant from 56 specimens collected from Minnesota, Iowa, North Dakota South Dakota, Nebraska and Kansas. Pleasants (1993) recorded mean values ranging from 7.0-9.4 flowers per plant from four sites in North Dakota and Minnesota. Flowers open from the base of the inflorescence to the top, a few each day (Figure 6). Bowles (1983) reported that an individual flower would persist for up to 10 days, however at the Sheyenne National Grassland in southeastern North Dakota, Pleasants and Moe (1993) reported that an individual flower would last for about seven days.

The flowers give off a sweet fragrance that becomes more intense in the late evening. The most striking visual characteristics of the flower are the large, deeply fringed, tri-lobed lower lip and long, slender spur. Measurements indicate that the lip may be 17-32 mm long and 20-39 mm wide, while the spur may be 36-55 mm long with a maximum diameter of 2.7 ± 0.5 mm (Sheviak and Bowles 1986). The pollinaria consists of pollinia (pollen), a caudicle and viscidium (Nilsson 1992). In the western prairie fringed orchid the minute grains of pollen are arranged into subunits called massulae (Pleasants and Moe 1993). These subunits form a bi-lobed mass that is attached to the column, which is then secured to the viscidium. The entire unit is sheathed within an anther pouch, with the exception of the viscidium, which is exposed and adapted to cement itself to the pollinator (Bowles 1983). Each flower has one pollinium located on either side of its stigmatic surface (Figure 6). This allows for a 6-7 mm separation of the viscidia (Sheviak and Bowles 1986). The opening to the nectar spur is located immediately below the stigmatic surface (Figure 6).

The floral characteristics indicate a moth pollination method (Fægri and van der Pijl 1979). While the small opening to the nectar spur restricts the position of the moth, it increases the likelihood that one or both viscidia will come into contact with either the proboscis or eyes of the moth (Sheviak and Bowles 1986). The length of the nectar spur and position of the viscidia further reduces the list of potential pollinators to those belonging to the Sphingidae or hawkmoth family. Members of this family possess long proboscis (tongues) that enable them to reach the nectar within the spur. Few, if any, observations of orchid pollination by swift-flying hawkmoths have been made in the field (Pleasant and Moe 1993; Sheviak and Bowles 1986; Bowles 1983). The proboscis length of various species of Sphingidae fall within the range of the nectar spur of *P. praeclara* and Sheviak and Bowles (1986) proposed the following species to be potential pollinators: *Eumorpha achemon* (Drury), *Hyles lineata* (F.), Sp*hinx drupiferarum* J.E. Smith and *Sphinx kalmiae* J.E. Smith.

In the absence of a flowering stem, the western prairie fringed orchid may produce a vegetative shoot. The shoot, arising from a tuberous root, may consist of a single leaf or as many three leaves. The year to year sequence of flowering stems and vegetative shoots does not follow a set pattern but Sieg and King (1995) determined that aboveground plants found on the Sheyenne National Grassland in North Dakota lived for three years or less. *Platanthera praeclara* may go dormant for a season or more, as is typical in other species of orchids (Nilsson 1992). Once absent, the chance that it will remain absent the following year is greater than 80% (Sieg and King 1995). Therefore, the recruitment of new plants is directly dependant upon seed production.

In the event that a flower is visited and pollinated, a seed capsule will develop. While relatively low to moderate levels of seed capsule development have been documented for fringed orchids (Bowles 1983; Pleasants and Moe 1993), a single capsule may contain thousands of dust like seeds (Bowles 1983). The minute size and buoyancy of the seed, suggests that it is suited for either wind (Bowles 1983) or water dispersal (Rasmussen 1995 in Hof *et. al.* 1999). The mechanism for seed dispersal is not fully understood. Timing of dispersal, such as late fall, winter, or spring, is unknown. The distance that seeds are carried will depend on a variety of environmental factors such as wind speed, natural flow of water as well as man-made diversions, flooding and periods of drought.

Following seed dispersal, germination is the next critical phase for this species. Germination will occur only if the seed makes contact with associated soil-based fungi (Bowles 1983). It is believed that these fungi work symbiotically with the root system of the plant, allowing it to absorb nutrients from the soil. Root systems containing fungi are referred to as mycorrhizae (Currah *et. al.* 1990). Zelmer and Currah (1995) found and described a new species of fungi, *Ceratorhiza pernacatena* Zelmer and Currah, within the root cortical cells of the western prairie fringed orchid. Very little is known about the amount of time required between seed germination and above ground growth. Estimates range from as little as two years for vegetative plants (Harley 1969 in Davis 1995) and up to 12 years for flowering plants (Curtis 1946 in Bowles 1983). Hof *et. al.* (1999) suggested that germinated seeds may spend one year as underground protocorms and appear the following year as an aboveground plant. The viability of *P. praeclara* seed is variable. Sharma *et al.* (2003) found seed viability, i.e. seeds with viable embryos, to be

as high as 36.5% and as low as 9.3%, with lower levels being observed in larger orchid populations. Furthermore, in vitro seedling growth studies indicate that only a fraction of viable seeds will result in leaf-bearing seedlings (Zettler *et al.* 2001, Sharma *et al.* 2003).

With many aspects of its biology unknown or only partially understood, conservation efforts for the western prairie fringed orchid are difficult to co-ordinate with those of the surrounding habitat. It is suggested that management of tall grass prairie habitat should include periodic fires on a three to five year cycle (Anderson 1990, Moore and Fortney 1994). This may lead to a reduction of encroachment by woody vegetation and improve the quality of native grasses (Moore and Fortney 1994). Pleasants (1995) reported that an early spring fire can have a positive effect on an orchid population if subsequent precipitation levels are near or above normal. However, the affect of fire on pollinator growth and development and the interaction between pollinators and the western prairie fringed orchid is unknown.

Lepidopteran characteristics important to orchid pollination. Rapid flight abilities and a long proboscis are two characteristics that make Lepidoptera effective and efficient pollinators for a variety of plant genera, including those within the Orchidaceae (Dressler 1981, Scoble 1992, Proctor *et al.* 1996). Rapid flight between flowers guarantees that a number of flowers will be visited in a relatively short time frame. When pollinators visit more flowers, there is a greater probability that they will be out-crossed, thus increasing the plant's reproductive output. In the case of the orchid, *D. ferrugine*, seed weight more than doubled in out-crossed flowers compared to self-pollinated ones, 24.3 mg and 10.0 mg, respectively (Johnson 1994). A long proboscis allows a pollinator to acquire nectar

concealed in a nectar spur; however, it also forces the pollinator to become aligned with the flower's reproductive structures to either remove the pollinaria or deposit pollen grains on the stigma (van der Pijl and Dodson 1966).

The Lepidopteran head and body are scaly and hairy, with the exception of the eyes and proboscis (Borror *et al.* 1989); which are primary attachment sites for orchid viscidia (Dressler 1981). The relationship between attachment site and morphology of orchid flowers has been documented for certain plant-pollinator pairs. A large column will separate the orchid's viscidia more distantly and contact is made with the eyes of the pollinators, as with the following orchid-pollinator pairs: *Platanthera chlorantha* (Cluster Reichb.) and *Deilephila porcellus* (L.) (Nilsson 1983), *Cynorkis uniflora* and *Nephele densoi* (Keferstein) (Nilsson *et al.* 1992) and *C. uniflora* and *Hippotion geryon* (Boisduval) (Nilsson *et al.* 1992). When the orchid possess a smaller column, the viscidia are positioned closer together and the contact site becomes the proboscis as with the following orchid-pollinator examples: *D. ferruginea* and *M. tulbaghia* (Johnson 1994), *Platanthera dilatata* (Pursh) Lindley ex Beck and *Discestra oregonica* (Grote) (Larson 1992) and *M. venosum* and *Nephele accentifera accentifera* (de Beauvois) (Luyt and Johnson 2001). A third site to which pollinaria may be attached includes the palps; however, this is uncommon and will occur in situations if the proboscis is shorter than the nectar spur and the scales have been lost from the palps (Nilsson 1983).

Sphingidae as pollinators. Moths in the Family Sphingidae (sphinx moths) include some of the fastest flying Lepidoptera in the world, capable of beating their wings at rates of 25 to 45 beats per second (Davidson 1965 in Schreiber 1978), comparable to

hummingbirds with 50 to 78 beats per second (Meise 1969 in Schreiber 1978). Along with rapid flight, sphinx moths are capable of traveling relatively long distances (Holland 1968, Dressler 1981, Hodges 1995); therefore, they have a greater potential to transfer pollinaria between isolated patches of orchids. This is vitally important to the plant because greater out-crossing leads to greater reproductive fitness.

The development of the proboscis is highly variable among the Sphingidae ranging from non-functional or rudimentary to those that are many times longer than the body of the individual (Hodges 1971, Nilsson 1998, Westwood and Borkowsky 2004). In a survey of Sphingidae from Central Madagascar, Nilsson *et al.* (1985) measured the proboscis length for 26 species. Five species had proboscis that measured less than 2 cm, including: *Temnora grandidiere* (Butler), *T. argyropeza* (Mabille), *Acherontia atropos* (L.), *Pseudoclanis grandidieri* (Mabille), and *Gynoeryx meander* Guenée. Four species had a proboscis length greater than 10 cm, including: *Xanthopan morgani* (Walker), *Coelonia solani* (Boisduval), *Agrius convolvuli* (L.) and *Panogena ligens* (Butler). Westwood and Borkowsky (2004) examined proboscis length of 15 species of sphinx moths collected from southeastern Manitoba and found six species that had mean lengths less than 3 mm, five species with lengths of 9-23 mm and four species with lengths greater than 30 mm. Among this last group, *Sphinx drupiferarum* and *Hyles gallii* (Rottenburg) were identified as pollinators and *Sphinx cheris* (Hubner) and *Sphinx kalmiae* were considered potential pollinators of the western prairie fringed orchid in Manitoba (Westwood and Borkowsky 2004). The variability in proboscis length may benefit moths within habitats by partitioning the available nectar resource, thus reducing competition. Furthermore, lengthening of the nectar spur in orchids could lead to the

establishment of one-to-one relationships between plant and pollinator species. When an orchid is pollinated by a single pollinator species, it reduces the risk of lost, misdirected or damaged pollinarium, a highly evolved structure that is energetically costly to produce (Benzing 1987).

The dense covering of long hairlike scales, that cover much of the Sphingidae body (Pittaway 2006), limits the availability of pollinaria attachment sites to the eyes and proboscis (Nilsson 1983). Much like the proboscis-nectar spur interaction, specific attachment sites for the pollinarium strengthen the plant-pollinator relationship. If the orchid pollinaria is not placed in the correct position on the pollinator such that it cannot make contact with the stigma of another flower, the reproductive output is reduced. It has also been observed that some moths may acquire considerable numbers of pollinaria that cover the eyes and may obscure their vision (Nilsson 1983 and Nilsson *et al.* 1985). Both Cuthrell (1994) and Westwood and Borkowsky (2004) collected sphinx moths with as many as eleven *P. praeclara* pollinaria attached to the eyes. To locate plants it has been suggested that sphinx moths follow the unique bouquet of floral odours produced by the flowers (Nilsson 1983, Nilsson *et al.* 1985, Nilsson *et al.* 1992). This hypothesis was confirmed by Raguso *et al.* (1996) when they studied the electroantennogram responses of *Hyles lineata* following exposure to various floral compounds. The electroantennogram results showed that these moths responded most strongly to aromatic esters such as benzyl acetate and methyl salicylate as well as oxygenated monoterpenoids including linalool and linalool oxides, all significant components of flower scents or odours. The ability to respond to specific floral compounds has been linked to greater

floral consistency in the interaction between *Sphinx ligustri* L. and *Platanthera biflora* (L.) Rich. (Nilsson 1983) and *Nephele densoi* and *Cynorkis uniflora* (Nilsson *et al.* 1992).

Pollinator energy requirements. There exists little information in the literature regarding adult sphinx moth energy requirements. Of the many Lepidopteran families, sphinx moths have some of the largest bodies (Hodges 1971), and the energy they expend for flight is considered large (Miller 1997), thus sphinx moths are considered to be high-energy demanders (Scoble 1992). Among the sphinx moths, Heinrich (1983) calculated the average cost of hovering to be approximately 1mg of sugar per gram of body weight per minute. Nilsson *et al.* (1985) examined the orchid-pollinator pair, *Angraecum arachnites* Schltr. and *Panogena ligens*, to determine the accuracy of Heinrich's cost of hovering. *Panogena ligens* weighs approximately one gram and was estimated to require 1.3 mg of sugar for each minute it spent hovering. As the nectar of *A. arachnites* was determined to be 13.3% sugar, the moth could ingest approximately 1.5 mg of sugar each time it visited a flower. The results of these calculations indicated that a single visit would sustain the moth for approximately 70 seconds of hovering flight. Considering that the duration of a flower visit may last fewer that three seconds (Heinrich 1983), sphinx moths are able depart a flower with a considerable energy surplus.

Sphingidae life history. In their respective reviews of the Sphingidae of North America North of Mexico and the Western Palearctic, neither Hodges (1971) nor Pittaway (2006) give specific details for the development time for each of the life cycle stages. Schreiber (1978) suggested that sphinx moth adult-plant associations and larval stages need further

examination; however, knowledge is still limited for many sphinx moths and their relationship with larval host plants and adult food sources (Duarte and Schlindwein 2005, Pittaway 2006). Pittaway (2006) indicated that the nutritional quality of larval food plants, a subject in which little work has occurred for sphinx moths, would be a valuable area to study. It is widely accepted that there is a highly specific relationship between larvae and their host plants; however, the relationship between adults and food plants is more general (Hodges 1971, Scoble 1992, Luyt and Johnson 2001, Raguso and Willis 2003, Duarte and Schlindwein 2005).

The Sphingidae are endopterygotes, undergoing complete metamorphosis in four stages – ovum, larva, pupa, and adult (Scoble 1992). Most species of Sphingidae are univoltine in northern climates (Hodges 1971). Development time of the ovum is variable between species and for some it is independent of temperature (Pittaway 2006). Typically, the eggs are cemented to the underside of leaves belonging to the larval food plant; though, Pittaway (2006) indicated that some species would oviposit on the flower heads, or on dead stems and stones at the base of the food plant. Once the larva has eaten its way out of the eggshell, it will rest, and then feed on the leaves of the host plant. Early instars may rest along the midrib of the leaf when not feeding (Pittaway 2006). As larvae mature, they move to fresh leaf material and may actively wander in search of a new host plant if food becomes scarce. Following four to six instars, the larva is mature and most species will burrow into the leaf litter or soil to form a small chamber in which the process of pupation will take place (Pittaway 2006).

During pupation, larval tissues are broken down and reorganized to from adult tissues and structures. When the tissues have been reduced to clusters of nuclear cells,

the insect may undergo diapause to survive the winter months (Pittaway 2006). When all of the tissues have been reorganized, a series of abdominal contractions allow the pupa to wriggle to the surface of the leaf litter or soil and the adult will emerge from the pupal case (Pittaway 2006). After 30 minutes, the wings are fully expanded and several hours more are needed for them to dry and harden before flight is possible.

There are two periods during their life cycle when the Sphingidae are most vulnerable. During the migration to pupation sites, the larvae face the greatest risk of exposure to predators (Pittaway 2006). They are completely exposed and any benefit of camouflaged colouration is lost as they move down the host plant to the soil. The second vulnerable period occurs when females search for oviposition sites and are most likely to encounter predatory species (Pittaway 2006).

The Sphingidae include approximately 1000 species worldwide, with the greatest diversity in the tropical regions (Hodges 1971). There are 124 species recorded for North America north of Mexico (Hodges 1983). Twenty-two species representing 10 genera have been collected from Manitoba (A. R. Westwood, pers. comm.). Many sphinx moths are both crepuscular and nocturnal and only a few are exclusively diurnal (Holland 1968, Hodges 1971, Scoble 1992).

Westwood and Borkowsky (2004) collected two *H. gallii*, each with two *P. praeclara* pollinaria, and four *S. drupiferarum* individuals with 3-11 pollinaria per moth during a three-year study of *P. praeclara* in Manitoba. During a study of the insects associated with *P. praeclara* and *P. leucophaea*, Cuthrell (1994) collected one male *S. drupiferarum* from the Sheyenne National Grasslands in North Dakota that had 11 *P. praeclara* pollinaria attached to the left eye and eight to the right eye. Cuthrell

(1994) also collected one male *E. achemon* that was collected with ten *P. praeclara* pollinaria attached to the left eye and seven on the right compound eye during the same study. Ralston *et al.* (2006) reported that *Hyles euphorbiae* (Linnaeus), an introduced species in North America, was collected from the Sheyenne National Grasslands in North Dakota with *P. praeclara* pollinarium attached to the eyes.

Plant nectar. Nectar serves an important function in plant reproduction by attracting and rewarding the pollinator for their visits to flowers. Nectar is the most common reward, with two-thirds of the orchid family using nectar as the main pollinator reward (van der Pijl and Dodson 1966), while the remaining third resort to nectar deceit, alternative rewards (e.g. pollen or floral fragrance), mimicry of prey species, or offer a resting place (Neiland and Wilcock 1998). Plant-pollinator relationships are determined by several nectar characteristics such as the accessibility of the nectar relative to floral morphology, sugar concentration, volume, viscosity and chemical composition (Proctor *et al.* 1996, Perret *et al.* 2001, Galetto and Bernardello 2004). Galletto *et al.* (1997) note that sugar composition has been determined for approximately 110 species of orchids or a small fraction of the estimated 19500 to 25000 species.

Nectar is a solution consisting primarily of sugar and water (Fahn 1979). There is considerable variation in the composition of nectar across plant species. Sugars dominate the solutes that can include sucrose, glucose, fructose, maltose, melibiose and raffinose in varying amounts (Shuel 1955, Perret *et al.* 2001, Galetto and Bernardello 2004). Nectars that are sucrose dominated are present in plants pollinated by hummingbirds and Lepidoptera, while glucose and fructose dominated nectars are found in bee pollinated

species (Southwick 1990, Perret *et al.* 2001, Galetto and Bernardello 2004). Other nectar constituents include minerals, enzymes, essential oils, and volatile organic substances, which can give the nectar a characteristic fragrance or taste (Shuel 1955, Southwick 1990).

Nectar is brought to the flower by means of the phloem (Fahn 1979). The phloem sap (pre-nectar) moves from the sieve elements to the cells of the nectariferous tissue where it is modified by enzyme activity and processes of resorption (Fahn 1979). Nectar is secreted by specific structures called nectaries, the structure of which can range from a few cells to elaborate organs (Shuel 1955). The location of nectaries is variable for different plant species, they can be on the receptacle, on the base or apex of the ovary, on the sepals, petals, or the stamens, as well as in petals modified as spurs or extrafloral locations (Shuel 1955). Nectar secretion is influenced by the physiologic state of the plant and environmental factors (Fægri and van der Pijl 1979, Galetto and Bernardello 2004). When a plant undergoes extensive growth, nectar secretion diminishes (Shuel 1955). Temperature thresholds must be attained before nectar is secreted (Shuel and Pederson 1952) and atmospheric humidity, high wind speeds and air temperatures can affect the sugar concentration of the nectar through accelerated rates of water evaporation (Shuel and Pederson 1952, Shuel 1955).

Nectar is an important energy source for insects. Sugar constituents and their ratios as well as the volume of nectar will determine the amount of energy available to the nectar-seeking insects. Disaccharides such as sucrose contain more energy than a monosacharide such as glucose (Fægri and van der Pijl 1979). Sugar concentration varies between 15-75% of the nectar (%, wt/ total wt) (Fægri and van der Pijl 1979, Galetto and

Bernardello 2004, Guerenstein *et al.* 2004, Tian *et al.* 2004). While providing large amounts of energy, high sugar concentrations can trigger a negative response by reducing the amount of water uptake by the pollinator (Fægri and van der Pijl 1979) and increasing the viscosity of the nectar, which may make extraction more difficult (Heyneman 1983).

The amount of nectar and energy produced by a flower is related to the characteristic rate of energy expenditure of the pollinator (Heinrich and Raven 1972). Since the average cost of hovering for sphinx moths is 1mg of sugar per gram of body weight per minute (Heinrich 1983), flowers pollinated by sphinx moths should provide an adequate amount of energy (Heinrich and Raven 1972), in the form of nectar, to cover the energy cost of feeding and flight to the next flower. Sphinx moths, with their rapid wing beat, are capable of hovering in front of flowers to collect nectar thus energy costs should be roughly correlated with the energy provided by nectar (Nilsson *et al.* 1985, Borror *et al.* 1989). Nectar spur length also has been correlated to proboscis length of the pollinators (Nilsson 1992, Nilsson 1998, Nilsson *et al.* 1992, Maad 2000) and examination of nectar volumes and sugar concentrations are common in pollination research (Perret *et al.* 2001, Galetto and Bernardello 2004, Tian *et al.* 2004). There is no published data for nectar production or sugar content for the western prairie fringed orchid. Measurement of these variables will give a better understanding of the orchid pollination syndrome and show whether the rewards offered by the flower match the energetic and nutrient requirements of the pollinator.

Light traps and moth attraction. Moths, like many adult insects, are attracted to various wavelengths of light (Hsiao 1972). Photoreceptors in the insect's eyes are

sensitive to a wide spectrum of light, ranging from the ultraviolet to red (Rutowski 2003), or in terms of measured wavelengths, approximately 250 to 600 nm (Borror *et al.* 1989, Brisco and Chittka 2001). The attraction to light sources has been termed light-compass orientation (Horn 1976). When an insect becomes fixated on a light source (real or artificial), it will then move at a constant angle toward it; among moths, this produces a spiral path (Horn 1976). Taking advantage of this behaviour, light traps are a well documented research tool for examining moth communities (Blomberg *et at.* 1976, Southwood 1978, Thomas 1996, Thomas 2001, Duarte and Schlindwein 2005). Assessments of light sources of differing wavelengths have shown that ultraviolet light is more attractive to moths than light of other wavelengths (Blomberg *et at.* 1976, Nabli *et al.*1999). Most species of Sphingidae can be readily collected using incandescent, mercury vapor and ultraviolet lights (Hodges 1971, Duarte and Schlindwein 2005).

CHAPTER 3

ENHANCING POLLINATION OF THE ENDANGERED WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*) BY SPHINX MOTHS (LEPIDOPTERA: SPHINGIDAE) IN TALL GRASS PRAIRIE IN SOUTHEASTERN MANITOBA.

ABSTRACT

The endangered western prairie fringed orchid, *Platanthera praeclara* Sheviak and Bowles, is found in remnant tall grass prairie in southeastern Manitoba. The western prairie fringed orchid has a lower level of seed capsule development, less than 6% for the current study, in comparison to more southern populations in the United States, as high as 39% in some cases. Pollination is limited to few select species of sphinx moths, Sphingidae, Lepidoptera, a group that is less abundant in comparison to other lepidopteran families. This study tested the hypothesis that the presence of ultraviolet light sources near orchids would attract more sphinx moths into orchid habitat and increase feeding activity of sphinx moth pollinators, thus increase seed capsule production. A significantly larger number of individual flowers and plants developed seed capsules in the ultraviolet light treatment plots (5.13 ± 0.42 % of available flowers; 35.12 ± 1.74 % of total plants) than the control plots (2.78 ± 0.42 % of available flowers; 21.76 ± 2.58 % of total plants), indicating that the level of seed capsule development observed in the Manitoba population may be linked to population levels of the sphinx moths pollinators. Under natural conditions, size of inflorescence did not influence frequency of pollinaria removal or seed capsule production. In ultraviolet light plots,

those plants with 11 or more flowers, had a significantly higher level of seed capsule production than plants with 10 or fewer flowers. Results also indicated that ultraviolet lights may be useful to manipulate seed capsule production for other research purposes.

INTRODUCTION

The endangered western prairie fringed orchid, *Platanthera praeclara*, is found in remnant tall grass prairie in southeastern Manitoba. When in flower, these orchids will grow 38-85 cm tall (Sheviak and Bowles 1986). The inflorescence of creamy white flowers makes this species highly visible during its flowering period of late-June to mid-July (Figure 6). The flowers give off a sweet fragrance that becomes more intense in the late evening. The most striking visual characteristics of the flowers are the large, deeply fringed, tri-lobed lower lip and long, slender nectar spur (Figure 6) (Sheviak and Bowles 1986). These floral characters limit pollination to a select few species of sphinx moths, (Sphingidae, Lepidoptera) (Cuthrell 1994, Westwood and Borkowsky 2004, Ralston *et al.* 2006).

Platanthera praeclara only occurs in wet sedge meadows within remnant tall grass prairie habitat in central North America. Loss of habitat is considered the leading cause for its' endangered status in Canada and the United States (Davis 1994, U.S. Fish and Wildlife Service 1996). Tall grass prairie is considered the most endangered ecosystem in North America (Samson and Knopf 1996, Hamilton 2005, Whiles and Charlton 2006) with less than 0.5% of the original habitat remaining in Manitoba (Joyce and Morgan 1989). Westwood and Borkowsky (2004) have recently described the pollination process for *P. praeclara* in Canada. Westwood and Borkowsky (2004) also

noted that there is a low level of seed capsule development in the Canadian population in comparison to more southern populations of *P. praeclara.*

This low level of seed capsule development may be related to the scarcity of pollinators (Westwood and Borkowsky 2004). Sphingidae are generally less abundant than species in many other lepidopteran families (Hodges 1971, Duarte and Schlinwein 2005). The area surrounding *P. praeclara* habitat in Manitoba has become fragmented by agricultural land use ranging from tame pasture development to conversion to cropland, with considerable insecticide and herbicide usage. The nocturnal pollinators may be drawn to the many light sources that now flood the previously dark countryside, such as farm lights and intersection lights at highway junctions and away from the less inhabited areas that support populations of western prairie fringed orchids. Other factors that may influence pollinator visitation may include lack of larval host plants or competition from alternate nectar sources.

To test the hypothesis that low production of seed capsules in *P. praeclara* in Manitoba is related to sphinx moth density an experiment was designed to attract sphinx moth pollinators into orchid habitat to attempt to increase pollination success. This study tested the hypothesis that attraction of sphinx moths pollinators using ultraviolet light sources will increase feeding activity thus increasing seed capsule production.

METHODS

Study area. The Manitoba Tall Grass Prairie Preserve is located within the Rural Municipality of Stuartburn in southeastern Manitoba near the Canada-United States border (49° 08' N, 96° 40' W) (Figure 7). The lands purchased for the Preserve

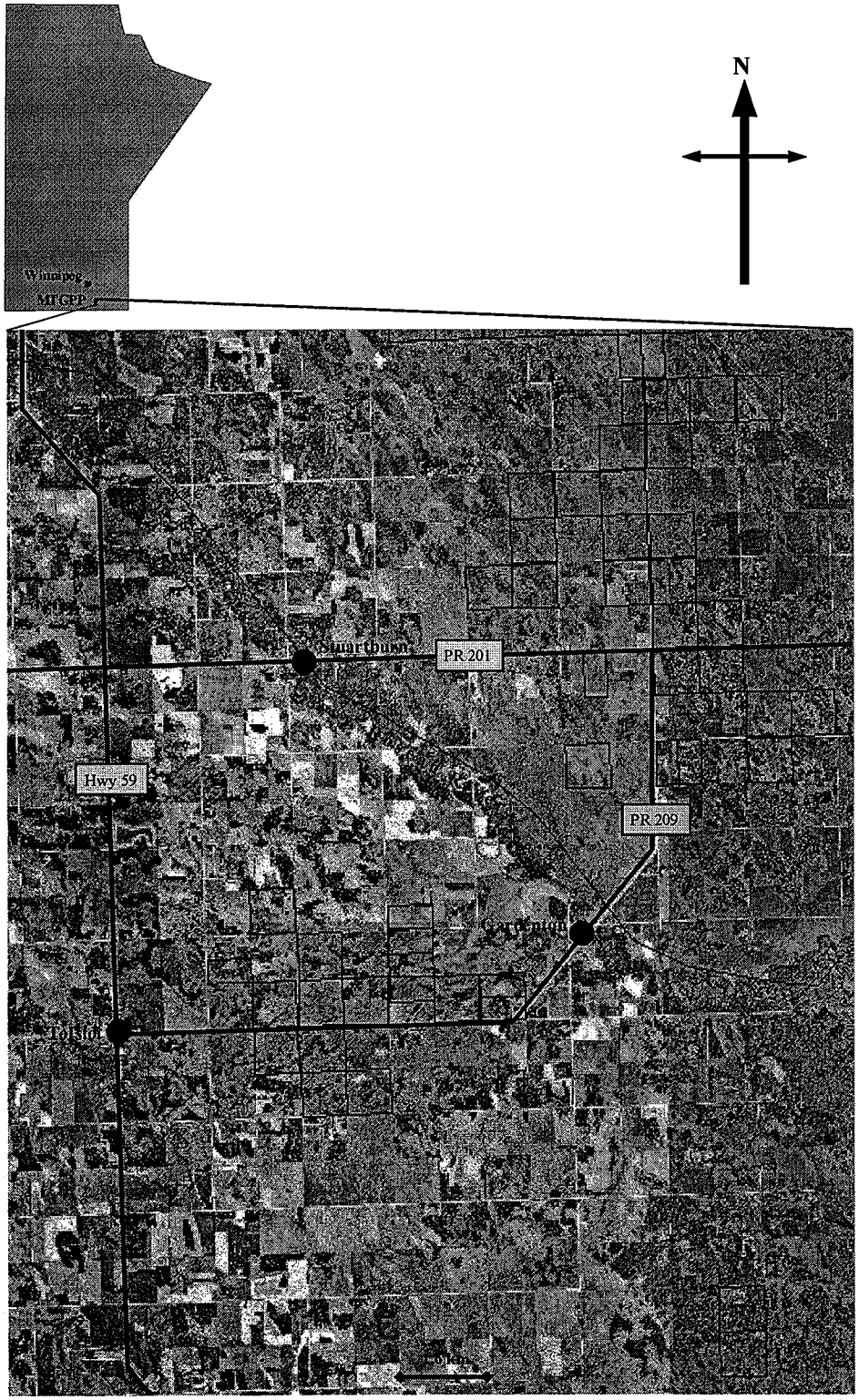

Figure 7. Location of the Manitoba Tall Grass Prairie Prairie Preserve in southeastern Manitoba. Property titles held by Preserve partners outlined in black.

represented the largest contiguous tracts of remnant tall grass prairie in Manitoba following a systematic search for tall grass prairie by the Manitoba Naturalists Society in the 1980s (Joyce and Morgan 1989). The Preserve and surrounding area represents the only known location in Canada in which *P. praeclara* naturally occurs, with approximately 63 % of the population occurring within the Preserve. The remaining plants are on private lands or in road allowances adjacent to the Preserve (Borkowsky and Jones 1998).

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 and Fortney 1994). The soil is a grey-wooded podzol, having a sandy-loam to clay-loam texture with frequent rock outcrops (Moore and Fortney 1994). The shallow slope of the landscape (1-3%), poor drainage and high water table (within 3m of the surface) generally inhibit agricultural productivity and potential within the Preserve (Moore and Fortney 1994).

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 oak (*Quercus macrocarpa* Michx.) and shrubs including saskatoon (*Amelanchier alnifolia* Nutt.), chokecherry (*Prunus virginiana* L.) and hazelnut (*Corulus* spp.). The herbaceous layer is dominated by poison-ivy (*Rhus radicans* L.), meadow rues (*Thalictrum* spp.), goldenrods (*Solidago* spp.), golden Alexander [*Zizia aurea* (L.) Koch] and various gramnoids. 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 potentilla (*Potentilla fruiticosa* L.) and rose (*Rosa* spp.) occur in the upland prairie. The sedge meadow is dominated by various sedge species (*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 and Best 1987, Moore and Fortney 1994).

Ultraviolet light and pollination. Prior to experimental plot selection, inventory assessments for *P. praeclara* from previous growing seasons and general orchid distribution maps for the Preserve (Davis 1994, Borkowsky and Jones 1998), were examined to establish a preliminary list of plot locations. When flowering stems became visible in late May 2000 (i.e. height of stems approximately 10 cm), potential sites were examined to determine the number of flowering stems to be produced during the growing season. Eight sites were selected each with a minimum of 30 orchid plants. Sites were separated by a minimum of 500 m. Each site was surrounded to some degree by aspen woodland and was not visible at 2 m above ground from an adjacent site. Within each site, two plots were established. Plots were chosen such that the adjacent plot was not visible at 2 m above the ground and they were separated by aspen woodland. Each plot within a site was randomly assigned one of two treatments, ultraviolet light or no ultraviolet light (i.e. the control). The four plots assigned to the ultraviolet light treatment were labelled UV-P1, UV-P2, UV-P3 and UV-P4 and the control plots CN -P1, CN -P2, CN -P3 and CN -P4. In 2001, replacement plots were established on 4 July for the ultraviolet light and control plots labelled UV-P5 and CN-P5 respectively. These

replacement plots were necessary following a brief but intense hailstorm that passed through the experimental area on 3 July 2001 and caused considerable damage to four of the eight plots (i.e. CN-P1, CN-P3, UV-P2 and UV-P4). The plants within these plots were so severely damaged that studies could not be continued and a single replacement plot was established in each treatment (i.e. CN-P5 and UV-P5) within three days of the storm.

All sites were located within the north block of the Preserve (Figure 8). In the second week of June, the center of each plot was marked with an orange pin flag and a 60 m radius, covering 1.13 ha, was marked with eight additional pin flags. In the third week of June, the four plots assigned to the ultraviolet light treatment had a small wooden shelter erected at the center (Figure 9). Each shelter was constructed by placing four 1.5 m fence posts 1 m apart to form a square. Fence posts were buried 0.5 m into the ground. A 1 m x 1 m piece of exterior grade, 6.25 mm thick plywood was nailed to the top of the posts. The ultraviolet light and its power source were located underneath this shelter to minimize/prevent rainfall damage to the electrical components. There were four plots each with an ultraviolet light and four control treatments that did not receive an ultraviolet light, thus each treatment was replicated four times. One orchid plant was considered the sampling unit within a plot.

Each plant in each plot was marked with a piece of uniquely numbered flagging tape tied loosely to its base. The location of the plant within the plot was recorded in 2000 and 2001 relative to the plot center by measuring the distance (m) and degree vector to facilitate the relocation of the plant after senescence. In 2002, plant locations were recorded in longitudinal and latitudinal degrees with a Garmin 12XL GPS unit.

Figure 8. Location of study plots in the northern block of the Manitoba Tall Grass Prairie Preserve.

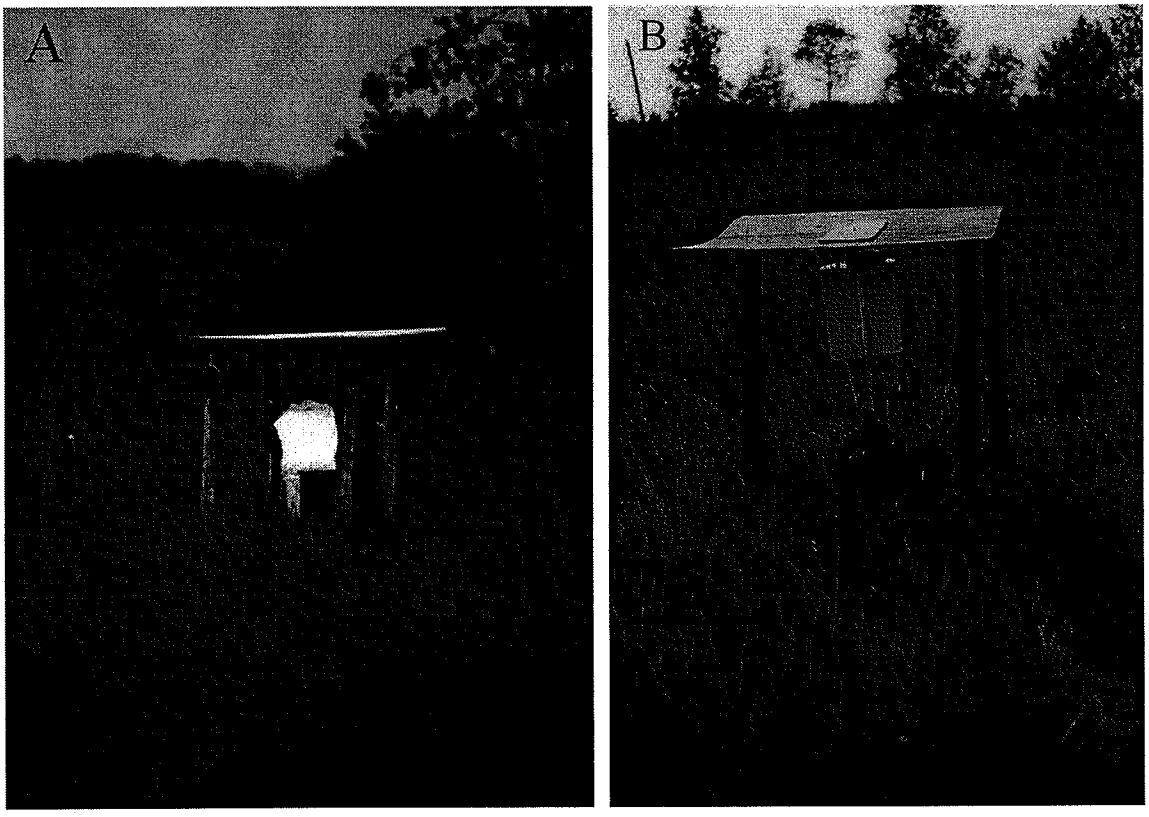

Figure 9. Shelters and placement of ultraviolet light equipment. A, 2000 and 2001; B, 2002.

The number of flowers, their individual condition, presence or absence of polliniaria per flower and development of seed capsules was recorded for each plant. Number of flowers per plant was determined by carefully examining the inflorescence, with the flowers numbered from the lowest to the uppermost ones. Herbivory was recorded for individual flowers by recounting the number of flowers per plant during the bloom period and tallying the number of clipped stems during the seed capsule survey. The number of absent flowers was deducted from the number of flowers recorded when plots were established to determine the number of flowers at the end of the season.

Flowers were examined for removal of pollinaria during the flowering period. The number of seed capsules per plant was recorded in the fall (Table 1).

The ultraviolet light utilized in this study was a Ward's ® All Weather Insect Bucket Trap with a single 8 watt florescent bulb located in the center of four baffles. A Motomaster ® 12 volt marine deep cycle battery was used to power the light by attaching a Tran-bal ® Transformer (Model 12FA, Bodine Inc. Collierville, TN) between the battery and trap. In 2000 and 2001, a white cloth cover was placed over the entire trap to prohibit entry by insects, while allowing the light to remain visible. The white cloth cover was also used to lower the intensity of the light emission in an effort to minimize the attraction from control plots. Traps were placed on the ground beneath the shelters and weighted down with several rocks to prevent displacement by wind or animals. In 2002, the trap's lower collecting pail was removed and the light was hung from the center of the shelter's plywood cover. The cloth cover was removed in 2002 to test the lights at their maximum intensity. The ultraviolet light was operated on alternate nights throughout the flowering period between the hours of 2000 hours and 0800 hours. The

Table 1. Survey dates for assessments of flower condition and number of seed capsules in 2000, 2001 and 2002.

Year	Treatment	Location	Plot labels[1]	Flower condition			Seed capsule
2000	Control	SW24-2-6E	CN-P1	11-Jul			16-Sep
		NE35-2-6E	CN-P2	17-Jul			
		NE26-2-6E	CN-P3	6-Jul			16-Sep
	Ultraviolet Light	SW25-2-6E	UV-P1	5-Jul			16-Sep
		NW36-2-6E	UV-P2	5-Jul			16-Sep
		SE2-3-6E	UV-P3	10-Jul			16-Sep
2001	Control	SE24-2-6E	CN-P1[2]				
		NW24-2-6E	CN-P2	20-Jun	26-Jun	5-Jul	4-Sep
		SE26-2-6E	CN-P3[2]				
		NE35-2-6E	CN-P4	6-Jul	10-Jul		7-Sep
		NW24-2-6E	CN-P5[3]	5-Jul			4-Sep
	Ultraviolet Light	SW24-2-6E	UV-P1	25-Jun	2-Jul	11-Jul	13-Sep
		SW25-2-6E	UV-P2[2]	21-Jun	29-Jun	5-Jul	
		NW36-2-6E	UV-P3	23-Jun	7-Jul		7-Sep
		SE2-3-6E	UV-P4[2]				
		SW24-2-6E	UV-P5[3]	6-Jul	8-Jul		14-Sep
2002	Control	SE24-2-6E	CN-P1	12-Jul	19-Jul	26-Jul	7-Sep
		NW24-2-6E	CN-P2	4-Jul	13-Jul	24-Jul	7-Sep
		SE26-2-6E	CN-P3	10-Jul	19-Jul	26-Jul	7-Sep
		NE35-2-6E	CN-P4	10-Jul	16-Jul	27-Jul	7-Sep
	Ultraviolet Light	SW24-2-6E	UV-P1	8-Jul	16-Jul	23-Jul	6-Sep
		SW25-2-6E	UV-P2	5-Jul	12-Jul	22-Jul	6-Sep
		NW36-2-6E	UV-P3	8-Jul	13-Jul	22-Jul	6-Sep
		SE2-3-6E	UV-P4	10-Jul	15-Jul	23-Jul	7-Sep

[1] Plot labels: UV = Ultraviolet light treatment, CN = Control treatment

[2] Severe hail damage to plants, plot eliminated from analysis

[3] Replacement plot established after 3 July 2001 hailstorm.

Table 2. Operation of ultraviolet lights in 2000, 2001 and 2002.

Year	Night of Operation		Plots[1]
2000[2]	6-Jul	7-Jul	UV-P1, UV-P2
	7-Jul	8-Jul	UV-P3
	8-Jul	9-Jul	UV-P1, UV-P2
	9-Jul	10-Jul	UV-P3
	10-Jul	11-Jul	UV-P1
	11-Jul	12-Jul	UV-P3
2001	25-Jun	26-Jun	UV-P1, UV-P2, UV-P3, UV-P4
	27-Jun	28-Jun	UV-P1, UV-P2, UV-P3, UV-P4
	29-Jun	30-Jun	UV-P1, UV-P2, UV-P3, UV-P4
	1-Jul	2-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	3-Jul	4-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	5-Jul	6-Jul	UV-P1, UV-P3, UV-P5
	7-Jul	8-Jul	UV-P1, UV-P3, UV-P5
	9-Jul	10-Jul	UV-P1, UV-P3, UV-P5
	11-Jul	12-Jul	UV-P1, UV-P3, UV-P5
	13-Jul	14-Jul	UV-P1, UV-P3, UV-P5
	15-Jul	16-Jul	UV-P1, UV-P3, UV-P5
	17-Jul	18-Jul	UV-P1, UV-P3, UV-P5
	19-Jul	20-Jul	UV-P1, UV-P3, UV-P5
2002	6-Jul	7-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	8-Jul	9-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	10-Jul	11-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	12-Jul	13-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	14-Jul	15-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	16-Jul	17-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	18-Jul	19-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	20-Jul	21-Jul	UV-P1, UV-P2, UV-P3, UV-P4
	22-Jul	23-Jul	UV-P1, UV-P2, UV-P3, UV-P4

[1] Plot: UV = Ultraviolet light treatment, CN = Control treatment

[2] Rotated two ultraviolet lights among three plots in 2000

ultraviolet light was operated for six nights beginning 5 July 2000, 13 nights beginning on 25 June 2001 and nine nights beginning on 6 July 2002 (Table 2).

Statistical analysis. The number of plants, number of flowers per plant, relative position of each flower within the inflorescence, number of available pollinaria and number of seed capsules were recorded for each site and all treatments in 2001 and 2002. The mean number of flowers per plant and standard error of the mean (SEM) was calculated for each plot in 2000, 2001 and 2002. Prior to analysis, all experimental variables were tested for departure from the normal distribution (Zar 1996). Within the 2001 data set seed capsule production per plant and flower were log transformed to meet the assumptions of a normal distribution (Zar 1996). Independent t-tests (α=0.05) were used to compare number of plants, number of flowers per plant, number of available pollinaria, and number of seed capsules between treatments. As the number of plants per plot ranged between 48 and 98 in 2000, 29 and 78 in 2001, and 25 and 151 in 2002, the level of seed capsule production was calculated as a percentage of the available plants and flowers for each site and treatment. The resulting values (± SEM) were compared for the two treatments to determine if there was a difference in seed capsule development. Pearson's correlation coefficient (α=0.05) was calculated to determine if seed capsule production could be correlated to pollinaria removal.

Due to the considerable variation in the number of flowers per plant, three plant size categories (i.e. small, medium and large) were established based on the number of flowers within the inflorescence. The mean number of flowers per plant and standard deviation of all plants was calculated from the pooled 2001 and 2002 data set (7.22 ± 2.96, $n = 1248$). The mean ± standard deviation was considered to be the

medium size category (i.e. 4 to 10 flowers per plant) with the small and large size categories being three or fewer flowers and 11 or more flowers in the inflorescence respectively. The mean number of seed capsules per plant and per available flowers and the standard error of these means (SEM) was calculated for 2001 and 2002. Analysis of variance (ANOVA) (α=0.05) was used to determine if there was a difference in seed capsule production among the three plant categories within each treatment. When a significant difference was identified among the plant size categories, Fisher's least significant difference (LSD) post hoc test was performed because of its consistency to determine differences between mean seed capsule production for plant size categories (Saville 1990).

To determine if herbivory of the flowers affected the overall reproductive level of the orchids, a paired sample t-test was performed. The percentage of seed capsule development was calculated first using the total number of flowers at the start of the season then using the total number of flowers remaining at the end of the bloom period. The percentages were compared for all plots combined, and then for each treatment group.

All statistical analyses were done with SPSS v. 11.0.1 (SPSS Inc. 2001).

RESULTS

Ultraviolet light. The mean number of flowers produced per plant was 6.66 ± 0.14 standard error of the mean (SEM), 6.87 ± 0.16 and 7.32 ± 0.09 for all plots in 2000, 2001 and 2002 (Table 3). In September 2000, the seed capsule development of many plants in plots UV-P2, UV-P3 and CN-P3 could not be determined due to

herbivory. Seed capsule production data was only complete for two undamaged plots and no further analysis could be performed on the 2000 data set. In 2001, mean percent pollinaria removal was not significantly different between the ultraviolet light treatment, 13.08 ± 2.43 %, and the control, 10.58 ± 3.36 %, ($t = 0.614$, df 4, $p = 0.573$). Mean percent pollinaria removal was significantly different between the two treatments, 7.75 ± 0.50 % in the ultraviolet light treatment and 6.21 ± 0.22 % in the control, ($t = 2.810$, df 6, $p = 0.031$) in 2002.

Seed capsule production in 2001 totalled 11 capsules and 226 capsules in 2002 (Table 3). In 2001, mean seed capsule production (Table 4) as a percentage of available flowers was not significantly different between the ultraviolet light treatment, 0.57 ± 0.57 %, and the control, 0.52 ± 0.27 % ($t = 0.08$, df 4, $p = 0.939$). However, in 2002 the difference in seed capsule production as a percentage of available flowers was significant ($t = 4.49$, df 6, $p = 0.004$), with mean percent seed capsule production in the ultraviolet light plots being nearly twice that of the control, 5.13 ± 0.42 % and 2.78 ± 0.32 % respectively (Table 4).

Mean seed capsule production, as a percentage of total plants, was not significantly different between the ultraviolet light and control treatments in 2001, 3.08 ± 3.08 % and 3.67 ± 1.67 % respectively ($t = -0.169$, df 4, $p = 0.874$) (Table 5). In 2002 seed capsule production as a percentage of all plants, the difference was significant between the treatments, 35.12 ± 1.74 % for the ultraviolet light plots and 21.76 ± 2.58 % for the control plots ($t = 4.613$, df 6, $p = 0.004$) (Table 5).

There was no correlation between pollinaria removal and seed capsule production by plants, r = -0.215, $p = 0.683$, or by flowers, r = -0.129, $p = 0.807$, in 2001. There was

Table 3. Plot summaries for measured variables of flowering western prairie fringed orchids sampled, 2000-2002.

Year	Location	Plot Pair	Plot[1]	No. Plants	No. Flowers	Inflorescence Size Mean ± SEM[2]	Min.	Max.	No. Pollinaria Available	No. Pollinaria Removed	No. Seed Capsules
2000	SW25-2-6E	1	UV-P1	53	431	8.13 ± 0.35	3	15	862	160	n/a[3]
	NW36-2-6E	2	UV-P2	67	455	6.79 ± 0.26	3	12	910	4	7
	SE2-3-6E	3	UV-P3	48	328	6.83 ± 0.54	1	18	656	151	37
	SW24-2-6E	1	CN-P1	87	578	6.64 ± 0.27	2	14	1156	280	n/a
	NE35-2-6E	2	CN-P2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	NE26-2-6E	3	CN-P3	98	584	5.96 ± 0.25	2	14	1168	35	n/a
Total/Mean[4]				353	2376	6.66 ± 0.14	1	18	4752	630	44
2001	SW24-2-6E	1	UV-P1	50	315	6.30 ± 0.49	1	16	630	108	0
	SW24-2-6E	2	UV-P5	65	352	5.42 ± 0.30	2	12	704	94	6
	NW36-2-6E	3	UV-P3	78	503	6.45 ± 0.29	3	12	1006	88	0
	NW24-2-6E	1	CN-P2	72	615	8.54 ± 0.37	3	16	1230	81	2
	NW24-2-6E	2	CN-P5	75	560	7.47 ± 0.39	2	18	1120	191	1
	NE35-2-6E	3	CN-P4	29	191	6.59 ± 0.52	2	13	382	31	2
Total/Mean				369	2536	6.87 ± 0.16	1	18	5072	593	11

Table 3. Continued.

Year	Location	Plot Pair	Plot[1]	No. Plants	No. Flowers	Inflorescence Size Mean ± SEM[2]	Min.	Max.	No. Pollinaria Available	Removed	No. Seed Capsules
2002	SW24-2-6E	1	UV-P1	87	532	6.11 ± 0.26	1	13	1064	71	28
	SW25-2-6E	2	UV-P2	91	706	7.76 ± 0.32	1	16	1412	123	32
	NW36-2-6E	3	UV-P3	151	1121	7.42 ± 0.22	1	18	2242	160	50
	SE2-3-6E	4	UV-P4	25	159	6.36 ± 0.42	2	11	318	27	10
	SE24-2-6E	1	CN-P1	150	1068	7.12 ± 0.25	2	20	2136	132	35
	NW24-2-6E	2	CN-P2	150	1200	8.00 ± 0.25	2	17	2400	141	33
	SE26-2-6E	3	CN-P3	91	631	6.93 ± 0.28	1	6	1262	75	12
	NE35-2-6E	4	CN-P4	106	810	7.64 ± 0.24	3	15	1620	111	26
Total/Mean				851	6227	7.32 ± 0.10	1	20	12454	840	226

[1] UV = Ultraviolet light treatment; CN = Control treatment

[2] SEM = standard error of the mean

[3] n/a = data not collected

[4] Mean values only for inflorescence size

Table 4. Comparison of seed capsule production for plot pairs as a percentage of the available flowers in 2001 and 2002.

Year	Plot Pair	Treatment	No. Flowers	No. Seed Capsules	% Produced
2001	1	Ultraviolet light	315	0	0.00
		Control	615	2	0.33
	2	Ultraviolet light	352	6	1.70
		Control	560	1	0.18
	3	Ultraviolet light	503	0	0.00
		Control	191	2	1.05
2002	1	Ultraviolet light	532	28	5.26
		Control	1068	35	3.27
	2	Ultraviolet light	706	32	4.53
		Control	1200	33	2.75
	3	Ultraviolet light	1121	50	4.46
		Control	631	12	1.90
	4	Ultraviolet light	159	10	6.28
		Control	810	26	3.21

Table 5. Comparison of seed capsule production for plot pairs as a percentage of total plants in 2001 and 2002.

Year	Plot Pair	Treatment	No. Plants	No. Plant with Seed Capsule(s)	% Produced
2001	1	Ultraviolet light	50	0	0.00
		Control	72	2	2.78
	2	Ultraviolet light	65	6	9.23
		Control	75	1	1.33
	3	Ultraviolet light	78	0	0.00
		Control	29	2	6.90
2002	1	Ultraviolet light	87	14	16.09
		Control	150	27	18.00
	2	Ultraviolet light	91	12	13.19
		Control	150	20	13.33
	3	Ultraviolet light	151	24	15.89
		Control	91	9	9.89
	4	Ultraviolet light	25	8	32.00
		Control	106	17	16.04

no correlation between pollinaria removal and seed capsule production in plants in 2002, $r = 0.532, p = 0.175$, however, there was a significant correlation within flowers, $r = 0.756, p = 0.030$.

Inflorescence size. There was no significant difference between mean percent seed capsule production by plant and by flowers for the two treatments for each of the plant size categories based on inflorescence numbers in either 2001 or 2002 (Table 6). When seed capsule production was compared within a treatment for the three size categories, a significant difference was observed for the ultraviolet light treatment, as significantly more seed capsules were recorded in 2002 versus the control (Table 7). No difference was observed in 2001 (Table 7). Post hoc tests indicated that orchids in the small and medium categories had significantly lower mean percent seed capsules per plant and per flower than large orchids (Table 7).

Herbivory. Seed capsule production derived from the number of flowers at end of season was slightly greater than seed capsule production by number of flowers at beginning of season, however, the loss of individual flowers during the flowering season did not significantly alter the mean seed capsule production within the treatments in either 2001 or 2002 (Table 8).

Table 6. Comparison of inflorescence class between treatments on mean seed capsule production (%) per plants and flowers for 2001 and 2002.

Year	Inflorescence Class	Treatment	n	% per Plants Mean ± SEM[1,2]	*t*	*df*	*p*	% per Flowers Mean ± SEM	*t*	*df*	*p*
2001	Small	Ultraviolet light	3	4.76 ± 4.76	1.000	4	0.374	1.80 ± 1.80	1.000	4	0.374
		Control	3	0.00 ± 0.00				0.00 ± 0.00			
	Medium	Ultraviolet light	3	2.72 ± 2.72	-0.862	4	0.437	0.46 ± 0.46	0.319	4	0.765
		Control	3	3.19 ± 0.73				0.48 ± 0.12			
	Large	Ultraviolet light	3	0.00 ± 0.00	-1.000	4	0.374	0.00 ± 0.00	-1.000	4	0.374
		Control	3	11.11 ± 11.11				0.90 ± 0.90			
2002	Small	Ultraviolet light	4	0.00 ± 0.00	-1.732	6	0.134	0.00 ± 0.00	-1.718	6	0.137
		Control	4	25.00 ± 14.43				9.17 ± 5.34			
	Medium	Ultraviolet light	4	21.78 ± 3.66	0.014	6	0.990	3.21 ± 0.58	-0.012	6	0.991
		Control	4	21.68 ± 7.04				3.23 ± 1.11			
	Large	Ultraviolet light	4	93.80 ± 22.78	0.716	6	0.501	7.80 ± 2.12	0.756	6	0.478
		Control	4	68.99 ± 26.10				5.55 ± 2.10			

[1] Mean and SEM are equal when only one replicate produces seed capsules

[2] SEM = standard error of the mean

Table 7. Comparison of inflorescence class within treatments on mean seed capsule production (%) as a portion of plants and flowers in 2001 and 2002.

		Inflorescence		% per Plants				% per Flowers			
Year	Treatment	Class[1]	n	Mean ± SEM[2,3,4]	F	*df*	*p*	Mean ± SEM	F	*df*	*p*
2001	Ultraviolet	Small	3	4.76 ± 4.76	0.520	2,6	0.624	1.80 ± 1.80	0.610	2,6	0.570
		Medium	3	2.72 ± 2.72				0.46 ± 0.46			
		Large	3	0.00 ± 0.00				0.00 ± 0.00			
	Control	Small	3	0.00 ± 0.00	1.180	2,6	0.368	0.00 ± 0.00	0.860	2,6	0.468
		Medium	3	3.19 ± 0.73				0.48 ± 0.12			
		Large	3	11.11 ± 11.11				0.90 ± 0.90			
2002	Ultraviolet	Small	4	0.00 ± 0.00a	13.584	2,9	0.002	0.00 ± 0.00a	9.508	2,9	0.006
		Medium	4	21.78 ± 3.66a				3.21 ± 0.58a			
		Large	4	93.80 ± 22.78b				7.80 ± 2.12b			
	Control	Small	4	25.00 ± 14.43	2.228	2,9	0.164	9.17 ± 5.34	0.789	2,9	0.483
		Medium	4	21.68 ± 7.04				3.23 ± 1.11			
		Large	4	68.99 ± 26.10				5.55 ± 2.10			

[1] Inflorescence Class: Small 1-3 flowers, Medium 4-10 flowers, Large >11 flowers.

[2] Means followed by the same letter in the same column, within the same year and treatment, are not significantly different, Fisher's LSD test ($p < 0.05$).

[3] Mean and SEM are equal when only one replicate produces seed capsules.

[4] SEM = standard error of the mean

Table 8. Herbivory and hail damage effect on seed capsule production in 2001 and 2002 by treatment.

			Mean seed capsule production (%) by flowers ± SEM[1]				
Year	Treatment	n	beginning of season[2]	end of season[3]	*t*	df	*p*
2001	Combined	6	0.54 ± 0.28	0.95 ± 0.42	-2.428	5	0.060
2002	Combined	6	3.96 ± 0.51	4.07 ± 0.52	-1.750	7	0.124
2001	Ultraviolet light	3	0.57 ± 0.57	0.83 ± 0.83	-1.000	2	0.423
	Control	3	0.52 ± 0.27	1.07 ± 0.41	-2.448	2	0.134
2002	Ultraviolet light	4	5.14 ± 0.42	5.30 ± 0.38	-1.393	3	0.258
	Control	4	2.78 ± 0.32	2.84 ± 0.32	-1.120	3	0.344

[1] SEM = standard error of the mean

[2] Date range: June 20 - July 6, 2001, July 4 - 12, 2002

[3] Date range: September 4 - 14, 2001, September 6 - 7, 2002

DISCUSSION

Size of Inflorescence. The mean number of flowers per plant varied during this study from 6.66 ± 0.14 in 2000 to 7.32 ± 0.09 in 2002. These values are similar to the range documented by Pleasants (1993), 7.0 – 9.4, but less than the 12.6 flowers per plant reported by Sheviak and Bowles (1986). Pleasants' (1993) study took place in Minnesota and North Dakota where as Sheviak and Bowles (1986) examined orchids from locations across the range of the western prairie fringed orchid in the United States that included relatively southern states such as Iowa, Nebraska and Kansas. The longer and warmer growing season in the southern part of the orchid's range may produce larger plants with more flowers.

Pollinaria removal. To be an effective pollinating agent, a hawkmoth must remove at least one of the pollinaria from an orchid flower. Increased feeding activity by hawk moths presumably should lead to an increased number of pollinaria removed. In 2001, the difference in the number of pollinaria removed between the two treatments was not significant, while in 2002 a significantly higher portion of pollinaria where removed in the ultraviolet light treatment versus the control. In 2001 and 2002, levels of pollinaria removal in both the ultraviolet and control treatments were lower than levels recorded under natural conditions in southeastern North Dakota (33 %) during a study that took place in 1989 (Pleasants and Moe 1993). In an examination of pollinaria removal during 1991 and 1992, Pleasants (1993) found that values ranged from 6.5 to 37 % at four sites in North Dakota and Minnesota.

Between 2001 and 2002 the number of pollinaria removed was higher in the first year (i.e. 13.08 and 10.58 %, ultraviolet and control respectively) than the second year

(i.e. 7.75 and 6.21 %, ultraviolet and control respectively). Pleasants (1993) found a similar difference between study years with overall site averages of 33% and 8% for 1991 and 1992, respectively. Hawkmoth populations fluctuate from year to year and the yearly difference in pollinaria removal may be a result of their local abundance (Westwood and Borkowsky 2004). Incidental observations during the current study, also found that pollinaria were attached to the ends of orchid's petals and other surrounding vegetation, particularly on the leaves of tall grasses such as big blue stem and Indian grass. This may have been due to rapid plant movement caused by wind. During windy periods, the inflorescence may contact stems and leaves of surrounding vegetation, especially grasses that exceeded 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. In 2001, the 3-July storm that produced hailstones was accompanied by strong winds that may have greatly enhanced orchid flower contact on adjacent vegetation. Extreme weather events (i.e. hail storms) did not occur during the 2002 flowering season when the ultraviolet light treatment significantly increased seed capsule production. In 2002, the increased seed capsule production in the ultraviolet light plots may have been due, in part, to removal of the cover sheet on the light sources to maximize the attractiveness of the plots although we did not test this effect. Future research should examine the intensity of light required to attract hawk moth pollinators into the plots.

Wind can also affect a pollinator's ability to travel between plants. Eisikowitch and Galil (1971) observed a correlation between wind speed and levels of pollination and seed set in an Israeli amaryllis, *Pancratium maritimum* L. The long tubular flower

requires the hawk moth pollinators to push its head into the flower and insert its proboscis into a narrow tube containing nectar, much like *P. praeclara*. Hawk moth flower visits were regular when wind speeds were below 2 m/sec, resulting in the highest levels of pollination and seed set (Eisikowitch and Galil 1971). Pollination did not occur when wind speeds were greater than 3 m/sec as the hawk moths did not travel between flowers, wind speeds between 2 and 3 m/sec reduced the flight activities of the hawk moth pollinators and resulted in lower levels of pollination and seed set (Eisikowitch and Galil 1971). Hawk moth visitations to the flowers of *Merremia palmeri* (S. Wats.) Hallier also end when winds are gusty or became moderately strong (Willmott and Búrquez 1996). Future research should examine the effects of wind on pollination success in *P. praeclara*.

Seed capsule production. As a measure of available flowers, seed capsule production was low in both treatments, less than 1 % in 2001 and less than 6 % in 2002 regardless of treatment. Pleasants (1993) reported levels ranging from 25 to 39 % in 1991 and 5.4 to 15 % in the following year for the western prairie fringed orchid at locations in North Dakota and Minnesota. A 1989 study from southeastern North Dakota found 30 % of the available western prairie fringed orchid flowers produced seed capsules (Pleasants and Moe 1993). Also in comparison to seed capsule production for other North American species, the results from the current study fall well below the 49.3 % average (range 13.6 to 79 %) for 11 other nectariferous orchids (Neiland and Wilcock 1998).

A correlation between pollinaria removal and seed capsule production for the western prairie fringed orchid was documented by Pleasants and Moe (1993). In the current study, there was a correlation between pollinaria removal and seed capsule

production by flowers only in the second season, which again may have been linked to greater light intensity. The group of orchids produced in the 2002 season was not exposed to a severe hail storm as the previous one had and this may contribute to the difference observed between years.

Inflorescence size. In this study the addition of ultraviolet light to increase the attractiveness of orchids to moths did not alter seed capsule production when comparing orchids within a particular inflorescence class. However, within the ultraviolet treatment plants classified as having a large inflorescence produced significantly more seed capsules by plants and by flowers than either small or medium sized inflorescences. This would suggest that under natural conditions pollinators were equally attracted to small, medium and large sized inflorescences but their visitation patterns within a patch may be altered by ultraviolet light. Under natural pollination conditions, Plesants and Moe (1993) found that seed capsule production is not correlated to inflorescence size; however, they did not use size categories as the current study does. There are few reported studies that test ultraviolet light as a means of attracting beneficial insects such as pollinators (Nabli *et al.* 1999). However, yellow fluorescent lamps have been tested and used as a means of repelling insects pests from vegetables, flowers, fruit trees and other economically important agricultural crops (Naba 2001, Tanaka 2001, Yano 2001, Yase 2001).

Herbivory. The removal of a few plants or flowers by herbivores did not affect seed capsule production in either 2001 or 2002. It is fortunate that the orchid population in Manitoba is one of the largest and that the removal of a few plants or individual flowers by herbivores is not likely to harm the overall reproductive output of this population.

However, in 2000, seed capsule counts could not be completed as many stems had been clipped near the base and reduced to pieces 10 cm or less in length. The inflorescence containing the seed capsules could not be located and was most likely consumed by small rodents. It is likely that the rodent population was substantially decreased during a series of management burns and wildfires that occurred during fall 2000 and spring 2001. Reduced rodent populations following burns are well documented in the literature (Kaufman *et al.* 1983, Vacanti and Geluso 1985, Kaufman *et al.* 1990). Plants examined during the seed capsule surveys in 2001 and 2002 did not display the same level of seed capsule clipping as 2000. When herbivory occurs at an extreme level, as observed in 2000, the sexual reproductive output of the orchid could be compromised.

CONCLUSION

The western prairie fringed orchid population in Manitoba has fewer flowers per plant than those in the southern parts of its range. The use of ultraviolet light as a method to increase the attractiveness of orchids to their nocturnal, sphinx moth pollinators was successful as measured by an increase in seed capsule development. Under natural conditions, size of inflorescence did not influence frequency of pollinaria removal or seed capsule production. In ultraviolet light plots, those plants with 11 or more flowers, had a significantly higher level of seed capsule production than plants with 10 or fewer flowers. Herbivory of individual flowers or plants did not affect seed capsule production in 2001 and 2002. It appears that the intensity of the ultraviolet light may affect the degree of attraction to orchid pollinators.

CHAPTER 4

NECTAR SUGAR CONCENTRATION AND RELATIVE QUANTITY DURING POLLINATOR FLIGHT PERIODS IN THE ENDANGERED WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*) IN MANITOBA.

ABSTRACT

The endangered western prairie fringed orchid, *Platanthera praeclara*, is found in remnant tall grass prairie in southeastern Manitoba. There exists no published data on nectar production in the western prairie fringed orchid or possible links between nectar quantity and sugar concentration to moth attraction and subsequent pollination success. This study was designed to measure nectar quantity and sugar concentration during the bloom period. Nectar sugar concentration decreased by approximately 6 % as the flowering period progressed over the season but did not change significantly over a 24-hour period. At night, the nectar spur became more elongated during which the nectar column height also increased. Within the inflorescence, lower positioned flowers had shorter nectar spurs and less nectar compared to higher positioned flowers. The size of the inflorescence was positively correlated to spur length and nectar column height but was not correlated to nectar sugar concentration.

INTRODUCTION

Western prairie fringed orchid. The endangered western prairie fringed orchid, *P. praeclara*, is found in small areas of remnant tall grass prairie in southeastern Manitoba. The Manitoba population is the only known location in Canada and fluctuates

widely from 2000 flowering stems to more than 20000 flowering stems annually (Borkowsky and Jones 1998). In the United States there are small isolated populations of the orchid (each consisting of only several hundred or less plants) in North Dakota, South Dakota, Minnesota, Nebraska and Iowa (Sheviak and Bowles 1986; Bray and Wilson 1992; U.S. Fish and Wildlife Service 1996).

Extensive agricultural activities throughout southern Manitoba and the central United States have radically changed the landscape including areas once characterized as tall grass prairie. In Manitoba, the extent of this once vast area of grasses, forbs, shrubs and accompanying wildlife has been reduced to a few small locations, with the majority of sites concentrated in the vicinity of Vita and Tolstoi, in the Rural Municipality of Stuartburn (Joyce and Morgan 1989). This Preserve was established through the cooperation of a number of partners including the Manitoba Naturalists Society, World Wildlife Fund, Wildlife Habitat Canada, Manitoba Conservation and Manitoba Habitat Heritage Corporation with the Nature Conservancy of Canada and Environment Canada joining the partnership shortly thereafter. In the United States less than 4% of the 60 million ha original tall grass prairie exists (Samson and Knopf 1994). The western prairie fringed orchid is confined to isolated patches within these last remnant tall grass prairie areas, and before being discovered in the Tolstoi area (now the Manitoba Tall Grass Prairie Preserve) it was not known to exist in Canada. The orchid has been placed on Canada's list of endangered plants and its endangered status is recognized on a worldwide basis (Collicutt 1993; Davis 1995). The government of Manitoba listed the species, as endangered under the Manitoba Endangered Species Act on April 19, 1996 (C. Hamel, pers. comm.).

The western prairie fringed orchid was first documented in Manitoba in 1987 (Catling and Brownell 1987). Previously collected specimens were identified as the prairie white fringed orchid (*Platanthera leucophaea*) (Johnson 1985). A distinction was made between the eastern and western plants of the prairie white fringed orchid when Sheviak and Bowles (1986) demonstrated that in addition to geographical displacement, the plants also possess different pollination mechanisms and floral characters. These differences are distinctive and suggest that hybridization is unlikely. The discovery of this perennial orchid and remnant parcels of tall grass prairie habitat lead to the formation of the Manitoba Tall Grass Prairie Preserve. The entire orchid population exists within an area of approximately 12 000 ha, including agricultural lands, such as pastures that may support orchids and cultivated cropland and upland prairie that do not). In Manitoba, the orchid is associated with sedge meadows that are dominated by various sedge species (*Carex* spp.) and rushes (*Juncus* spp.) along with prairie cord grass (*Spartina pectinata*), swamp birch (*Betula glandulosa*) and several species of willows (*Salix* spp.) (Looman and Best 1987, Moore and Fortney 1994). It is estimated that 63% of the flowering population in Manitoba occurs within the Preserve. The population found in Manitoba is the largest in North America (Davis 1994).

To date most research on the biology of the western prairie fringed orchid has occurred in the most southern parts of its range in the United States (Pleasants and Moe 1993; Sieg and King 1995; Hof *et al.* 1999, Sharma *et al.* 2003). Little is known about the biology of the orchid specific to the Manitoba population. In Manitoba, several organizations including The Manitoba Museum, local universities and other non-profit groups have investigated certain life cycle aspects of the orchid, but the results of most of

these studies have not been published in the general literature to date. However, it has become evident that the population of western prairie fringed orchids in Manitoba may have low seed capsule production compared to populations in the United States (Sheviak and Bowles 1986, Westwood and Borkowsky 2004). The role that varied seed capsule production between northern and southern populations may play in maintaining healthy populations in the few remaining areas where the orchid is found, is unknown. Although higher levels of seed production may ensure adequate reproduction over the long term and help maintain core orchid numbers in the southern populations, the significance of lower seed capsule production in the northern population is unknown.

Flowering stems of the western prairie fringed orchid will grow 38-85 cm in height (Sheviak and Bowles 1986). The inflorescence of creamy white flowers makes this species highly visible during its flowering period of mid-June to mid-July. The number of flowers contained within the inflorescence is highly variable. Sheviak and Bowles (1986) reported a mean of 12.6 flowers per plant from 56 specimens collected from Minnesota, Iowa, North Dakota South Dakota, Nebraska and Kansas. Pleasants (1993) recorded mean values ranging from 7.0-9.4 flowers per plant from four sites in North Dakota and Minnesota during a two-year study. Flowers open from the base of the inflorescence to the top, a few each day (Figure 6). Bowles (1983) reported that an individual flower would persist for up to 10 days, however at the Sheyenne National Grassland in southeastern North Dakota, Pleasants and Moe (1993) reported that an individual flower would last for about seven days.

The flowers give off a sweet fragrance that becomes more intense in the late evening. The most striking visual characteristics of the flower are the large, deeply

fringed, tri-lobed lower lip and long, slender spur. Measurements indicate that the lip may be 17-32 mm long and 20-39 mm wide, while the spur may be 36-55 mm long with a maximum diameter of 2.7 ± 0.5 mm (Sheviak and Bowles 1986). The pollinaria consists of pollinia (pollen), a caudicle and viscidium (Nilsson 1992). In the western prairie fringed orchid the minute grains of pollen are arranged into subunits called massulae (Pleasants and Moe 1993). These subunits form a bi-lobed mass that is attached to the column, which is then secured to the viscidium. The entire unit is sheathed, with the exception of the viscidium, which is exposed and adapted to cement itself to the pollinator (Bowles 1983). Each flower has one pollinium located on either side of its stigmatic surface. This allows for a 6-7 mm separation of the viscidia (Sheviak and Bowles 1986). The opening to the nectar spur is located immediately below the stigmatic surface.

The floral characteristics indicate a moth pollination method (Faegri and van der Pijl 1979). While the small opening to the nectar spur restricts the position of the moth, it increases the likelihood that one or both viscidia will come into contact with either the proboscis or eyes of the moth (Sheviak and Bowles 1986). The length of the nectar spur and position of the viscidia further reduces the list of potential pollinators to those belonging to the Sphingidae or hawkmoth family. Members of this family possess long proboscis that enable them to reach the nectar within the spur. Few, if any, observations of pollination of the orchid by swift-flying hawkmoths have been made in the field (Pleasant and Moe 1993; Sheviak and Bowles 1986; Bowles 1983). The proboscis length of various species of Sphingidae fall within the range of the nectar spur of *P. praeclara* and Sheviak and Bowles (1986) proposed the following species to be potential

pollinators: *Eumorpha achemon*, *Hyles lineata*, Sphinx *drupiferarum* and *Sphinx kalmiae*.

In the event that a flower is visited and pollinated, a seed capsule will develop. While relatively low to moderate levels of seed capsule development have been documented for fringed orchids (Bowles 1983; Pleasants and Moe 1993), a single capsule may contain thousands of dust like seeds (Bowles 1983). The minute size and buoyancy of the seed, suggests that it is suited for either wind (Bowles 1983) or water dispersal (Rasmussen 1995 in Hof *et. al.* 1999). The mechanism for seed dispersal is not fully understood. Timing of dispersal, such as late fall, winter, or spring, is unknown. The distance that seeds are carried will depend on a variety of environmental factors such as wind speed, natural flow of water as well as man-made diversions, flooding and periods of drought.

Plant nectar. Nectar serves an important function in plant reproduction by attracting and rewarding the pollinator for their visits to flowers. Nectar is the most common reward, with two-thirds of the orchid family using nectar as the main pollinator reward (van der Pijl and Dodson 1966), while the remaining third resort to nectar deceit, alternative rewards (e.g. pollen or floral fragrance), mimicry of prey species, or offer a resting place (Neiland and Wilcock 1998). Plant-pollinator relationships are determined by several nectar characteristics such as the accessibility of the nectar relative to floral morphology, sugar concentration, volume, viscosity and chemical composition (Proctor *et al.* 1996, Perret *et al.* 2001, Galetto and Bernardello 2004). Galletto *et al.* (1997) note that sugar composition has been determined for approximately 110 species of orchids or a small fraction of the estimated 19 500 to 25 000 species.

Nectar is a solution consisting primarily of sugar and water (Fahn 1979). There is considerable variation in the composition of nectar across plant species. Sugars dominate the solutes that can include sucrose, glucose, fructose, maltose, melibiose and raffinose in varying amounts (Shuel 1955, Perret *et al.* 2001, Galetto and Bernardello 2004). Nectars that are sucrose dominated are present in plants pollinated by hummingbirds and Lepidoptera, while glucose and fructose dominated nectars are found in bee pollinated species (Southwick 1990, Perret *et al.* 2001, Galetto and Bernardello 2004). Other nectar constituents include minerals, enzymes, essential oils, and volatile organic substances, which can give the nectar a characteristic fragrance or taste (Shuel 1955, Southwick 1990).

Nectar is an important energy source for insects. Sugar constituents and their ratios as well as the volume of nectar will determine the amount of energy available to the nectar-seeking insects. Disaccharides such as sucrose contain more energy than a monosacharide such as glucose (Faegri and van der Pijl 1979). Sugar concentration varies between 15-75% of the nectar (%, wt/ total wt) (Faegri and van der Pijl 1979, Galetto and Bernardello 2004, Guerenstein *et al.* 2004, Tian *et al.* 2004). While providing large amounts of energy, high sugar concentrations can trigger negative responses by reducing water uptake by the pollinator (Faegri and van der Pijl 1979) or increasing the viscosity of the nectar, thus making it more difficult to extract from the flower (Heyneman 1983).

The amount of nectar and energy produced by a flower is related to the characteristic rate of energy expenditure of the pollinator (Heinrich and Raven 1972). Flowers pollinated by sphinx moths need to provide an adequate amount of energy

(Heinrich and Raven 1972), in the form of nectar, to cover the energy cost of feeding from the flower and the energy cost of flight to the next flower. Sphinx moths are strong flyers with a rapid wing beats and are capable of hovering in front of flowers without landing platforms while extending their proboscis into the nectar spur (Nilsson *et al.* 1985, Borror *et al.* 1989). Nectar spur length has been correlated to proboscis length of the pollinators (Nilsson 1992, Nilsson 1998, Nilsson *et al.* 1992, Maad 2000) and examination of nectar volumes and sugar concentrations are common in pollination research (Perret *et al.* 2001, Galetto and Bernardello 2004, Tian *et al.* 2004). There is no published data for nectar production or sugar content for the western prairie fringed orchid. Measurement of these variables will give a better understanding of the orchid pollination syndrome and show whether the rewards offered by the flower match the energetic and nutrient requirements of the pollinator.

The objective of the study was to determine how nectar sugar concentration and quantity of nectar in *P. praeclara* varied over the flowering season. Orchid nectar spur length, nectar height and nectar sugar concentration were measured over the flowering season, over several 24-hour periods, among flowers on the same plant and among small, medium and large plants to determine if these variables may vary during the overlap period of moth flight and orchid flowering.

METHODS

Study area. The Manitoba Tall Grass Prairie Preserve is located within the Rural Municipality of Stuartburn in southeastern Manitoba near the Canada-United States border (49° 08' N, 96° 40' W) (Figure 10). The lands purchased for the Preserve

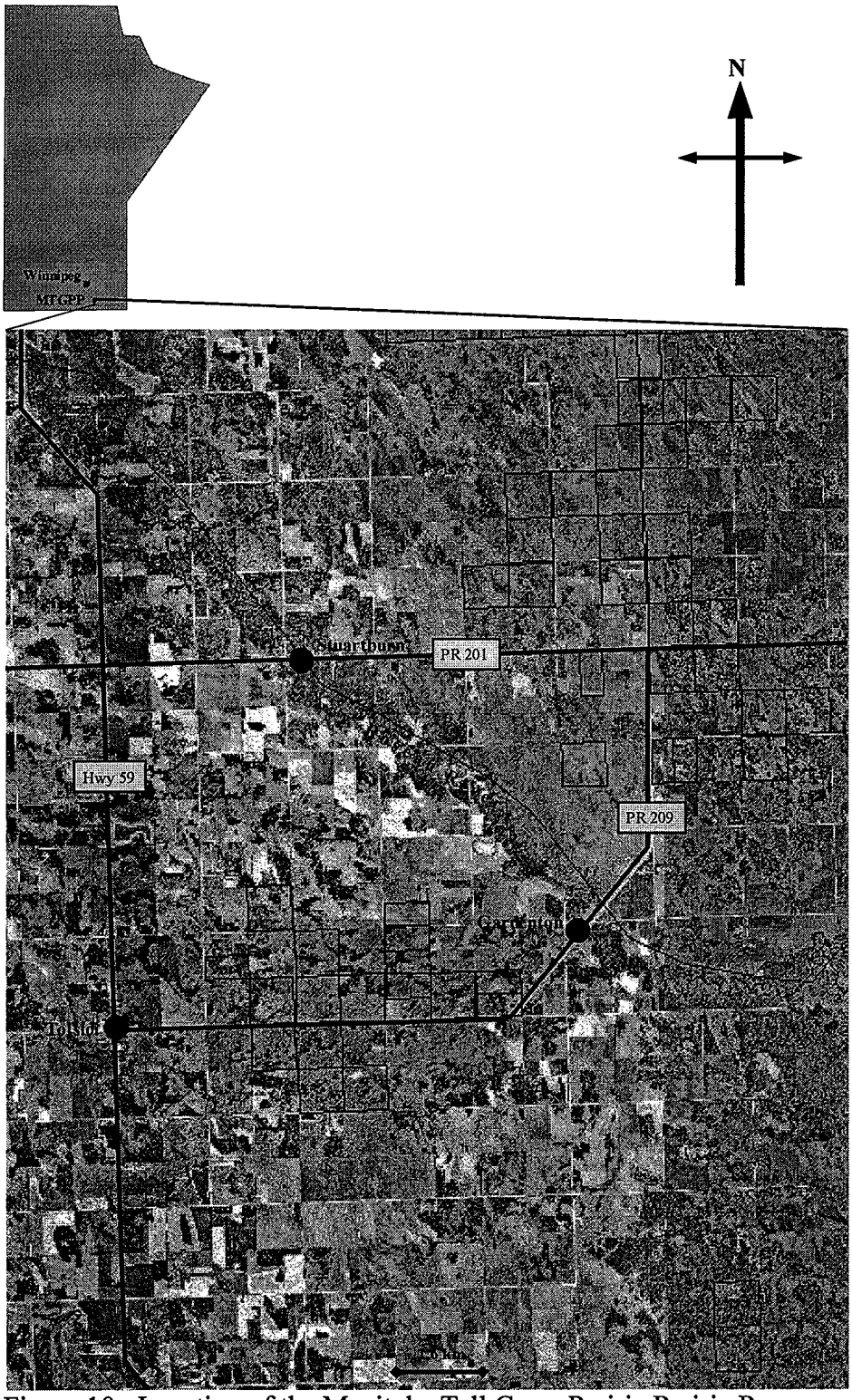

Figure 10. Location of the Manitoba Tall Grass Prairie Prairie Preserve in southeastern Manitoba. Property titles held by Preserve partners outlined in black.

represented the largest contiguous tracts of remnant tall grass prairie in Manitoba following a systematic search for tall grass prairie by the Manitoba Naturalists Society in the 1980s (Joyce and Morgan 1989). The Preserve and surrounding area represents the only known location in Canada in which *P. praeclara* naturally occurs, with approximately 63 % of the population occurring within the Preserve. The remaining plants are on private lands or in road allowances adjacent to the Preserve (Borkowsky and Jones 1998).

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 and Fortney 1994). The soil is a grey-wooded podzol, having a sandy-loam to clay-loam texture with frequent rock outcrops (Moore and Fortney 1994). The shallow slope of the landscape (1-3%), poor drainage and high water table (within 3m of the surface) generally inhibit agricultural productivity and potential within the Preserve (Moore and Fortney 1994).

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*), interspersed with oak (*Quercus macrocarpa*) and shrubs including saskatoon (*Amelanchier alnifolia*), chokecherry (*Prunus virginiana*) and hazelnut (*Corulus* spp.). The herbaceous layer is dominated by poison-ivy (*Rhus radicans*), meadow rues (*Thalictrum* spp.), goldenrods (*Solidago* spp.), golden Alexander (*Zizia aurea*) and various gramnoids. The upland prairie is dominated by big blue stem (*Andropogon gerardi*) and Indian grass (*Sorghastrum nutans*) and forbs such as purple prairie clover

(*Petalostemum purpureum*), wild strawberry (*Fragaria virginiana*), goldenrod (*Solidago* spp.) and sunflower (*Helianthus* spp.). Shrubs such as shrubby potentilla (*Potentilla fruiticosa*) and rose (*Rosa* spp.) occur in the upland prairie. The sedge meadow is dominated by various sedge species (*Carex* spp.) and rushes (*Juncus* spp.) along with prairie cord grass (*Spartina pectinata*), swamp birch (*Betula glandulosa*) and several species of willows (*Salix* spp.) (Looman and Best 1987, Moore and Fortney 1994).

Nectar sugar concentration and quantity. The quantity and sugar concentration of the nectar of *P. praeclara* was examined over the flowering season, 8-19 July 2002, by examining 1506 flowers from 172 plants. Twelve groups of orchids, containing between 4-34 individual plants, were randomly chosen from Preserve properties and adjacent roadsides at least 100m from plots utilized in the pollination study. All groups were located in the northern portion of the Preserve (Figure 11).

Each plant was marked with a piece of flagging tape, uniquely numbered, tied loosely to its base. The specific location of each plant was recorded in longitudinal and latitudinal degrees with a Garmin 12XL GPS unit. The number of flowers per plant was determined by carefully examining the inflorescence, identifying the flowers sequentially, beginning from the lowest then progressing to the uppermost one. Flower condition was recorded for each flower within the inflorescence on a scale of 1 to 5 as follows:

1 – flower bud, tightly closed petals, flower center not visible;

2 – partially opened, flower center visible with fringes of petals remaining curled; obscuring access to nectar spur and stigma;

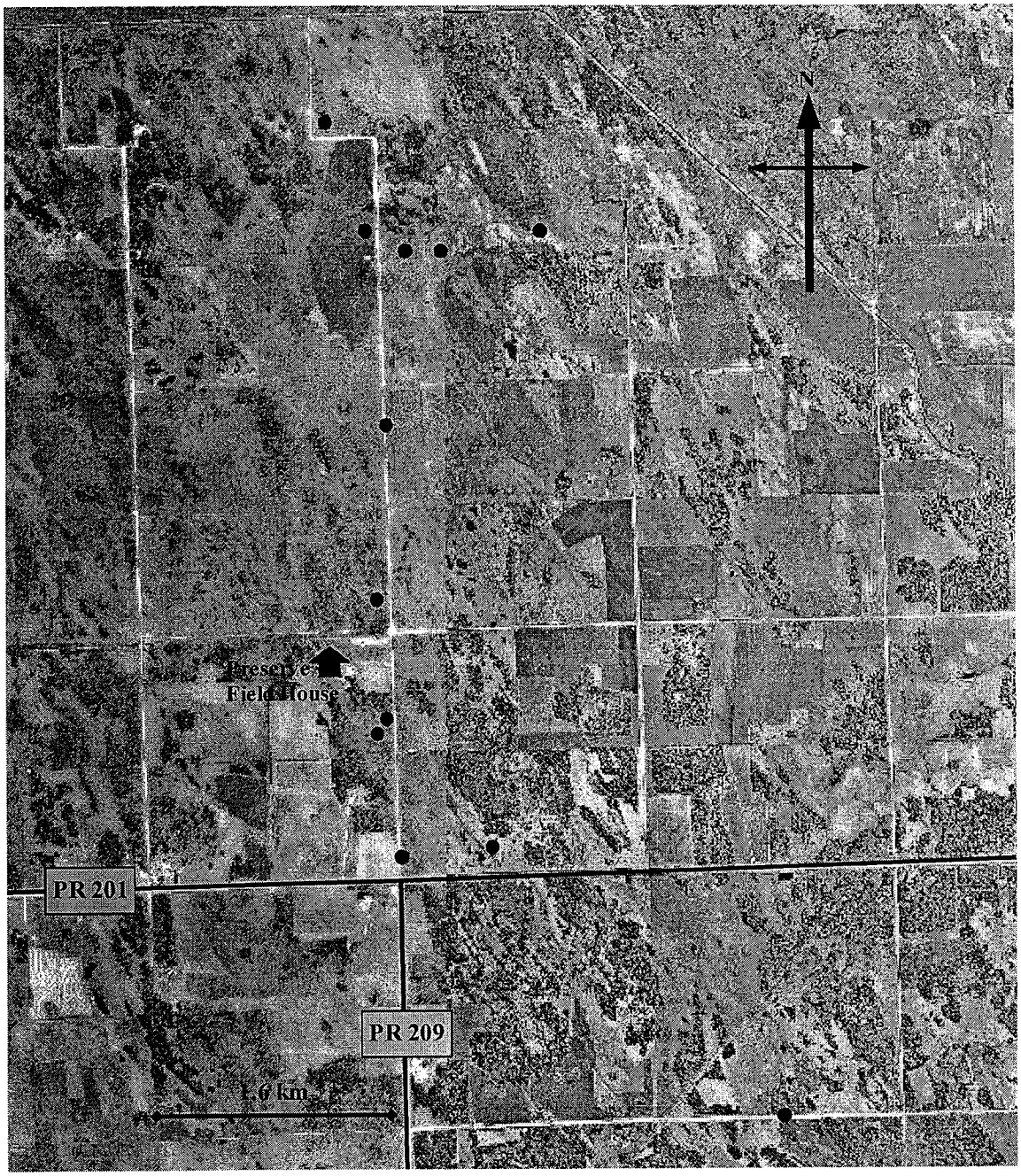

Figure 11. Location of spur and nectar sampling sites in the northern block of the Manitoba Tall Grass Prairie Preserve in 2002.

3 – full flower;

4 – wilting, petals beginning to turn brown along edges;

5 – wilted, petals entirely brown.

Flowers were examined for the presence and removal of the pollinaria.

The quantity of nectar was determined by first measuring the overall length of the nectar spur from its base to tip in millimeters with a ruler, then measuring the height of the nectar from the tip of the spur to the bottom of the meniscus, also in millimeters, providing a nectar column measurement. The estimated proboscis length reflects the difference between spur length and nectar column height. The opaque quality of nectar spur tissue easily facilitated the recording of the level of the nectar in the spur.

Extraction of the nectar from the nectar spur was completed through trial and error using several techniques. Initially, a microcapillary tube was to be inserted into the nectar spur to withdraw the nectar. This proved impracticable, as the tube did not fit into the spur opening. A second method tested was to cut a small slit into the spur with a scalpel and withdraw the nectar with a microcapillary tube. However, too much damage was inflicted on the spur while forming the slit and the nectar could not be successfully extracted. Finally, nectar was removed by inserting an insulin syringe (Becton-Dickinson, Micro-fine IV, 28 guage, 12.7mm needle) into the side of the nectar spur immediately above the level of the nectar. The nectar was removed and the nectar from three flowers was pooled for each measurement of sugar concentration. The nectar sample was placed onto a hand-held refractometer (Manufacturer: W. S. R. Tokyo) with a Brix scale 0-50% to determine the percentage of sugar in the nectar. The nectar spur was considered the sampling unit in this experiment.

Statistical Analysis. Prior to analysis, all experimental variables were tested for departure from the normal distribution (Zar 1996). Within the data set for spur length (n = 1013), estimated proboscis length needed by a moth to reach the nectar (n = 1001) and sugar concentration (n = 287), 4, 6 and 8 outliers were removed respectively, to improve the distribution of the data. Nectar height (n = 1013) was transformed using the log 10 (nectar height + 1) to normalize the distribution of the data. Eight outliers were removed from the nectar height data. Estimated proboscis length was calculated by subtracting nectar height from spur length.

An analysis of variance (ANOVA) was used to determine if there was a difference in spur length, estimated proboscis length, nectar height and sugar concentration by sampling day (α=0.05). Pearson correlations were also used to examine the relationship of the four variables with sampling day over the flowering period. An ANOVA was used to determine if there was a difference in spur length, estimated proboscis length, nectar height and sugar concentration over a 24-hour period, using six 4-hour periods (i.e. 0001-0400, 0401-0800, 0801-1200, 1201-1600, 1601-2000, 2001-0000 hours), and for light levels (i.e. none, dawn/dusk, full) (α=0.05). When an ANOVA result was significant, a Fishers Least Significant Difference (LSD) test was used to determine differences between means based on its consistancy in providing a reasonable trade off between the Type I and Type II error rate (Saville 1990, Dytham 2003). Due to the large number of comparisons Bonferroni correction was used to lower the level of significance required for a significant difference to be detected within the test (Zar 1996). A paired t-test was used to compare nectar sugar concentration between different flower positions within a plant. There were 26 plants for which a comparison was made

between lower and middle positioned flowers, 60 plants with nectar sugar concentration values from lower and upper positioned flowers and 29 plants in which a comparison was made between middle and upper positioned flowers (α=0.05). Pearson correlation was used to examine the relationship of nectar spur length, estimated proboscis length, nectar height and sugar concentration with size of inflorescence (α=0.05).

All statistical analyses were completed with SPSS v. 11.0.1 (SPSS Inc. 2001).

RESULTS

Over the sampling period, the mean spur length (± SEM) was 45.3 ± 0.1 mm, nectar column height was 12.4 ± 0.2 mm and estimated proboscis length needed by a moth to reach the nectar was 33.0 ± 0.2 mm (Table 9). The mean sugar concentration was 23.9 ± 0.2 % which included a wide range of values from a low of 13 % to high of 34 % (Table 9). The mean flower condition was 3.13 ± 0.01 (n = 1013), indicating the individual flowers sampled for this study were in peak condition.

Seasonal flowering period. There was a significant difference in mean nectar spur length per flower over the nine-day sampling period ($F_{8,1000} = 12.290, p < 0.001$) (Table 10) and it was correlated with sampling day, whereby spur length generally increased during the sampling period ($r = 0.195, p < 0.001$). There was a significant increase in nectar column height during the sampling period ($F_{8,996} = 35.303, p < 0.001$) (Table 10) and there was a strong positive correlation with increase in sampling day ($r = 0.427, p < 0.001$). Estimated proboscis length required to reach the nectar and sugar concentration differed over the sampling period and decreased with sampling day (Table 10) ($r = -0.237, p < 0.001$ and $r = -0.253, p < 0.001$, respectively).

Table 9. Mean spur length, nectar height, estimated proboscis length and sugar concentration for western prairie fringed orchids in 2002.

Variable	n[1]	Mean ± SEM[2]	Minimum	Maximum
Spur length (mm)	1009	45.3 ± 0.1	31	56
Nectar height (mm)	1005	12.4 ± 0.2	2	42
Estimated proboscis length (mm)	995	33.0 ± 0.2	12	46
Sugar concentration (%)	279	23.9 ± 0.2	13	34

[1] n = number of flowers

[2] SEM = standard error of the mean

Table 10. Mean spur length, nectar height, estimated proboscis length and sugar concentration by sampling day.

Sampling Day	Spur Length (mm)		Nectar Height (mm)		Estimated Proboscis Length (mm)		Sugar concentration (%)	
	n	mean ± SEM[1,2]	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM
8-Jul	77	42.2 ± 0.3a	77	8.0 ± 0.4a	77	34.2 ± 0.5b	18	24.5 ± 0.5b,c
9-Jul	91	44.5 ± 0.4b,c	89	9.1 ± 0.5a,b	88	35.2 ± 0.5b	25	24.7 ± 0.6b,c
11-Jul	97	45.8 ± 0.4c,d	97	10.5 ± 0.4b,c,d	96	35.1 ± 0.6b	29	25.9 ± 0.6b,c
12-Jul	42	44.8 ± 0.6b,c	43	11.2 ± 0.6c,d	42	33.4 ± 0.7b	12	26.0 ± 0.7c
15-Jul	97	44.6 ± 0.4b,c	96	9.9 ± 0.4b,c	96	34.7 ± 0.5b	24	24.2 ± 0.8b,c
16-Jul	194	45.0 ± 0.3b,c,d	195	12.5 ± 0.4d,e	193	32.5 ± 0.4b	53	24.3 ± 0.6b,c
17-Jul	271	46.4 ± 0.2c,d	271	13.9 ± 0.3e,f	269	32.6 ± 0.3b	81	23.4 ± 0.4a,b,c
18-Jul	112	46.8 ± 0.4d	112	17.1 ± 0.6f	111	29.6 ± 0.6a	31	21.7 ± 0.7a,b
19-Jul	28	43.3 ± 0.7a,b	25	16.5 ± 1.9f	23	28.0 ± 1.5a	6	20.1 ± 1.5a
Total/Mean	1009	45.3 ± 0.1	1005	12.4 ± 0.2	995	33.0 ± 0.2	279	23.9 ± 0.2
df		8, 1000		8, 996		8, 986		8, 270
F		12.290		35.303		13.062		4.674
p		< 0.001		< 0.001		< 0.001		< 0.001

[1] SEM = standard error of the mean

[2] Means within each column followed by the same letter are not significantly different (Fisher's LSD test, Bonferroni correction $p > 0.006$.)

24-hour four-hour period. Spur length, nectar column height and estimated proboscis length values were significantly different over the 4-hour time periods while sugar concentration did not differ (Table 11). Comparing the means for the 4-hour intervals, spur length varied by 1.8 mm, nectar column height varied by 4.6 mm and estimated proboscis length varied by 3.2 mm, while sugar concentration varied by 1.3 %. Maximum means for spur length, 46.0 ± 0.5 mm, and nectar column height, 14.5 ± 0.9 mm, were measured during the 0401-0800 hour period. Nectar column height was lowest during 1201-1600 hours and 2001-0000 hours at 10.7 ± 0.3 and 9.9 ± 0.7 mm respectively. The nectar column height was highest during the 0401-0800 hour period, the same period during which spur length was greatest. Estimated proboscis length was greatest during the 2001-0000 hour interval, the same period when nectar column height was at its lowest.

Light levels. Spur length, nectar column height and estimated proboscis length were significantly different among light levels (Table 12). Spur length values were similar during no light and dawn/dusk periods and greater during full light periods (Table 12). Nectar column height and estimated proboscis height were similar during dawn/dusk and full light periods and less than the no light periods (Table 12). There was no significant difference in sugar concentration among the three light levels (Table 12).

Flower position. Of the four variables measured, spur length, nectar column height and sugar concentration were significantly different among flower positions within the inflorescence for different plants over the flowering season (Table 13). Lower positioned flowers had shorter nectar spurs and less nectar compared to flowers at other positions, but had higher sugar concentrations than flowers located in the upper region of the

Table 11. Mean spur length, nectar height, estimated proboscis length and sugar concentration by 4-hour sampling periods.

Sampling Period	Spur Length (mm) n	Spur Length (mm) mean ± SEM[1,2]	Nectar Height (mm) n	Nectar Height (mm) mean ± SEM	Estimated Proboscis Length (mm) n	Estimated Proboscis Length (mm) mean ± SEM	Sugar concentration (%) n	Sugar concentration (%) mean ± SEM
0001-0400	127	45.9 ± 0.4b	128	14.0 ± 0.5b	127	32.0 ± 0.5a	38	24.6 ± 0.4
0401-0800	65	46.0 ± 0.5b	65	14.5 ± 0.9b	63	32.3 ± 0.8a	20	23.4 ± 0.8
0801-1200	208	45.4 ± 0.3a,b	207	13.2 ± 0.4b	206	32.2 ± 0.4a	59	23.6 ± 0.5
1201-1600	282	44.2 ± 0.2a	278	10.7 ± 0.3a	274	33.5 ± 0.3a,b	71	24.7 ± 0.4
1601-2000	268	45.9 ± 0.3b	268	12.7 ± 0.3b	266	33.2 ± 0.4a,b	77	23.3 ± 0.5
2001-0000	59	45.1 ± 0.4a,b	59	9.9 ± 0.7a	59	35.2 ± 0.6b	14	24.1 ± 0.9
Total/Mean	1009	45.3 ± 0.1	1005	12.4 ± 0.2	995	33.0 ± 0.2	279	23.9 ± 0.2
df		5, 1003		5, 999		5, 989		5, 273
F		5.977		13.660		4.080		1.450
p		< 0.001		< 0.001		< 0.001		0.207

[1] SEM = standard error of the mean

[2] Means within each column followed by the same letter are not significantly different (Fisher's LSD test, Bonferroni correction $p > 0.008$)

Table 12. Mean spur length, nectar height, estimated proboscis length and sugar concentration by light levels over the flowering season.

Light Levels	Spur Length (mm) n	Spur Length (mm) mean ± SEM[1,2]	Nectar Height (mm) n	Nectar Height (mm) mean ± SEM	Estimated Proboscis Length (mm) n	Estimated Proboscis Length (mm) mean ± SEM	Sugar concentration (%) n	Sugar concentration (%) mean ± SEM
None	192	45.9 ± 0.3b	193	14.2 0.4b	190	32.1 ± 0.4a	58	24.2 ± 0.4
Dawn/Dusk	126	46.2 ± 0.3b	126	11.4 0.5a	126	34.8 ± 0.5b	33	24.0 ± 0.7
Full	691	44.9 ± 0.2a	686	12.0 0.2a	679	32.9 ± 0.2a	188	23.9 ± 0.3
Total/Mean	1009	45.3 ± 0.1	1005	12.4 0.2	995	33.0 ± 0.2	279	23.9 ± 0.2
df		2, 1006		2, 1002		2, 992		2, 276
F		7.950		12.595		8.794		0.158
p		< 0.001		< 0.001		< 0.001		0.854

[1] SEM = standard error of the mean

[2] Means within each column followed by the same letter are not significantly different (Fisher's LSD test, $p > 0.05$)

Table 13. Mean spur length, nectar height, estimated proboscis length and sugar concentration by flower position for different plants over the flowering season.

Flower Position	Spur Length (mm)		Nectar Height (mm)		Estimated Proboscis Length (mm)		Sugar concentration (%)	
	n	mean ± SEM[1,2]	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM
Lower	369	44.4 ± 0.2a	369	11.0 ± 0.3a	366	33.3 ± 0.30	111	24.6 ± 0.35b
Middle	272	45.5 ± 0.2b	269	12.6 ± 0.3b	268	33.0 ± 0.30	65	24.0 ± 0.45a,b
Upper	368	46.0 ± 0.2b	367	13.6 ± 0.3c	361	32.6 ± 0.31	103	23.2 ± 0.35a
Total/Mean	1009	45.3 ± 0.1	1005	12.4 ± 0.2	995	33.0 ± 0.2	279	23.9 ± 0.2
df		2, 1006		2, 1002		2, 992		2, 276
F		14.956		24.514		1.576		4.439
p		< 0.001		< 0.001		0.207		0.013

[1] SEM = standard error of the mean

[2] Means within each column followed by the same letter are not significantly different (Fisher's LSD test, $p > 0.05$)

inflorescence. Estimated proboscis length did not differ among the lower, middle and upper positions (Table 13).

Sugar concentration was compared among different flower positions within an inflorescence of a specific plant (Table 14). A significant difference was observed in the sugar concentration between the lowest positioned, 24.8 ± 0.5 %, and uppermost flowers, 23.8 ± 0.3 % ($t = 2.043$, df = 59, $p = 0.046$); however, there was no significant difference in the sugar concentration between the lower and middle positioned flowers and the middle and upper positioned flowers.

Size of inflorescence. Pearson correlation coefficients were calculated for spur length, nectar column height, estimated proboscis length and sugar concentration in relation to the size of the inflorescence. There was a slight increase in spur length as the size of inflorescence increased, r = 0.092, $p = 0.003$, n = 1009, and a similar increase in nectar column height with larger inflorescences, r = 0.077, $p = 0.015$, n = 1005. There was no correlation between either estimated proboscis length and size of inflorescence, r = -0.002, $p = 0.950$, n = 995, or sugar concentration and size of inflorescence, r = -0.002, $p = 0.969$, n = 279.

DISCUSSION

Spur length. *Platanthera praeclara* mean spur length in this study was 45.3 ± 0.1 mm, which is similar to that observed in herbarium specimens (mean = 45.7 mm, standard deviation = 5.9 mm) from a wide range of sites across its range in the United States by Sheviak and Bowles (1986). Among the 15 species of sphinx moths collected in the vicinity of the Manitoba Tall Grass Prairie Preserve, none had a mean proboscis length

Table 14. Sugar concentration compared at three different flower positions within an orchid plant.

		Sugar concentration (%)			
Flower Position	n	mean ± SEM[1]	*t*	df	*p*
Lower	26	25.1 ± 0.6	0.617	25	0.543
Middle		24.7 ± 0.5			
Lower	60	24.8 ± 0.5	2.043	59	0.046
Upper		23.8 ± 0.3			
Middle	29	24.1 ± 0.6	0.060	28	0.953
Upper		24.1 ± 0.5			

[1] SEM = standard error of the mean

greater than 40.3 ± 0.5 mm (Westwood and Borkowsky 2004). In addition to the two species identified as pollinators, Westwood and Borkowsky (2004) found only two other species, *Sphinx chersis* and *Sphinx kalmiae* had mean proboscis lengths, 40.3 ± 0.5 mm and 33.6 ± 1.3 mm respectively, longer than the minimum spur length, 31 mm, recorded in the present study. The positive relationship between spur length and pollinator proboscis length, with the former being greater than the latter, has been documented in other orchid-sphinx moth pairs (Nilsson *et al.* 1985, Johnson 1995, Johnson and Liltved 1997, Wasserthal 1997). Orchid-sphinx moth pairs have co-evolved to maintain a close relationship such that the orchid maintains a reliable pollinator and the sphinx moth a reliable food source.

Nectar column height. Over the flowering season, nectar column height increased from less than 10 mm during the first two days to more than 13 mm on the last two days of flowering. This was expected as most flowers produce nectar during the bloom period and flowers not visited by pollinators continue to store nectar in the spur. A larger quantity of nectar was available to pollinators when there was no sun light compared to the dawn/dusk and full sun periods. The differences observed in nectar column height over the 4-hour intervals as well as light levels, may be comparable to other plants. Groman and Pelmyr (1999) found greater nectar production in the evening compared to the late morning and afternoon in a non-orchid, *Manfreda virginica* L. Luyt and Johnson (2001) observed that nectar height in *Mystacidium venosum* increased during the day and reached a peak in the evening hours, coinciding with hawkmoth activity. When the 24-hour cycle was evenly divided into 4-hour intervals, nectar column heights were at the lowest for 1210-1600 hours and 2001-0000 hours, although the choice of hourly intervals

was somewhat arbitrary. The decrease in nectar quantity during the early afternoon period (1210-1600 hours) was significant and may be attributed to evaporation of water from the nectar as suggested by Shuel and Pederson (1952) and Shuel (1955). During the same 4-hour interval, sugar concentration rose to 24.7%, an increase of 1.1 % from the previous interval, indicating that evaporation of water was likely. The second interval, 2001-0000 hours, for which a decrease in nectar column height was observed may be the result of nectar feeding by sphinx moths as most species are crepuscular or nocturnal, although the low pollination success recorded in 2001 and 2002 from Part I of this study does not support extensive hawk moth activity.

Estimated proboscis length. The estimated proboscis length reflects the difference between spur length and nectar column height. Moths with a proboscis measuring a minimum of 33 mm in length would be able to reach nectar stored in the spur in the earlier portion of the flowering period; however, over time flowers continue to produce nectar which may cause the high levels toward the end of the flowering season. At the end of the flowering period, moths with a short proboscis, approximately 28 mm or more in length, would be able to extract nectar from the spur. Based on measurements of sphinx moths collected in Manitoba, only two more species, *Sphinx chersis* and *Sphinx kalmiae* may be potential pollinators in addition to *Hyles gallii* and *Sphinx drupiferarum* as they have mean proboscis' lengths that would enable them to reach the nectar in *P. praeclara*. The collection dates listed by Westwood and Borkowsky (2004) suggest that *Sphinx chersis* would likely to be encountered only during the early portion of the orchid bloom period while *Sphinx kalmiae* may be on the wing throughout the flowering period. Additional study of the flight period and distribution of these species in relation

to the orchid bloom period is needed to confirm if either species is in fact a pollinator of the western prairie fringed orchid in Manitoba.

Nectar sugar concentration. Mean nectar sugar concentration was 23.9 % and never exceeded 35 % for any plant. In other orchids, pollinated by sphinx moths, mean sugar concentrations are variable: 16 % for *Mystacidium venosum* (Luyt and Johnson 2001), 17.3-19.5 % for *Cynorkis uniflora* (Nilsson *et al.* 1992), 26.3-28.3 % for *Bonatea speciosa* (L. f.) Willd. (Johnson and Liltved 1997), 13.3 % for *Angraecum arachnites* (Nilsson *et al.* 1985, Nilsson *et al.* 1987), 16.5 % for *Angraecum sesquipedale* Thou. (Wasserthal 1997), 34.9 % for *Disa cooperi* Reichb.f. (Johnson 1995). Among other sphinx moth pollinated flowers, mean sugar concentrations are also variable: 22.1 % for several plant species (Pyke and Waser 1981), 20-28 % for *Merremia palmeri* (Willmont and Búrquez 1996) and 17.5 % for *Manfreda virginica* (Groman and Pelmyr 1999).

The insignificant difference in sugar concentration among the 4-hour intervals and light levels suggests that nectar quality in *P. praeclara* was consistent over 24-hours; however, as the blooming period progresses nectar sugar concentration becomes more dilute. Using sugar concentration as an indicator of the nectar quality, the nectar available to pollinators in the earlier portion of the bloom season has more sugar, thus most likely to provide more energy. The later portion of the known pollinator species flight period generally overlaps with the beginning of the bloom season for *P. praeclara* (Westwood and Borkowsky 2004). There may be a benefit to *P. praeclara* to have higher nectar sugar concentrations during the early portion of the bloom season; first to lure pollinators away from other nectar sources in the vicinity and then encourage pollinators to make repeated visits.

There is no discernable trend for nectar sugar concentration among the Orchidaceae. Luyt and Johnson (2001) found mean nectar sugar concentrations to increase during the day from approximately 12 % at 0800 hours to 16.5 % at 2000 hours for *Mystacidium venosum*. However, Johnson and Liltved (1997) did not find a significant change in sugar concentration over a 16-hour period for *Botatea speciosa*.

Flower position. Flowers in the lower portion of the inflorescence had shorter nectar spurs with a lower amount of nectar, as indicated by a shorter nectar column height, but had higher sugar concentrations, in comparison to flowers in other portions of the inflorescence. Flowers in the lower portion of the inflorescence are the first to open and have a greater overlap with pollinator flight periods, thus they may receive more pollinator visits. While pollinators have access to less nectar, the increased sugar concentration in lower positioned flowers may offer a sufficient amount of energy to maintain the relationship between the orchid and the pollinator.

The difference in sugar concentration among different flower positions within a plant may also be linked to aging of the lower flowers. As the lower flowers are the first to open, they may have been opened for more than a week before the uppermost flowers are open and available to pollinators. Nectar in the lower flowers may have less water content due to evaporation of water, which would increase the sugar concentration of the nectar. Alternately, the nectar may be reabsorbed as the flowers age, which could result in an increase in the nectar sugar concentration in lower positioned flowers.

Maintaining higher nectar sugar concentrations in lower flowers may also take advantage of moth behaviour to fly upwards along an inflorescence as apposed to a downward direction. After feeding from *Angraecum sesquipedale* flowers, hawk moths

fly backward and upward (Wasserthal 1997). Production of higher quality nectar in the lower flowers may increase the chance that the moth will remain feeding within the inflorescence and remove additional pollinaria.

Size of inflorescence. Plants producing an inflorescence with many flowers creates a larger floral display and may be more attractive to pollinators, as it creates a superior visual cue and a stronger contrast against the background (Nilsson *et al.* 1985, Raguso and Willis 2003). A pollinator attracted to plants with a larger inflorescence may be rewarded with a greater quantity of nectar or a greater sugar concentration. In the case of *P. praeclara*, the results of this current study suggests that pollinator visitation to plants with larger inflorescences are rewarded with a greater quantity of nectar per flower.

CONCLUSION

The mean nectar spur length of *P. praeclara* flowers exceeded the proboscis length of the sphinx moth pollinators in southeastern Manitoba reported by Westwood and Borkowsky (2004), as well as two potential pollinator species that share similar morphological characteristics and flight periods with the recorded pollinator species. Nectar spur length increased over the bloom season, as did nectar column height, which resulted in a decrease in the estimated proboscis length that would be required to reach the nectar. Nectar sugar concentration decreased by approximately 6 % as the flowering period progressed over the season.

At night, the nectar spur became more elongated during which the nectar column height also increased. It is possible that the weight of the additional nectar may cause

the spur to stretch or there may be a physiological response that elongates the spur. Nectar sugar concentration did not change significantly over a 24-hour period.

Lower positioned flowers in the inflorescence had shorter nectar spurs and less nectar compared to flowers at higher positions. Over the flowering season, lower flowers had higher sugar concentrations than upper flowers. Comparing flowers within the same inflorescence, lower flowers had a higher sugar concentration than upper flowers.

The size of the inflorescence was positively correlated to spur length and nectar column height but was not correlated to nectar sugar concentration.

CHAPTER 5

GENERAL DISCUSSION

The western prairie fringed orchid population in Manitoba has on average fewer flowers per plant than those in the southern parts of its range (Sheviak and Bowles 1986). Seed capsule production in control plots, which are more reflective of natural conditions, was less than 0.032 capsules per flower and less than seed capsule production in more southern populations (Sheviak and Bowles 1986, Pleasants 1993, Pleasants and Moe 1993).

The use of ultraviolet light as a method to increase the attractiveness of orchids to their nocturnal sphinx moth pollinators was successful as measured by an increase in seed capsule development in 2002. This ability to manipulate seed capsule production to a certain degree may prove beneficial in several instances. As an endangered species in Manitoba, it is unlawful to disturb the orchid in its natural habitat, thus removal of plants or seeds from the wild is not permitted (Manitoba Conservation – Endangered Species Act 2006). The ultraviolet lights could temporarily be used to increase seed production and perhaps allow a harvest of seed capsules for use in research or establishment efforts elsewhere given the difficulty of seed germination. Given the effectiveness of the lights in increasing seed capsule production, the lights could be employed strategically within the Manitoba Tall Grass Prairie Preserve to encourage orchid establishment in areas where few orchids are presently found. As wind and air temperature influence flight activity of sphinx moths, the ultraviolet lights could be employed in patches of orchids when weather conditions during the orchid bloom period are less than ideal for moth flight. The ultraviolet light would attract and retain the pollinators in the vicinity of the

orchids to encourage moth-feeding activity among the orchids as opposed to other plant species. Alternately, ultraviolet light could be used during optimal weather conditions to maximize moth-feeding activity in orchid patches as the pollinating species are not abundant in southern Manitoba.

Using the ultraviolet light in the southern portion of the orchids range may result in greater levels of seed capsule production as the pollinator guild is larger and the individual species have greater population levels than that found in Manitoba. Furthermore, the intensity of the ultraviolet light could be tested to determine the distance at which moth attraction would be possible as well as the number of light sources required for patches of orchids of differing densities.

In control plots, the size of inflorescence did not influence frequency of pollinaria removal or seed capsule production; however, in ultraviolet light plots, plants with 11 or more flowers, had a significantly higher level of seed capsule production than plants with 10 or fewer flowers. Individuals producing many flowers create a larger floral display and may be more attractive to pollinators, by creating a superior visual cue and a stronger contrast against the background (Nilsson *et al.* 1985, Raguso and Willis 2003). Perhaps larger inflorescences also produce a large scent plume to attract moths seeking nectar. Under ultraviolet light, white objects appear to glow and have a stronger contrast against the background. It is possible that the visual cue of a western prairie fringed orchid with a large inflorescence near ultraviolet light presents as a superior visual cue to the pollinators. If ultraviolet light is used as a regular management tool there could be an unintentional selection for more robust plants producing more flowers per stem over time.

Two aspects of seed capsule production that was not tested in this study was the viability of the seed and the number of seeds per capsule. Sharma *et al.* (2003) found that seed viability was lower in seed capsules collected from larger orchid populations relative to smaller ones. It would be interesting to determine if greater seed capsule production is linked in any way to seed viability at both the population and plant levels. As for the number of seeds per capsule, we do not know if plants within the ultraviolet light treatment produced more seeds than the controls

Herbivory of individual flowers or plants did not affect seed capsule production in 2001 and 2002. The low number of seed capsules observed in 2001 may be the result of inadequate overlap between orchid bloom and pollinator flight period resulting in few flower visits and less pollination. In Manitoba, hawkmoth populations fluctuate from year to year (Westwood and Borkowsky 2004) and there may have been fewer moths flying in 2001. Although plots were visited following the hailstorm in 2001, there may have been residual effects from the hail on flowers and stems, undetectable to observers at the time. The severe bruising of flowers, leaves and stems may have stressed the orchids to such a degree that reproductive output was lowered. The severity of the hailstorm may have affected the moth population by damaging or even killing individuals while resting in trees or shrubs. Following the storm, many aspen and oak trees were missing leaves and small branches on their northern side, the direction from which the storm approached. In a study like this, it would have been advantageous to monitor the pollinator flight period as was done by Westwood and Borkowsky (2004). The time and labour to carry out an adult survey were not available during this study. In addition, using a light trap for an adult survey is destructive and removes pollinators permanently

from the population which is undesirable. However, the many technological advances in GPS transmitters could one day make it possible to track the movements of individual moths within and between patches of orchids and assist with our understanding of the flow of genetic material or the lack thereof.

It may be possible that the flower-pollinator relationship is still evolving between the western prairie fringed orchid and the sphinx moths that currently feed from and pollinate the flowers. In time, the overlap between flowering and flight periods may become more synchronized, resulting in more flower visits by the moths and increased seed capsule production by the orchid. Alternately, the massive loss and fragmentation of North America's tall grass prairie during the past century may have resulted in the removal of pollinators from the orchid habitat.

The mean nectar spur length of *P. praeclara* flowers exceed the proboscis length of the documented sphinx moth pollinators in the Manitoba Tall Grass Prairie Preserve as well as two potential species that share similar morphological characteristics and flight period overlap. It may be useful in the future to study the prevalence of the pollinators and determine the relationship of moth density in the Manitoba Tall Grass Prairie Preserve and the number of seed capsules produced. Nectar spur length increased over the bloom season, as did nectar column height, resulting in a decrease for the required estimated proboscis length needed to reach the nectar. Nectar sugar concentration decreased by 6 % as the flowering period progressed. It appears moths with shorter proboscis lengths could utilize flowers in the later portion of the bloom season although the energy reward may be less.

At night, the nectar spur became more elongated during which the nectar column height also increased. It is possible that the weight of the additional nectar may cause the spur to stretch or there may be a physiological response that elongates the spur. Nectar sugar concentration did not change significantly over a 24-hour period. It appears that changes in sugar concentration may take more than 24-hours to take effect.

Lower positioned flowers in the inflorescence had shorter nectar spurs and less nectar compared to flowers at higher positions. Lower flowers had higher sugar concentrations than upper flowers within an inflorescence. The size of the inflorescence was positively correlated to spur length and nectar column height but was not correlated to nectar sugar concentration. The differing sugar concentrations and spur characteristics between flowers within an inflorescence is intriguing. There are studies that examine the evolutionary importance of such differences (Stebbins 1970, Johnson *et al.* 1998, Maad and Nilsson 2004) for some orchid species but no research exists regarding the importance of intraspecific pollination competition for *P. praeclara*, either between plants or within flowers themselves.

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