Does Insect Pollination Limit Seed Production in White Cockle (*Silene latifolia* Poir.)?

A Thesis Submitted to the College of Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon

By

Jay Anderson

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying, publication, or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Request for permission to copy or to make other use of material in this thesis, in whole or part, should be addressed to:

Head of the Department of Plant Sciences
51 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan (S7N5A8)

ABSTRACT

Silene latifolia Poir. (white cockle or white campion) is an important weed in western Canadian agriculture. White cockle is an indeterminate flowering dioecious species having staminate and pistillate flowers on separate plants. New plants originate almost exclusively from seed. Therefore, both male and female plants are required in order for seed production to occur. Due to the dioecious nature of the species, seed production may be limited. Experiments were conducted in 2009 and 2010 at or around Saskatoon, Saskatchewan. Floral morphology and anatomy of both staminate flowers and pistillate flowers were examined. Specifically, anther and stigma development, floral nectaries, floral nectary stomata, and staminodes and pistillodes were observed and characterized in this species, using both scanning electron microscopy and light microscopy. Furthermore, field experiments were designed to evaluate whether *S. latifolia* relies solely on insect pollinators for seed production and if so, determine *when* pollination is occurring, and to establish if seed production in this species is limited due to pollination limitation. It was found that *S. latifolia* was predominantly insect-pollinated and pollination occurred both day and night; however, in 2010 pollination occurred mainly at night. Furthermore, female plants that were further than 4m from a compatible pollen source experienced reduced pollination levels and thereby seed production was reduced. Results of the pollination experiments suggested that seed production in *S. latifolia* may be limited by insect-pollination. Our results help to illustrate the role of pollination in the establishment of *S. latifolia* in Saskatchewan. There were clear pollination limitations for *S. latifolia* as a weed, however, the unique floral biology of this species, such as indeterminate flowering, quick pollen release, and potentially large seed yields, has allowed it to establish in western Canada and become an important weed on forage and minimum tillage farms.

ACKNOWLEDGEMENT

Many individuals have helped to bring to pass the outcome and success of my Masters research and without them; this project would not be what it is today. I would like to give thanks to the Agriculture Development Fund of the Saskatchewan Ministry of Agriculture for funding this research project. I would also like to thank University of Saskatchewan field staff: Shaun Campbell, Jill Ewanishin, Matt Leisle, Gerald Stuber, and Ken Sapsford, summer staff: Caitlin Labach, Shanna George, graduate students: Dilshan Benaragama, Hema Sudhakar Duddu, Teketel Haile, Nicole Seerey, Lauren Schewe, and Danielle Stephens. I would also like to thank Perri Ruszkowski (Prince Albert), Garry Podbielski (Meath Park), Curtis Braaten at the Conservation Learning Centre, and Scott McCaig from the City of Saskatoon whose support and cooperation in this project has been invaluable. I wish to acknowledge and thank Adam Harrison and Dennis Wulff at the University of Saskatchewan phytotron for their assistance and cooperation while I grew many plants for this research project. I would like to thank the Department of Biology at the University of Saskatchewan, for use of their microscopy lab instruments and to Dr. Guosheng Liu for his guidance and patience while I did the imaging for my research. I would also like to thank Dr. Doug Waterer who allowed me the use of lab equipment in a situation of urgency and to Dr. Eric Lamb whose expertise in statistics was a great help in the analysis of the data for this project. Furthermore, I would like to express my appreciation to Linda Matthews whose generosity, friendship, and support has been extraordinary. Many thanks to Department of Plant Sciences Head Dr. Bruce Coulman and Department of Plant Sciences office staff Carolyn Ouellete, Mary Lee, Marlene Freeman, and Sharon Stevens, who have been very helpful and provided extensive assistance over the duration of my graduate studies. I am also indebted to my advisory committee members, Emeritus Professor Rick Holm and Dr. Art Davis whose expertise and guidance has been a great help over the duration of this project. In addition, I would like to express my sincere appreciation to the external examiner for my thesis defense, Dr. Vipen Sawhney, from the Department of Biology at the University of Saskatchewan, who made time for my seminar and exam and who brought to my attention important insights and ideas to further the strength of my thesis. Finally, I wish to express my deep and sincere appreciation to my supervisor on this

project, Dr. Steve Shirtliffe, whose patience and long-suffering through this project has been paramount—obviously without him I would not be where I am today. Steve has put great trust and confidence in me notwithstanding my inexperience. Thank you Dr. Shirtliffe.

It is also important that I thank my wife Stephanie and other family members who have given endless strength and support throughout this project. Thank you.

DEDICATION

To both of my maternal grandparents, who passed away during the completion of my Masters research. They consistently asked me about my schooling, showing genuine interest in my education. I think of them often and know they are proud of my completion of this work. And to my two children, Jacob and Gema, who were born during this period of time.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

ANOVA	**Analysis of Variance**
h	**Hour(s)**
LM	**Light microscope**
SEM	**Scanning electron microscope**
μmol	**Micromoles**

1.0 INTRODUCTION

Silene latifolia Poir. (Caryophyllaceae), also known as white cockle or white campion, is an important dioecious weed across prairie regions of southern Canada and the northern United States (Royer & Dickinson, 1999). *Silene latifoia* can be found in a variety of cropping systems and is a concern on no-till farms and forage pastures in the prairie provinces of Canada. It is native to Eurasia and was introduced to North America from Europe in the early 1800s (McNeill, 1977). Little is known about the pollination ecology and pollination biology of *S. latifolia* unique to western Canadian agriculture and how its dioecious nature affects its reproductive ability as a weed.

Plant invasions are an important consideration in weed management (Cousens & Mortimer, 1995; Booth et al., 2003; Dekker, 2005; Radosevich et al., 2007). They can cause significant ecologic and economic losses (Booth et al., 2003). However, the invasiveness of a species may be somewhat limited by its breeding system. To help understand the ecologic or economic risks associated with potential plant invasions, research to characterize the ecological behaviour (i.e. breeding system) of a weed should be conducted (Dekker, 2005).

In general, the invasiveness of dioecious weeds has received little scientific attention (Costea et al., 2005). In populations of dioecious species where the ratio of males to females is approximately 1:1 (as is the case in *S. latifolia*), only half of the plants (females) can produce seeds. Thus, colonization of an area by dioecious species must begin with a minimum of two individuals (male and female) (Baker, 1955; Costea et al., 2005). Pollination limitation has been reported in dioecious species because both male and female plants, as well as their pollinators, must live within relatively close proximity in space and time (Baker, 1955). In addition, seed set in insect-pollinated dioecious plants has been observed to decrease with distance from a compatible pollen source (de Jong et al., 2005). Therefore, *S. latifolia* may have pollination limitation (Baker, 1955).

A detailed investigation of the floral biology and pollination ecology of *S. latifolia* ecotypes found in western Canada, may indicate its potential invasiveness and may also contribute to better weed management.

The main hypothesis of this thesis is that seed production in *S. latifolia* can be pollen limited. To test this hypothesis, the following objectives were developed: (1) verify that *Silene latifolia* is primarily insect-pollinated, (2) determine *when* pollination occurs, and (3) determine the effect of distance on pollen limitation. Another set of objectives were developed to explore the floral biology of *S. latifolia*, specific to western Canadian agriculture: (1) observe changes in anther and stigma surface structure during floral phenology, (2) characterize floral nectary tissue and nectary stomata in staminate and pistillate flowers, and (3) determine if vestigial reproductive structures of the opposite sex are present in staminate and pistillate flowers of *S. latifolia*, a dioecious species.

2.0 LITERATURE REVIEW

2.1 Species introduction

2.1.1 Species description

Silene latifolia is a member of the family Caryophyllaceae and behaves as an annual, biennial, or a short-lived perennial (McNeill, 1977). Its common name is white cockle or white campion. The species is native to Eurasia and was introduced to North America from Europe in the early nineteenth century. *Silene latifolia* is dioecious, with either staminate (male) flowers or pistillate (female) flowers on separate plants. The diploid number of chromosomes in *S. latifolia* is 2n=22 + XY in males and 2n=22 + XX in females (Lengrerova, et al., 2004). Therefore, hybridization is possible between this species and other members of the *Silene* genus (Mrackova, et al., 2008) as all *Silene* species have chromosome number 2n=24 (Lengrerova, et al., 2004). *Silene latifolia* has sex chromosomes with “maleness” caused by an XY chromosome and “femaleness” caused by an XX chromosome. Furthermore, the Y chromosome in male plants will suppress gynoecium development in male flowers and vise-versa in female plants.

The species has indeterminate flowering and prolific seed production. Flowers have a white corolla and a fused bladder-like calyx. Shoots grow from a thick, almost woody base into flowering and non-flowering stems. Stems exhibit dichotomous branching and have swollen nodes, which is common in species belonging to Caryophyllaceae (Hickey & King, 1988). Individual plants may grow to 100cm tall (Royer & Dickinson, 1999). However, males are generally shorter with more flowers than females (Delph & Meagher 1995). Leaves are larger and lance-shaped near basal regions of the plant and become gradually smaller higher on the stem (Douglas, 1998). Both stems and leaves are pubescent. Roots start as a taproot and eventually thick fleshy horizontal roots extend radially from the plant in the soil. Vegetative clones arise from buds on horizontal spreading roots or root fragments (McNeill, 1977). *Silene latifolia* is often confused with *Silene noctiflora* (night-flowering catchfly) and *Silene vulgaris* (bladder campion). However, *S. latifolia* has distinguishing characteristics that set it apart from both species. *S. latifolia* is a dioecious perennial with rather large white aromatic flowers, whereas *S. noctiflora* is an annual with a slightly pink calyx and hermaphroditic flowers. Also, *S. noctiflora* has sticky hairs on upper regions of the plant. *Silene vulgaris* is an annual with hermaphroditic flowers and is an entirely glabrous plant (Royer & Dickinson, 1999).

2.1.2 Prairie weed survey for *Silene latifolia*

Field surveys conducted by the Alberta, Saskatchewan, and Manitoba governments indicate that *S. latifolia* populations have been declining in annual cropping systems since the 1970s (Leeson et al., 2005). In the 1970s, *S. latifolia* was found in 6 of 8 ecoregions (Boreal Transition, Aspen Parkland, Fescue Grassland, Moist Mixed Grassland, Lake Manitoba Plain, and Interlake Plain) in western Canada; in the 1980s in 5 of 8 ecoregions (Boreal Transition, Aspen Parkland, Moist Mixed Grassland, Lake Manitoba Plain, and Interlake Plain); in the 1990s in 3 of 8 ecoregions (Aspen Parkland, Fescue Grassland, and Interlake Plain); and in the 2000s in only half of the ecoregions (Boreal Transition, Aspen Parkland, Fescue Grassland, and Interlake Plain) (Leeson et al., 2005). The surveys show that *S. latifolia* has not advanced into all 8 ecoregions but has been geographically reduced to only 3 ecoregions in the 1990s from 6 ecoregions in the 1970s and gaining back only one region (Boreal Transition) from the 2000s surveys.

2.1.3 Flower description

Silene latifolia flowers have distinct morphological features. Flowers are radially symmetrical (actinomorphic) with a superior ovary position (Hickey & King, 1988). Corollas are made up of five white bifid petals. Petals have ligular appendages that form a circle at the centre of the flower. At the appendage, petals bend at a 90° angle and extend to the base of the ovary. This extension forms a tubular-shaped corolla. Sepals are fused and surround the corolla tube. The calyx seems inflated and makes up the majority of the visible portion of the flower. Staminate flowers have ten stamens, five of which are epipetalous. The remaining five stamens are antipetalous and borne from the receptacle of the flower. Anthers exhibit longitudinal dehiscence. Staminate flowers have a 10-veined calyx, whereas pistillate flowers have a 20-veined calyx. In addition, veins on staminate flowers are reddish-purple compared to green veins on pistillate flowers. Furthermore, staminate flowers are generally smaller than pistillate flowers. Pistillate flowers have five stigmas that emerge from the styles attached above the superior ovary (Royer & Dickinson, 1999). The ovary has free-central placentation and an open locule (centrospermae) (Hickey & King, 1988).

2.1.4 Reasons for Concern

2.1.4.1 Invasive potential

Silene latifolia has become an important weed across prairie regions of southern Canada and the northern United States (McNeill, 1977; Royer & Dickinson, 1999). Typically, invasive plants establish and spread in new areas following their introduction. As a result, native flora can be displaced by unwanted, even weedy species (Radosevich et al., 2007). Blair and Wolfe (2004) suggest North American ecotypes of *S. latifolia* may have evolved to become considerably more aggressive than their European ancestors. They observed that plants grown from *S. latifolia* seed collected in North America had earlier germination, faster growth, more flowers, better survival, and less resource investment into important defense mechanisms compared to their European ancestors. They concluded that the North American ecotypes invest more energy into growth and reproduction and less resources into defense against predators (Blair & Wolfe, 2004).

However, no investigation of potential for North American ecotypes of *S. latifolia* to advance or spread in their non-native range has been conducted. Furthermore, the level of vegetative reproduction occurring in this species is not well documented (McNeill, 1977), but may be considerable in its invasive potential.

Silene latifolia has an annual to short-lived perennial life-history pattern (McNeill, 1977). Therefore, a population of *S. latifolia* can be simultaneously composed of seedlings, perennial rosettes, and mature flowering plants. Plants typically flower 40 days following germination. The indeterminate flowering of *S. latifolia* encourages pollinator visits for as long as weather conditions permit. After pollination, capsules ripen and dehisce in roughly 35 days (McNeill, 1977). As a result, *S. latifolia* can produce and disperse seed before most crops are ready for harvest. *Silene latifolia* plants that reach fruiting prior to freezing can disperse seed and germinate that fall or delay germination until the following spring.

A single female plant of *S. latifolia* has an average seed potential of up to 24,000 seeds in a growing season (Pearson, 1969; Thompson, 1970). Germination tests performed under controlled conditions have shown over 90% germination within 8-15 days. Experiments conducted provide no evidence of seed dormancy in the species (Pearson, 1969).

Silene latifolia populations pose a considerable risk to farmers in Canada. Minimal information is found in the literature on the invasiveness or invasibility of *S. latifolia* specific to western Canadian agriculture. Therefore, our knowledge of the invasive potential of *S. latifolia* is limited.

2.1.4.2 Pollination ecology

Little is known about insect pollination of *S. latifolia* specific to western Canada. The dioecious breeding system of *S. latifolia* makes the species somewhat unique as a weed. It is thought that *S. latifolia* reproduces almost exclusively by seed (McNeill, 1977). Thus, there must be some reliance on insect pollination for success of the species.

On the contrary, the pollination ecology of *S. latifolia* in Europe has received considerable attention. In Europe, a noctuid moth species, *Hadena bicruris*, has a highly specialized relationship with *S. latifolia* preferring only *S. latifolia* flowers (Dötterl et al., 2005). *Silene latifola* is a 'night-blooming' species, producing flowers that open at dusk and close shortly after dawn. Floral emissions released during the night attract adults of *H. bicruris*. Floral scent helps insect pollinators find specific flowers especially night-blooming species (Dötterl et al., 2005; Ashman, 2009; Hossaert-McKey et al., 2010). Floral aromatics are important in plant-insect communication (Bruce et al., 2005). In *S. latifolia,* male moths visited male and female flowers for a nectar reward (Dötterl et al., 2005). As a result, moths collected pollen from anthers and deposited pollen on stigmas, inducing pollination in *S. latifolia*. Female moths then lay eggs exclusively in female flowers where eventually larvae emerge to feed on maturing seeds. As a result, approximately 25% of seed produced is lost. This specialized relationship has both advantages and disadvantages for *S. latifolia* fitness in Europe. For example, *H. bicruris* is an important insect in pollination of European ecotypes of *S. latifolia*, while its larvae feed on developing seeds within the maturing capsule of *S. latifolia.* This insect-host relationship does not exist in North America as *H. bicruris* is found only in Europe (Blair & Wolfe, 2004).

The most important pollinators for North American ecotypes of *S. latifolia* are lepidopterans such as noctuid, geometrid, and sphingid moths (Young, 2002). Studies conducted near Golden, Colorado (Jefferson County), found lepidopterans to exclusively pollinate flowers but not predate seeds of *S. latifolia*. The floral features of *S. latifolia* suit the moth pollination syndrome (Baker & Hurd, 1968). These features include the white corolla, deep corolla tube, sweet floral scent, nocturnal anthesis, and concealed nectar. Some studies suggest *S. latifolia* flowers attract only night-flying insects and claim *S. latifolia* is pollinated exclusively by nocturnal moth species (Witt et al., 1999). However, contradicting studies suggest that both diurnal and nocturnal pollinators will visit flowers that are opened for longer than 12 hours (Miyake et al., 1998; Arizaga et al., 2000; Slauson, 2000; Young, 2002). Young (2002) concluded that both diurnal and nocturnal pollinators visit *S. latifolia* flowers. Moths were never observed early in the morning or before dusk and could not be observed visually after dark due to lack of sufficient light.

In that study, bees, wasps, and flies were observed early in the morning up to just before dusk. Temporal distribution among diurnal and nocturnal visits was clearly segregated and did not overlap (Young, 2002). Sphingid and noctuid moths were found to be the most effective and efficient visitors compared to daytime visitors. For example, moths were found to transfer more pollen from male flowers to female flowers based on seed production, when compared to bees and wasps, which transferred considerably less pollen onto female flowers.

2.1.4.3 Problematic cropping systems

Silene latifolia is an important weed in forage and pasture cropping systems in North America. Specifically, *S. latifolia* is problematic in areas where annual grains and perennial forages are grown in rotation. The majority of research done on *S. latifolia* control has been conducted in forages (eg., Dearborn, 1959; Hastings & Kust, 1970a; Hastings & Kust, 1970b; Kapusta, 1973; Kapusta & Strieker, 1975; Wyse & McGraw, 1987). Cultural practices associated with alfalfa production provide ideal conditions for the short-lived perennial life cycle of *S. latifolia* and this weedy species is problematic in forages other than alfalfa as well (Royer & Dickinson, 1999). *Silene latifolia* is well adapted to growing conditions where the soil is subject to little or no disruption as roots must build adequate carbohydrate reserves during the late summer and early fall in order to develop cold hardiness for temperatures during the winter (Hastings & Kust, 1970b). These requirements are easily met where forages are grown, as there is no soil disruption during the critical carbohydrate storage period. Furthermore, the presence of *S. latifolia* plants in hay samples moderately dilutes the digestible crude protein levels (Hastings & Kust, 1970a).

Silene latifolia seed is similar in size and appearance to alfalfa and other leguminous forage seeds (McNeill, 1977). This makes seed cleaning difficult for seed growers where *S. latifolia* plants contaminate forage stands. Seed impurities are considered to be a major source of *S. latifolia* dispersal (Royer & Dickinson, 1999).

No-till farming also provides ideal soil conditions for species having a perennial life cycle (Blackshaw et al., 2006). Due to low soil disturbance in no-till systems, perennial plants are allowed to store ample carbohydrates in root tissue during the growing season and eventually for overwintering (Hastings & Kust, 1970a). Where small grain crops are grown under no-till conditions, *S. latifolia* can become easily established. As a result, annual grain crops that are grown in conjunction with reduced tillage may be at risk of *S. latifolia* contaminations. Small grain farmers who practice reduced tillage must resort to methods such as herbicides rather than tillage for control (Hastings & Kust, 1970a).

2.2 Breeding systems

Angiosperms dominate present day terrestrial flora in both diversity and biomass with roughly 300,000 different species (Hickey & King, 1988; Richards, 1997). Scholars have grouped angiosperms into approximately 300-400 families (Hickey & King, 1988). Within these families there are a variety of flower characteristics that affect pollination. These characteristics define the *breeding system* of the flower or plant species (Kwak & Bekker, 2006). A breeding system is a method used by a plant to control the genetic structure of a community and patterns of evolution (Richards, 1997).

2.2.1 Dioecism

Dioecism has evolved from hermaphroditic species many times among many unrelated taxa (Proctor et al., 1996; Freeman et al., 1997). In angiosperms, the incidence of dioecy is somewhat rare occurring in only about 4-6% of plant species (Renner & Ricklefs, 1995; Richards, 1997). However, dioecy is relatively common in the woody tropical tree forests on Hawaii and New Zealand making up 28 and 12-13% of species on those islands respectively. A large proportion of dioecious species are animal pollinated (zoophilous), though many are wind-pollinated (anemophous) as well. In a review of dioecy and pollination systems, moth pollination made up 9% of pollination occurring in dioecious species (Bawa, 1980). Herbaceous plants have the lowest incidence of dioecy, with dioecy being restricted to perennials.

2.2.2 Floral characteristics

2.2.2.1 Floral nectary functions and types

Floral nectaries are a type of secretory gland contained within a flower (Weberling, 1989). This secretory tissue is located at a variety of different positions within the flower and is the site of nectar secretion. Nectar is a fluid that contains mainly sugars and is generally an attractant for potential insect pollinators. Floral nectaries generally have some connection with the pollination biology of a flower; however, this is not always the case.

There are three main types of nectary: mesophyllary, epithelial, and trichomatic (Weberling, 1989). Mesophyllary type nectaries are composed of mesophyll cells that are glandular in form. In mesophyllary nectaries, nectar is secreted through nectar slits, which are a stomata-like opening in the glandular tissue. Epithelial type nectaries originate in the epidermal cells. These cells have oversized nuclei and serve a secretory function. Trichomatic nectaries are specialized trichome hairs that secrete nectar.

2.2.2.2 Staminode origin and function

A staminode is a sterile stamen borne in the position where a fertile stamen on the flower would have been (Watson & Dallwitz, 1992). Therefore, when a stamen aborts, but retains similar characteristics to a perfect stamen, it becomes a staminode (Decraene & Smets, 2001). In angiosperms, a functional stamen is divided into two parts, the filament and the microsporangia-bearing anther (Weberling, 1989). However, staminodes may differ in size, shape, and branching when compared to fertile stamens. In addition, staminodes may be vascularized or unvascularized depending on the species (Decraene & Smets, 2001).

In some plant species, stamens of a flower are transformed to fulfill some other biological necessity in response to an evolutionary shift and thereby become a functional staminode (Decraene & Smets, 2001). Functional staminodes may fulfill a role in biological nutrition, structure, or attraction. As a nutritional function, staminodes may

serve a nutritive role by providing a food supply for flower visitors in the form of sterile pollen or nectar. Staminodes may also become a collecting structure for nectar in association with floral nectary tissue. As an attractive agent, staminodes may serve as an attractant for pollinators by producing desirable colours or odours.

2.2.3 Pollen-pistil interaction

The interaction between stamen and pistil is a highly specialized event in angiosperms (Edlund et al., 2004). The stigmatic surface of the pistil provides a favourable environment for pollen germination (Shivanna & Sawhney, 1997). Pollen must be genetically compatible with the stigma in order for successful germination and fertilization to occur. Furthermore, it is critical for pollen to be viable and stigmas to be receptive synchronously.

2.3 Pollination in angiosperms

One major adaptation of angiosperms is pollen travel, and subsequent pollination, in the absence of water (Richards, 1997). Pollination in angiosperms generally has three phases (1) pollen release from the male part of flower, (2) transfer from paternal part to maternal part, and (3) deposition of pollen to recipient surface on maternal part. In angiosperms, there are abiotic and biotic mechanisms for pollen transfer (phase 2). Biotic pollination involves an organism, other than the plant, that acts as a vector for pollen (Faegri & van der Pijl, 1971). The majority of angiosperms rely heavily on biotic, rather that abiotic vectors, for pollination (Waser, 2006).

2.3.1 Insect-pollination and pollination syndromes

Plant-insect interactions are crucial for sexual reproduction in many flowering plants (Faegri & van der Pijl, 1971). Typically, when insects reach the reproductive phase in their life cycle they are in search of an energy rich food source. Insects often harvest essential nutritional requirements from plants in the form of nectar or sometimes pollen. In the event that an insect visits a flower for nectar or pollen, pollination can occur. The

majority of insect pollinators belong to orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera (Proctor et al., 1996). The interaction between these insects and the flowers they visit can be highly specialized or very generalized (Waser, 2006).

In general, flowers have a particular syndrome of characteristics that correspond to each type of pollinator (Baker & Hurd, 1968; Faegri & van der Pijl, 1971). Flowers of *S. latifolia* closely follow a moth pollination syndrome (Baker & Hurd, 1968). A few key features of moth pollinated flowers include night-blooming flowers with nocturnal anthesis, strong nocturnal scent emissions, white or faintly coloured petals, and deeply hidden nectar (Faegri & van der Pijl, 1971).

2.3.1.1 Night-blooming species

Nocturnal anthesis has developed to ensure the reproductive success of certain plant species (Miyake et al., 1998; Arizaga et al., 2000). It is suggested that plants have evolved certain characteristics to favour pollination by more reliable, high-quality pollinators. Pollinators that are considered to be reliable, efficient, or high quality are those that have a high pollen removal to deposition ratio per visit (Miyake et al., 1998). This high rate of pollen transfer could be especially so in obligate out-crossers, such as the dioecious night-blooming species *S. latifolia*. In *Lonicera japonica*, diurnal bees deposit a higher number of pollen grains on stigmas than nocturnal hawkmoths on a per visit basis (Miyake et al., 1998). However, bees remove over 10 times more pollen during one visit compared to hawkmoths. Therefore, nocturnal anthesis in *L. japonica* maximizes pollen transfer. Under the stress of finite resources, plants develop characteristics, such as nocturnal anthesis, to select against pollinators like diurnal bees that consume high amounts of pollen.

2.3.1.1.1 Nocturnal floral scent emissions

Floral scent is thought to have evolved to select preferential flower visitors. Plants advertise the availability of floral rewards, like nectar, to insects through floral scent (Jürgens et al., 2002a). In *S. latifolia*, flowers accumulate nectar during the day until

flowers open at night. Then, freshly opened flowers are nectar-filled for nocturnal visitors like moths (Witt et al., 1999). Floral scent compounds of *S. latifolia* follow the general characteristics of moth-pollinated flowers emitting a strong, sweet odour around dusk and into the night (Jürgens et al., 2002a). It is around this time that flight activity of adult lepidopterans start (Dreisig, 1986). Moths are a more reliable pollinator of *S. latifolia* (Young, 2002), which is supported even further by findings on timing and composition of floral scent emissions (Jürgens et al., 2002a) and timing of nectar accumulation in flowers (Witt et al., 1999).

2.3.1.2 Phalaenophily (moth pollination)

Most moths remove pollen from flowers while visiting flowers to collect nectar (Faegri & van der Pijl, 1971; Proctor et al.,1996). The specific flight pattern of a moth species while visiting flowers affects where pollen is collected on their bodies. Moths that hover over flowers collect pollen on their proboscis and head, whereas moths that flutter around flowers collect pollen on their legs. Moths are important for the reproductive success of many plant species. Richards et al. (1999) suggest that nocturnal moths are strong flyers and will disperse pollen great distances (up to 640m).

2.3.1.2.1 Sensory reception in moths

Moths use chemoreception to locate host plant species that offer nectar or pollen as a reward (Ramaswamy, 1988). Moths can often locate host plants even when clouded by an array of other non-host plant signals using olfactory receptor neurons (ORNs) located primarily on insect antennae (Bruce et al., 2005). In nocturnal moths, olfaction is particularly important for detecting insect attractants such as floral scent, which lead to host plants when vision becomes impaired due to darkness (Ramaswamy, 1988; Dötterl et al., 2005). Investigations show that lilac aldehyde compounds (monoterpenoids) are powerful antennal stimulants for lepidopterans and attract noctuid moths (Dötterl et al., 2007). Lilac compounds are found abundantly in *S. latifolia* flowers (Dötterl et al., 2007; Dötterl et al., 2005; Meagher, 2002).

2.3.2 Pollination limitation in dioecious plants

There are many mechanisms that contribute to the incidence of pollination failure in plants. The event of pollination can be impeded during pollen removal, transport, or deposition onto the stigma (Wilcock & Neiland, 2002). Pollination failure is even further exaggerated in dioecious species where cross-pollination is obligatory; pollination failure can occur >95% of the time due to lack of sufficient pollen transport between the sexes (Martinez-Palle & Aronne, 2000). The low frequency of dioecy (4-6%) may be a result of pollen limitation of seed production (Charlesworth, 1993). The magnitude of pollination limitation generally increases with increasing distance between male and female individuals (Kay et al., 1984; de Jong et al., 2005).

Pollination limitation has been observed in many dioecious plant species (Kay et al., 1984; Campbell, 1985; de Jong et al., 2005). In one experiment, plants were tested for distance-dependent pollination limitation. Female plants were placed from 0 m up to 25 m from a compatible pollen source (Campbell, 1985). All species tested experienced pollination limitation with increasing distance from the pollen source. In another experiment, plants were tested for pollination limitation in two co-flowering species (de Jong et al., 2005). Two dioecious species in the same area reach anthesis at the same time and thereby compete for pollinators during anthesis. As a result, one species was visited less frequently by insect-pollinators and experienced pollination limitation.

2.4 Invasion biology

Plant invasion biology has been given considerable attention in the literature (Cousens & Mortimer 1995; Booth et al., 2003; Dekker, 2005; Inderjit & Colautti, 2005; Murrell, 2006; Radosevich et al., 2007). Invasion can be defined as the geographical expansion of a species into an area previously uninhabited by that species. Plant species that have the same traits as resident species or have traits that allow them to inhabit vacant niches in an area may become invasive.

2.4.1 Characteristics of invasive species

In general, plant invasions are very difficult to predict (Booth et al., 2003). To be classified invasive, a plant must successfully (1) establish in a new area, (2) colonize that area, and (3) endure occupation of that habitat for more than one generation (Dekker, 2005) without human intervention (Radosevich et al., 2007).

Many factors influence the invasiveness or invisibility of a plant species in a locality. These factors might include community structure, evolutionary history, propagule pressure, environmental conditions, nearby disturbance, and environmental stress (Radosevich et al., 2007). Williamson (1996) defines propagule pressure as being the probability that a seed, fruit, or vegetative clone produced by a plant species would disperse, establish, and survive in adequate quantities to sustain the species in an area. The spatial advancement of a plant species, once introduced to an area, relies on the reproductive ability of that species and subsequent propagule dispersal. Invading plant populations generally start as an individual plant, then form patches, and may eventually move as advancing fronts into new areas of a field. Plants that disperse their seed great distances often put larger geographic areas at risk of an invasion; whereas, plants that disperse their seed very short distances often advance much slower (Radosevich et al., 2007). In this way, propagule pressure becomes important in understanding the potential for a plant population to invade an area.

2.4.2 'Long-distance' dispersal effect

There is a well-established concept known as "Baker's rule" that helps to understand, in part, the invasion biology of plant species (Baker, 1955). In self-compatible species, one seed is sufficient to start a colony of plants. However, one seed of a self-incompatible species is not sufficient to do the same. It requires two seeds of a self-incompatible, but cross-compatible nature, to grow and mature to reproductive stages in order to initiate a colony of plants. Furthermore, those sexually reproducing plants must be in relatively close proximity in time and space (Baker, 1955). Therefore, plant invasiveness is accelerated by self-compatibility and not self-incompatibility (Baker, 1955; Petanidou et al., 2011). In dioecious species, cross compatible means male and female plants. This necessity makes colonization of dioecious species even less likely due

to a 50% chance that if two seeds fall together, they must yield male and female plants. Given the pollination limitation that occurs with dioecious plants, the probability of a single distant *S. latifolia* plant successfully producing seed is reduced. Because of this restriction, the species may have limited ability as a weed.

3.0 FLORAL MORPHOLOGY AND ANATOMY OF *Silene latifolia*

3.1 Introduction

Silene species contribute a diversity of reproductive systems to Caryophyllaceae (Desfeux et al., 1996). Flowers in *Silene* can be hermaphroditic or imperfect, such that *Silene* species can be gynodioecious, gynomonoecious, andromonoecious, trioecious, subdioecious, or dioecious. There are over 60 *Silene* species, six of which reportedly exhibit dioecy including *S. acaulis, S. diclinis, S. dioica, S. latifolia*, *S. otitis*, and *S. pseudotites* (Desfeux et al., 1996).

Silene latifolia is a dioecious perennial weed. Dioecious species have staminate (male) flowers only on certain plants and pistillate (female) flowers only on the other plants. Dioecism is a rare trait among weedy species. It is generally associated with woody tropical species (Bawa, 1980; Matallana et al., 2005) so it is uncommon to have herbaceous dioecious species as problematic weeds of the Canadian prairies. In spite of that, Canada thistle is dioecious and is an aggressive weed in Canadian agriculture (Royer & Dickinson, 1999). In general, dioecious weed species have received little attention and their pollination biology on an ecosystem-to-ecosystem basis should be more widely investigated (Baker, 1984).

Silene latifolia has been of interest to many biologists and as a result, many studies have been conducted to examine a diversity of its characteristics. Experiments have been conducted in *S. latifolia* to identify floral scent composition (Jürgens et al., 2002a), nectar dynamics and sugar composition (Witt et al., 1999), pollen-ovule ratios (Jürgens et al., 2002b), and effect of stigma age on receptivity (Young & Gravitz, 2002). The nectar composition of *Silene* species is hexose-dominant (glucose and fructose) analyzed by chromatography (Percival, 1961). These consistent analyses of nectar sugar composition in various *Silene* species, were supported by Witt et al. (1999) who reported that nectar of pistillate flowers in *S. latifolia* had only glucose and fructose (and no sucrose). However, in staminate flowers higher content of both hexose sugars were found in nectar when compared with pistillate flowers, and the former also contained small amounts of sucrose.

Floral vascular anatomy and floral surface morphology have also been investigated in *S. latifolia*, although, only briefly in pistillate flowers (Thomson, 1942). Therefore, information is lacking on floral morphology and floral anatomy for *both* staminate (e.g anthers) and pistillate (e.g. stigmas) flowers of this species. No information exists to characterize aspects of its floral anatomy (other than vascular tissue), such as the location and abundance of nectary tissue.

Little is known of the floral morphology and anatomy of nectary tissue in *S. latifolia*. Numerous examinations that identify tissues and structures important in pollination biology, such as floral nectary tissue, have been conducted on non-dioecious Caryophyllaceae species (Zandonella, 1966, 1967a, 1967b, 1970a, & 1970b). These studies make general assumptions about other species in Caryophyllaceae; therefore, it is important to conduct the research on this species (Rohweder, 1967). Studies that characterize nectary tissue in dioecious *Silene* species are scarce. It is reported that stoma-like structures may be present on the surface of nectary tissue in dioecious species *S. diocia* (Rohweder, 1967). However, details on nectary morphology and anatomy in staminate and pistillate flowers of the dioecious *S. latifolia* do not exist.

Furthermore, no work has been done to determine if vestigial reproductive organs are present in *S. latifolia*. In some dioecious species, remnants of reproductive organs of the opposite sex have been found in staminate and pistillate flowers (Proctor et al., 1996). For example, rudimentary stamen-like structures were observed in pistillate flowers of *S. dioica* (Thomson, 1942; Rohweder, 1967). Aborted stamens can develop into glandular secretory tissue, which produce nectar, therefore becoming functional staminodes that may serve a function in pollination biology (Weberling, 1989; Decraene & Smets, 2001). However, vestigial reproductive structures in staminate and pistillate flowers for *S. latifolia* have not been thoroughly examined. Observing the presence or absence of staminodes and/or pistillodes in this species may be an indication of the level of suppression caused by the sex chromosome in males (Y) and females (X). The male fertility gene has a gynoecium-suppressing function whereas the female fertility gene as an androecium-suppressing function (Mrackova, et al., 2008).

General information does exist for members of Caryophyllaceae and Sileneae; however, our knowledge of *S. latifolia* is incomplete. Investigations to characterize reproductive organ morphology and floral nectary structure in *S. latifolia* will add to our knowledge of dioecious plant species. Such investigations on *S. latifolia* would help to clarify uncertainties and deepen our understanding of its pollination and reproductive biology.

The objectives of this study were (1) to observe changes in anther and stigma surface structure during floral phenology, (2) to characterize floral nectary tissue and nectary stomata in staminate and pistillate flowers, and (3) to determine the presence and structure of vestigial reproductive structures of the opposite sex in staminate and pistillate flowers of *S. latifolia,* a dioecious species.

3.2 Materials and methods

3.2.1 Plant material and growth conditions

Plant material for the following experiments was grown and prepared for examination from January to May 2010 at the University of Saskatchewan, Saskatoon, SK. All plant material used for observation in these studies was grown in a controlled growth environment located in the College of Agriculture and Bioresources. Seeds for this study were obtained in 2009 from a naturally growing population of *S. latifolia* Poir. located near Meath Park, SK (53°18’36.53” N, 105°20’17.74” W). Seeds were planted on January 12th, 2010, and grown until flowering. Plants were grown in 15 cm pots containing No. 4 Sunshine® Potting Mix under 18 h of light and 6 h of darkness at 22°C light and 16°C dark. Light intensity in the chamber was 720μmol m^{-2} s^{-1}. Plants were watered daily or as needed. Slow release starter fertilizer (14-14-14) was added at seeding. Fifteen male and 15 female plants were used for source material in these experiments. Plant material for this study was examined using a dissection microscope, light microscope, and scanning electron microscopy.

3.2.2 Observation of fresh plant material

The location of floral nectaries and origins of nectar in *S. latifolia* in fresh staminate and pistillate flowers were viewed using a stereoscopic dissection microscope (SMZ-1B Nikon Japan: 8X-35X magnification). Digital images were captured using a microscope mounted DinoXcope Version 1.1 (304_1637- Universal) AnMo Electronics Corporation. Based on these initial observations, floral tissues were prepared for scanning electron microscopy (SEM) and light microscopy (LM).

3.2.3 Scanning electron microscopy (SEM)

3.2.3.1 Growth stages

Five staminate flowers and 5 pistillate flowers at various stages of development were selected (Figure 3.1). Flower age treatments for both flower sexes were: Stage 1 – 24 h pre-anthesis; Stage 2 – 12 h pre-anthesis; Stage 3 – 12 h post-anthesis; Stage 4 – 24 h post-anthesis; and Stage 5 – 36-48 h post-anthesis. Anthesis was defined as the opening of the flower bud (Usher, 1966).

Both staminate and pistillate flowers were measured (mm) prior to examination. Calyx and corolla lengths were measured separately on three flowers at each of the 5 stages. For staminate flowers, Stage 1 – calyx: 16±1 mm; corolla: n/a, Stage 2 – calyx: 16±1 mm; corolla: 4±1mm, Stage 3 – calyx: 17±1 mm; corolla: 13±1 mm, Stage 4 – calyx: 22±0.5 mm; corolla: 13±1 mm, Stage 5 – calyx: 22±1 mm; corolla 13±1 mm. For pistillate flowers, Stage 1 – calyx: 22±0.5 mm; corolla: n/a, Stage 2 – calyx: 22±1 mm; corolla: 4±1mm, Stage 3 – calyx: 22±1 mm; corolla: 8±0.5 mm, Stage 4 – calyx: 23±0.5 mm; corolla: 14±1 mm, Stage 5 – calyx: 24±0.5 mm; corolla 14±1.5 mm.

Silene latifolia is indeterminate and can have flowers at multiple stages of development on a single plant (immature buds to totally ripened capsules). In order to estimate staminate and pistillate flower age, a preliminary experiment was conducted. Staminate and pistillate buds were labeled and revisited every 2 h to determine when the flower reached anthesis and, to observe any morphological changes associated with floral

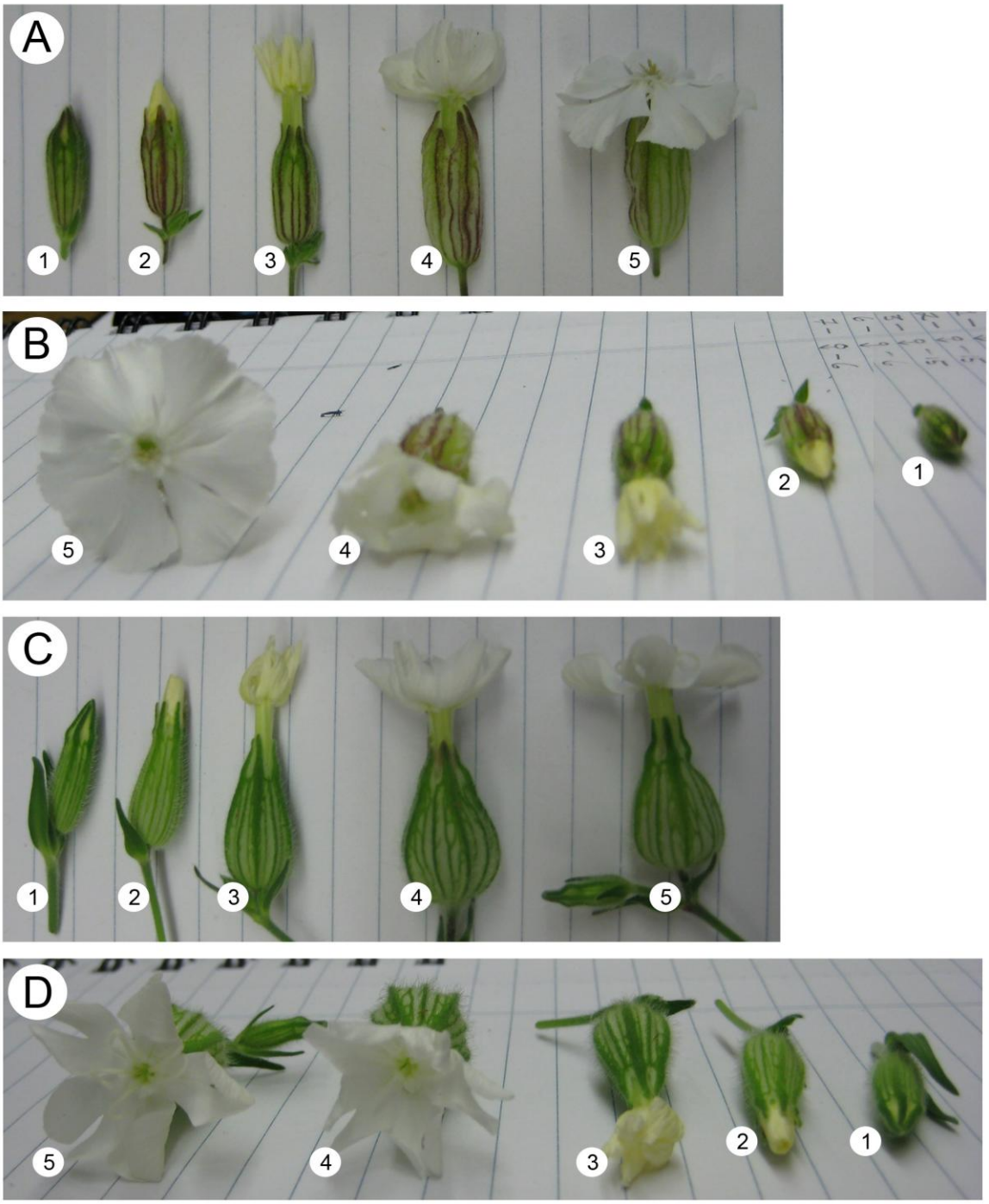

FIGURE 3.1 Images of staminate (A-B) and pistillate (C-D) *S. latifolia* flowers using Canon digital camera. **(A)** Lateral view of stages 1-5 (left to right). **(B)** Anterior view of stages 1-5 (right to left). **(C)** Lateral view of stages 1-5 (left to right). **(D)** Anterior view of stages 1-5 (right to left). Stage 1 – 24 h pre-anthesis; Stage 2 – 12 h pre-anthesis; Stage 3 – 12 h post-anthesis; Stage 4 – 24 h post-anthesis; and Stage 5 – 36-48 h post-anthesis

development. Based on these observations, visual rating parameters were established so that the time (h) to anthesis and time after anthesis could be estimated.

Flowers were randomly selected from plants with the appropriate stages of development on March 4th, 2010. Epipetalous anthers and entire styles/stigmas were removed from selected flowers for SEM. Tissue samples were fixed using 2% glutaraldehyde (GA) in 25mM sodium phosphate (Na_2HPO_4 and NaH_2PO_4) buffer, pH 6.8. The GA was then rinsed away using three changes of 25mM buffer. Samples were then placed in a post-fixation solution of 1% osmium tetroxide (OsO_4) in the same buffer for 2 h. The post-fixative (OsO_4) solution was rinsed away with another three changes of buffer, followed by three changes of distilled water. Samples were then dehydrated through a graded acetone series and plant material was gradually introduced into a 100% acetone solution (Echlin, 2009). Plant tissues were critical point dried using liquid CO_2 (Polaron Instruments E3000 Series II, Watford, UK), and specimens were mounted onto 12mm diameter aluminum stubs using Scotch® double-sided adhesive. Samples were then coating with gold particulate in S150B Gold Sputter Coater and observed by SEM (Philips 505 1983 model) at 25-30 kV. Images were captured using Fuji Film 400 positive/negative instant film. Positives were scanned using an Epson 3200 Photo scanner and images were arranged into plates using Adobe Photoshop® CS5.

Longitudinal sectioning of staminate and pistillate flower samples, following critical point drying and gold coating, was necessary to observe stomatal pores on nectary surfaces. Samples were hand sectioned using a razor under the dissection microscope. Once samples were repositioned on aluminum stubs, they were recoated with gold for viewing under the SEM.

3.2.4 Light microscopy (LM)

3.2.4.1 Experimental procedures

Nectary tissue for LM utilized flowers of identical stages that had been examined by SEM, following removal of epipetalous anthers and styles. Removal of only anthers and styles left nectary tissue intact in these flowers. Samples were dehydrated prior to

paraffin wax embedding and subsequent sectioning using procedures of Jensen (1962). Nectary samples were fixed using 2% GA in 25mM sodium phosphate buffer. Glutaraldehyde was rinsed away using three changes of buffer. Nectary samples were then rinsed three times with a 50% ethanol solution, before dehydration through a graded n-butanol series until 100% n-butanol solution was reached. Samples were then infiltrated with chips of paraffin wax (Paraplast®, Fisher Scientific) before evaporation of n-butanol followed by three changes of pure liquid paraffin to remove any remaining n-butanol from samples. Liquid paraffin was then cooled leaving nectary samples embedded in solid paraffin. Samples were mounted on small wood blocks in preparation for sectioning with the rotary microtome (Type 1212; Leitz Wetzlar, Germany). Cross- and longitudinal-sections of 7.5 μm were made of the floral nectary tissues. Sections were mounted on glass slides and stained with 0.05% toluidine blue O in 20 mmol/L sodium benzoate buffer, pH 4 (O'Brien & McCully, 1981) for 15 minutes. Following staining, slides were rinsed twice in xylene (1 h and >12 h respectively) to dissolve wax from the sections. Sections were then mounted in Permount™ (Fisher Scientific, Fair Lawn, NJ, USA), covered with a glass cover slip, and allowed to set for 24 h. Samples were examined using a Zeiss West Germany III RS Universal light microscope. Images of cross- and longitudinal-sections were taken using a microscope-mounted camera using FujiFilm X-tra Superia (400 ASA) and imaged electronically using an auto-negative film carrier (Noritsu QSS-3001).

3.3 Results and discussion

3.3.1 Observation of fresh material

Staminate flowers of *S. latifolia* had an inflated, cylindrical shaped calyx made up of fused sepals and having purple venation (Figure 3.2A, B). There were ten veins on the calyx of each staminate flower. There were also five bifid heart-shaped petals on each flower, which opened perpendicular to the calyx after blooming (Figure 3.2C). The base of all petals together formed a corolla tube, which surrounded the entire androecium before anthers dehisced and filaments grew past the length of the corolla tube. There were ten stamens arranged in two alternating whorls of five. Five stamens were borne on the receptacle where they alternated with petals (i.e. antipetalous) and the other five

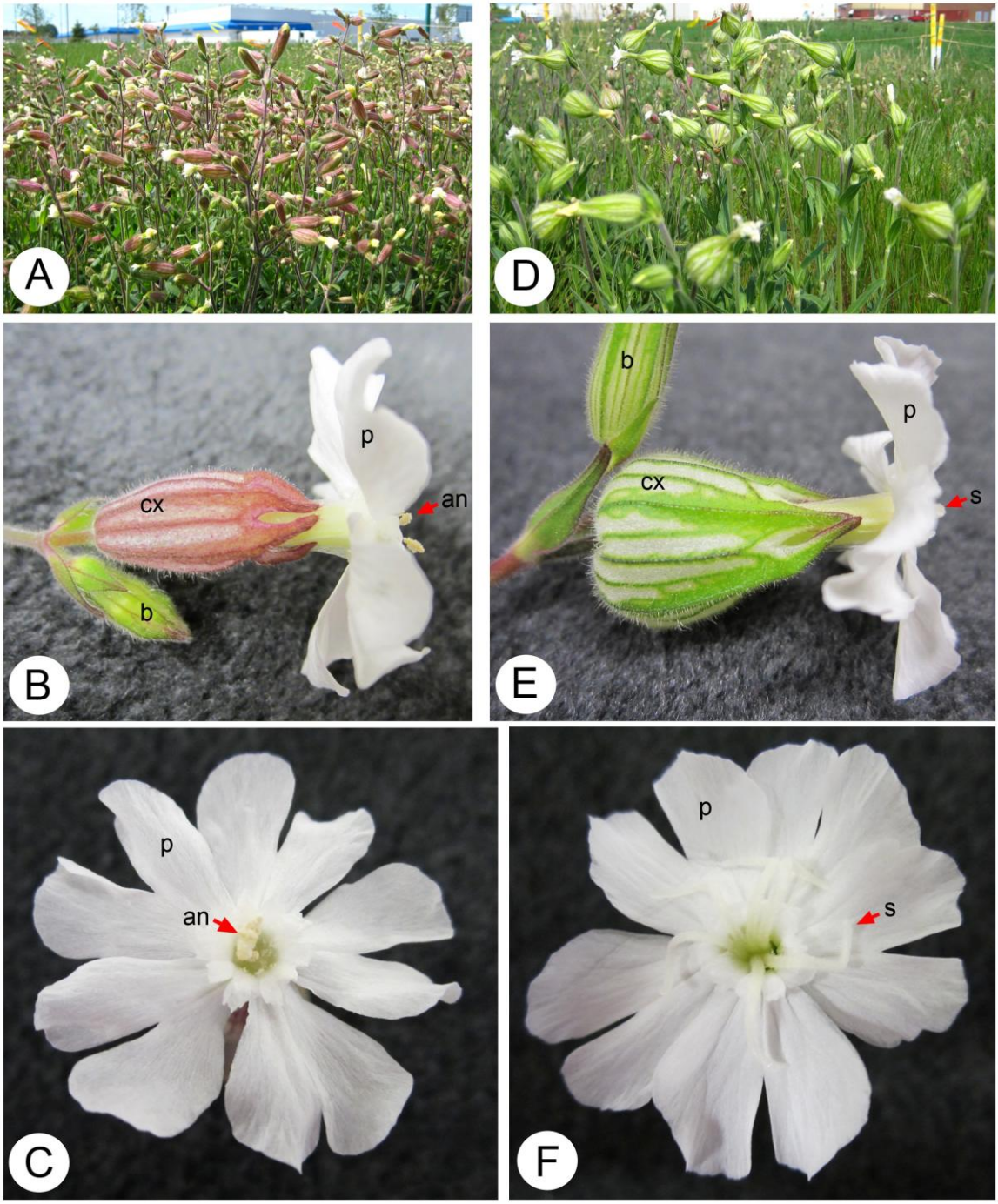

FIGURE 3.2 Images of staminate (A-C) and pistillate (D-F) flowers of *S. latifolia* using Canon digital camera. **(A and D)** Naturally occurring male (A) and female (D) *S. latifolia* plant populations located in Saskatoon. **(B and E)** Lateral view of staminate (B) and pistillate (E) flowers. Anthers seen on staminate flowers and stigmas seen on pistillate flowers. **(C and F)** Anterior view of staminate (C) and pistillate (F) flowers. Anthers seen in centre of corolla in staminate flowers and stigmas seen in centre of corolla in pistillate flowers. *Abbreviations:* an, anther; b, flower bud; cx, calyx; p, petal; s, stigma

stamens were borne one on each petal (epipetalous). Anthers dehisced along longitudinal slits in the thecae.

Pistillate flowers of *S. latifolia* had an inflated, teardrop-shaped calyx made up of fused sepals having green venation (Figure 3.2D, E). There were twenty veins on the calyx of each pistillate flower. Each flower had five bifid heart-shaped petals on each flower (Figure 3.2F) and open the same as in staminate flowers. Pistillate flowers had a corolla tube surrounding the gynoecium of the flower. Styles eventually elongated past the length of the corolla, thus exposing their stigmas. There were five styles/stigmas in each pistillate flower (but sometimes 4 or 6 were observed). Styles had a dry stigmatic surface and only one stigma lobe covered in many stigmatic papillae. The ovary in *S. latifolia* had free central placentation, but appeared to have the remnant of five equally divided locules in each ovary. However, there was no physical structure remaining to partition regions of the ovary into locules. Nectary tissue and nectar was observed in staminate and pistillate flowers under the dissection microscope. In staminate flowers, nectar was observed to originate from the centre of the flower from the area surrounded by filaments (Figure 3.3A-D). Nectar smothered the base of each filament and filled the spaces between a network of filamentous hairs. However, it was difficult to observe the exact origin of the nectar in staminate flowers.

In pistillate flowers, nectar was observed to originate from the area surrounding the base of the ovary (Figure 3.3E). Accumulation of obvious droplets of nectar was observed between petals. In some flowers, nectar filled a small portion in the bottom of the inflated calyx. Again, it was difficult to determine the exact origin of nectar under the dissection microscope due to the abundance of nectar observed near the base of the ovary.

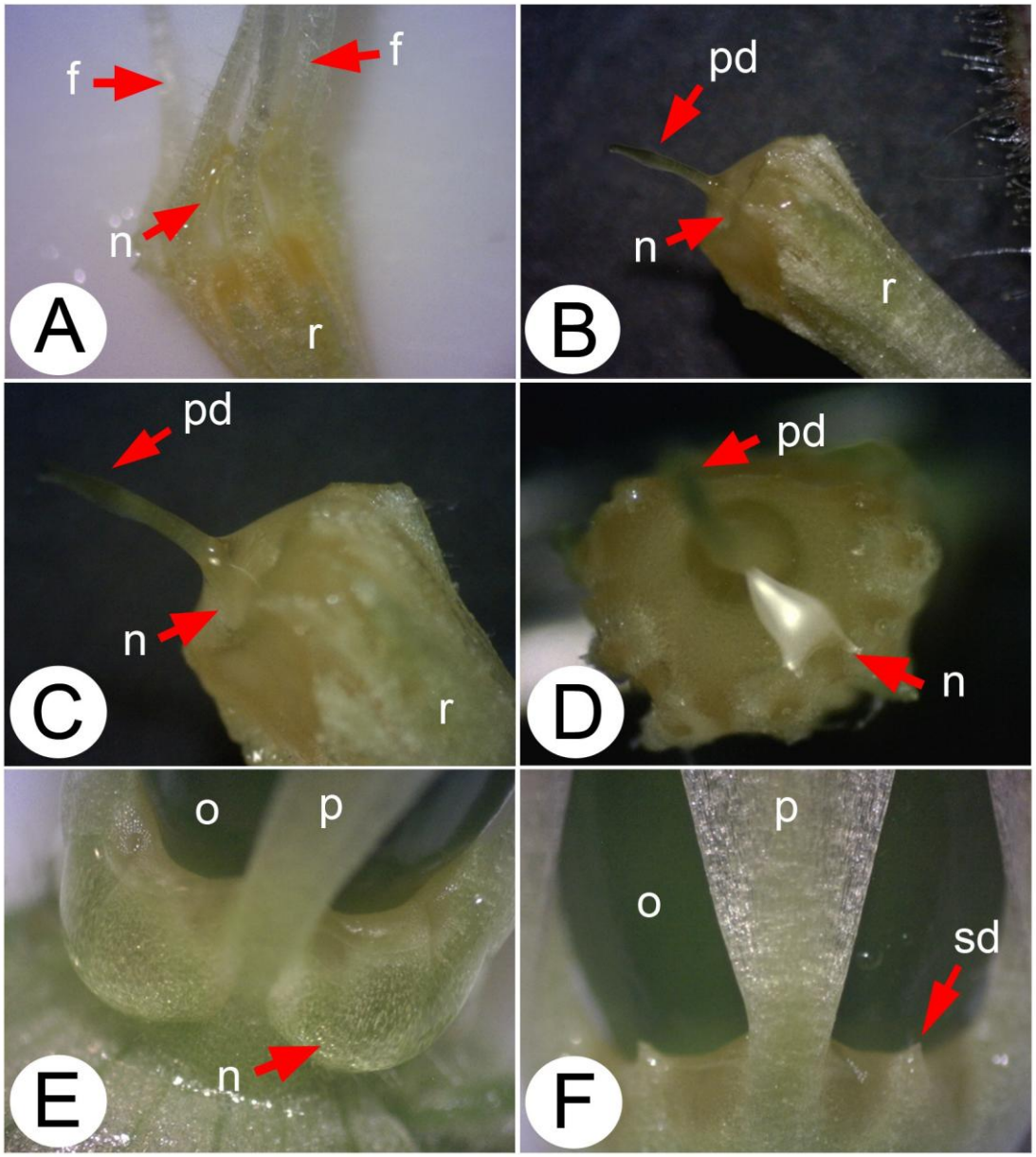

FIGURE 3.3 Images of staminate (A-D) and pistillate (E-F) *S. latifolia* flowers using stereoscopic dissection microscope. Staminate *S. latifolia* flowers A-D: **(A)** Nectar at the base of filaments. Corolla and calyx were removed from the flower. **(B)** Nectar and pistillode are more visible with filaments removed. **(C)** Higher magnification of nectar and pistillode with corolla, calyx, and stamens removed. **(D)** Top view of nectar and pistillode. Pistillate *S. latifolia* flowers E-F: **(E)** Calyx was torn away to view ovary and receptacle. Nectar observed to flow between petals forming large droplets. **(F)** Diminutive staminodes alternating with petals. *Abbreviations:* f, filament; n, nectar; o, ovary; p, petal; pd, pistillode; r, receptacle; sd, possible staminode

3.3.2 SEM of changes in anthers and stigmas

In staminate flowers, changes in anther morphology were observed with increasing anther age (Figure 3.4A-D). Anther dehiscence of the epipetalous stamens occurred approximately 12 h prior to anthesis (Figure 3.4B) and pollen had almost completely escaped from anthers by 24 h after anthesis (Figure 3.4D). Anthers dehisced by longitudinal slits (stomia) along the lateral region of each theca.

Studies on *Lilium longiflorum* L. indicate that pollen had poor germination prior to anthesis in the species. It was concluded that some level of pollen desiccation is an important final step in maturation of *L. longiflorum* pollen (Lin & Dickinson, 1984). Anther dehiscence in staminate flowers of *S. latifolia* prior to anthesis seems dangerous for a dioecious species due to potential pollen losses prior to insect visitation. However, anther dehiscence prior to anthesis may allow time for pollen drying to occur before buds open (Castellanos et al., 2006). This process may ensure that pollen grains are "ready" when insect pollinators visit flowers following anthesis.

Due to the indeterminate nature and staggered opening of the anthers of the 10 stamens (antipetalous followed by epipetalous) (Personal observation) in *S. latifolia*, there are always new buds emerging and new anthers dehiscing. As a result, pollen release is sustained over a period of several weeks or even months. Therefore, if a single staminate flower depletes its pollen over a 36 h period, there is more pollen available in another flower on the plant. This type of pollen dosing is common in species with frequent and wasteful pollinators (Castellanos et al., 2006) as is the case in *S. latifolia* (Young, 2002). This method of pollen release may be an advantage for the reproductive ability of the species.

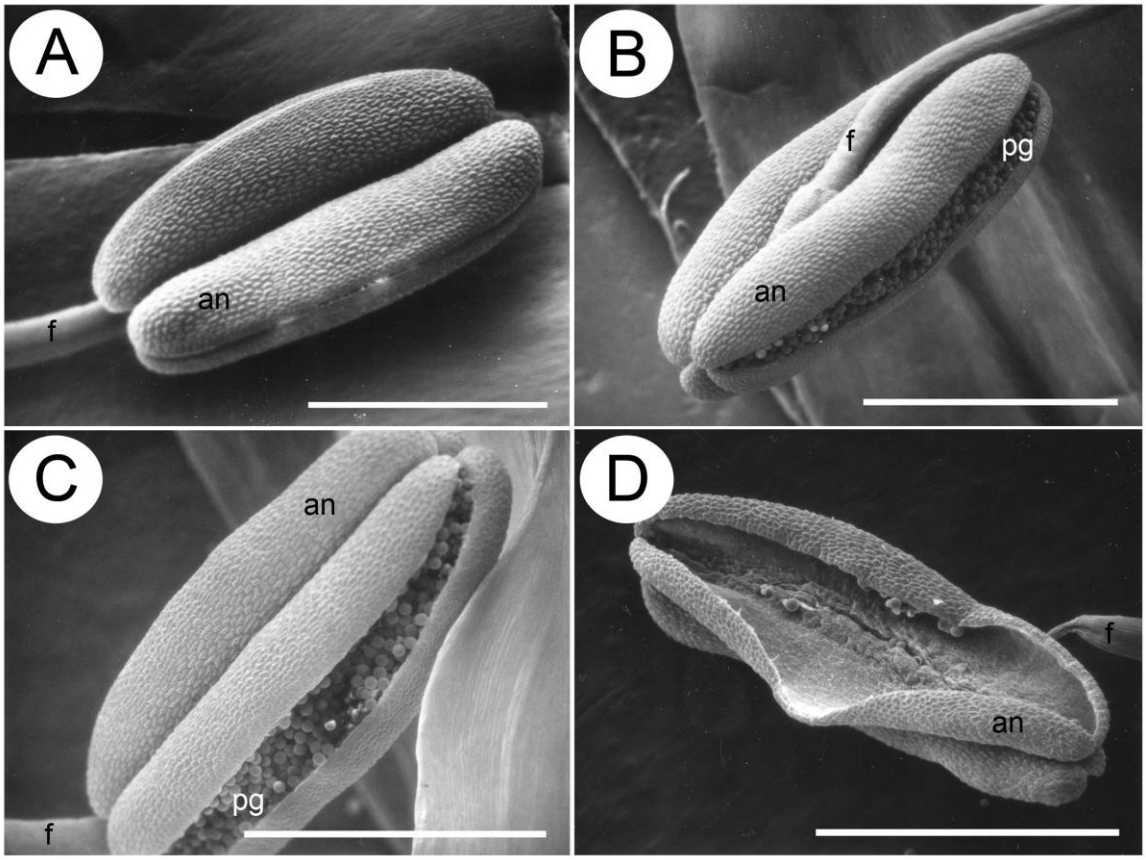

FIGURE 3.4 Scanning electron micrographs of gradual anther dehiscence in staminate flowers of *S. latifolia* at four developmental stages. **(A)** Stage 1 (24 h pre-anthesis): Epipetalous anther and filament. **(B)** Stage 2 (12 h pre-anthesis): Epipetalous anther and filament. **(C)** Stage 3 (12 h post-anthesis): Anther has dehisced by longitudinal slits (stomia) along thecae and pollen grains are visible. **(D)** Stage 4 (24 h post-anthesis): Few pollen grains remain within the anther's locule. Bars = 1mm in A-D. *Abbreviations:* an, anther; f, filament; pg, pollen grain(s)

Finally, it is reported that the pollen-ovule ratio (P/O) in *S. latifolia* is 55/1 (Jürgens et al., 2002b), which is quite low for dioecious species (Cruden, 2000) compared to 8300/1 in some species (Kubitzki & Kurz, 1984). However, these P/Os are compared flower-to-flower and not plant-to-plant. In the case of *S. latifolia,* it is reported that male plants produce a higher number of flowers compared to female plants. Therefore, P/Os in the species per plant are much higher than the literature suggests (Jürgens et al., 2002b). If pollen is readily available for visiting insects to remove from many flowers and over a long period of time, there may be a better chance for successful pollination in the species. It has been reported that *S. latifolia* has the potential to produce up to 24,000 seeds per plant (McNeill, 1977) which, in ideal conditions may partially be due to high P/Os (per plant) and readily available pollen.

In pistillate flowers, changes in stigma morphology were observed with increasing stigma age (Figure 3.5A-I). Stigmatic papillae were observed to elongate, become denser, and in some cases papilla surfaces became uneven and wavy as flower age increased up to 48 h after anthesis. The entire stigma was also observed to elongate and curl as pistillate flowers aged (Figure 3.2F). In self-incompatible species, it is important that the surface structure of the stigma (i.e. stigmatic papillae) facilitates the cohesion of compatible pollen grains to give the species the best possible chance for reproduction. In *Arabidopsis thaliana*, stigma and papillae extension combined allows the stigma to be more physically receptive to pollen (Edlund et al., 2004). Furthermore, in *Clintonia borealis* a perennial forest plant found in eastern North America, stigma adhesion and receptivity was observed to gradually increase with stigma age (Galen et al., 1986).

Young and Gravitz (2002) considered the effect of stigma age on stigma receptivity in *S. latifolia* by measuring the level of pollen germination on stigmas of increasing age (up to 120 h). It was found that pollen germination declined with stigma age, while seed production and seed weight stayed the same. They concluded that stigmatic age (up to 120 h) was not negatively correlated with receptivity and that the low levels of seed set found in a previous study may be due to a difference in pollinator effectiveness rather than stigma age (Young & Gravitz, 2002). Although our study only made observations of

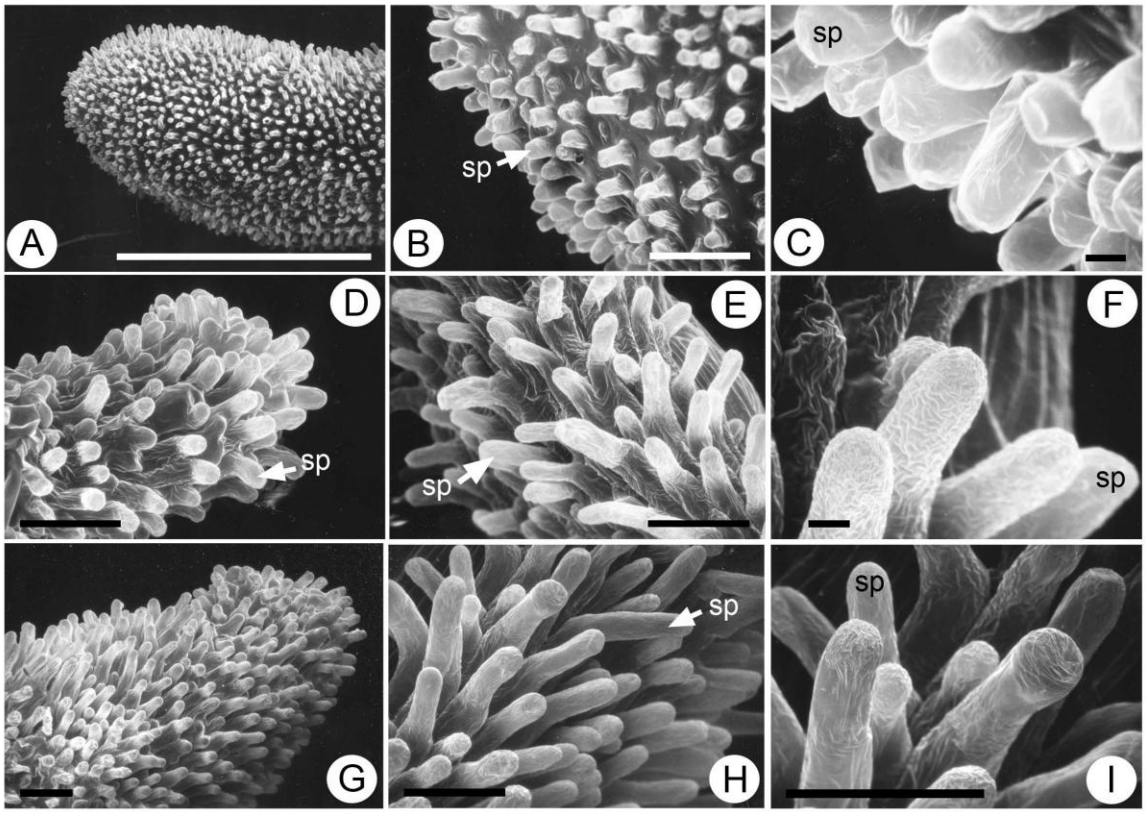

FIGURE 3.5 Scanning electron micrographs of papillae elongation on stigmas of pistillate flowers in *S. latifolia* at three developmental stages (Stages 1, 3, 5). A-C: Stage 1 (24 h pre-anthesis): Papillae on stigma. **(A)** Low magnification. Minimal papillae protrusion from stigmatic surface at this stage. **(B)** Medium magnification and **(C)** High magnification. Smooth texture on papillae surface at this stage; D-F: Stage 3 (12 h post-anthesis): Papillae on stigma. **(D)** Low magnification. Gradual elongation of stigmatic papillae at this stage. **(E)** Medium magnification and **(F)** High magnification. More surface texture on papillae surface at this stage; G-I: Stage 5 (36-48 h post-anthesis): Papillae on stigma. **(G)** Low magnification. Stigmatic papillae reach maximum length for stigma ages. **(H)** Medium magnification and **(I)** High magnification. Some lobing and surface texture on papillae at this stage. Bars = 10μm in C and F; 0.1mm in B, D, E, G-I; 1mm in A. *Abbreviations:* sp, stigmatic papillae

structural changes on the stigmatic surface up to 48 h, our assumptions are supported by findings of Young and Gravitz (2002), who found that pollen germination decreased, whereas stigma receptiveness did not. Our findings suggest that stigma.receptivity does increase with age based on the morphological changes that occurred in stigmatic papillae length, texture, and shape up to 48 h after anthesis These physical changes are believed to help ensnare pollen grains so pollen germination can occur (Edlund et al., 2004).

3.3.3 SEM of floral nectary morphology and structure

3.3.3.1 Nectary location and structure

Floral nectary tissue in staminate flowers was found to consist of the receptacle and the basal staminal ring to which the antipetalous whorl of stamens were directly attached (Figure 3.6A). Nectary tissue was concealed by hairs, which protrude from the basal region of each filament. In general, the receptacle in staminate flowers is reduced in size compared to pistillate flowers probably because there is no functional pistil in staminate flowers. Further observations suggest that floral nectaries in staminate flowers were positioned in the gynoecial region of the flower (where the carpel would have been, were it a pistillate flower) (Figure 3.6A). The receptacle appeared to be developed into a cup- or bowl-shaped structure (similar to pistillate flowers) surrounding a pistillode. This occurrence has not been reported for this species (Rohweder, 1967), but supports other exploratory work which indicate that floral nectaries in most members of the tribe Sileneae exist as a ring surrounding the base of reproductive organs in respective flowers (Thomson, 1942; Rohweder, 1967). Due to the reduced size of the receptacle in staminate flowers, there is less nectary tissue present when compared to pistillate flowers. It is believed that the gynoecial region of a staminate flower can function as a nectary in dioecious species (Decraene & Smets, 1999). This situation appears to be the case in *S. latifolia* as the region surrounding the pistillode is almost exclusively nectary tissue. However, nectary tissue was not observed in the pistillode in staminate flowers.

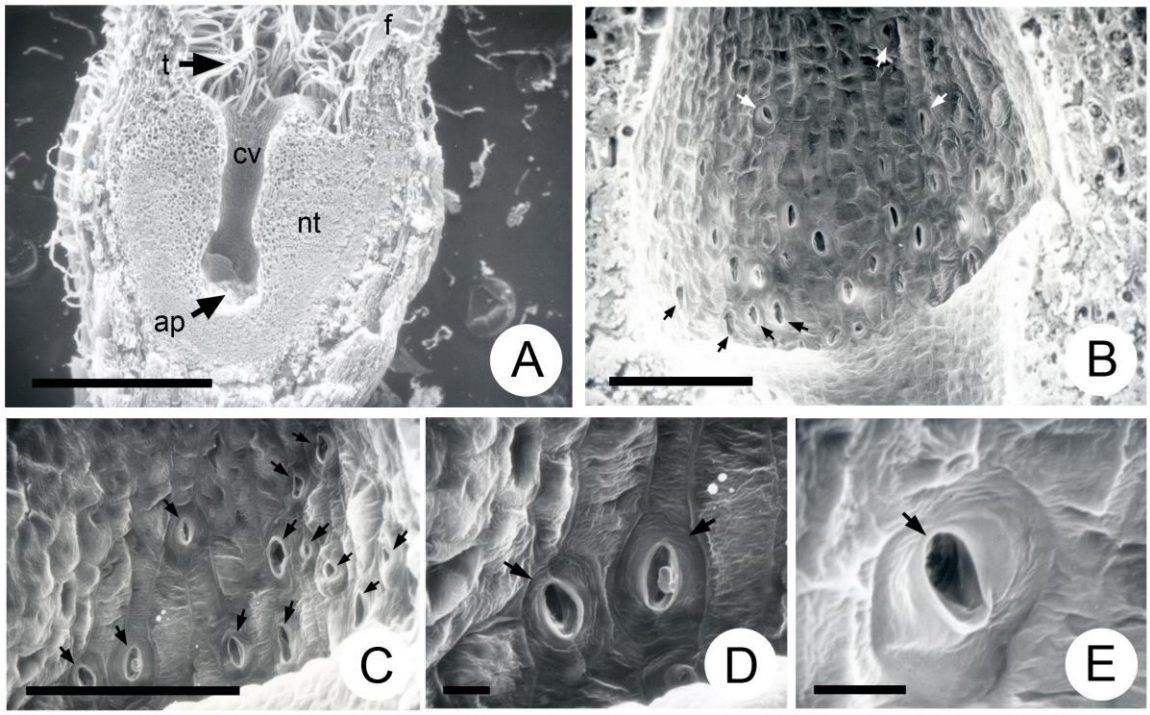

FIGURE 3.6 Scanning electron micrographs of staminate flowers of *S. latifolia*. **(A)** Pistillode removed from central region of the flower leaving a central cavity. Nectary tissue is located in the receptacular region of the flower. **(B)** Higher magnification on base of cavity where pistillode was removed. Black and white arrows indicate nectar slits or stomata. **(C)** Higher magnification of region near base of cavity where pistillode was removed. Arrows indicate nectar slits or stomata. **(D)** Higher magnification. Arrows indicate a pair of nectar slits or stomatal pores. **(E)** Higher magnification. Arrow indicates stomatal pore surrounded by guard cells. Bars = 10μm in D and E; 0.1mm in B and C; 1mm in A. *Abbreviations:* ap, attachment point of pistillode; cv, cavity (pistillode removed); f, filament; nt, nectary tissue; t, filament trichomes. Black and white arrows with no abbreviation indicate stomata.

In pistillate flowers, floral nectaries develop as an outgrowth of the receptacle in a ring-shaped structure (Figure 3.7A-B). The nectary ring, into which the 5 petal bases insert, surrounds the base of the ovary. Nectary location within individual flowers plays a role in pollination biology (Bernardello et al.,2000). In the case of *S. latifolia*, functional reproductive organs are in the direct path of insects, which may be searching for nectar. Nectar placement in *S. latifolia* is ideal as insects visiting staminate flowers could insert their proboscides at the top of the corolla tube to collect the nectar at the base of the flower and could subsequently remove pollen from the anthers in the process. Furthermore, insect visitors could then transport removed pollen grains and deposit them on stigmas when reaching to the base of compatible pistillate flowers to collect nectar.

3.3.3.2 Nectary stomata

Floral nectary stomata were observed in staminate flowers (Figure 3.6B-E). Stomata were found lining the inner base of the cup-shaped nectary tissue that surrounds the pistillode. As a general observation, nectary stomata were more prevalent on nectary surfaces in staminate flowers compared to pistillate flowers. The presence of nectar slits within the flower is a good indicator of nectary tissue under the surface (Weberling, 1989). It is assumed that nectar is at least partially secreted through stomata in *S. latifolia* (Rohweder, 1967) indicating the presence of a mesophyllary nectary type (Weberling, 1989). This placement of stomata corresponds with observations concerning nectar originating from the central region of staminate flowers Figure 3.3A-D.

Floral nectary stomata were also observed in pistillate flowers (Figure 3.7C-E). Stomata were found lining the walls of the paired cavities observed to alternate with petals (Figure 3.7A-B). This finding suggests that nectar might exude from the nectar slits and then drain from cavities to cover the adaxial face of the region surrounding the base of ovary observed in Figure 3.3E-F. Again, these results support findings that nectary tissue in the tribe *Sileneae* surrounds the basal area of the gynoecium and androecium in respective flowers (Thomson, 1942). Nectary stomata have not been reported in the literature on this species. However, our findings are similar to

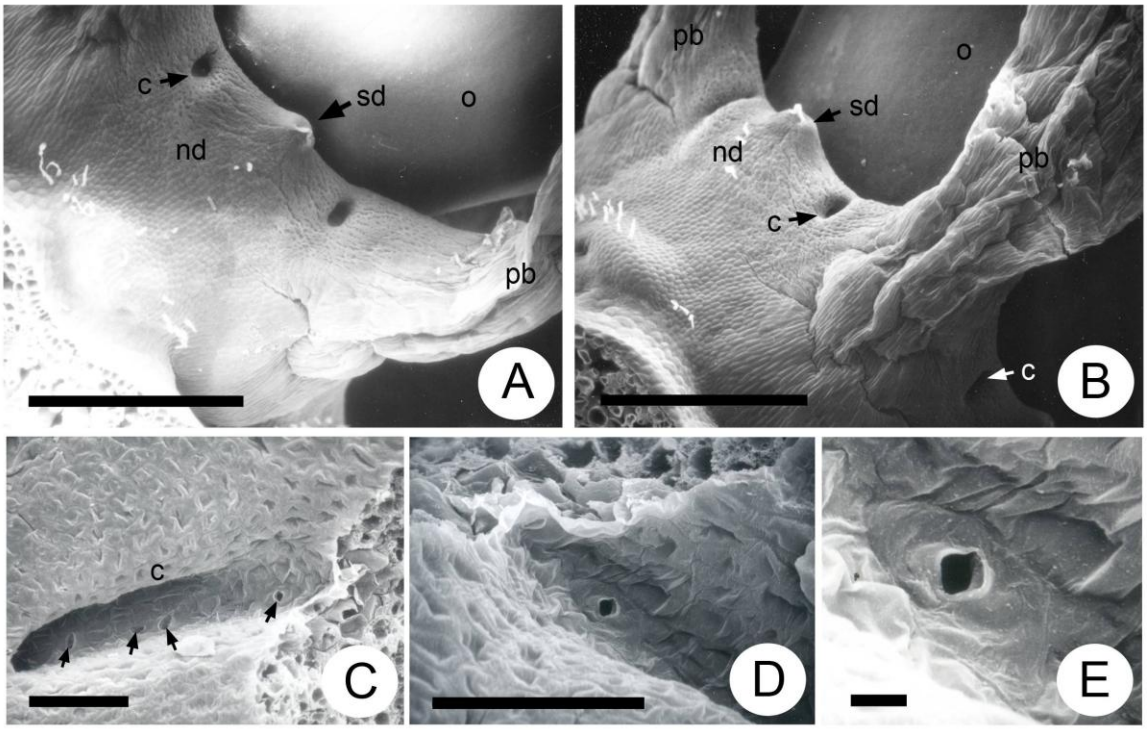

FIGURE 3.7 Scanning electron micrographs of pistillate flowers of *S. latifolia.* **(A)** Receptacle forms an outgrowth around the base of the ovary in a ring-shaped structure. Paired cavities or canals located on nectary disk. Possible staminode alternating with petals around the base of the ovary. **(B)** Paired cavities on nectary disk that alternate with petals around the base of the ovary on the nectary disk. Possible staminode alternating with petals around the base of the ovary. **(C)** Longitudinal section of canal lined with nectar slits or stomata (indicated by arrows). **(D)** Higher magnification of nectar slits or stomata in canal (indicated by arrows). **(E)** Higher magnification of stomatal pore found in canal. Bars = 10μm in E; 0.1mm in C and D; 1mm in A and B. *Abbreviations:* c, canal; nd, nectary disk; o, ovary; pb, petal base; sd, possible staminode. Black arrows indicate stomata.

observations that were made in pistillate flowers of *S. dioica.* In *S. dioica*, nectar was observed to fill the staminal ring, which served as a type of reservoir at the base of each flower (Rohweder, 1967). When nectar slits are present on the nectary surface, it is a strong indication of mesophyllary type nectaries when the mesophyll is glandular in form. Nectar is likely secreted partially through nectary stomata (Weberling, 1989).

3.3.4 LM of floral nectary anatomy and structure

3.3.4.1 Nectary location and anatomy

In cross- and longitudinal-sections of staminate flowers, nectary tissue was secluded to a very small central region in the receptacle (Figures 3.8 and 3.9). Furthermore, nectary tissue was reduced in size and appeared to be less abundant when compared to pistillate flowers (compare Figure 3.8A to 3.10A). The width of all the nectary tissue in staminate flowers was slightly greater than 0.5mm. Whereas, in pistillate flowers one side of the nectary cells (lateral to ovary) in longitudinal-section was greater than 1mm. These findings may support those of Witt et al. (1999) who reported that in *S. latifolia*, staminate flowers contained lower nectar volumes compared to pistillate flowers. It seems reasonable that a smaller region of nectary tissue might produce less nectar; however, that is entirely speculative.

In pistillate flowers, nectary tissue was an outgrowth of the receptacle, which surrounded the entire base of the ovary (Figure 3.10, 3.11A and 3.11D). This pattern is characteristic of receptacular type nectaries (Weberling 1989). Furthermore, nectary tissue was observed at the base of each petal in pistillate plants (Figure 3.10E). This tissue faced inward and was immediately adjacent to the ovary. The nectar-producing cells filled the staminal ring surrounding the base of the gynoecium with nectar. Nectar was observed to drain from canals borne in the staminal ring surrounding the base of the ovary. Within these canals, stoma-like structures were observed (Figure 3.11B, C, E, F), which were presumed to secrete nectar to the outer region of the staminal tube and fill the staminal ring (Rohweder, 1967).

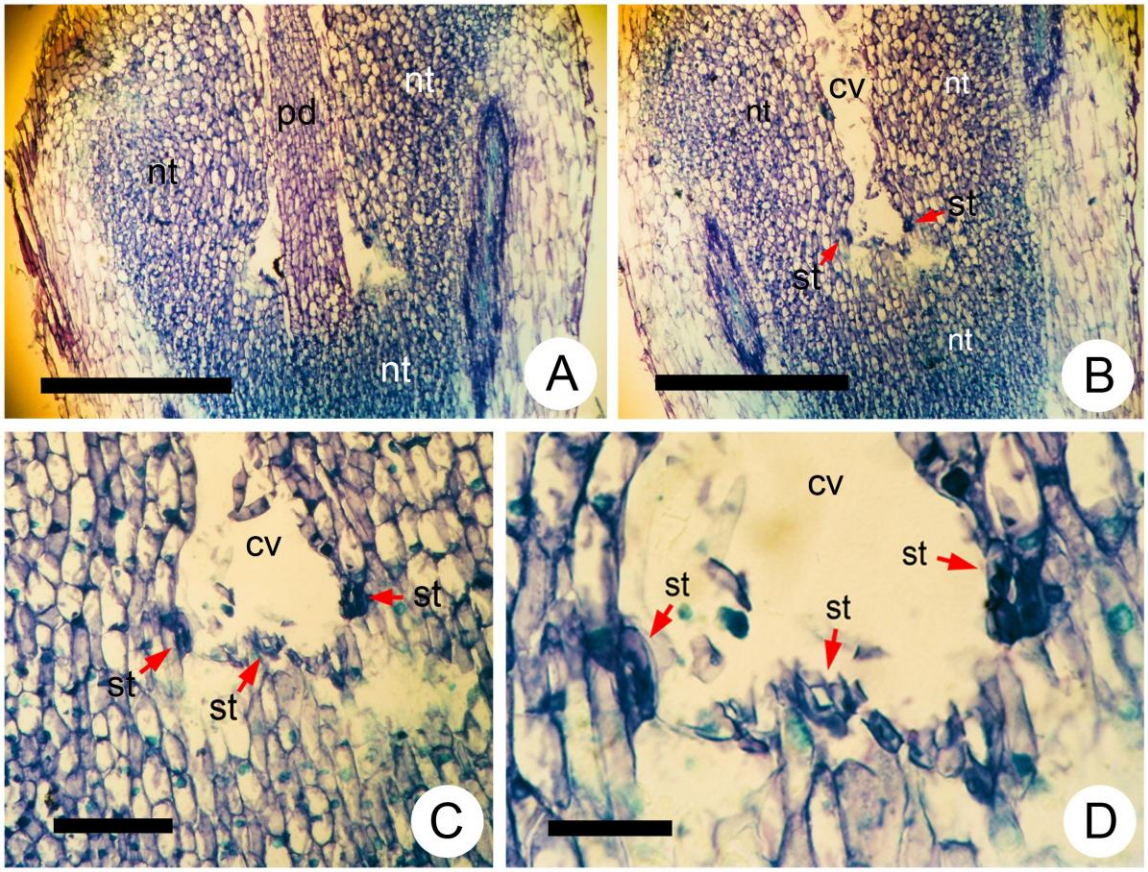

FIGURE 3.8 Light micrographs of staminate flowers of *S. latifolia*. Images are of nectary tissue with stomata in longitudinal-section. **(A)** Nectary tissue surrounding the pistillode. **(B)** Receptacular nectary tissue without the pistillode. Stomata near the base of the cavity. **(C)** Higher magnification of stomata lining inner surface of cavity. **(D)** Higher magnification of stomata lining inner surface of cavity. Bars = 0.05mm in D; 0.1mm in C; 0.5mm in A and B. *Abbreviations:* cv, cavity (pistillode absent); nt, nectary tissue; pd; possible pistillode, st, stomata

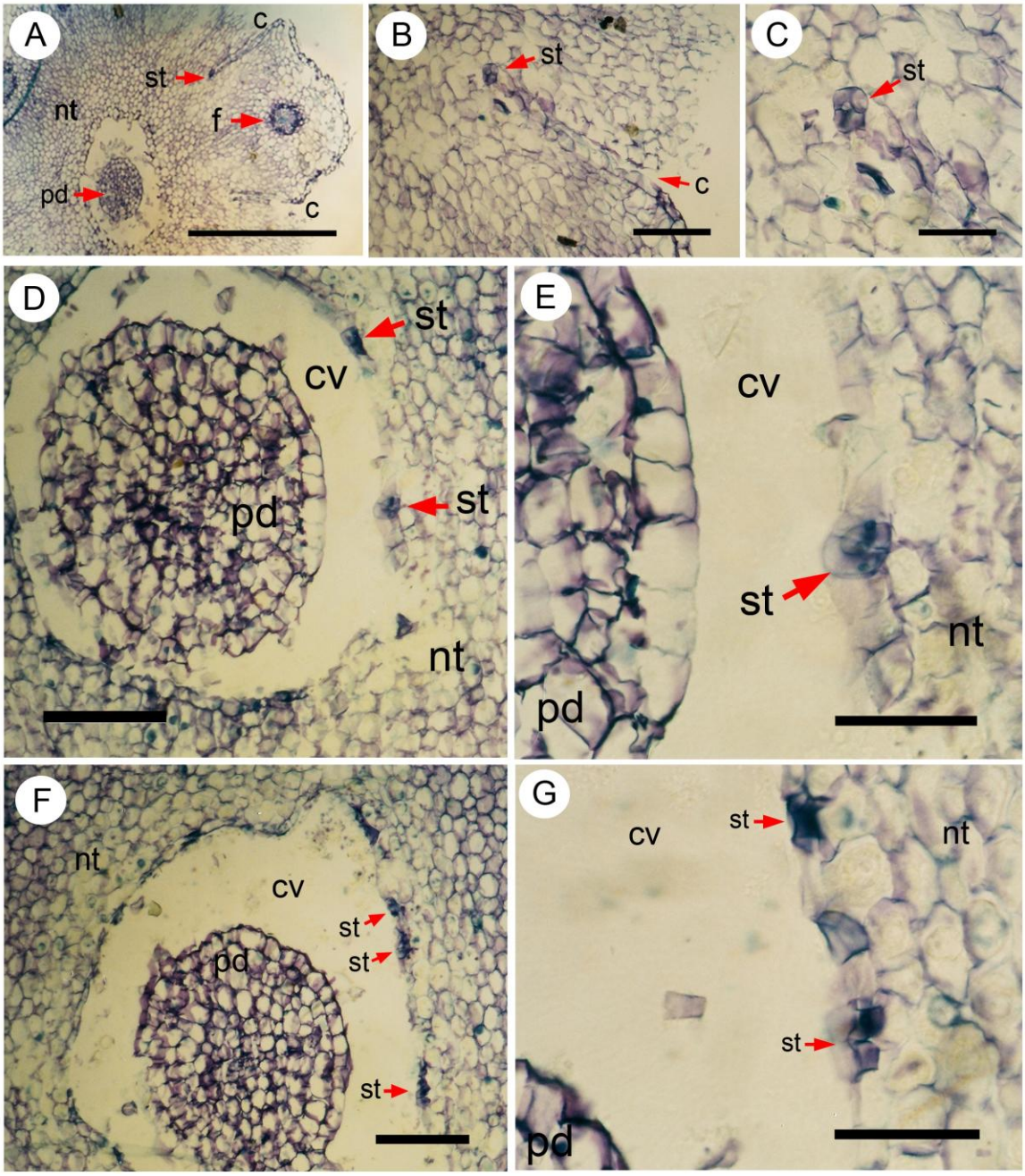

FIGURE 3.9 Light micrographs of staminate flowers of *S. latifolia*. Nectary tissue with stomata in cross-section. **(A)** Section through receptacle of flower. Nectary tissue surrounding the pistillode. Stoma in canal on the receptacle. **(B)** Higher magnification of stoma in canal. **(C)** Higher magnification of stoma in canal. **(D)** Stomata lining the inward facing wall of the receptacular nectary tissue. This inward facing wall is immediately adjacent to the pistillode. **(E)** Higher magnification of stomata lining the inward facing wall of the receptacular nectary tissue. The pistillode is observed as well. **(F)** Stomata lining the inward facing wall of the receptacular nectary tissue. This inward facing wall is immediately adjacent to the pistillode. **(G)** Higher magnification of stomata lining the inward facing wall of the receptacular nectary tissue. Bars = 0.05mm in C, E and G; 0.1mm in B, D, and F; 0.5mm in A. *Abbreviations:* c, canal; cv, cavity; f, filament; nt, nectary tissue; pd, possible pistillode; st, stomata

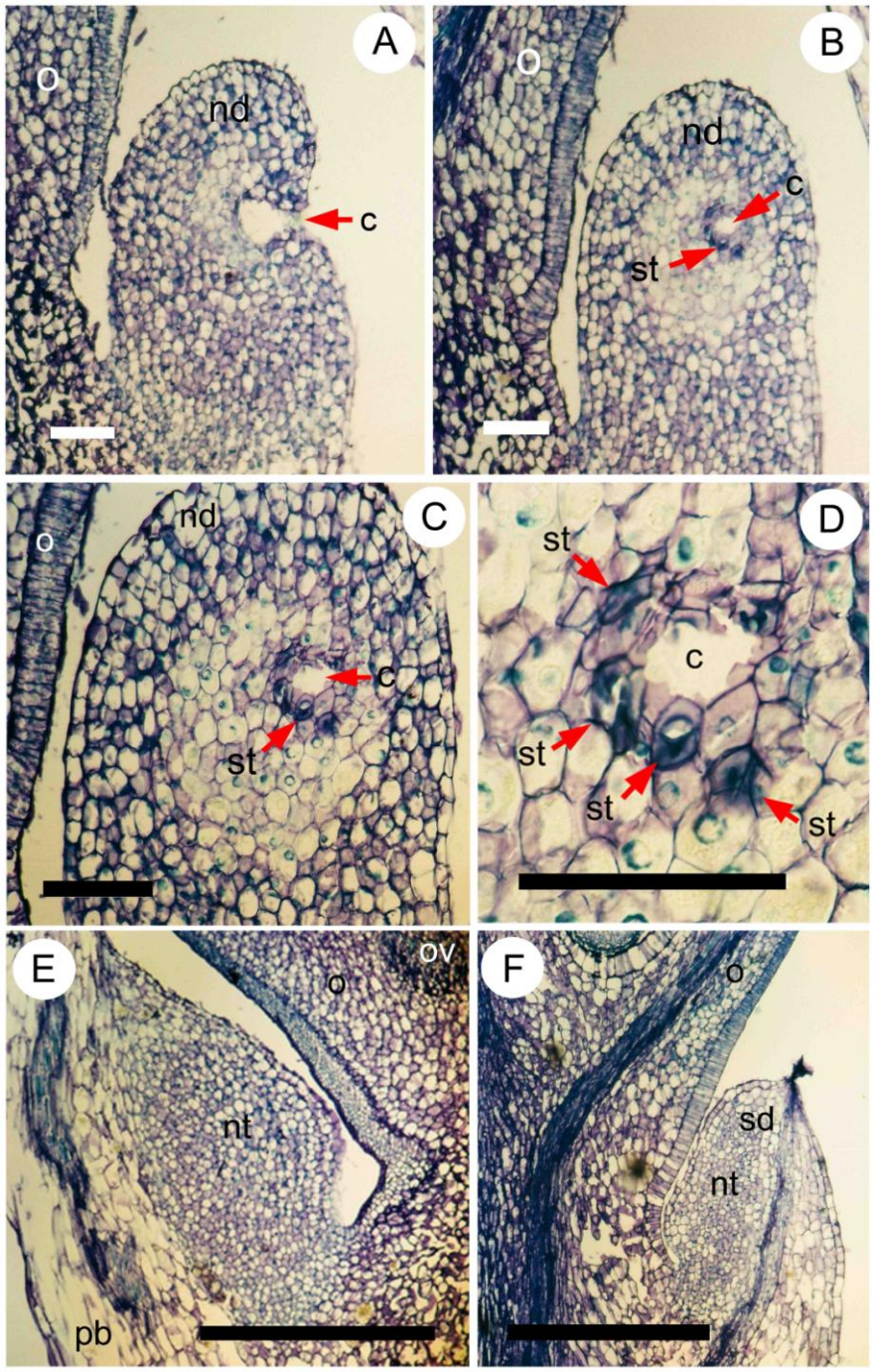

FIGURE 3.10 Light micrographs (LM) of pistillate flowers of *S. latifolia*. Nectary tissue with stomata in longitudinal-section. **(A)** Section through receptacular nectary tissue surrounding the base of the ovary. Canal in outward facing wall. **(B)** Further sectioning through the flower shows canal without opening to outward facing region with stomata in the canal. Nectary tissue is immediately adjacent to the ovary. **(C)** Higher magnification of stomata within canal found in receptacular nectary tissue. Nectary tissue is immediately adjacent to the ovary. **(D)** Higher magnification of stomata in canal. **(E)** Section through petal with nectary tissue at base. Nectary tissue faces in towards the ovary. **(F)** Section through a possible staminate vestigial structure (i.e. staminode) protruding from the nectary disk. Bars = 0.05mm in C, D and F; 0.1mm in E; 0.5mm in A and B. *Abbreviations:* c, canal; o, ovary; ov, ovule; nt, nectary tissue; nd, nectary disk; pb, petal base; sd, possible staminode; st, stomata

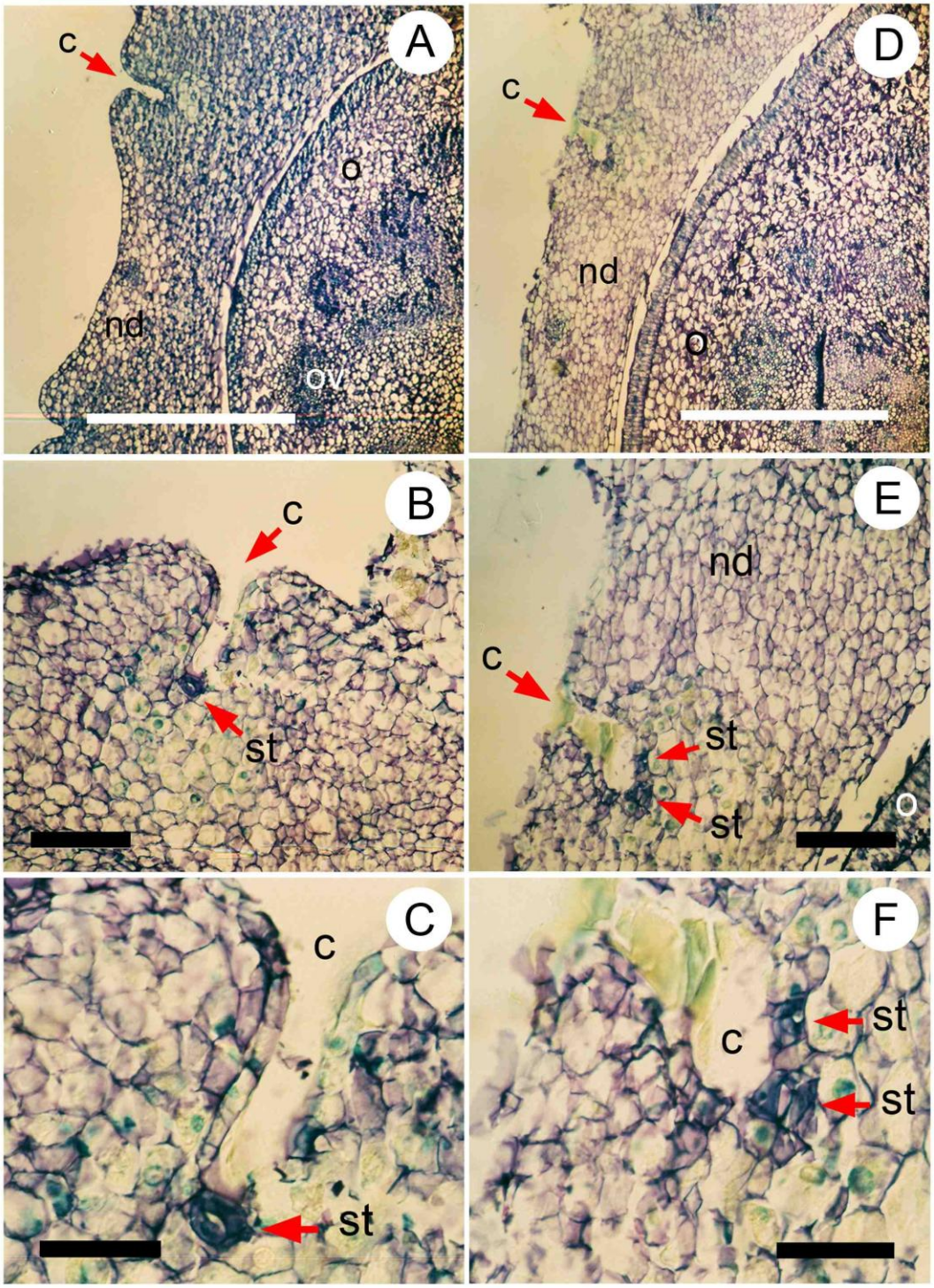

FIGURE 3.11 Light micrographs of pistillate flowers of *S. latifolia*. Nectary tissue with stomata in cross-section. **(A)** Section through receptacle of flower. Nectary tissue surrounding the ovary. Paired canals as indents on the outward facing surface of the nectary disk. **(B)** Higher magnification of stomata in canal on nectary disk. Stoma found near deepest region of the canal. **(C)** Higher magnification of stoma in canal on nectary disk. **(D)** Similar to (A), section through receptacle of flower. Nectary tissue surrounding the ovary. Stomata in canal found in nectary disk. **(E)** Higher magnification of stomata in canal on nectary disk. Stomata found lining the walls of the canal. **(F)** Higher magnification of stomata in canal on nectary disk. Bars = 0.5mm in E; 1mm in C, D, and F; 2mm in A and B. *Abbreviations:* c, canal; o, ovary; ov, ovule; nd, nectary disk; st, stomata

3.3.4.2 Nectary stomata

Stomatal pores in staminate flowers were observed in cross- and longitudinal sections of the nectary tissue (Figure 3.8B-D and 3.9B-G). They were located in two general locations on nectary surfaces. First, stomata were seen lining the epidermal wall of the cup-shaped nectariferous tissue lining the canal that surrounded the pistillode. Glandular tissues were observed immediately adjacent to the nectar slits. Second, stomata were found in paired canals on the receptacle that occurred at the margins of each staminal filament base.

Stomatal pores in pistillate flowers were observed in cross- and longitudinal-sections of the nectary tissue (Figure 3.10B-D and 3.11B-F). They were located in canals or cavities on the receptacle. These cavities occur in pairs and alternate with petals on the flower. Similar observations have been made in pistillate flowers of dioecious *S. dioica* (Rohweder, 1967), which also has paired canals that resemble those found in *S. latifolia*. It was concluded that the stoma-like slits in *S. dioica* secreted nectar to the area surrounding the base of the ovary. However, this observation was made only in pistillate flowers of *S. latifolia*. Our findings in *S. latifolia* support what has been found in pistillate flowers of *S. dioica*.

3.3.5 Vestigial reproductive organs

There was a single pistillode present in staminate flowers; however, there were no fertile carpels in staminate flowers (Figure 3.12A & B). The pistillode was a narrow filamentous structure protruding upwards from the central region of the receptacle in staminate flowers. Terminally, the structure resembled a stigma with possible papillae-like hairs near the tip (Figure 3.12E). The elongate, non-papillate part resembled the style of a pistillate flower. This structure was surrounded by nectary tissue and respective floral whorls (stamens, petals, and sepals) (Figure 3.12D). This finding supports observations made in fresh staminate flowers where a possible pistillode was observed to originate from the centre of the staminate flowers (Figure 3.3B-D). In *S. latifolia*, the

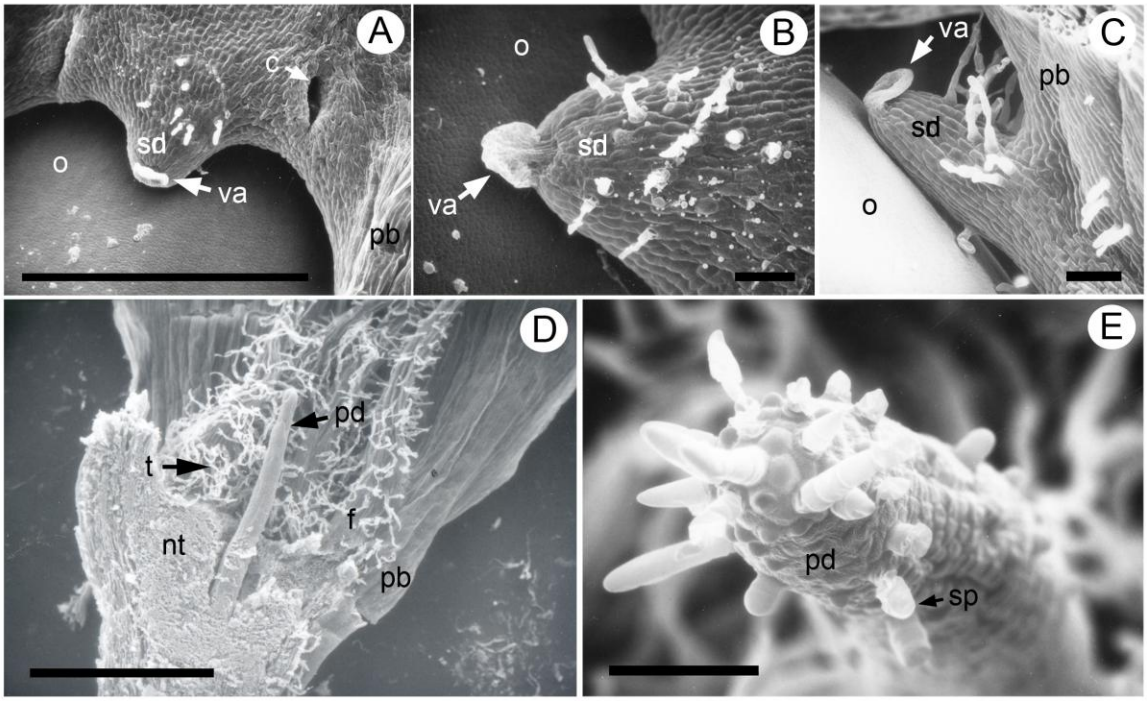

FIGURE 3.12 Scanning electron micrographs of staminodes (A-C) and pistillode(s) (D-E) in *S. latifolia*. A-C: Pistillate flowers—staminode. **(A)** Possible staminode borne between petals in a whorl around the base of the ovary. Paired cavities also observed. **(B)** Higher magnification of possible staminode. **(C)** Possible staminode borne at the base of the petal facing inward and adjacent to the ovary. D-E: Staminate flowers—pistillode. **(D)** Flower dissected in longitudinal-section—stamen and corolla partially removed. Pistillode protruding upward from the centre of the receptacle. The base of the pistillode is surrounded by nectary tissue. **(E)** Higher magnification of a pistillode. Possible stigmatic papillae near the tip of the structure. Bars = 0.1mm in B, C and E; 1mm in A and D. *Abbreviations:* c, canal; f, filament; nt, nectary tissue; o, ovary; pb, petal base; pd, possible pistillode; sd, possible staminode; sp, possible stigmatic papilla; t, filament trichomes; va, possible vestigial anther

pistillode is not made up of nectariferous tissue as is sometimes believed to be the case in dioecy (Decraene & Smets, 1999).

There were ten staminodes present in pistillate flowers (Figure 3.12C, D, E). Pistillate flowers do not have fertile stamens. Two whorls of five rudimentary stamen-like structures were observed on pistillate flowers. One whorl was antipetalous around the base of the ovary (Figure 3.12A-B) and was comprised of nectary tissue on its inner surface (Figure 3.10F), whereas the other whorl was epipetalous and occupies the inward facing side of each petal base adjacent to the ovary (Figure 3.12C). This series of epipetalous staminodes also contributed nectary tissue at the petal bases opposite the ovary base (Figure 3.10E). These conspicuous structures were located in the androecial region of pistillate flowers and are therefore assumed to be aborted stamens (Thomson, 1942). Furthermore, trichomes were observed on the surface of staminodes (Figure 3.12A-C) resembling the filaments of stamens of staminate flowers. Possible vestigial anthers were also observed on staminodes. These features suggest that the vestigial structures observed on pistillate flowers are aborted stamens (i.e. staminodes). Moreover, staminate flowers have two whorls of five stamens, five stamens are arranged in a whorl alternating with petals (antipetalous) on the receptacle, whereas the other five stamens are borne on petals (epipetalous). Staminodes on pistillate flowers were positioned where fertile stamens are within staminate flowers of this species and slightly resemble anthers, but contained no pollen. However, the staminodes in *S. latifolia* did contain nectariferous tissue. These findings support the belief that rudimentary reproductive organs in flowers can become specialized into nectar secreting tissue and thereby, serve a function in pollination biology (Decraene & Smets, 2001).

Both staminate and pistillate flowers of *S. latifolia* have remnants of the opposite reproductive organ in the form of sterile pistillodes or staminodes. These observations support findings in four dioecious species where all flowers (staminate and pistillate) had rudimentary reproductive organs of the opposite sex. In that study, pistillodes sometimes had stigmas that wilted and staminodes often opened but contained no pollen (Kubitzki & Kurz, 1984). Furthermore, the presence of rudimentary female reproductive organs in

staminate flowers may be the result of incomplete suppression of the gynoecium by the Y chromosome in male plants and vise-versa in females.

3.4 Conclusion

Changes in surface morphology of anthers and stigmas occurred over time in *S. latifolia*. In anthers, each theca split longitudinally approximately 12 h before anthesis. In stigmas, papillae elongated, became denser, and in some cases papilla surfaces became uneven and wavy as flower age increased up to 48 h after anthesis. Moreover, staminate and pistillate flowers formed receptacular nectaries, meaning that nectary tissue made up a portion of the receptacle in flowers. Nectar slits (modified stomata) were present on the surface of nectaries in both staminate and pistillate flowers. Finally, staminate flowers had a central rudimentary pistil (pistillode) and pistillate flowers formed rudimentary stamens (staminodes).

The morphological changes that occurred in stigmas of *S. latifolia* over time are a new discovery. Furthermore, nectary tissue location and presence of nectary slits (modified stomata) have not been documented in this species until now. Finally, the observation and characterization of vestigial organs in both staminate and pistillate flowers of *S. latifolia* are new discoveries.

4.0 POLLINATION EXPERIMENTS

4.1 Introduction

Pollination is important for the success of *S. latifolia* as a weed (McNeill, 1977). Dioecious plant species are self-incompatible obligate out-crossers (Richards, 1997) and many are insect-pollinated (Proctor et al., 1996). *Silene latifolia* relies almost exclusively on seed production for reproduction (McNeill, 1977) and is mainly pollinated by nocturnal moths and diurnal bees (Young, 2002).

The ability of a plant species to colonize an area is related to its mating system. A well-established hypothesis known as "Baker's rule" suggests that self-compatible plants are more successful invaders compared to self-incompatible species (e.g. dioecism). In self-compatible plant species, one plant can initiate a sexually reproducing colony; whereas in self-incompatible plants, one plant cannot initiate a sexually reproducing colony on its own—it needs a partner. This makes successful establishment of self-incompatible species less likely (Baker, 1955). Clearly, the reason for this is the inability of a self-incompatible plant species to self-pollinate. Therefore, invasiveness of self-incompatible plant species may be limited due to pollination restrictions (Baker, 1955; Petanidou et al., 2011).

Pollen limitation occurs when plants produce less seed than they would if sufficient pollen quantity were deposited on receptive stigmas (Knight et al., 2005; Ashman et al., 2004). Pollination limitation may hinder seed production, and as a result slow population growth rate (Davis et al., 2004). The risk of pollination limitation can be especially high in fragmented habitats where individual plants are isolated from a larger population. The amount of pollination limitation that occurs as a result of habitat fragmentation depends on the area of habitat loss and/or isolation of the individual plants (Knight et al., 2005; Wilcock & Neiland, 2002). Pollen limitation was observed in four dioecious plant species dependent on insect pollination (de Jong et al., 2005). They concluded that the occurrence of pollination decreased when the distance between male and female plants increased. However, the furthest distance between females and the pollen source in all studies was only measured to a maximum of 15m. Kay et al. (1984) obtained similar results in the

dioecious species, *Silene dioica*. Based on the results of both studies, *S. latifolia* may also be subject to pollination limitation due to its dioecious breeding system.

Few studies exist that consider the effects of distance on pollen limitation in potentially invasive dioecious weed species such as *S. latifolia*. Young (2002) determined that noctuid moths were the most effective pollinator of *S. latifolia* in Colorado. However, nothing is known of the distance-dependent pollen limitation in this species or in dioecious plants in general. Characterizing the pollination ecology of *S. latifolia* specific to western Canada may provide a model for other dioecious plants and help to evaluate the effect of diocey on pollination limitation and its potential to affect invasiveness.

The hypothesis of this study is that *Silene latifolia* is pollen limited due to the dioecious nature of the species. The objectives of this study were (1) to verify that *Silene latifolia* is primarily insect pollinated, (2) to determine *when* pollination occurs, and (3) determine the effect of distance on pollen limitation.

4.2 Materials and methods

4.2.1 Pollinator exclusion trial

4.2.1.1 Experiment design and location

This experiment was conducted in 2009 and 2010 near Meath Park, SK (53°18’36.53” N, 105°20’17.74” W). Treatments were set up in a randomized complete block design. Insect exclusion treatments were applied to single female plants. Insects were excluded, non-excluded, and sham-excluded. Treatments were replicated eight times in 2009 and four times in 2010. In 2010, a fourth treatment was added where single male and female plants were excluded from insect visits together. Male and female plants in this experiment were part of a naturally occurring *S. latifolia* population within a farmer’s field where *Pisum sativum* L. (peas) and *Brassica napus* L. (canola) were grown in 2009 and 2010, respectively.

4.2.1.2 Experimental procedures

Plants to be used for treatments were identified as female. Then, any open flowers were removed before treatments were applied in order to ensure that no pollination occurred before treatment application. Female plants were selected based on their relatively close physical proximity to one another (within 4m).

Exclosures were built around female plants for exclosure, sham-exclosure, and male and female combined exclosure (in 2010) treatments. These exclosures were constructed using four wooden stakes, measuring 125cm (height) by 4cm (width) by 4cm (width), as the frame. Stakes were forced approximately 30cm into the ground to form a 100cm (height) by 50cm (width) by 50cm (width) wooden frame centered on individual female plants.

For the exclosure treatment, female plants were fully surrounded by black fibreglass insect screening (mesh size 0.51 x 0.67mm) to exclude possible insect pollinators. The screen was stapled to the wood along the height of the exclosures and stapled together around the perimeter of the exclosure top in order to effectively exclude large insects. The edge of the screen was then buried 15-20 cm under soil to prevent insect intrusions near the exclosure base.

For the sham-exclosure treatment, the north facing side of the exclosure was left uncovered. These exclosures were designed to expose plants to insect pollinators, while partially controlling for shading as a limiting factor in seed production. In addition, sham-exclosures could eliminate the physical presence of the exclosure as a possible deterrent for pollinators.

For the non-exclosure treatment, female plants were left fully exposed.

On July 9th, 2010, between 14:00 and 16:00 h, incident light was measured using a Quantum Meter® at all experimental sites both under the fiberglass screen (where applicable) and in direct sunlight.

4.2.1.3 Data collection

In 2009, exclosures were constructed June 24th and plants were harvested on August 27th. In 2010, treatment exposure commenced June 25th and plants were harvested August 24th. This protocol allowed adequate time for pollination to occur and for flowers to set seed and ripen within respective treatments. Following the treatment period, both ripe and immature seed bearing capsules were removed and placed in envelopes. Following removal of capsules, entire plants were removed at the soil surface. Plants were dried in an oven at 70°C, within 3 h of harvest, for approximately 48 h to allow for adequate drying of the plant material. Following drying, whole dried plants were weighed for biomass readings. Seed capsules were air-dried and seeds were separated from respective capsules and counted by hand, then weighed and recorded.

4.2.2 Pollination timing trial

4.2.2.1 Experiment design and location

This experiment was conducted in 2009 and 2010 in Saskatoon, SK (52°06’31.36” N, 106°42’25.11” W). Treatments were arranged in a randomized complete block design replicated four times. Exclosure treatments were applied to individual female plants. Treatments were exclosure, non-exclosure, night-exclosure, and day-exclosure (Figure 4.1). The experimental site was a grassy area composed partly of a naturally occurring population of both male and female *S. latifolia* plants. Male plants served as the pollen source during treatment application. Female plants for the experimental treatments were transplanted approximately 2m from the pollen source.

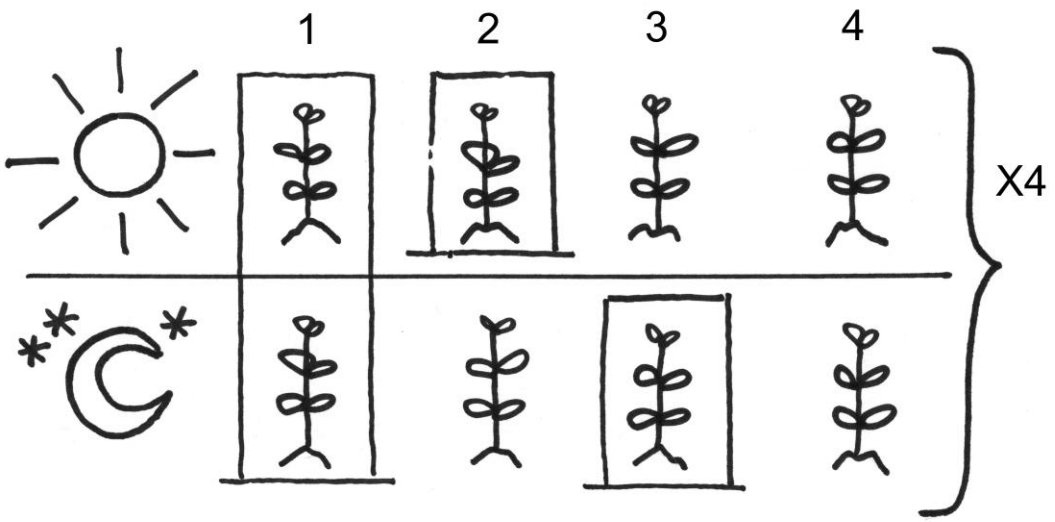

FIGURE 4.1 Pollination timing trial design: Depiction of pollination timing trial treatments. Treatments to the right of the sun are female plants during the day and treatments to the right of the crescent moon are female plants during the night. Plants vertically adjacent to each other (separated by the long horizontal line) represent the same plant at different times (day or night). Boxes surrounding plants represent a structure designed to exclude insect visitors. Moving from left to right are (1) exclusion, (2) day-exclusion, (3) night-exclusion, and (4) non-exclusion treatments.

4.2.2.2 Experimental procedures

Unpollinated female plants were grown in a controlled growth environment prior to introduction to the experimental location. These female plants were grown from seed and allowed to reach flowering so identification of sex could be made prior to introduction into the experimental environment. Each female plant was grown in a single 15 X 18 cm pot using No. 4 Sunshine® Potting Mix. Plants were grown under 18 h of light at 22°C and 6 h of dark at 16°C for approximately 35 days. Light intensity was 1185μmol m^{-2} s^{-1} in the chamber and was measured using a Quantum Meter® at the top of the plant canopy. Soil cores were removed from the experimental site to fit the potted plants. Each pot bottom was removed and plants were transplanted into the site. This transplant procedure was done to allow plants to derive moisture and nutrients from the soil. Watering was done weekly at the site for approximately 3 weeks until plants were acclimated to outdoor conditions and roots were presumably established in the soil. Plants for the exclosure treatment had the exclosures built (see section 4.2.1.2) at the time of transplanting. Plants for the day-exclosure treatment were introduced then covered with moveable exclosures. Four moveable exclosures were built for this experiment for day-exclosure and night-exclosure treatments. The moveable exclosures prevented insect visits during the day (day-exclosure) and during the night (night-exclosure). These treatments required twice-daily moving of the exclosures just prior to twilight and one hour prior to sunrise (Dreisig, 1986). Moveable exclosures that covered female plants during the day were moved in the evening to cover female plants during the night plants and vise-versa to uncover the opposing treatment. Exclosure move times were adjusted daily to account for shortening day length. Following the treatment application period, exclosures were built around the non-exclosure and night-exclosure treatments to discontinue pollinator visitations to pistillate flowers and allow ripening to occur. Prior to exclosure construction, all open flowers exposed to the treatments were affixed with tags around the flower stem base for later harvesting. Tagging flowers prevented confusion during capsule harvest, as *S. latifolia* flowers indeterminately and new flowers would have opened after caging. Flowers were left to ripen until visual indications of physiological maturity were present i.e. capsule hardening and subsequent colour change.

4.2.2.3 Data collection

In 2009, treatments were applied commencing July 10th and all plants were fully excluded on July 22nd and in 2010, on July 9th and July 24th, respectively. This treatment period allowed adequate time for pollination to occur. Sufficient time was then allowed for seeds to ripen in the fully exclosed, experimental plants of respective treatments. Flowers and entire plants were harvested following the maturation period as mentioned in the previous experiment. In addition, seed count and biomass data were recorded as previously described (see section 4.2.1.3).

4.2.3 Pollination distance trial

4.2.3.1 Experiment design and location

This experiment was conducted during the 2009 and 2010 growing seasons near Prince Albert, SK at the Conservation Learning Centre (53°01'43.17" N, 105°45'53.12" W). There were six distance treatments for this experiment. Distance treatments were the distance between females and the pollen source (males). Distances were measured along a linear interval at 4m, 8m, 16m, 32m, 64m, and 128m transects. The linear interval was measured running directly south in a commercial annual grain crop field. The linear interval began at the edge of a perennial forage crop, which consisted partly of a natural population of male and female *S. latifolia* plants. The natural population served as a both a reference point and pollen source for the trial.

4.2.3.2 Experimental procedures

Thirty female plants were introduced into this site from the controlled growth environment as previously described. In 2009 and 2010, female plants were transplanted into canola and *Avena sativa* L. (oat) crops, respectively. Introduced females were not exposed to pollen or pollinators prior to treatment application in the natural setting. Five female plants were transplanted at each of the six distance transects. Transplants at each distance were spaced one metre apart and arranged in a single file row perpendicular to the linear interval. This design was used in order to maintain accurate distance from the

pollen source at each distance. Each female plant was grown as previously described. In both years, a 60 cm area surrounding each transplant was cleared by uprooting the canola or crop to reduce interspecific competition. The transplanted plants were covered with clear plastic bags for less than 24 h to prevent damage during herbicide application to the grain crop. Furthermore, the annual cropping area was surveyed weekly to ensure no other pollen source was present (i.e. other *Silene* species). This surveying procedure ensured pollen traveling to respective distances was from the designated pollen source.

4.2.3.3 Data collection

In 2009, female plants were transplanted on July 8th and plants were harvested August 22nd. In 2010, female plants were transplanted on June 23rd and plants were harvested August 26th. This protocol allowed sufficient time for pollinators to remove, transport, and deposit pollen from staminate to pistillate flowers given the experimental conditions. Once capsules started ripening (approximately 28 d after treatment initiation), they were removed weekly to prevent seed loss. Following the treatment period, both ripe and immature seed bearing capsules were removed and placed in coin envelopes. Entire plants were also harvested for biomass determination. Methodology for data collection was the same as described earlier in the pollinator exclusion trial.

4.2.4 Statistical analysis

Analysis for both the pollinator exclusion trial and the pollination timing trial was very similar. Seed counts were log transformed to equalize variance. Analysis was performed using Analysis of Variance (ANOVA) using SAS Proc Mixed (SAS Institute, 2008). All exclosure treatments were considered fixed in the analysis, whereas block and year were considered random factors. For the pollinator exclusion trial, 2009 and 2010 were analyzed separately because a fourth treatment was added in 2010; therefore, years could not be combined. The pollination timing trial was analyzed by year due to differences in the environment (Table 4.1).

Data for the pollination distance trial was tested for significance by year using nonlinear regression analysis of curves and model parameters using the *multdrc* and *compParm* extension packages in R (Version 2.6.1). In this analysis, global regression and parameters were compared to individual years for each variable tested. This analysis was done by combining years for each variable and then comparing years individually to the global values. The relationship between variables and distance was fit using the 2-parameter power relationship shown:

$$y=a\chi^{b} \qquad [4.1]$$

In this equation, *y* is the dependent variable (seed number or capsule number), *a* is the y-intercept, χ is the independent variable (distance), and *b* indicates the slope of the line (negative in this case). Where no difference was observed between years for the variable tested, years were combined. A line of best fit was then calculated using parameters from the global model to predicted values to describe both years of data.

4.3 Results and discussion

4.3.1 Pollinator exclusion trial

In both years, there was no seed production when pollinators were excluded (Figure 4.2A-F). In contrast, all other treatments resulted in seed production and differed from the exclosure treatment ($P<0.05$). This finding establishes that *S. latifolia* relies on insects to carry pollen from staminate flowers to pistillate flowers and supports findings by Young (2002) who found that in Colorado, *S. latifolia* is pollinated by insects.

The sham-exclosure treatment reduced seed number per plant and seed number per capsule compared to the non-exclosure treatment in 2009 only (Figure 4.2A and C). Furthermore, the sham-exclosure treatment did not significantly reduce the number of flowers pollinated per plant compared to the non-exclosure treatment (Figure 4.2E and F). It is possible that this treatment may have partially impeded pollinators, as only the north facing sides of sham- exclosures were open. If pollinators approached from all directions,

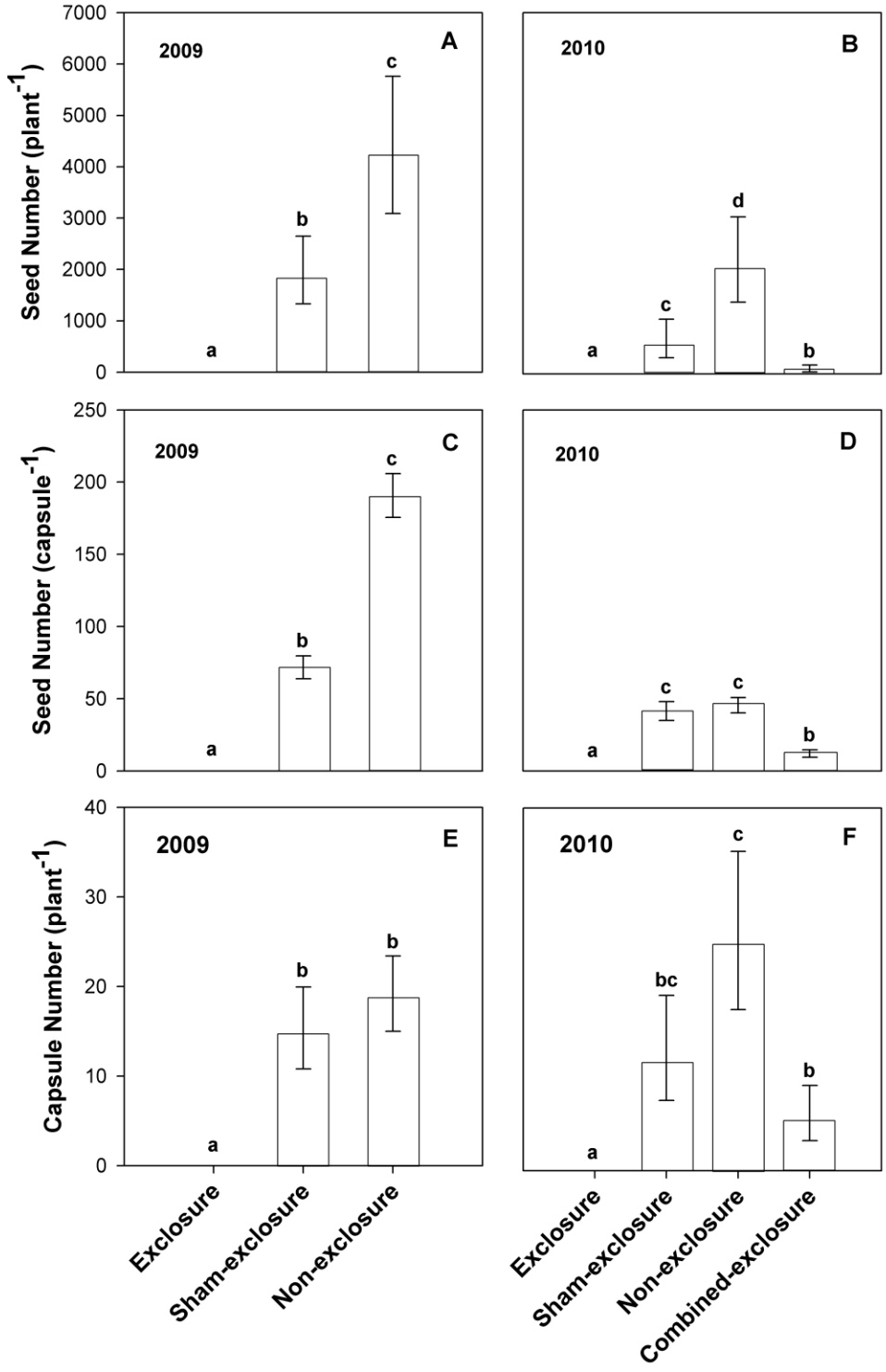

FIGURE 4.2 Pollinator exclusion trial: Seed number per plant, seed number per capsule, and capsule number produced per plant produced according to each exclosure treatment in 2009 and 2010. Within each histogram, bars sharing a letter are not significantly different (P>0.05) (least square means). Data and error bars are back transformed.

sham-exclosures may have obstructed three-quarters of pollinator visits. This obstruction may have reduced seed production in sham-exclosure plants.

Combined-exclosure treatment (male and female combined) resulted in fewer seeds per plant and per capsule than sham-exclosure and non-exclosure treatments, but similar capsule number per plant as the sham-exclosure treatment (Figure 4.2B, D, and F). In 2010, when male and female plants were in the same exclosure, some pollination occurred and seed set differed significantly from the exclosure treatment. It was assumed that any seed production that occurred in the combined-exclosure treatment was the result of wind pollination. The incidence of wind pollination was minimal and therefore the number of capsules pollinated per plant in the combined-exclosure treatment was lower than the number of capsules pollinated in the non-exclosure treatment, where insect visitation to flowers was permitted.

Reduced seed production per flower between sham-exclosure and non-exclosure treatments in 2009 may be an indication of reduced deposition of pollen by pollinators (Wilcock & Neiland, 2002). A pollinator must effectively remove, transfer, and deposit pollen onto a receptive stigma in order for pollination and subsequent ovule fertilization to occur (Faegri & van der Pijl, 1971). With bees, the frequency and duration of floral visits may determine how much pollen is removed (Davis, 1997) or in this case deposited. Fewer pollen grains deposited would result in fewer seeds produced per flower. This finding was otherwise undetectable by analyzing differences in seed number per plant between exclosure treatments alone. In general, this result agrees with seed produced per plant, as higher or lower seed production per flower would increase or decease total seed production per plant, respectively.

Capsule number per plant was comparable between years; however, there was less seed production per plant and per flower in 2010 when compared to 2009. This reduction in seed production may be due to the heavy rainfall that occurred in 2010 that may have reduced pollinator activity and effectiveness (Table 4.1).

TABLE 4.1 Weather data table: 1971-2000 data obtained from Environment Canada (2010).

		Rainfall			Temperature		
Location	Month	**2009**	**2010**	**30-yr average**	**2009**	**2010**	**30 yr-average**
		----------mm----------			----------°C----------		
Saskatoon	April	2.8	72.6	15	2.9	6.9	4.7
	May	6.9	128.5	41.5	8.7	9.7	11.8
	June	75.5	169.0	60.5	14.8	15.3	16.0
	July	50.3	46.0	57.3	15.8	17.6	18.3
	August	82.4	43.7	35.4	15.9	16.2	17.6
	Total	217.9	459.8	194.7	-	-	-
Prince Albert	April	2.8	105.2	16.6	2.0	6.0	3.1
	May	37.7	81.2	44.3	7.9	9.6	10.5
	June	70.4	128.0	72.5	14.6	15.7	15.2
	July	92.4	92.2	76.8	16.3	18.0	17.5
	August	67.8	26.4	58.0	15.8	16.5	16.3
	Total	271.1	433	251.6	-	-	-

4.3.2 Pollination timing trial

Pollinator exclosures affected the number of seeds produced per plant, and per capsule, and capsule number per plant (P=<0.001; Figure 4.3). As observed in the previously described pollinator exclusion trial, there was no seed production in either year when pollinators were excluded. In 2009, the exclosure treatment differed from all other treatments whereas in 2010, the exclosure treatment differed from all other treatments except the night-exclosure treatment.

Excluding pollinators during the day did not affect seed production compared to non-exclosed plants in both years (Figure 4.3). The timing of pollinator exclusion affected seed production in 2010 where almost no seeds were produced in the night pollination exclosure treatment. In contrast, seed production in the day-exclosure treatment did not differ from the non-exclosure treatments in 2009 and 2010 for all variables tested indicating that night pollination occurred almost exclusively (Figure 4.3). This suggests night pollinators were responsible for the majority of pollination in 2010 only.

In 2009, seed production per capsule was significantly lower in the night-exclosure treatment compared to the non-exclosure treatment (Figure 4.3C). This outcome may indicate lower pollinator efficiency in only night-exclosure treatments (i.e. day-pollinated flowers). This result supports findings by Young (2002) who concluded that nocturnal moths to be the most effective pollinators of *S. latifolia* compared to diurnal bees and flies.

In 2009, more seed produced was per plant and per capsule but from fewer capsules when compared to 2010 (Figure 4.3A and C). Therefore, pollinators in 2009 may have visited flowers more frequently and for longer periods of time when compared to 2010 (Davis, 1997).

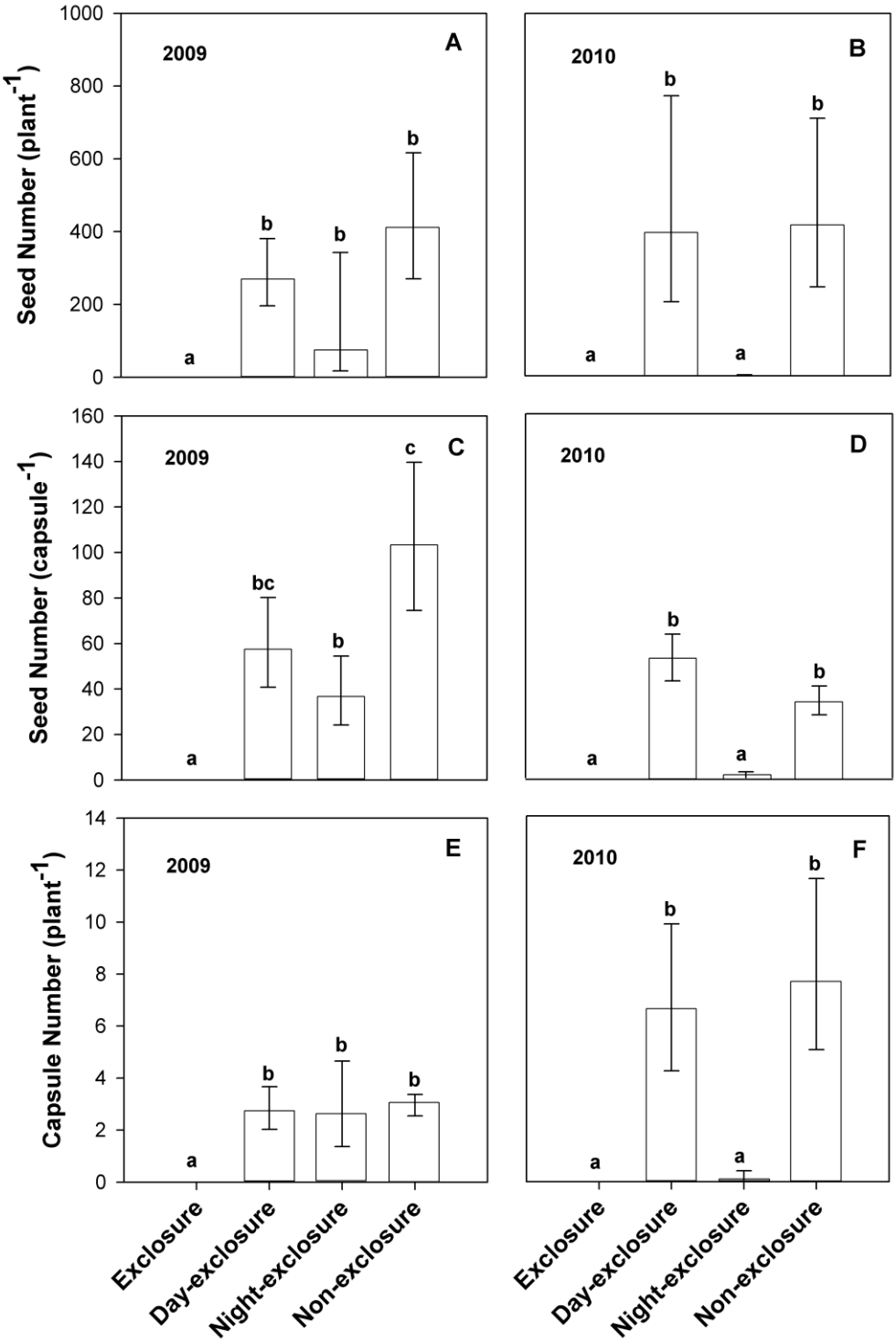

FIGURE 4.3 Pollination timing trial: Seed number per plant, seed number per flower, and capsule number produced per plant produced according to each exclosure treatment in 2009 and 2010 (left to right respectively). Within each histogram, bars sharing a letter are not significantly different (P>0.05) (least square means). Data and error bars are back transformed.

Excessive precipitation in 2010 may have reduced pollinator effectiveness, thereby reducing pollination and seed production. In 2010, total rainfall was more than double the average and during the treatment period (July 9th – July 24th) rainor severe thunderstorms were observed on 50% of the days (Table 4.1). Excess rainfall may have restricted the activity of daytime pollinators (Corbet, 1990), as there was a considerable reduction in seed production for night-exclosed plants (i.e. day-exposed). Rainfall can cause irreparable damage to anthers and pollen thereby negatively affecting pollen removal, deposition, or germination (Corbet, 1990). Furthermore, dilution of nectar by free water may interfere with important plant-insect interactions. However, given that seed production was reduced only in the night-exclosure (i.e. day-exposed) treatment, reduced daytime pollinator activity probably caused reduced seed production.

Finally, noctuid moths were captured at the pollination timing experimental site. Moths were observed visiting *S. latifolia* plants. Observation of moths at this site helped to solidify that *S. latifolia* is insect-pollinated.

4.3.3 Pollination distance trial

In both years, seeds per female plant declined with distance from the pollen source patch (Figure 4.4A). There was a difference between years in the non-linear regression that described the relationship (P= 0.0044). The slope of decline in the number of seeds per plant (parameter b) was greater in 2009 than in 2010 (P= 0.0036). The intercept (parameter a) also differed between years (P= 0.0016), possibly indicating that there were differences in pollination close to the edge of the patch. However, this regression predicted that in 2009 and 2010 plants at 0 m (next to the pollen source) would produce 18,693 and 3214 seeds, respectively. It seems evident that insects were less likely to carry pollen great distances from staminate to pistillate flowers.

The number of seeds produced per capsule did not differ between years (P= 0.1338) and was described by a power law non-linear regression (Figure 4.4b). The regression predicts that the number of seeds per capsule will decline slightly with distance from the pollen source. The lack of difference between years for number of

seeds produced per flower may indicate that pollinators were equally effective at all distances and in both years. As there was a decline in the number of seeds produced per flower with distance, seeds produced per flower may not have been affected by pollinator competition. Thus, once *S. latifolia* flowers had been located by pollinators, duration of visit would be independent of distance or crop. This result suggests that pollinators deposited equal quantities of pollen at each distance regardless of other factors (such as crop). One way this outcome could have been accomplished is by comparable visit duration of insect pollinators (Davis, 1997) at each distance (i.e. the act of landing versus hovering moths). Therefore, night-flying, settling moths, may be what is important here.

The number of capsules per plant was lower in 2009 than in 2010 and declined with distance at a greater rate ($P = 0.0078$; Figure 4.4c). Thus, the number of flowers pollinated per plant resulted in differences between the years in seed number per plant because seed number per capsule did not differ.

The different crops present in 2009 and 2010 may explain the difference between years for seed number per plant and capsule number per plant. In 2009, the crop surrounding the experimental female plants of *S. latifolia* was canola, which flowers indeterminately and is primarily pollinated by honeybees (Sabbahi et al., 2005). In contrast, in 2010 the crop was oat, which is self-pollinated. The presence of canola co-flowering insect-pollinated species, could have created pollinator competition in 2009 (Campbell, 1985; Knight et al., 2005). Co-flowering competition likely occurred during the day because flowering of *S. latifolia* and canola was observed to overlap. Furthermore, honeybees are diurnal insect pollinators and have previously been observed to be mainly responsible for day-pollination of *S. latifolia* (Young, 2002). The previously described pollination timing trial found no difference between seed production in day- and night-exclosed plants. Therefore, having a co-flowering insect-pollinated species present may have decreased pollination of *S. latifolia* overall during the day. There are no reports of nocturnal pollination of canola flowers, so pollinator competition may not have occurred at night. Overall, it is possible that diurnal pollinators neglected *S. latifolia* in the presence of numerous canola flowers causing less seed production in *S. latifolia* at further distances from the pollen source.

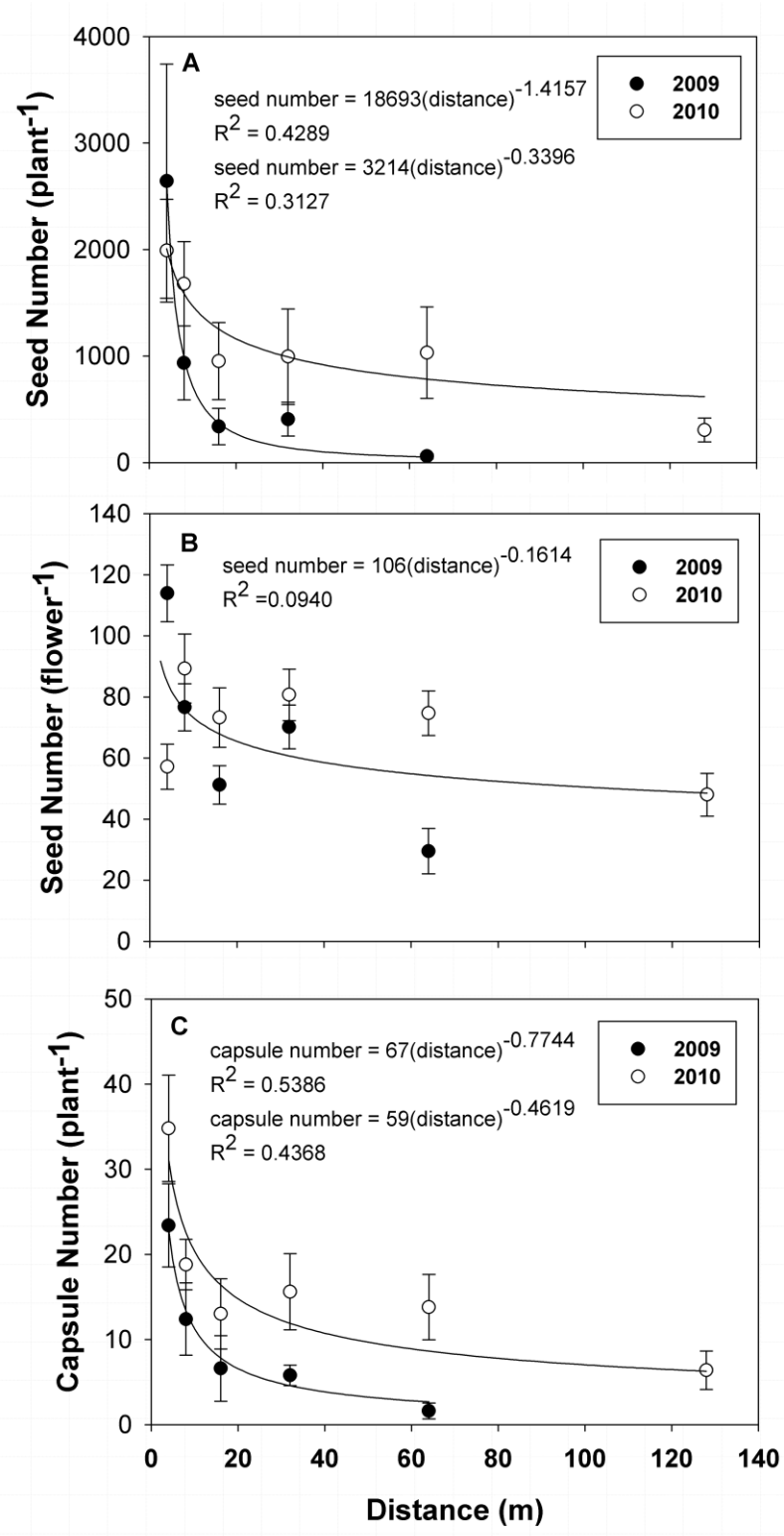

FIGURE 4.4 Pollination distance trial: Distance-dependent effects on pollination in *S. latifolia*. (A) Seed number per plant, (B) seed number per capsule, and (C) capsule number per plant produced at each distance in 2009 and 2010.

Pollination limitation has been reported where two dioecious species in the same area reach anthesis simultaneously (Campbell, 1985). As a result, separate species compete for pollinators. Campbell (1985), reported pollinator sharing in the understory herbaceous species *Stellaria pubera*. Pollinators of *S. pubera* are also known to visit *Claytonia virginica*. When populations of both species were in the same area, seed production of *S. pubera* was decreased. This result suggests some preferential selection of flowers by insects. When flowers of *C. virginica* were removed from the area, seed production in *S. pubera* increased. It was concluded that lower seed production in *S. pubera* in the presence of *C. virginica* was a result of pollinator sharing and not competition for physical resources. A similar situation could have occured in *S. latifolia* in 2009, where canola was the co-flowering species.

Pollination limitation has also been reported in the dioecious plants, *Valeriana dioica*, *Salix repens*, *Asperagus officinale*, and *Bryonia dioica* (de Jong et al., 2005). In that study, plant species were tested for distance-dependent pollination limitation. Female plants were placed from 0 m up to 25 m from a compatible pollen source. In all species, at increasing distance from the pollen source, pollination was limited, possibly due to fewer visits or visits of lower quality by insects.

Lower seed production per plant at further distances in 2009 may also be explained by pollination failure due to the presence of heterospecific pollen from the canola crop. Heterospecific pollen can reduce fertilization and seed production because of chemical or physical inhibitors present during pollen germination (Wilcock & Neiland, 2002). As oat is a self-pollinated cereal crop lacking conspicuous flowers and floral rewards, there should have been no pollinator competition in 2010.

TABLE 4.2 *P*-values from non-linear regression ANOVA in **R**. Site year data compared to global data for each variable. Equation $y=ax^b$

	Model compared	Parameters compared	
		Intercept (a)	Slope (b)
Variable	Site-year : global	Site-year : global	Site-year : global
Seed number ($plant^{-1}$)	0.0044	0.0016	0.0036
Seed number ($capsule^{-1}$)	0.1338	0.0558	0.1154
Capsule number ($plant^{-1}$)	0.0078	0.2122	0.8023

4.4 Conclusion

Silene latifolia is pollen limited due to the dioecious nature of the species. It is mainly insect pollinated with only minimal occurrence of wind pollination when males and females are in very close proximity. *Silene latifolia* is mostly dependent on night pollination but it can be pollinated during the day. Finally, female plants at further distances from the pollen source produce less seed compared with female plants closer to the pollen source probably because insects were less likely to carry pollen further distances.

From the results of these experiments it seems evident that *S. latifolia* may exhibit limited invasiveness as a weed. Due to the self-incompatible nature of the species, seed production depends on the relative proximity of male and female plants. In a natural setting, where males and females are separated by distances greater than 8 m, seed production can be limited.

5.0 GENERAL DISCUSSION

Seed production in *S latifolia* is pollen limitated. Pollination, and thereby seed set, is limited with greater than 4 m of spatial separation between sexes in this species. Our study verifies the longstanding hypothesis known as "Baker's rule" which indicates the improbability of a single individual of a self-incompatible plant species establishing a sexually reproducing colony (Baker, 1955). Thus, sexual reproduction requires two self-incompatible individuals (in our case male and female) in order to establish a colony. Baker's rule suggests that floral self-compatibility, rather than self-incompatibility, promotes the invasiveness of a plant species. Therefore, according to Baker (1955) and the findings of our study, *S. latifolia* may exhibit limited invasiveness. Male and female plants must be in close proximity spatially and temporally for any seed production to result. The reliance of this species on insect pollinators and the dioecious nature of the species, limit its invasiveness in the event of chance long-distance separation between individual seeds.

Another reason for the relatively poor success of *S. latifolia* as a weed, in addition to the presence of pollination limitation, may be its lack of an effective seed dispersal mechanism. Most new invasions in the species are from contaminated forage seed such as alfalfa (Hastings & Kust, 1970a; McNeill, 1977; Royer & Dickinson, 1999). Therefore, seed dispersal is passive in *S. latifolia* and occurs mainly as a result of human intervention through movement of its seed with forage seed. This factor may limit the ability of *S. latifolia* to actively invade new areas.

The findings of our research indicate that *S. latifolia* has some important reproductive limitations. However, there is evidence in the literature to suggest that *S. latifolia* is an aggressive weed (McNeill, 1977; Blair & Wolfe, 2004). *Silene latifolia* is an obligate out-crosser and thereby relies almost entirely on insect pollinators for the proliferation of its species. In wet and cool years when the weather is unsuitable for regular pollinator movement, there may be reduced seed production. Because the reproductive success of *S. latifolia* relies heavily on the activity of pollinators that can be restricted by cool wet weather, there are important reproductive limitations that will limit its success—this constraint is especially so for diurnal pollination of the species. Furthermore, seed production per flower was very inconsistent in the species. These findings are possibly a manifestation of inconsistencies in pollen removal and pollen deposition by pollinators of *S. latifolia*.

In contrast, important morphological changes occur over time in anthers and stigmas to encourage reproductive success in the species. Pollen is released from anthers within a 36 h time period and structural changes occur in stigmatic papillae to facilitate successful pollen capture (Edlund et al., 2004). In addition, pollen release in male plants is an continual event due the indeterminate nature of the species. This fact could be advantageous for dioecious species where successful pollination may be limited and seed set is important for successful reproduction of the species.

Vestigial reproductive structures of the opposite sex were found in staminate and pistillate flowers of *S. latifolia.* Upon further observation, it seems evident that the region where female reproductive organs would have developed in staminate flowers and the

region where male reproductive organs would have formed in pistillate flowers have become specialized to perform functions in pollination biology (eg. nectary tissue).

Moreover, nectary slits (modified stomata) are present in both staminate and pistillate flowers. In staminate flowers, nectary stomata are located on the receptacle in the epidermal layer in two general areas. In pistillate flowers, nectary stomata line the walls of paired cavities on the receptacle. Nectary stomata have not previously been documented in this species. Images taken using the dissection microscope provide initial evidence to suggest nectar secretion was occurring from the regions heretofore mentioned. However, it cannot be concluded that *all* nectar is secreted though the observed slits.

Our findings make an important contribution to our understanding of the dynamics of nectar production in *S. latifolia*. It is hypothesized in the literature that certain floral characteristics attract a certain spectrum of insects. This concept is known in the scientific community as pollination syndrome (Fenster et al., 2004). Research has been done to build a conceptual framework to match specific floral characteristics with pollinator type (Fenster et al., 2004). For example, day- and night-active Lepidoptera are generally attracted to flowers that produce large amounts of nectar near the base of a long corolla tube in flowers (eg. Caryophyllaceae) (Witt et al., 1999). Our findings may help to support the pollination syndrome hypotheses by our locating nectary stomata in *S. latifolia.* Nectar slits (i.e. modified stomata) generally secrete nectar (Weberling, 1989) to regions of the flower accessible to insects. However, not all floral nectaries (or nectary stomata) serve a function in pollination biology (Weberling, 1989). In *S. latifolia,* nectary stomata were located near the base of both staminate and pistillate flowers of *S. latifolia* and likely serve an important role in pollination biology.

Although *S. latifolia* is pollen limited and may exhibit limited invasiveness, it has been successful in establishing itself in a non-native range. Therefore, *S. latifolia* could be classified as an invasive plant species, as it has met certain criteria outlined for successful plant invaders (Dekker, 2005; Radosevich et al., 2007). It is believed that it has been successful partially due to the indeterminate nature of the species. Pollen release is rapid on a per flower basis but is prolonged over the duration of the growing season.

Pistillate flowers are receptive to pollen for 120 h (Young & Gravitz, 2002), but a female plant can remain receptive during the entire flowering period. It is also believed that these characteristics are an important adaptation in the species because they may allow the species to successfully reproduce even though there are important reproductive limitations present (eg. dioecism). Nectar collects near the base of the inflated calyx in both staminate and pistillate flowers. This occurrence is ideal for nectar seeking insects, like lepidoperans, which utilize their long proboscis to reach near the base of the flower to collect nectar and remove pollen from anthers or deposit pollen on stigmas in the process. There are some important pollination limitations in the species; however, the species has found some balance between limitations in its pollination ecology and strengths in its floral biology.

Overall, our examination of the pollination ecology and floral biology of *S. latifolia* yielded new discoveries. These discoveries include, presence of pollination limitation in the species, presence of nectary slits on nectary surfaces in both staminate and pistillate flowers, and the existence of vestigial reproductive organs of the opposite sex in both flower genders. These findings have not been documented in this species until now.

5.1 Future research

This research is the first to report on the presence of pollination limitation in *S. latifolia* in western Canada. However, there were certain limitations to this research. The common method of measuring pollen limitation in plants is to pollinate flowers on one plant by hand and compare seed set of hand pollinated flowers to naturally pollinated flowers on other plants (Wesselingh, 2007). In our experiments, efforts to hand-pollinate stigmas with compatible pollen, and then bag hand-pollinated flowers, at each distance in the pollination distance trial were unsuccessful in both years. In 2009, random flowers were hand-pollinated at all distances in the pollination distance trial. However, flowers at some of the distances fell from plants and seed set in hand-pollinated flowers was inconsistent. In 2010, heavy rainfall prematurely dislodged pollen grains from flowers at the experimental site prior to transplanting of female plants. Therefore, hand pollination was not possible due to lack of available pollen at time of transplanting. Future research

may involve conducting a thorough investigation of pollen limitation in the species in conjunction with quantifying the degree to which colonization of the species is affected by these limitations. To test pollination limitation using that method in this species might have given more insight into quantifying actual pollination limitation at each distance (Wesselingh, 2007).

Seed production levels for *S. latifolia* have been estimated (Pearson, 1969) as a potential for the species but were not thoroughly quantified. Potential for seed dispersal was not considered either. These may also be potential avenues for future research.

More details are also required with respect to the invasiveness of this species—not just the potential for such. A study of this type would require more detailed field measurements of population dynamics (new annuals and returning perennials) and population growth in the species within a farmer's field. It is known that *S. latifolia* has horizontally growing roots (McNeill, 1977). An investigation into how vegetative reproduction contributes to the spread of the species may also be an important consideration for future research. An examination of the potential for population growth in the species as a result of vegetative reproduction may also be useful in a study of its invasiveness. Again, measurements of growth of the population and of individual plants once established in a field would provide evidence of presence or absence of vegetative reproduction in *S. latifolia.*

Another potential for research is to identify the species of diurnal and nocturnal pollinators of *S. latifolia*. Though it has been done in other areas of North America, it is important to observe conditions unique to western Canada. In these experiments, attempts at using infrared light to record insect visits at night were unsuccessful. Visual observation of moths pollinating flowers would have provided more substantial evidence for night pollination (which occurred predominantly in 2010 of the pollination timing trial) and is important to record for western Canadian ecotypes of *S. latifolia.*

One aspect of pollination biology that warrants consideration is pollen release in *S. latifolia.* For instance, close examination of the timing of pollen release (dehiscence) in

antipetalous vs. epipetalous anthers in staminate flowers would provide more evidence for the current hypothesis that pollen release is actually prolonged due to the staggered release of pollen in the two stamen whorls per staminate flower. Furthermore, quantifying how much and how fast pollen is released could shed more light on the passive or aggressive nature of pollen release in the species and how it compares to other dioecious species.

This study is the first to characterize floral biology in staminate and pistillate flowers of *S. latifolia* in western Canada. However, many aspects of floral biology were left undiscovered due to the complexity of its biology. Changes in stamens and pistils could be considered over a wider range of ages. For example, up to 5 d or 120 h after anthesis might provide more insight into what happens structurally to stigmas during that period of time. Such investigations could more fully support findings by Young and Gravitz (2002) who tested stigma receptiveness up to 5 d or 120 h after anthesis. In addition, structural changes were only observed in epipetalous anthers and not antipetalous. All anthers should be considered in future research. These investigations may provide greater insight into the unique biology that exists in this species and in dioecious species in general.

6.0 LITERATURE CITED

Arizaga, S., E. Ezcurra, E. Peters, F. R. de Arellano, and E. Vega. 2000. Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. I. Floral biology and pollination mechanisms. Am. J. Bot. 87:1004-1010.

Ashman, T., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85:2408-2421.

Ashman, T. 2009. Sniffing out patterns of sexual dimorphism in floral scent. Funct. Ecol. 23:852-862.

Baker, H. G. and P. D. Hurd. 1968. Intrafloral ecology. Annu. Rev. Entomol. 13:385-414.

Baker, H. G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. Evolution 9:347-349.

Baker, H. G. 1984. Some functions of dioecy in seed plants. Am. Nat. 124:149-158.

Bawa, K. S. 1980. Evolution of dioecy in flowering plants. Annu. Rev. Ecol. Syst. 11:15-39.

Blackshaw, R. E., A. G. Thomas, D. A. Derksen, J. R. Moyer, P. R. Watson, A. Légère and G. C. Turnbull. 2006. Examining Tillage and Crop Rotation Effects on Weed Populations in the Canadian Prairies. p. 189-201. *In* H. P. Singh 2006. Handbook of Sustainable Weed Management. CRC.

Blair, A. C. and L. M. Wolfe. 2004. The evolution of an invasive plant: An experimental study with *Silene latifolia*. Ecology 85:3035-3042.

Bernardello, G., L. Galetto, and G. J. Anderson. 2000. Floral nectary structure and nectar chemical composition of some species from Robinson Crusoe Island (Chile). Can. J. Bot. 78:862-871.

Booth, B. D., S. D. Murphy, and C. J. Swanton. 2003. p. 235-255. *In* Weed Ecology in Natural and Agricultural Systems. CABI Publishing.

Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: A volatile situation. Trends Plant Sci. 10:269-274.

Campbell, D. R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: Competition for pollination. Ecology 66:544-553.

Castellanos, M. C., P. Wilson, S. J. Keller, A. D. Wolfe, and J. D. Thomson. 2006. Anther evolution: Pollen presentation strategies when pollinators differ. Am. Nat. 167:288-296.

Charlesworth, D. 1993. Why are unisexual flowers associated with wind pollination and unspecialized pollinators? Am. Nat. 141:481-490.

Corbet, S. 1990. Pollination and the weather. Israel J. of Bot. 39:13-30.

Costea, M., S. E. Weaver, and F. J. Tardif. 2005. The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. Can. J. Plant Sci. 85:507-522.

Cousens, R. and M. Mortimer. 1995. p. 55-85. *In* Dynamics of Weed Populations. Cambridge University Press.

Cruden, R. W. 2000. Pollen grains: Why so many? Plant Syst. Evol. 222:143-165.

Davis, A. R. 1997. Pollination efficiency of insects. p. 87-120. *In* K. R. Shivanna and V. Sawhney eds. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press.

Davis, H. G., C. M. Taylor, J. G. Lambrinos, and D. R. Strong. 2004. Pollen limitation causes an allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). Proc. Natl. Acad. Sci. 101:13804-13807.

Dearborn, C. H. 1959. Weeds in Alaska and some aspects of their control. Weeds 7:265-270.

Decraene, L. P. R. and E. Smets. 2001. Staminodes: Their morphological and evolutionary significance. The Bot. Rev. 67:351-402.

Decraene, L. P. R. and E. Smets. 1999. The floral development and anatomy of *Carica papaya* (Caricaceae). Can. J. Bot. 77:582-598.

de Jong, T. J., J. C. Batenburg, and P. G. L. Klinkhamer. 2005. Distance-dependent pollen limitation of seed set in some insect-pollinated dioecious plants. Acta. Oecol. 28:331-335.

Dekker, J. 2005. Biology and anthropology of plant invasions. p. 235-250. *In* M. W. C. Inderjit. Invasive Plants: Ecological and Agricultural Aspects.

Delph L. F. and T. R. Meagher. 1995. Sexual dimorphism masks life history trade-offs in the dioecious plant *Silene latifolia*. Ecology 76:775-785.

Desfeux, C., S. Maurice, J. P. Henry, B. Lejeune, and P. H. Gouyon. 1996. Evolution of reproductive systems in the genus *Silene*. P. Roy. Soc. Lond. B Bio. 263:409-414.

Dötterl, S., D. Burkhardt, A. Jürgens, and A. Mosandl. 2007. Stereoisomeric pattern of lilac aldehyde in *Silene latifolia*, a plant involved in a nursery pollination system. Phytochemistry 68:499-504.

Dötterl, S., L. M. Wolfe, and A. Jürgens. 2005. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66:203-213.

Douglas, G. W. 1998. *In* Cody W. J. "Illustrated flora of British Columbia volumes 1-8". Can. Field Nat. 117:329.

Dreisig, H. 1986. Timing of daily activities in adult Lepidoptera. Entomol. Gen. 12:25-43.

Echlin, P. 2009. Sample stabilization for imaging in the SEM. Handbook of Sample Preparation for Scanning Electron Microscopy and X-Ray Microanalysis. p.137-183.

Edlund, A. F., R. Swanson, and D. Preuss. 2004. Pollen and stigma structure and function: The role of diversity in pollination. The Plant Cell 16:84-97.

Faegri, K. and L. Van der Pijl. 1971. p. 53-138. *In* The principles of pollination ecology. Pergamon Press, Oxford.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annu. Rev. Ecol. Evol. S. 35:375-403.

Freeman, D. C., J. L. Doust, A. El-Keblawy, K. J. Miglia, and E. D. McArthur. 1997. Sexual specialization and inbreeding avoidance in the evolution of dioecy. Bot. Rev. 63:65-92.

Galen, C., J. Shykoff, and R. Plowright. 1986. Consequences of stigma receptivity schedules for sexual selection in flowering plants. Am. Nat. 127:462-476.

Hastings, R. and C. A. Kust. 1970a. Control of yellow rocket and white cockle in established alfalfa. Weed Sci. 18:329-333.

Hastings, R. E. and C. A. Kust. 1970b. Reserve carbohydrate storage and utilization by yellow rocket, white cockle, and hoary alyssum. Weed Sci. 18:140-148.

Hickey, M. and C. King. 1988. p. 75-79. *In* 100 Families of Flowering Plants. Cambridge University Press.

Hossaert-McKey, M., C. Soler, B. Schatz, and M. Proffit. 2010. Floral scents: Their roles in nursery pollination mutualisms. Chemoecology 20:75-88.

Inderjit, M. W. C. and R. I. Colautti. 2005. The ecology of biological invasions: past, present and future. p. 19-43. *In* The ecology of biological invasions: Past, present and future. Invasive Plants: Ecological and Agricultural Aspects. Germany.

Jensen, W. A. 1962. p. 408. *In* Botanical Histochemistry. WH Freeman & Co. San Francisco & London.

Jürgens, A., T. Witt, and G. Gottsberger. 2002a. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochem. Syst. Ecol. 30:383-397.

Jürgens, A., T. Witt, and G. Gottsberger. 2002b. Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: Correlation with breeding system, pollination, life form, style number, and sexual system. Sex Plant Reprod. 14:279-289.

Kapusta, G. 1973. Common chickweed control in established alfalfa. Weed Sci. 21:119-122.

Kapusta, G. and C. F. Strieker. 1975. Selective control of downy brome in alfalfa. Weed Sci. 23:202-206.

Kay, Q., A. Lack, F. Bamber, and C. Davies. 1984. Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. New Phytol. 98:515-529.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. Ashman. 2005. Pollen limitation of plant reproduction: Pattern and process. Annu. Rev. Ecol. Evol. S. 36:467-497.

Kubitzki, K. and H. Kurz. 1984. Synchronized dichogamy and dioecy in neotropical Lauraceae. Plant Syst. Evol. 147:253-266.

Kwak, M. M. and R. M. Bekker. 2006. Ecology of plant reproduction: Extinction risks and restoration perspectives of rare plant species. p. 362-387. *In* N.M. Waser and J. Ollerton Plant-Pollinator Interactions: From Specialization to Generalization. University of Chicago Press.

Leeson, J. Y. 2005. *In* Prairie Weed Survey of Cereal, Oilseed and Pulse Crops from the 1970s to the 2000s. Agriculture and Agri-Food Canada, Saskatoon Research Centre

(Canada), and Saskatchewan. Saskatchewan Agriculture, Food and Rural Revitalization. Agriculture and Agri-Food Canada, Saskatoon Research Centre.

Lengerova, M., E. Kejnovsky, R. Hobza, J. Macas, S. R. Grant, and B. Vyskot. 2004. Multicolor FISH mapping of the dioecious model plant, *Silene latifolia*. Theor. Appl. Genet. 108:1193-1199.

Lin, J. J. and D. B. Dickinson. 1984. Ability of pollen to germinate prior to anthesis and effect of desiccation on germination. Plant Physiol. 74:746-748.

Martínez-Pallé, E. and G. Aronne. 2000. Pollination failure in Mediterranean *Ruscus aculeatus* L. Bot. J. Linn. Soc. 134:443-452.

Matallana, G., T. Wendt, D. S. D. Araujo, and F. R. Scarano. 2005. High abundance of dioecious plants in a tropical coastal vegetation. Am. J. Bot. 92:1513-1519.

McNeill, J. 1977. The biology of Canadian weeds. 25. Silene alba (Miller) E.H.L. Krause. Can. J. Bot. 57:1103–1114.

Meagher, R. L. 2002. Trapping noctuid moths with synthetic floral volatile lures. Entomol. Exp. Appl. 103:219-226.

Miyake, T., R. Yamaoka, and T. Yahara. 1998. Floral scents of hawkmoth-pollinated flowers in Japan. J. Plant Res. 111:199-205.

Mrackova, M., M. Nicolas, R. Hobza, I. Negrutiu, F. Moneger, A. Widmer, B. Vyskot, and B. Janousek. 2008. Independent origin of sex chromosomes in two species of the genus *Silene*. Genetics. 179:1129-1133.

Murrell, D. 2006. Local interactions and invasion dynamics: Population growth in space and time. p. 147-168. *In* M.W. Cadotte, S. M. McMahon, T. Fukam. Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature. Springer. Netherlands.

O'Brien, T. P. and M. E. McCully. 1981. The study of plant structure: Principles and selected methods. Melbourne, Termarcarphi Pty. LTD.

Pearson, J. O. 1969. A life history study of the white cockle (*Lychnis alba* Mill.) and some competitive effects in alfalfa (*Medicago sativa* L.). PhD thesis, Michigan State University, East Lansing, MI.

Percival, M. S. 1961. Types of nectar in angiosperms. New Phytol. 60:235-281.

Petanidou, T., R. C. Godfree, D. S. Song, A. Kantsa, Y. L. Dupont, and N. M. Waser. 2011. Self-compatibility and plant invasiveness: Comparing species in native and invasive ranges. Perspect. Plant Ecol. Evol. Syst. 1-10.

Proctor, M., P. Yeo, and A. Lack. 1996. p. 24-49, 80-98. *In* The natural history of pollination. Timber Press. Portland, Oregon.

Radosevich, S. R., J. S. Holt, and C. Ghersa. 2007. p. 3-35. *In* Ecology of Weeds and Invasive Plants: Relationship to Agriculture and Natural Resource Management. LibreDigital.

Ramaswamy, S. B. 1988. Host finding by moths: Sensory modalities and behaviours. J. Insect Physiol. 34:235-249.

Renner, S. S. and R. E. Ricklefs. 1995. Dioecy and its correlates in the flowering plants. Am. J. Bot. 82:596-606.

Richards, A. J. 1997. p. 10-65. *In* Plant breeding systems. Chapman & Hall. London.

Richards, C. M., S. Church, and D. E. McCauley. 1999. The influence of population size and isolation on gene flow by pollen in *Silene alba*. Evolution 53:63-73.

Rohweder, O. 1967 Centrospermen-Studien. 3. Blutenentwicklung und Blutenbau bei Silenoideen (Caryophyllaceae). Bot. Jb. 86:130-85.

Royer, F. and R. Dickinson. 1999. p. 328-329. *In* Weeds of the Northern United States and Canada.

Sabbahi, R., D. De Oliveira, and J. Marceau. 2005. Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Cruciferae: Brassicaceae). J. Econ. Entomol. 98:367-372.

SAS Institute. 2008. SAS user's guide. Version 9.2. SAS Inst. Cary, NC.

Shivanna, K. R. and V. Sawhney. 1997. p. 22-23, 28-29. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press.

Slauson, L. A. 2000. Pollination biology of two chiropterophilous agaves in Arizona. Am. J. Bot. 87:825-836.

Thomson, B. F. 1942. The floral morphology of the Caryophyllaceae. Am. J. Bot. 29:333-349.

Thompson, P. 1970. A comparison of the germination character of species of Caryophyllaceae collected in central Germany. J. Ecol. 58:699-711.

Waser, N.M. 2006. Specialization and generalization in plant-pollinator interactions: a historical perspective p. 3-19 *In* N.M. Waser and J. Ollerton Plant-Pollinator Interactions: From Specialization to Generalization. University of Chicago Press.

Watson. L. and M. J. Dallwitz. 1992. The families of flowering plants: Descriptions, illustrations, identification, and information retrieval. Version: 14th December 2000. http://biodiversity.uno.edu/delta/

Weberling, F. (and translated by R. J. Pankhurst). 1989. p. 129-130, 192-195. *In* Morphology of Flowers and Inflorescences. Cambridge University Press.

Wesselingh, R. A. 2007. Pollen limitation meets resource allocation: Towards a comprehensive methodology. New Phytol. 174:26-37.

Wilcock, C. and R. Neiland. 2002. Pollination failure in plants: Why it happens and when it matters. Trends Plant Sci. 7:270-277.

Williamson, M. H. 1996. Biological Invasions. London: Chapman & Hall.

Witt, T., A. Jürgens, R. Geyer, and G. Gottsberger. 1999. Nectar dynamics and sugar composition in flowers of *Silene* and *Saponaria* species (Caryophyllaceae). Plant Biol. 1:334-345.

Wyse, D. L. and R. L. McGraw. 1987. Control of white campion (*Silene alba*) in birdsfoot trefoil (*Lotus corniculatus*) with dinitroaniline herbicides. Weed Technol. 1:34-36.

Young, H. J. 2002. Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). Am. J. Bot. 89:433-440.

Young, H. J. and L. Gravitz. 2002. The effects of stigma age on receptivity in *Silene alba* (Caryophyllaceae). Am. J. Bot. 89:1237-1241.

Zandonella, P. 1966. Morphologie vegetale.- Les nectaries des Caryophyllaceae: Presence d'un systeme de drainage dans la tribu des Lychnideae. C. R. Acad. Sc. Paris, Series D: 262:2035-2038.

Zandonella, P. 1967a. Morphologie vegetale.- Les nectaires des Alsinoideae: *Stellaria* et *Cerastium sensu lato*. C. R. Acad. Sc. Paris, Series D: 264:2466-2469.

Zandonella, P. 1967b. Stomates des nectaries floraux chez les Centrospermales. Bulletin de la Société botanique de France 114:11-20.

Zandonella, P. 1970a. Infrastructure des cellules du tissu nectarigène floral de quelques Caryophyllaceae. C.R. Acad. Sci. Paris, Series D: 270:1310-1313.

Zandonella, P. 1970b. Le nectaire floral des Caryophyllaceae: L'appereil nectarifere des Sperguleae. Bulletin Mensuel de la societe Linneenne De Lyon. 39:253-260.

7.0 APPENDIX

7.1 Appendix A: Images of Pollinator exclusion trial, pollination timing trial, and pollination distance trial treatments

Figure A.1 Pollinator exclusion trial: exclusion structures. Full exclusion (left) and sham-exclusion with north facing side open (right).

Figure A.2 Pollination timing trial: Exclusion structures. Full exclusion (left) and movable day-exclusion. Sandbags were used to anchor exclusion structures.

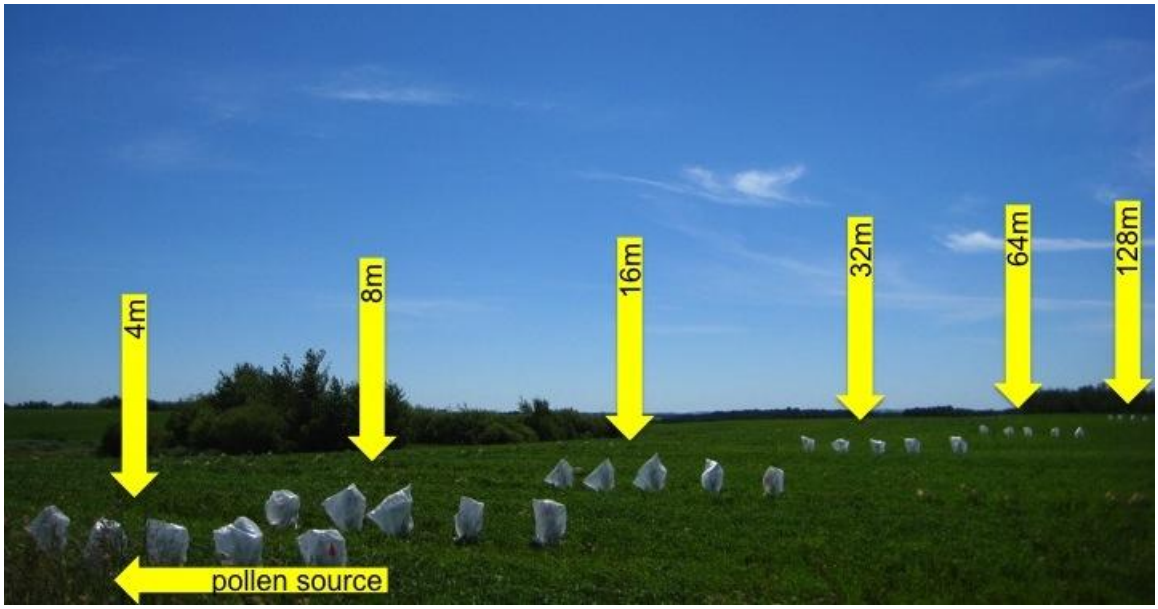

Figure A.3 Pollination distance trial: distance treatment structure. Pollen source at bottom left with distance treatments in linear interval with transects of 4m, 8m, 16m, 32m, 64m, and 128m. Bags were used to cover female plants while the annual crop was sprayed.