## HABITAT CHARACTERIZATION AND BIOLOGY OF THE THREATENED DAKOTA SKIPPER (*Hesperia dacotae*) in Manitoba

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## ABSTRACT

The Dakota Skipper, *Hesperia dacotae*, is a threatened butterfly restricted to fragmented prairies in the Interlake, southwestern Manitoba and southeastern Saskatchewan in Canada. Currently there is limited data on the life history and habitat requirements in Canada to implement effective conservation measures. The purpose of this study was to determine key biological and physical habitat requirements within existing Dakota Skipper sites in Manitoba. Four Dakota Skipper sites in the Interlake and four sites in southwestern Manitoba were selected to measure the composition of vegetation and flowers in bloom during the flight period, and soil characteristics. Adult populations were also surveyed at each site. Sites were generally subject to annual fall haying and several sites exhibited adverse impacts associated with flooding, succession, encroachment by invasive plants, vehicle damage, cattle grazing or wildfire.

There was considerable overlap in vegetation and flower composition between sites, and between the Interlake and southwest Manitoba regions. The most abundant plant species at sites in the vegetation surveys included: Agropyron repens, Andropogon gerardii, Andropogon scoparius, Deschampsia caespitosa, Poa compressa, Poa cusickii, Scolochloa festucacea and Sonchus arvensis. In the flower surveys the most abundant plants included: Zygadenus elegans, Melilotus alba, Petalostemon candidum, Petalostemon purpureus, Campanula rotundifolia, Lobelia spicata, Crepis runcinata, Rudbeckia hirta and Solidago ptarmicoides. Four previously documented and ten possible larval food plant species were recorded as well as 34 previously documented or possible adult nectar plant species. There was no significant difference between regions in plant species diversity measures (Shannon diversity, Shannon eveness, Simpson reciprocal and Berger-Parker reciprocal indices). Indicator Species Analysis identified ten species in the vegetation surveys and 15 species in the flower surveys as significant regional indicators. Principal Component Analyses (PCA) showed several species of plants and flowers associated with either a region or site. Soil and soil-related parameters measured at sites included: bare ground, duff layer, soil pH, soil moisture, soil compaction, soil bulk density, soil particle size and texture, soil organic matter content, soil available calcium, magnesium and sodium cation content, and air temperature and relative humidity at the soil level. There were significantly higher levels of the mean duff

layer, soil pH measured at the University of Winnipeg laboratory, soil moisture measured in the field, mean soil compaction and compaction at 20, 30 and 40 cm, clay content, silt content, organic matter content and available magnesium content in the Interlake region and higher levels of sand content in southwestern Manitoba. The air temperature during the "larval period" was also significantly higher in the Interlake in 2010 and the mean of 2010/2011, while the air relative humidity was higher in the southwest in 2010, 2011 and the mean of both years. Redundancy Analyses (RDAs) showed associations between the soil parameters and vegetation or flower species, explaining considerable amounts of variation (58 to 74% on first three axes), and with clay content and available magnesium cation content significantly higher in the Interlake.

The Dakota Skipper flight period ranged from 13 to 19 days, with up to 15 days difference between the start date in 2010, 2011 and 2012. Populations of Dakota Skipper appear to be on the decline at all eight sites when compared to past surveys, and Dakota Skipper were absent from three of the sites where they were previously documented. The population estimate for the site with the most skippers (Site H) using Insect Count Analyzer (INCA) software ranged from 277 to 332 individuals in 2011. Dakota Skipper adults were observed nectaring upon twelve species of flowering plants, six of which are new records for North America: *Melilotus alba, Petalostemon purpureus, Oenothera biennis, Lobelia spicata, Crepis runcinata* and *Solidago ptarmicoides*. The majority of nectaring observed was upon *Rudbeckia hirta*. Amplification of a region of cytochrome *c* oxidase I (COI) gene from a single Dakota Skipper mid-leg sampled in a non-lethal manner was demonstrated to be as effective as conventional lethal sampling and identified two polymorphic sites.

Certain floral species are required by Dakota Skipper for shelter, and larval and adult feeding. A set of key vegetation and soil attributes that characterize Dakota Skipper sites were developed as a guide to identify critical habitat in Manitoba. Comparisons of present land use at Dakota Skipper sites, flora, edaphic factors and adult densities were examined and potential relationships are discussed. Management recommendations and future research direction based on these results are provided.

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## **1.0 GENERAL INTRODUCTION**

In the past two centuries almost all the tallgrass and mixed grass prairie in North America has been lost due to human disturbance, leaving very little habitat for native prairie plant and animal species (Samson and Knopf 1994). One butterfly species in particular, the Dakota Skipper (Hesperia dacotae Skinner 1911) (Figure 1), was once found throughout much of the tallgrass and eastern mixed grass prairies of Manitoba, Saskatchewan, North Dakota, South Dakota, Minnesota, Iowa and Illinois (Cochrane and Delphey 2002; COSEWIC 2003). In Canada, up to 99.9% of tallgrass and mixed grass prairie has been lost, and as a result the Dakota Skipper is now restricted to fragmented patches of native prairie in the Interlake and the southwest regions of Manitoba, and southeastern Saskatchewan (Samson and Knopf 1994; Environment Canada 2007). Dakota Skipper is now considered to be at-risk of extinction due to habitat destruction and a poor understanding of how key life history characteristics are affected by management activities in remnant habitats (Environment Canada 2007). The Dakota Skipper is one of a number of species of grassland skippers experiencing decline in North America, and the loss of this species would mean the disappearance of another important butterfly in North American grassland ecosystems.



Figure 1. Female Dakota Skipper nectaring upon Rudbeckia hirta (black-eyed Susan)

While habitat loss and disturbance have had significant impacts on the decline of Dakota Skipper populations, poor knowledge of fundamental elements of life history and habitat requirements have contributed to further decline (Dana 1991; Cochrane and Delphey 2002; COSEWIC 2003; Environment Canada 2007). There is a need to better understand the role that the structure and composition of the vegetation communities in prairie habitats have on skipper survival. Improved skipper habitat characterization is required to increase our understanding of what constitutes suitable Dakota Skipper habitat and which plant species can be used as indicators of areas most suitable as long term skipper refugia. Critical plant species include those species that the Dakota Skipper requires for larval or adult food sources, larval shelters (residence) and other plants for adult shelter, perching and mating.

The type of land use activity in Dakota Skipper habitat also influences the composition of vegetation. In addition to improved vegetative characterization of Dakota Skipper habitat, observations of adult behaviour are also required to gather life history data related to nectar plant use, mating and oviposition. Further understanding of the length of the flight period and population densities in Canadian habitats is also required (Environment Canada 2007). Improved descriptions of optimal habitat components for Dakota Skipper survival will allow conservation agencies, land managers and policy makers to better direct suitable conservation and recovery measures for the Dakota Skipper.

With these research requirements in mind the objectives of this study were to:

- develop an understanding of the land management history (i.e. haying, pasture land, cropland) at Dakota Skipper study sites;
- develop a Dakota Skipper site character profile based on floristic and edaphicrelated factors;
- 3) determine the adult flight period of Dakota Skipper in Manitoba;
- 4) estimate the population size at study sites based on adult counts;
- contribute additional life history data of Manitoba Dakota Skipper based on incidental observations of adults (i.e. nectaring, predation, egg laying, etc.);

- amplify a partial region of the mitochondrial cytochrome *c* oxidase I gene (COI) from Dakota Skipper DNA isolated from a single mid-leg sampled in a non-lethal manner; and,
- examine potential correlations between management history, site character profile and population size.

The following null hypotheses were tested:

- Dakota Skipper study sites share similar floristic and edaphic-related characters;
- adult Dakota Skipper use the same nectar food plants as documented in the United States; and,
- DNA can be successfully isolated from a live sampled single mid-leg and used as a template for polymerase chain reaction amplification of the COI mitochondrial gene partial sequence.

Sequencing of COI from a single leg (Objective 6) was implemented to demonstrate that tissue samples for molecular analysis can be obtained in a non-lethal manner from Dakota Skipper. Previous molecular research done on Lepidoptera has used 10 mm<sup>2</sup> wing clips or whole bodies but this is less desirable for small Lepidopteran species-at-risk because it may be too traumatic or lethal (Hamm et al. 2010; Koscinski et al. 2011). Some researchers have extracted DNA from insect legs, however none appear to have done so with the express purpose of minimizing harm to species of conservation concern with a small range and life history similar to Dakota Skipper (Châline et al. 2004; Keyghobadi et al. 2009; Vila et al. 2009; Hamm et al. 2010; Koscinski et al. 2011). Hence, the intention of Objective 6 is to show that relatively non-traumatic tissue sampling from Dakota Skipper can be performed for molecular purposes and that the live sampled product is compreble to samples from dead vouchers.

## 2.0 LITERATURE REVIEW

### 2.1 Status and Conservation

The Dakota Skipper is designated as Threatened federally by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and Schedule 1 under the Canadian *Species at Risk Act* (SARA) (Table 1) (COSEWIC 2003; Government of Canada 2011). In Manitoba Dakota Skipper is designated as Threatened under the *Manitoba Endangered Species Act* (MESA). In Saskatchewan Dakota Skipper currently has no designation under the *Saskatchewan Wildlife Act* (Saskatchewan Conservation Data Centre 2011). The legal status and status ranks for Dakota Skipper throughout its range are provided in Table 1. The species is listed as G2 (imperilled) globally and the World Conservation Union (ICUN) has designated the species as Vulnerable (NatureServe 2011).

Jurisdiction	Legal status*	Status rank
Canada	Threatened, Schedule 1	N2 (imperilled)
Manitoba	Threatened	S2 (imperilled)
Saskatchewan	None	S1 (critically imperilled)
United States	Candidate	N2 (imperilled)
North Dakota	None	S2 (imperilled)
South Dakota	None	S2 (imperilled)
Minnesota	Threatened	S2 (imperilled)
owa Endangered		S1 (critically imperilled)
Illinois	Extirpated	SX (presumed extirpated)

Table 1. Dakota Skipper status across the North American range (Government of Canada 2011; NatureServe 2011; Saskatchewan Conservation Data Centre 2011)

\*Legal Status under jurisdiction's Endangered Species Act unless otherwise indicated.

COSEWIC (2003) produced an Assessment and Status Report on the Dakota Skipper outlining the known information on the distribution, habitat, biology, population and threats to the Dakota Skipper followed by the Environment Canada (2007) release of a Recovery Strategy for the Dakota Skipper. The short-term recovery objectives for the Dakota Skipper are to:

- establish reliable population estimates for all Dakota Skipper populations and assess viability under current conditions;
- identify, secure and enhance significant habitat for the Dakota Skipper; and,
- increase knowledge of the Dakota Skipper in Canada, including distribution, abundance, biology and management practices.

The broad strategies to implement Dakota Skipper recovery and address threats include:

- a stewardship approach to secure important habitat;
- population monitoring to more accurately estimate population sizes, trends and area of occupancy;
- habitat management to maintain the plant community required for the Dakota Skipper survival and reproduction;
- research to fill gaps in knowledge of life history; and,
- a communication program to increase public awareness.

Thus in order to conserve and manage this species, further information is required on life history, habitat and population size.

## 2.2 Critical Habitat

Critical habitat as a concept is defined under the Canadian *Species at Risk Act* as "the habitat that is necessary for the survival or recovery of a listed wildlife species" (Government of Canada 2002). Critical habitat includes all the critical elements that an organism requires for survival. In the case of the Dakota Skipper this may include all the habitat required for feeding (larval and adult food plants), shelter, reproduction, dispersal, etc. Once critical habitat has been defined for a species it is then detailed in a Recovery Strategy report or Action Plan report for the species. At present the critical habitat for Dakota Skipper has not yet been determined. As an ultimate product of this study it is hoped that Dakota Skipper critical habitat can be better determined from the site character profile (floral and edaphic-related factors), with consideration of preferred nectar flower species and land use.

### 2.3 Study Organism

The Dakota Skipper (*Hesperia dacotae* Skinner 1911) is a skipper type butterfly belonging to the Order Lepidoptera. It is a tallgrass prairie obligate which has experienced dramatic range reductions due to habitat loss over the last 150 years (Vaughan and Shepherd 2005). The Dakota Skipper belongs to the family Hesperidae within the subfamily Hersperinnae (Branded Skippers) (COSEWIC 2003). The species was first described in 1911 from specimens taken from Volga, South Dakota and Grinnell, Iowa (Cochrane and Delphey 2002).

#### 2.3.1 Description

Like many North American skippers, the Dakota Skipper is a smaller butterfly with a wingspan ranging from 2.1 to 3.2 cm (Layberry et al. 1998; Cochrane and Delphey 2002). Adults have hooked antennae, stout bodies and fly in a rapid, skipping flight pattern (Layberry et al. 1998; Cochrane and Delphey 2002). The males and females are generally similar in appearance with dusty yellow, orange and brown wings, though they do have different markings (Appendix X). The dorsal surface of the male wings are orange and brown with a brown border and a distinct black mark (stigma) in the centre of the forewing. The ventral surface is a lighter dusty yellow-orange. The female differs from the male in that the dorsal surface of the wing is darker brown and orange with no stigma marks and with distinct white spots (hyaline) on the forewing that do not blend together (Appendix X) (Cochrane and Delphey 2002; COSEWIC 2003; Environment Canada 2007).

Adults lay eggs singly with eggs being hemispherical, 1.2 mm in diameter and 0.95 mm in height (Appendix X). Eggs are gleaming, semi-translucent white when deposited, becoming slightly duller a few hours later (MacNeill 1964; Dana 1991). The larvae of the Dakota Skipper are light brown or flesh coloured and 1.9 to 2.2 cm long. Larvae have a large black head capsule with pits, typical of many *Hesperia*, but the head capsule is pitted throughout, including on the ventral (face) portion (MacNeill 1964; McCabe 1981). The prothoracic shield, thoracic legs and spiracles are black and late instar larvae have a white waxy substance on the venter of the 7th and 8th abdominal segments (MacNeill 1964; McCabe 1981; Dana 1991). The pupae is poorly described

and thought to be reddish brown and similar to other *Hesperia* (MacNeill 1964; Cochrane and Delphey 2002).

The adult skipper is similar in appearance to the Ottoe Skipper (*Hesperia ottoe* W.H. Edwards) which is larger with a greater wing span, and the Indian Skipper (*Hesperia sassacus* Harris) which is darker with larger spots (Klassen et al. 1989; Cochrane and Delphey 2002). Dakota Skipper also resembles the Long Dash Skipper (*Polites mystic* W.H. Edwards) which bears a broader and differently shaped forewing stigma that extends to the wing tip (Klassen et al. 1989; COSEWIC 2003). The Pawnee Skipper (*Hesperia leonardus pawnee* Dodge), Plains Skipper (*Hesperia assiniboia* Lyman), Peck's Skipper (*Polites peckius* W. Kirby) and Garita Skipper in Canada (Klassen et al. 1989; Environment Canada 2007).

#### 2.3.2 Biology and Natural History

Dakota Skipper have one generation per year, overwintering in the larval stage (Figure 2). Adults live two to four weeks and have been previously recorded in Canada from June 23 to July 29 with peak emergence between June 27 and July 8 (COSEWIC 2003; Environment Canada 2007). In the United States, adult emergence occurs earlier in June and the flight period is typically longer in duration (McCabe 1981; Dana 1991; Swengel and Swengel 1999a; Cochrane and Delphey 2002). Most research indicates that males emerge several days earlier than females as the larval development of females is longer then males (Dana 1991; Swengel and Swengel 1999a; Cochrane and Delphey 2002). McCabe (1981) reported that both sexes emerge at about the same time, however this is likely an error associated with the difficulties in detecting adults at low densities and precisely 'aging' adults (Dana 1991; Swengel and Swengel 1999a; Cochrane and Delphey 2002).

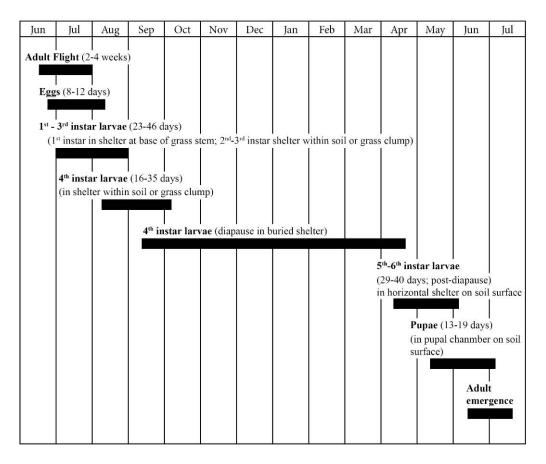


Figure 2. Dakota Skipper life history stages and approximate seasonal phenology in Canada (reproduced from Environment Canada, 2007)

Nectar is a vital food source for adult Dakota Skippers, and in Canada it has been previously reported as feeding on *Apocynum* spp. (dogbane), *Campanula rotundifolia* (harebell), *Echinacea angustifolia* (purple coneflower), *Rudbeckia hirta* (black-eyed Susan), and possibly *Lilium philadelphicum* (wood lily) and *Zygadenus elegans* (smooth camas) (Webster 2003; Webster 2007).

In the United States adults have also been recorded feeding from Astragalus crassicarpus (ground plum), Astragalus striatus (ascending purple milk-vetch), Medicago sativa (alfalfa), Melilotus officinalis (yellow sweet-clover), Oxytropis lambertii (purple locoweed), Petalostemon candidum (white prairie-clover), Trifolium hybridum (alsilk clover), Trifolium pratense (red clover), Oenothera serrulata (shrubby evening-primrose), Oenothera spp. (evening-primrose), Asclepias speciosa (showy milkweed), Asclepias syriaca (silky milkweed), Phlox pilosa (downy phlox), Verbena stricta (hoary vervain), Galium spp. (bedstraw), Achillea millefolium (yarrow), Agoseris glauca (false

dandelion), *Carduus nutans* (nodding thistle), *Chrysopsis* spp. (golden aster), *Cirsium flodmanii* (Flodman's thistle), *Erigeron annuus* (whitetop), *Gaillardia aristata* (blanketflower), *Lactuca* spp. (lettuce), and *Ratibida columnifera* (long-headed coneflower) (McCabe 1981; Dana 1991; Swengel and Swengel 1999a).

The mating strategy of the Dakota Skipper appears to be scramble competition polygyny and most male activity during the day is dominated by mate-seeking behaviour (McCabe 1981; Dana 1991). Females generally mate only once, though multiple matings do occur, and males mate more than once during their life. Males use a wait, perch and pursue courtship strategy, and do not seem to show territoriality based on a lack of perch fidelity. When a female is encountered they fall to the ground and copulate for up to 45 minutes, if the female is receptive (McCabe 1981; Dana 1991; COSEWIC 2003). The female begins to lay eggs shortly after mating and continues to do so throughout her life. Females lay approximately 20 to 30 eggs in the first few days and then egg production decreases to a few eggs per day near the end of her life. Females may lay from 180 to 250 eggs in total (McCabe 1981; Dana 1991; Cochrane and Delphey 2002). During oviposition the female crawls along grass leaves from one to four centimetres above the ground and deposits eggs singly on the underside of the leaves or on the upper surface (Dana 1991). Females may not select larval host plants for oviposition, but prefer to lay eggs on various grass species adjacent or intermixed with larval host plants. Females have been previously documented as ovipositing on Andropogon gerardii (big bluestem), Andropogon scoparius (little bluestem), Bouteloua curtipendula (side-oats grama), Sporobolus heterolepis (prairie dropseed), Stipa spartea (porcupine grass), and other smooth, wide grasses and forbs in the United States (Dana 1991; COSEWIC 2003).

Eggs incubate for 7 to 20 days (10 days on average) and the larvae eat the egg chorion during emergence (MacNeill 1964; McCabe 1981; Dana 1991). Larvae immediately crawl to the base of a bunch grass and construct a silken shelter amongst the blades of grass. The larvae are presumed to go through six instars in Canada and seven instars further south in their range (Dana 1991; Cochrane and Delphey 2002). Each instar typically lasts 6 to 21 days. As they grow, larvae construct successively larger angled shelters of silk on grass stems at the base of grass clumps or just below the soil surface. Often a blade or stem of grass or a silk tube extends from the entrance. Larvae prefer

bunchgrass species such as *Andropogon scoparius*, likely because they provide good structure for the shelters and are probably a food source (Dana 1991; COSEWIC 2003). The larvae generally forage for food at night, cutting off blades of grass and carrying them back to their shelters to feed upon, tending to forage relatively close to their shelters (McCabe 1981; Dana 1991; Cochrane and Delphey 2002).

Larvae diapause in the fourth instar during the winter months in Canada and in the fifth instar in the southern part of the range. Larvae overwinter in their shelters just below the surface of the soil (Dana 1991; Cochrane and Delphey 2002). The following spring (late April to May in Canada) larvae emerge and complete the remaining two instars. During the remaining two instars larvae construct elongate horizontal shelters on the soil surface (Dana 1991; COSEWIC 2003). In the last instar the larvae line their chambers with a waxy hydrofuge substance from the patch at the end of their abdomen which protects the pupae from high humidity (McCabe 1981; Dana 1991). The pupal stage lasts 13 to 19 days in the United States, with the adults emerging in late June to early July (Dana 1991).

Dakota Skipper larvae feed on a variety of grasses, preferring bunchgrasses. In field plots in the United States in enclosures where larvae were placed with multiple naturally occurring plants, the larvae fed largely upon *Andropogon gerardii*, *Andropogon scoparius, Bouteloua curtipendula, Sporobolus heterolepis* and occasionally upon *Panicum wilcoxianum* (sand millet), *Poa pratensis* (Kentucky blue grass), *Stipa spartea* and *Carex pensylvanica* var. *digyna* (sun-loving sedge) (Dana 1991). Only late instar, post-diapause larvae fed upon *Stipa spartea*, probably because the leaves were too tough for earlier instars (Dana 1991). In no-choice experiments, larvae also fed upon the introduced forage plant *Bromus inermis* (smooth brome). Larvae may prefer feeding on shorter and more fine-stemmed bunchgrass species. These grass species are also closer to the ground, whereas other species may be too high off the ground, too hairy or smooth, or undergo early senescence at a time when larvae require a food source (Dana 1991; Cochrane and Delphey 2002).

#### 2.3.3 Distribution and Population

The Dakota Skipper is a prairie obligate preferring dry-to-mesic mixed grass to wet-to-mesic tallgrass prairies. Historically, this skipper was likely distributed throughout the prairies of North America, however the distribution will never be precisely known since over 99% of the tallgrass prairie habitat and a similar amount of mixed grass habitat have been converted to agricultural use since the 1850's (COSEWIC 2003). Today the Dakota Skipper is restricted to prairie remnants in southern Manitoba, southeastern Saskatchewan, North Dakota, eastern South Dakota, and western Minnesota. The Dakota Skipper is believed to be extirpated from Iowa, Illinois, and the Tall Grass Prairie Preserve in southeastern Manitoba (Morden 2006; Environment Canada 2007).

In Manitoba there were seven known historic isolated populations of Dakota Skipper; currently only two of these locations support populations of Dakota Skipper. The Interlake region north of Winnipeg (between Lake Manitoba and Lake Winnipeg) holds the largest population in Canada, consisting of up to 17 sites where the skipper is locally common - in some years reaching densities of up to 25 adults/ha (Environment Canada 2007). The smaller second population is found at up to 14 sites near Griswold and Oak Lake in southwestern Manitoba (Webster 2007). In 2001 and 2002 three more sites were discovered in southeastern Saskatchewan along the Souris River (Hooper 2003; Environment Canada 2007). Coarse estimates conducted in 2002 suggest the population of Dakota Skipper in Manitoba may range from 25,000 to 35,000 individuals. Caution should be taken when considering this estimate due to the difficulties in calculating the density of this species and the limited nature of the 2002 survey (COSEWIC 2003; Environment Canada 2007). The population in Saskatchewan has been estimated to be at least 250 individuals (COSEWIC 2003). United States data provides no overall population estimates though researchers estimated a population of 2,000 to 3,000 individuals or 25 adults/hectare during the peak flight period at one site in Minnesota, and 40 adults/hectare at sites in North Dakota (COSEWIC 2003). Research also suggests that only one third to one half of adults are alive simultaneously (Cochrane and Delphey 2002).

#### 2.3.4 Habitat Requirements

In Manitoba the skippers prefer low, wet-to-mesic tallgrass prairies and mixed grass prairies, while in Saskatchewan they are in upland, dry-to-mesic mixed grass prairies. In North Dakota wet-to-mesic tallgrass sites support more dense populations than drier mixed grass sites (Cochrane and Delphey 2002; COSEWIC 2003). The tallgrass sites in Manitoba are generally found in openings of Populus spp. (aspen) and Quercus macrocarpa (bur oak) groves, consisting of depressed, wet areas and higher, dry areas. Adult Dakota Skippers tend to be found in the higher, dry areas, which are dominated by Andropogon gerardii, Andropogon scoparius, Sporobolus heterolepis, Lilium philadelphicum, Zygadenus elegans, Campanula rotundifolia and Rudbeckia hirta (Webster 2003). In Saskatchewan, the Dakota Skipper habitat along the Souris River consists of ridges and hillsides is dominated by bluestems and needlegrasses. Echinacea angustifolia is considered a good indicator plant of Dakota Skipper habitat in Saskatchewan (COSEWIC 2003). In the United States Dakota Skipper habitat is also dominated by Astragalus striatus, Gaillardia aristata, Ratibida columnifera and other composites (Cochrane and Delphey 2002; COSEWIC 2003). Many of the remaining North American Dakota Skipper habitats exist because they are poorly suited for agriculture due to wet conditions, steep topography and alkaline soils. Many sites are haved for cattle feed and some sites in the United States are subject to periodic burning (Cochrane and Delphey 2002; COSEWIC 2003).

#### 2.3.5 Threats to Survival

Dakota Skipper is dependent upon the need for suitable larval shelter and food plants, adult nectar plants, and mating sites, which are all related to the structure and compositon of native prairie vegetation communities (Environment Canada 2007). Adults are considered to have a poor dispersal ability and a short life span, which in a fragmented landscape may further limit dispersal opportunities (Environment Canada 2007). Historic conversion of prairies to agricultural lands by settlers is the most significant cause of loss of habitat for Dakota Skipper. Today, the primary threats are still related to habitat loss and disturbance. Threats include grazing, haying, accidental and controlled burning, habitat fragmentation, succession, competition by exotic plant species, occasional conversion to cultivated lands, use of hog manure, drainage alteration, and harvest of skipper specimens or harvest of *Echinacea angustifolia* (Cochrane and Delphey 2002; COSEWIC 2003; Environment Canada 2007).

#### 2.4 Past and Current Research on Dakota Skipper

Much of the survey work conducted in Canada in recent years has been driven directly or indirectly by the research requirements for the Status Assessment and Recovery Strategy reports (COSEWIC 2003; Environment Canada 2007). In 2002 and 2007 Reginald Webster was retained to conduct surveys for Dakota Skipper in Manitoba and Saskatchewan (Webster 2003; Webster 2007). In 2002, Webster visited all previously known sites to confirm their presence/absence, identify new sites, conduct coarse population estimates, and compile an overview of the species biology, limiting factors and threats (Webster 2003). The majority of Webster's time in 2002 was spent at sites in the Interlake, though he did visit the few known sites in southwestern Manitoba and identified additional sites. In 2001, Hooper (2003) collected three Dakota Skippers near Oxbow, Saskatchewan confiming its presence in that province. In 2007, Webster focused his efforts on southwestern Manitoba and southeastern Saskatchewan to further assess the distribution and population in that region. As in 2002, Webster identified several new Dakota Skipper sites and recommended areas to be further investigated (Webster 2007).

Under the supervision of Dr. R. Westwood, University of Winnipeg, Morden (2006) and Bates (2007) studied habitat related factors associated with the Dakota Skipper in Manitoba. Morden (2006) investigated the potential to reintroduce the Dakota Skipper to the Tall Grass Prairie Preserve (TGPP) in southeastern Manitoba, where it is believed to have been extirpated prior to 2000. Morden (2006) conducted vegetation surveys at three sites in the Interlake region where there were small populations of Dakota Skipper and at three sites in the TGPP. The intention was to examine the link between larval and adult food plants, and the presence of Dakota Skipper. The study found significant differences in the vegetation composition between the TGPP and Interlake plots (Morden 2006). It was thought that the Dakota Skipper's extirpation from the TGPP was due to disturbances such as controlled and unmanaged burns and cattle grazing. In the TGPP, Bates (2007) investigated the vegetation composition of four

disturbance categories (three burn site age groups plus grazed sites) in relation to larval and adult food plants. The results indicated that the more recently burned sites have higher plant diversity and higher occurrence of adult and larval food plants making them more suitable for Dakota Skipper, as compared to the other sites (Bates 2007).

A survey of listed bird species was undertaken in Riding Mountain National Park in 2002 (Walleyn 2002). During this survey Walleyn reported observing one Dakota Skipper each at two different locations, however vouchers were not collected so the record cannot be verified (Walleyn 2002).

As part of the United States species-at-risk legal evaluation process a status assessment was conducted for the Dakota Skipper throughout its current range (including Canada) by Cochrane and Delphey (2002). This report was compiled based on previous Dakota Skipper fieldwork and publications. It detailed the knowledge to date on the species biology, distribution, population, conservation efforts, status and threats, and provided conservation recommendations. This document was the first comprehensive Dakota Skipper Status Report, and it recommended that favourable land management practices be implemented, particularly in relation to agricultural land uses, and that further research be conducted on the species (Cochrane and Delphey 2002).

A systematic study of the life history of all *Hesperia* of North America, particularly those of California, was undertaken by MacNeill (1964). MacNeill (1964) provided detailed descriptions of all life stages of *Hesperia*, many of which are applicable to Dakota Skipper, and he also reported those attributes that were different in the Dakota Skipper (i.e. differences in male genitalia). The study examined much of the lower taxonomy of the *Hesperia* and stated that Dakota Skipper exhibited a great deal of taxonomic divergence from its nearest relative. MacNeill (1964) examined the habitat associations and distributions of the North American skipper species, and reported the Dakota Skipper's range to be "primarily prairie" with some overlap with the "north" boreal forest transition zone (MacNeill 1964).

McCabe (1981) was among the first to study the life history of the Dakota Skipper at sites in North Dakota and reported many key life history events including the dates and duration of the flight period, a description of the larva and larval behaviour, larval food plants and larval shelter construction. McCabe (1981) also described adult behaviour, courtship, dispersal, nectar plant use, predation, habitat, soil and vegetation composition. Management recommendations and remarks also suggested that Dakota Skipper site distribution may be associated with historical glacial lakes (McCabe 1981).

Dana (1991) investigated the life history and impacts of spring prescribed burns on Dakota and Ottoe Skippers at one site in southwest Minnesota from 1978 to 1983. The results of this study provided additional details on the adult and larval behaviour of Dakota Skipper, flight period, habitat use, food plants, fecundity, life span and population estimates. Dana (1991) found that plant fuel load in relation to burn intensity strongly influenced larval mortality with higher plant fuel loads causing greater mortality of larvae. Dana (1991) also suggested that the date of spring burns may affect mortality rates. Later, Dana (1997) studied the characteristics of three Dakota Skipper sites in Minnesota. Sites were characterized based on landform and topography, soils, vegetation and land use (management). The Dakota Skipper survey history for each site was also provided in addition to skipper distribution within sites and apparent habitat preferences. The population size and dynamics, threats and prognosis for each site were also estimated and a preliminary local level estimation of site characters was developed (Dana 1997).

Ann and Scott Swengel have spent the past three decades conducting surveys and research on rare grassland butterflies in Illinois, Iowa, Minnesota, Missouri, North Dakota and Wisconsin (Swengel 1996; Swengel 1998; Swengel and Swengel 1999a; Swengel and Swengel 1999b; Swengel and Swengel 2001; Schlicht et al. 2009). Swengel (1996) documented the effects of fire and hay management on the abundance of Dakota Skipper and other prairie specialists, and showed that Dakota Skipper was negatively affected by fire across the United States range and that these effects may persist for three to five years. Abundance of prairie specialists were also much higher in hayed sites as compared to burned sites, thus it was concluded that haying is a more favourable management approach for prairie specialist butterflies, including the Dakota Skipper (Swengel 1996). Swengel (1998) also compared various prairie management options: including single occasional wildfires, regular rotational burning, cutting (including haying) and cattle grazing. The specialist butterflies, including Dakota Skipper, were found to be more numerous after wildfires versus rotational burns and cutting over grazing (Swengel 1998). Swengel and Swengel (1999b) examined the correlation in the abundance of three

specialist grassland songbirds to prairie butterflies. They found that specialist prairie butterflies strongly correlated with the occurrence of the specialist songbirds, suggesting that conservation programs for specialist grassland birds may benefit prairie butterflies and that they may serve as indicators of each other's presence.

Swengel and Swengel (1999a) also studied adult Dakota Skipper density relative to flight period, adult sex ratios, geographic factors (latitude and longitude), time of day, weather and habitat factors. They found that habitat tended to show the greatest correlation to density and that Dakota Skipper occurred at lower densities at idle sites and burned sites. Dakota Skipper was also absent from sites with heavy grazing, but mowing/haying in September showed a positive effect on density. An increased proportion of females were observed later in the fight period (Swengel and Swengel 1999a). Swengel and Swengel (2001) compared the effects of different management approaches to three ecological subgroups of butterflies (native vegetation specialists, grassland and generalist species). Similar to their previous findings, their results indicated each of the subgroups had different degrees of sensitivity to various management approaches. The specialist subgroup, which included the Dakota Skipper, did not consistently favour one management type over another, and the authors advised conservation managers to use caution and not strictly employ only one management type over other approaches (Swengel and Swengel 2001).

Schlicht and Saunders (1995) surveyed Minnesota skippers between 1993 and 1997 (including Dakota Skipper) and found that populations have decreased, potentially in part due to rain and flooding in 1993, and several management recommendations were made in an effort to allow populations to recover. Schlicht et al. (2009) performed a meta-analysis of surveys from 1979 to 2005 to assess trends in prairie butterflies in Minnesota (including Dakota Skipper) and found that both transect and non-transect surveys provided consistent and reliable results. Populations of five specialist species, including Dakota Skipper, significantly declined over the 26 year sample period, while the five most common non-specialist species remained unaffected. It was also noted that the areas surveyed were all managed by fire and that the adjacent areas with different management methods did not exhibit the same trends and they concluded that management approaches need to consider other methods suitable to individual species' needs, in addition to ecosystem-based management approaches (Schlicht et al. 2009).

Characterization of non-biotic features of Dakota Skipper habitat across the United States range was undertaken by Rover et al. (2008). GIS analysis had suggested that historic Dakota Skipper sites showed a close association with the location of nearshore glacial lake features and their related soil associations. Royer et al. (2008) hypothesized that "such edaphic features as soil moisture, soil compaction and soil bulk density, as well as related non-biotic factors such as temperature and relative humidity at or near the soil surface... may be significant factors in larval survival potential". The study measured the topography, relief, soil compaction, soil pH, soil moisture, soil temperature, soil bulk density, soil texture and near-surface humidity within three sites in each of the following states: Minnesota, North Dakota and South Dakota. The results indicated that Dakota Skipper prefers two distinct substrate types: 1) "a relatively low surface relief with dense but relatively less compact soils; and, 2) another of relatively high relief with less dense but more compact soils" (Royer et al. 2008). Long-term cattle grazing was also attributed to higher levels of soil compaction at some sites and it was suggested that this may impact the vertical water distribution in the soils and in turn increase larval desiccation in late summer.

### 2.5 Molecular Research

To date only one study has examined the genetics of Dakota Skipper. The genetic population structure of Dakota Skipper in Manitoba, Minnesota and North Dakota was examined by Britten and Glasford (2002). Nine populations of Dakota Skippers were sampled and starch gel electrophoresis was employed to assay 21 isozyme loci. Genetic distance analysis indicated that the populations in Manitoba are somewhat distinct from those in Minnesota and North Dakota. Range-wide and regionally, the effective immigration rates were small and the effective population size was also low which indicates that the Dakota Skipper populations are genetically isolated from each other (Britten and Glasford 2002). No analysis has been done comparing adult populations at a smaller scale to examine genetic variability and movement between adjacent sites.

Prior to commencement of the present study, very few studies had been undertaken to test non-lethal sampling methods to remove body parts from living insects, particularly species of conservation concern. Châline et al. (2004) was among the first to investigate non-lethal tissue sampling methods upon Honey Bees (*Apis mellifera*; Apidae). They asserted that leg removal was too harmful to bees (as bees are dependent upon their legs for a variety of activities both when foraging and in the colony) and instead tested the efficacy of removing wing tips ( $\sim 1.3 \text{ mm}^2$ ). When compared to the removal of other tissues or body parts they found that removal of wing tips provided good quality DNA sufficient for amplification of microsatellite loci (Châline et al. 2004).

Vila et al. (2009) tested the quality of DNA and effects of fitness upon sampling of either one mid-leg (~ 15 mm long) or both hind-wing tails (~ 130 mm<sup>2</sup>) in a large, protected moth, Graellsia isabelae (Graëlls; Saturniidae). The DNA from both sources was of high quality and sufficient in quantity for amplification of microsatellite loci. The highest concentrations of DNA were obtained from the largest wing clips (~ 130 mm<sup>2</sup>) as compared to half a leg. They also found that while neither sampling method appeared to affect male fitness or female survivorship, leg sampling negatively affected female mating success. Vila et al. (2009) advised that wing-tail clippings from males would be the best sampling procedure in this species. Keyghobadi et al. (2009) also tested the effectiveness of removing wing clips ( $\sim 10 \text{ mm}^2$ ) relative to whole bodies from the at-risk Behr's Hairstreak (Satyrium behrii W.H. Edwards; Lycaenidae) and Mormon Metalmark (Apodemia mormo Felder and Felder; Riodinidae) for use in the generation and analysis of amplified fragment length polymorphism (AFLP) profiles. They found that samples from wing clips yielded results comparable to whole body sampling and reported that this was an effective form of non-lethal sampling for this type of species (Keyghobadi et al. 2009).

Hamm et al. (2010) evaluated the impact upon behavior and survival following removal of a wing clip (~ 2 to 3 mm<sup>2</sup>) from Painted Lady (*Vanessa cardui* Linnaeus; Nymphalidae) and Eyed Brown, (*Satyrodes eurydice* Linnaeus; Nymphalidae) butterflies. They did not remove legs for this study as Nymphalids use all four legs for walking which they require for stabilization (the front legs are greatly reduced). Butterflies were observed in a greenhouse setting and in a field setting following removal of tissue. DNA extracted from wing clips was amplified using PCR for the COI region using the primers employed in this study (Hamm et al. 2010). Hamm et al. (2010) found that removal of

wing clips had no significant impact upon the species studied. They did acknowledge that their species had among the lowest wing loading rates observed in Lepidoptera and that in species with higher wing loading (e.g. skippers), wing clipping may have more adverse effects (Hamm et al. 2010).

Recently Koscinski et al. (2011) evaluated the fitness effects of the removal of hind legs and wing clips from both wings (~ 25 mm<sup>2</sup>) from the Cabbage White (*Pieris rapae* Linnaeus; Pieridae) and the Common Ringlet (*Coenonympha tullia* Fabricius; Nymphalidae) butterflies in natural habitats. This study did not evaluate the quality and concentration of DNA obtained from the tissue samples. There were no differences in flight behaviour (short-term response) and survival (long-term response) in either species, though it was observed that differences in flight behaviour existed between the sexes of each species (Koscinski et al. 2011). Cabbage White males lived longer (survivorship) than females, while male Common Ringlet spent more time flying than females and travelled shorter distances (flight behaviour). Koscinski et al. (2011) also acknowledged that smaller species of butterflies (i.e. skippers) may be more negatively affected by wing clippings.

Although several studies have been undertaken to demonstrate that high quality DNA can be obtained from butterflies in a non-lethal manner, very few studies have expressly been designed to reduce impact of body part removal to species-at-risk and none have been undertaken upon skippers (Hesperidae). Removal of body parts may have significant affects on survival as many skippers have a very short flight period, are at-risk of extinction and occur at low population densities (COSEWIC 2003; Hamm et al. 2010). Dakota Skipper wings are each approximately 10 mm x 10 mm (100 mm<sup>2</sup>) and skippers have very high wing loading relative to other butterflies (Layberry et al. 1998), potentially making removal of wing clips ill-advised, as most studies required an area equivalent to approximately 5% or more of a Dakota Skipper wing, through a major vein. Dakota Skipper adults exhibit significant wing wear relatively quickly following emergence such that, after only a few days, adults appear very worn (*personal observ*.). Wing clipping may promote additional wing wear by exposing more surface to wear and disturbing intact surfaces. In addition, clipping reduces the amount of wing area available for flight.

## 3.0 GENERAL METHODS

Four candidate sites in central Manitoba (Interlake region) and four candidate sites in southwestern Manitoba with known populations of Dakota Skippers were selected for this study (Figure 3). These sites were chosen as the majority of the Dakota Skipper population in Canada has been previously recorded from these areas. The two regions are separated by approximately 200 km. Attempts were made to select sites in each area in general proximity to each other with the highest number of individuals based on previous surveys (Webster 2003; Webster 2007).

Interlake research sites were identified as Sites A to D and the southwest sites were identified as Sites E to H (Figure 3). The sites were located on privately owned land (Table 2) and permission to work on the properties was obtained from each parcel owner. Each land parcel (quarter section) containing a site was approximately 64 ha in size (0.8 x 0.8 kilometers), with the exception of Site E. Site E spanned two abutting parcels of land as there was insufficient suitable habitat on either parcel individually to carry out the research protocol.

Research sites*		Geographic			
Site ID	Field code	region	Ownership General land us		
А	IL19	Interlake	Private	Fall haying	
В	IL24	Interlake	Private with crown lease	Fall haying	
С	IL39	Interlake	Private	Fall haying	
D	IL50	Interlake	Private with industrial operation elsewhere on section	Fall haying & wildfire	
Е	OK1	SW Manitoba	Private	Fall haying	
F	OK6	SW Manitoba	Private	Fall haying & occasional cattle grazing	
G	OK7	SW Manitoba	Private	Fall haying	
Н	OK14	SW Manitoba	Private	Fall haying	

1 ubie 2. Site descriptions	Table 2.	Site	descriptions
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\*Site coordinates available on request.

Two 250 m long transects were established in each research site (Figure 4) to facilitate plant, soil and adult surveys (see Section 5.0 and 6.0 for detailed description).

Transects were nestled into the landscape over suitable Dakota Skipper habitat since the sites are a matrix of lower wetland areas and higher prairies and aspen groves. At Site E, one transect was placed in the southwest corner of one parcel and the second transect was placed in the northwest corner of the parcel immediately to the south.

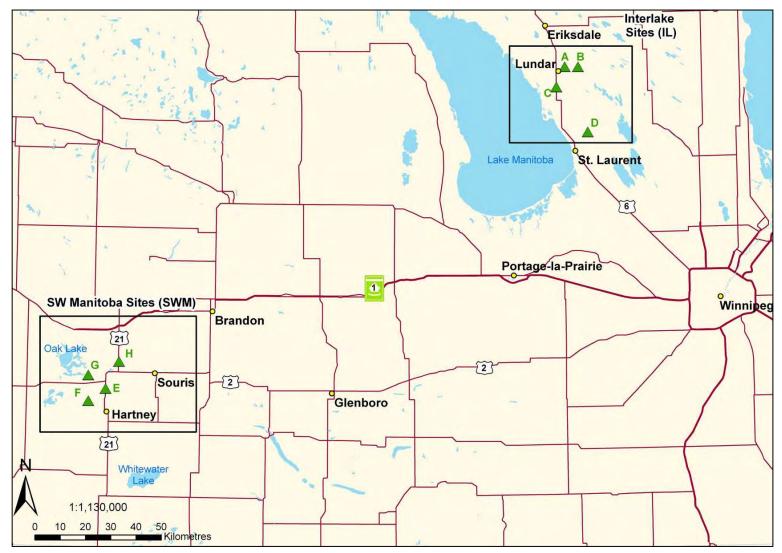


Figure 3. Location of Dakota Skipper research sites in Manitoba



Figure 4. Airphoto of Site C illustrating placement of transects within suitable habitat

## 4.0 SUMMARY OF LAND MANAGEMENT HISTORY

This section addresses objective 1: develop an understanding of the land management history (i.e. pasture land, haying, cropland) at Dakota Skipper study sites.

#### 4.1 Methods

In fall 2010, property owners and tenants of the study sites were contacted to obtain a history of land use for the past five years (commencing in 2006). A survey was sent to owners and/or tenants asking for specific details on the type of agricultural use (e.g. haying, pasture, cropland, etc.) and specific annual timing of agricultural activities for each of the past five years at the sites. A history of burning, pesticide use and other activities (e.g. installation of communication tower, etc.) was also requested.

## 4.2 Results

Responses were received from all the property owners and/or tenants. Generally all the sites were used as "wild hay" and subject to fall having (Table 2 and 3), when the land is sufficiently dry to permit access by farm machinery. In recent years, several of the sites were not haved due to wet conditions, particularly those in the Interlake region. Site A was previously cultivated and was also overgrown with Agrostis stolonifera (redtop). Site D experienced a wildfire in late September 2012 and additional back burning and firebreak ditching was employed on site. There was a loss of habitat during the study period at Site F in 2011 and 2012, caused by spring flooding as a result of poor drainage. The wetland areas at Site F appeared to be expanding during the study period. Additionally, in early summer 2012 cattle were pastured on Site F, causing considerable soil compaction and rutting. Cattle grazing was heavy enough at Site F such that by the Dakota Skipper flight period there were almost no nectar flowers present, few plants over 50 cm in height and the overall plant density was lower than previously observed. Site G also experienced a loss of habitat in 2011 and 2012 due to the flooding of Plum Creek which resulted in standing water on site through portions of each summer. All other sites appear to

have never been cultivated or seeded, though some drainage management appeared to have been completed at several sites. No utilities or other structures were present at the sites, nor were there any known application of fertilizers or pesticides at the sites. A full summary of the land use and impacts is provided in Table 3.

During the site selection, another potential site (OK13/15) in the parcel directly northwest from Site H was considered as it had previously had very high counts of Dakota Skipper (26 individuals in 2002 and 98 individuals in 2007) (Webster 2002, 2007). However, upon inspection in early summer 2010, the site was found to be heavily grazed by cattle and the habitat appeared to now be unsuitable for Dakota Skipper, thus this site was not selected.

Table 3.	Summary	of study	site land	use and	impacts

Site ID	Recent Haying	Ditching	Other Events	Historic Use	Impacts
A	Cut late Aug. or mid-Sept. 2006, 2008 to 2011. Not cut in 2007.	None on-site, only roadside ditching.	Cultivated (tilled) in Fall 2006 and 2007. Site has very high amount of redtop ( <i>Agrostis stolonifera</i> ) crop plant but has never apparently been seeded into site.	No known other agricultural activities on the site aside from haying and tilling.	Site is overgrown with redtop and only southeast corner remains as high quality prairie.
В	Cut in late Aug. or Sept. 2006, 2007 and 2011. Not cut in 2008 to 2010 as site too wet.	Ditching present through western portion of site and significant roadside ditching done in 2010 and 2011.	Site has some vehicle trails used by ATVs and hunters.	Ditching installed through western portion of site in 1990's to improve drainage from wetland to the south. Old pond present in northwest corner and excavated a while ago.	Some prairie patches to the extreme east (beyond ditches and up aspen corridors) are not being hayed with the rest and experiencing more aspen succession. Ditching through site appears to improve drainage on site. Roadside drainage excavations in 2010 and 2011 resulted in loss of a small amount of habitat. Recreational vehicle trails causing some local compaction and destruction of habitat.
С	Cut mid to late Sept. 2006, 2009 to 2011. Not cut in 2007 and 2008.	None on-site, only roadside ditching.	Field to the north (next quarter section) is seeded with alfalfa ( <i>Medicago sativa</i> ), so there is a bit of alfalfa and other invasive plants infiltrating at the property limit.	No known other agricultural activities on the site aside from haying.	No apparent impacts.

Table 3. Summary of study site land use and impacts
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Site ID	Recent Haying	Ditching	Other Events	Historic Use	Impacts
D	Cut in late Aug. or Sept. 2006 to 2008. Not cut in 2009 to 2011 as site too wet.	None on-site until fall 2012 for wildfire control. Roadside ditching present.	Site has a network of low use trails for dog-running and hunting. Wildfire occurred on site from Sept. 15 to 28, 2012, and back burning and fire guard ditches were employed. Ditching extends through middle of site in areas. Light and moderate sized machinery (pick-up truck & backhoe) were driven on-site.	No known other agricultural activities on the site aside from haying. Infrequently hayed as too difficult to access in wetter years.	Site experiencing aspen succession due to lack of regular haying however wildfire may have slowed this. Impacts of 2012 wildfire are unclear in regards to Dakota Skipper mortality and loss of habitat. Ditching and use of machinery on- site will have caused some compactions and loss of habitat.
E – North side	Cut in late Aug. 2007 to 2009. Not cut in 2006, 2010 and 2011 as site too wet.	None on-site, only roadside ditching.	Field to the east (on parcel) is seeded with alfalfa ( <i>Medicago</i> <i>sativa</i> ), so there is a bit of alfalfa infiltration.	No known other agricultural activities on the site aside from haying.	Low, wet area expands into a wetland during years with high precipitation overtaking more prairie habitat. Prairie patch at site is very small and may become overgrown with invasive species.
E – South side	Cut in late July or early Aug. 2006 to 2009 and early Sept. 2011. Not cut in 2010 as site too wet.	None on-site, only roadside ditching.	Controlled burn on site in Oct. or Nov. 2007.	No known other agricultural activities on the site aside from haying and controlled burn.	Controlled burn may have encompassed entire habitat on property and caused a decline in Dakota Skipper population. No other apparent impacts.

Table 3.	Summary	of study	site land	use and	impacts
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Site ID	Recent Haying	Ditching	Other Events	Historic Use	Impacts
F	Cut in Sept. or early Oct. 2006 to 2009. Not cut in 2010 or 2011 as site too wet.	None on-site, only roadside ditching.	Significant flooding of site in fall 2010, summer 2011 and 2012 associated with drainage obstructions northwards resulting in a large wetland area. Cattle intensely grazed on site in early summer 2012 causing soil compaction/rutting and devegetation such that, during the flight period, there were virtually no nectar flower heads present, and vegetation was very short and at much lower densities.	No known other agricultural activities on the site aside from haying.	Site is undergoing detrimental habitat alterations associated with the wetland expansion and cattle grazing. Wetland expansion caused loss of habitat through increased footprint. Cattle caused soil compaction/ rutting and devegetation resulting in loss of nectar flowers, shelter plants and larval food plants. Drainage alterations likely due to use of fill and build up of adjacent road to the north.
G	Cut in late Aug. or early Sept. 2006 to 2010. Not cut in 2011 as site too wet.	Ditching present through central portion of site to connect adjacent roadside ditching to Plum Creek.	Massive flooding of site in fall 2010 and through 2011.	Occasional seasonal flooding associated with Oak Lake and Plum Creek. Municipal road extends west through parcel and is subject to periodic improvements but is low use.	Periodic flooding reduces available habitat and causes larval mortality. Massive flooding in 2011 caused a significant loss of habitat that year; the long-term impacts are unknown at present.
H	Cut in late Aug. or early Sept. 2006 to 2011.	None on-site, only roadside ditching.	None.	No known other agricultural activities on the site aside from haying.	No apparent impacts.

#### 4.3 Discussion

The management history of the research sites was similar with fall having predominating. However, there were additional activities occurring at some sites which may have further impacted site quality. Research sites were largely used for "wild hay" production. As reported by Swengel (1996; 1998) having appears to be an appropriate alternative for the maintenance of Dakota Skipper habitat as it acts somewhat like fire in removing woody species, excessive thatch and organic matter build up while allowing continued growth of native flora.

Excessive flooding and subsequent ponding of water at sites, particularly Sites D, F and G, caused loss of habitat and also inhibited haying operations. The impacts of the wildfire at Site D are unknown at this time and may have caused a decline in the Dakota Skipper population at the site or improved the habitat. The impacts of cattle grazing at Site F are also unknown and may have adversely affected the Dakota Skipper populations present. Sites D, F and G are likely suffering the greatest impacts associated with the land use. Sites B is also at risk of some impacts associated with flooding, succession and recreational vehicle footprints. Sites A, C and E have minimal land use associated impacts. Lastly, Site H has no apparent on-going impacts.

# 5.0 CHARACTERIZATION OF DAKOTA SKIPPER HABITAT IN MANITOBA

This section addresses objective 2: develop a Dakota Skipper site character profile based on floristic and edaphic-related factors.

### 5.1 Methods

#### 5.1.1 Floristic Surveys

## 5.1.1.1 Vegetation Surveys

To implement vegetation surveys sampling points were established at 50 m intervals along transects (see Section 3.0), within five metres of either side of the interval (Figure 5). Two 250 m transects were established at each site. Vegetation surveys were conducted once in June and August 2010 and included an assessment of the herbaceous and woody species present. At each 50 m interval along each transect a 1 m x 1 m quadrat was randomly placed within five metres of one side or the other of the transect using a random numbers table (Figure 5). Six quadrats were placed per transect during each sample period. All plants within the quadrat area were identified to species (or the lowest taxonomic level) and the percent cover of each species was estimated. Samples of all species inventoried were taken to the laboratory and placed in a plant press for future species verification and reference. Plants were identified in both vegetative and flowering form using Best et al. (1977), Johnson et al. (1995), Semple et al. (1999), Vance et al. (1999), Semple et al. (2002) and Reaume (2009), and nomenclature was based on Looman and Best (1981) with synonyms provided in Appendix I.

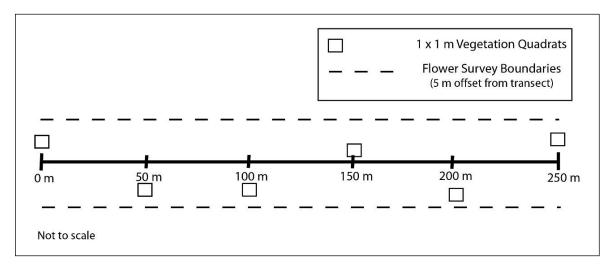


Figure 5. Diagram of the vegetation and nectar flower survey methods along a transect

Vegetation inventories in June 2010 were intended to capture species that may not have been present or difficult to identify later in the summer. The second vegetation survey was conducted in August 2010, after the Dakota Skipper flight period, when grasses and late blooming flowers were easier to identify. Sedges (Cyperacea) and rushes (Juncacea) were not identified in August as they were inventoried in the June survey. All vegetation surveys were conducted prior to any haying.

### 5.1.1.2 Nectar Flower Surveys

Surveys of nectar flower species were conducted along each transect in early and mid-July 2010 to coincide with the start and end of the Dakota Skipper flight period. A five metre offset was established on both sides of each transect and the entire 2500 m<sup>2</sup> area within these limits was surveyed. All species in flower were identified to the lowest taxonomic level possible and the number of stems for each species were counted. Samples of all species inventoried were taken to the laboratory and placed in a plant press for future species verification and reference.

#### 5.1.1.3 Data Analysis

The mean percent cover/m<sup>2</sup> for vegetation and mean number of stems/m<sup>2</sup> for nectar flower survey data were calculated for all species at the transect, site, and regional level for the June and August surveys. In addition, vegetation was grouped into higher taxonomic levels (Graminae, Cyperaceae, Juncaceae, "Other Monocots", the Composites, and "Other Dicots") as particular vegetative groupings may be significant indicators of

Dakota Skipper habitat (see Appendix I for synonyms). Known or potential larval and adult food plant species were also analyzed individually in the same manner as the taxonomic groupings. As data sets were not normally distributed the non-parametric Mann-Whitney U test was used to test for differences between sites and region. The level of significance ( $\alpha$ ) to reject the null hypothesis was set at *P*<0.05.

Several measures of diversity were calculated for each site for the vegetation and nectar flower surveys. For the vegetation surveys, the maximum value (mean percent cover/m<sup>2</sup>) of the two surveys was used in the diversity calculation. The higher mean value of the two surveys was chosen to ensure all species were represented in the analysis as some species may not have been identified, especially in the June survey due to small size or lack of developed taxonomic characteristics available to confirm identification. For the nectar flower surveys, the mean value (mean number of stems/m<sup>2</sup>) of the two survey dates was averaged for use in the diversity calculations. In this instance the two nectar flower surveys were separated by less then two weeks so there was very little difference in phenology and a high probability of recounting the same flower stems.

Diversity at sites was calculated using the Abundance Curve Calculator (Danoff-Burg and Chen 2005) to derive the Shannon diversity (H) index, Shannon eveness (E) index, Simpson reciprocal index (1/D) and the Berger-Parker reciprocal index (1/d) measures. Shannon H index is a combined evaluation of species richness and evenness, while Shannon E index is a measure of species evenness (Magurran 1988). The Shannon diversity index is calculated as follows:

 $H' = -\sum p_i \ln p_i$  (Equation 1)

where  $p_i$  is the proportion of individuals in the *i*<sup>th</sup> species. Shannon H index typically ranges between 1.5 to 3.5 and rarely exceeds 4.5 (Magurran 1988). The Shannon eveness index is calculated as follows:

$$E = H' / \ln S$$
 (Equation 2)

where *S* is the number of species. The Shannon E index ranges from zero to one, with one indicating that all species present are equally abundant (Magurran 1988).

The Simpson index is a measure of species diversity and heterogeneity. The reciprocal of the Simpson index is used such that an increase in the value represents an increase in species diversity. The Simpson reciprocal index is calculated as follows:

 $1/D = \sum \{ [n_i (n_i - 1)] \div [N (N - 1)] \}$  (Equation 3)

where  $n_i$  is the number of individuals in the  $i^{th}$  species and N is the total number of individuals. As the Simpson reciprocal index increases the species diversity increases, and is weighted towards the most abundant species in the sample and less sensitive to species richness (Magurran 1988).

The Berger-Parker reciprocal index is a measure of species dominance and is calculated as follows:

$$1/d = N_{max} / N$$
 (Equation 4)

where  $N_{max}$  is the number of individuals of the most abundant species in the sample. As the Berger-Parker reciprocal index increases the diversity increases and dominance decreases (Magurran 1988). The diversity analysis provides an ecosystem overview in terms of species richness, evenness and diversity. Differences in diversity indices between the two regions were evaluated for each measure using a *t*-test (*P*<0.05).

Indicator Species Analysis (ISA) was performed in PC-ORD (McCune and Mefford 1999) to detect if the presence of individual plant species may be significant indicators of a sampling unit (Dufrene and Legendre 1997; McCune and Grace 2002). ISA considers the abundance and fidelity of a species to a particular grouping, in this case site or region. Indicator species values range from 0 to 100 where zero is no strong association with site or region and 100 is a "perfect" indicator of a particular site or region (McCune and Grace 2002). PC-ORD allows for testing of significance with a Monte Carlo test (P<0.05). ISA was performed on the vegetation survey data and the nectar flower survey data to determine if particular species were indicative of a given region (Interlake or southwest Manitoba) or site.

Principal Component Analysis (PCA) was performed using Canoco for Windows (Ter Braak and Smilauer 2002). The ordination analysis was used to reduce the data to a few axes in N-dimensional space and show the data graphically (Legendre and Legendre 1998; Kenkel 2006). Ordination analysis enables analysis of trends by reducing the variation within large multivariable data sets. PCA extracts the data into as few ordination axes as possible such that as much of the variation as possible is explained in the first axis, followed by the second axes, then the third, etc. PCA generates an eigenvalue equivalent to the amount of variation held by each axis and the higher the

eigenvalue of an axis relative to the sum of all the eigenvalues, the more the variability is explained by that axis (Kenkel 2006). The result is a "biplot" or "triplot" that visually demonstrates through proximity the relationship between variables (Legendre and Legendre 1998; Kenkel 2006). Using the maximal mean of the two vegetation surveys a PCA was performed grouped by the eight research sites and all the plant species observed. The position of the larval food plant species and the "indicator species" indentified in the ISA by region were then highlighted by editing the output diagrams to create figures for those species alone. PCA was also performed on the flower survey data using the mean of the two flower surveys organized, and then the nectar flower species and ISA species were filtered out similar to the vegetation diagrams. A detrended analysis was first performed on the original data sets to ensure the data was linear (and not clumped), rationalizing the use of a PCA (Kenkel 2006). Data were log-transformed in Conoco prior to analysis.

## 5.1.2 Edaphic and Edaphic-Related Characterization

## 5.1.2.1 Edaphic and Edaphic-Related Surveys

Edaphic features related to soils and microclimate were measured at each transect at the soil surface. The term edaphic is defined as "of or relating to the soil" as in "resulting from or influenced by the soil rather than the climate" (Merriam-Webster Incorporated 2013). Conditions at and immediately below the soil surface were of primary interest because this is the habitat of the Dakota Skipper for much of their life particularly during the larval stage. The following soil and soil-related parameters were measured:

- amount of bare ground;
- depth of duff layer;
- soil pH to 10 cm depth;
- soil moisture to 10 cm depth;
- soil compaction at 10 cm, 20 cm, 30 cm and 40 cm depth;
- soil apparent bulk density;
- soil particle size content (amount of sand, silt and clay) and soil texture class;
- amount of soil organic matter and calcium, magnesium and sodium cations;

- air temperature at the soil surface; and
- air relative humidity at the soil surface.

During the June and August 2010 vegetation surveys, the percentage of bare ground in each 1 m x 1 m quadrat was estimated along with the depth (cm) of the duff layer. In August 2011, soil compaction, soil pH and soil moisture was measured in the field at 50 m intervals along transects using a Field Scout SC 900 Soil Compaction Meter with 19 mm tip (<sup>3</sup>/<sub>4</sub> inch) (Spectrum Technologies Inc., Aurora, Illinois) and a Kelway Soil pH and Moisture Meter (Kel Instruments Co. Inc, Wyckoff, New Jersey). Soil compaction was measured in pounds-per-square inch (PSI) at depths of 10, 20, 30 and 40 cm and then later converted to kilopascals (kPa). Soil pH and soil moisture measured as the percent saturation.

At each site soil was gathered from three randomly selected locations among the two transects and combined into a single sample for analysis. Soil samples were collected in early August 2011 from the first 10 cm of the soil horizon. At Sites B, C, D and H there appeared to be additional suitable Dakota Skipper habitat or prominent Dakota Skipper activity during the flight period greater than 50 m off the transects, thus additional bags of soil were gathered from these selected patches. One additional bag was collected at Sites B, C and D, while at Site H two additional bags were collected. Where multiple samples were collected at a site, the mean of the values for each site were obtained for the analysis.

In the laboratory the pH, moisture and soil texture of each sample was measured. The 0.01 M CaCl<sub>2</sub> method was used to measure soil pH in the laboratory, while the gravimetric oven drying method was used to determine the soil moisture (as percent gravimetric water content). The apparent texture of the soil was determined by the feel method. It should be noted that some data parameters (pH, moisture and texture) were measured multiple times and/or with different methods in different units so that the accuracy of the measurements could be checked or to enable comparison with other studies where different methods were employed. All soil samples were then sent to Exova Group Limited (Edmonton, Alberta) for additional soil analysis (soil moisture, sand, silt and clay composition, soil texture, organic matter, calcium, magnesium and sodium content, and apparent bulk density). Moisture was measured as percent moisture content using the gravimetric method for oven drying. The particle size content was determined by the hydrometer method which gives the percentages of sand, silt and clay in the soil. The proportion of the particles was then used to determine the actual soil texture class. The percent by weight of organic matter was determined with the loss of ignition method, while the available nutrients (calcium, magnesium and sodium) in the soil were determined using the ammonium acetate extractable cations method and measured in mg/kg. Lastly, the apparent bulk density of the soil was measured in kg/L with the core method.

Temperature and relative humidity at the soil surface were recorded continuously using a HOBO data logger installed adjacent to one transect at each research site from mid-June to mid-November 2010 and early May to late November 2011. The HOBO loggers (Onset Computer Corporation 2011, Bourne, Massachusetts) were programmed to record the temperature and relative humidity once per hour over a 24 hour period for the entire time they were placed in the field. Temperature was recorded in degrees Celsius and relative humidity was recorded as the percent relative humidity of the air. The loggers were placed in a 15 cm long section of 5 cm diameter white plastic central vacuum pipe (to serve as a sun baffle) directly on the soil surface. The data recorded by the HOBO loggers was downloaded from loggers to a portable laptop computer using HOBOware Pro Version 3.2.1 (Onset Computer Corporation 2011, Bourne, Massachusetts).

The soil analysis component of the present study was implemented in part for comparative purposes with results obtained by Royer et al. (2008) who evaluated similar soil parameters at Dakota Skipper sites in Minnesota, North Dakota and South Dakota. Additional information on Royer et al. (2008) sites can be found in Appendix VI. Royer et al. (2008) also measured the temperature and relative humidity at the soil from July 5 to September 23, 2000 and referred to this period as the "larval period" as this is the approximate time when Dakota Skipper larvae develop from the first instar to the winter diapause stage. Temperature and relative humidity soil data collected in this study was

converted to Julian weeks to enable comparison between years and studies. Royer et al.'s (2008) "larval period" therefore corresponds to Julian weeks 28 to 39.

#### 5.1.2.2 Data Analysis

The mean and standard error of each soil parameter was calculated for the research sites and regions. The mean temperature and relative humidity at the soil surface was calculated for each Julian week, research site and region, as well as the mean for the entire year. A Student's *t*-test was used to detect significant differences between the two regions. For illustrative purposes, points were smoothed into a spline when the temperature and relative humidity data were graphed by Julian week. Where the soil parameters were not normally distributed the data was logarithmically transformed for the *t*-test. The following variables were log transformed: bare ground, duff layer, soil moisture measured in the field, soil compaction at 20 cm, 30 cm and 40 cm and the mean of depth, sand particle size, and sodium cation.

A Redundancy Analysis (RDA) was performed using Canoco for Windows (Ter Braak and Smilauer 2002) to examine relationships of physical and species data. An RDA is very similar to a PCA (see Section 5.1.1.3) in that it is a type of ordination analysis that enables analysis of multivariate data by reducing the variation to a few axes and displaying the data graphically. The RDA is regarded as an extension of PCA but differs in that it allows the incorporation of environmental data along with the species data. The ordination of the environmental data (i.e. soils data) is under the constraint of the species data (i.e. species in vegetation or flower surveys) as it relates to the research sites (Legendre and Legendre 1998). The RDA also permits identification of any significant correlations between an environmental variable and the species data. An RDA was performed with the edaphic data and the following data sets pooled by site: the maximal mean of the two vegetation surveys, the maximal mean of the documented or possible larval food plant species from the vegetation surveys, the mean of the two flower surveys, and the mean of the documented or possible nectar flower species from the flower surveys. Eighteen of the edaphic data sets were used in the redundancy analyses as they were the only data or the most reliable data for that variable. Edaphic data used in the RDA consisted of: bare ground (% cover per m<sup>2</sup>); duff layer (cm); soil pH at University of Winnipeg lab; soil moisture (%) at University of Winnipeg lab; compaction

at 20 cm depth (PSI); mean compaction of 10, 20, 30 and 40 cm depth (PSI); bulk density (kg/L); sand particle size (%); clay particle size (%); silt particle size (%); organic matter (% by weight); available calcium (mg/kg); available magnesium (mg/kg); available sodium (mg/kg); mean temperature (°C) of the air at the soil level in 2010; mean temperature (°C) of the air at the soil level in 2011; mean relative humidity (%) of the air at the soil level in 2010; and mean relative humidity (%) of the air at the soil level in 2011. Data were log-transformed in Conoco prior to analysis. Physical variables were standardized to minimize the effects of collinearity.

#### 5.2 Results

Weather, specifically precipitation, greatly affected the selected Dakota Skipper sites during the 2010 and 2011 field season. In 2010 it rained quite frequently throughout the summer and the water table was high at sites by the end of the summer season. Soil surveys could not be undertaken in 2010 because in August and September the soil was very saturated. A combination of the high water table through the winter and heavy rains in spring 2011 caused considerable flooding throughout much of southwestern Manitoba and the Interlake (Environment Canada 2011). Most research sites in the southwest are associated with wetlands and/or tributaries of the Souris River. In the Interlake most of the research sites are also associated with the tributaries of Lake Manitoba or adjacent to large drainage ditches. Both the Souris River and Lake Manitoba experienced unprecedented flood levels in 2011 (Environment Canada 2011). As a result, there was extensive flooding at most sites. Standing water remained at sites B, F and G throughout the early and mid summer 2011, and the remaining sites dried by mid-June 2011. Therefore soil samples were not collected until August 2011.

#### 5.2.1 Vegetation and Flower Surveys

Vegetation communities in Interlake sites fall within the Aspen Parkland ecozone, and more specifically the Terrestrial system, Herbaceous Vegetation class (V) and Perennial Graminoid Vegetation (grasslands) subclass (A) (Greenall 1996). Wooded areas were present throughout all the Interlake research sites and were classified as *Populus tremuloides* Tallgrass Prairie Wooded Herbaceous Vegetation Type (V.A.6.c). The classification of the prairie communities at each site is provided in Table 4.

Site ID	Field code	Region	TNC – UNESCO code	Vegetation community type	Comments
A	IL19	Interlake	V.A.5.a	Tall Sod Temperate Grassland Formation	Site dominated by Agrostis stolonifera which is not a community type option.
В	IL24	Interlake	V.A.5.a	Tall Sod Temperate Grassland Formation; <i>Andropogon gerardii</i> – (Sporobolus heterolepis) – Andropogon scoparius* Herbaceous Vegetation Type	Sporobolus heterolepis is not present at site but this type best describes community present.
С	IL39	Interlake	V.A.5.a	Tall Sod Temperate Grassland Formation; <i>Andropogon gerardii – (Sorghastrum nutans)</i> Herbaceous Vegetation Type	Sorghastrum nutans is not present at site but this type best describes community present.
D	IL50	Interlake	V.A.5.a	Tall Sod Temperate Grassland Formation; <i>Andropogon gerardii</i> – (Sporobolus heterolepis) – Andropogon scoparius* Herbaceous Vegetation Type	Sporobolus heterolepis is not present at site but this type best describes community present.
E	OK1	SW Manitoba	V.A.5.c.	Medium-tall Sod Temperate or Subpolar Grassland Formation; Schizachyrium scoparium* - (Bouteloua spp.) – (Carex filifolia) Herbaceous Vegetation Type	<i>Bouteloua</i> spp. and <i>Carex filifolia</i> not present at site, though other <i>Carex</i> spp. abundant.
F	OK6	SW Manitoba	V.A.5.c.	Medium-tall Sod Temperate or Subpolar Grassland Formation; <i>Andropogon scoparius* - (Bouteloua</i> spp.) – (Carex filifolia) Herbaceous Vegetation Type	<i>Bouteloua</i> spp. and <i>Carex filifolia</i> not present at site, though other <i>Carex</i> spp. abundant.
G	OK7	SW Manitoba	V.A.5.a	Tall Sod Temperate Grassland Formation; <i>Andropogon gerardii</i> – (Sporobolus heterolepis) – Andropogon scoparius* Herbaceous Vegetation Type	Sporobolus heterolepis is not present at site but this type best describes community present.
Н	OK14	SW Manitoba	V.A.5.c.	Medium-tall Sod Temperate or Subpolar Grassland Formation; Andropogon scoparius* - (Bouteloua spp.) – (Carex filifolia) Herbaceous Vegetation Type	<i>Bouteloua</i> spp. and <i>Carex filifolia</i> not present at site, though other <i>Carex</i> spp. abundant.

## Table 4. Vegetation classification at research sites (Greenall 1996)

\* Andropogon scoparius is a synonym for Schizachyrium scoparium (little bluestem).

Classification of vegetation communities was per Greenall (1996), however some of the communities present at sites are not adequately described by Greenall (1996) or lack certain dominant species, and are noted in Table 4.

In southwest Manitoba sites were generally characterized as tallgrass prairie in early transition to mixed grass prairie (Table 4). The wetland communities found at sites E and H were classified as Semi Permanently Flooded Temperate or Subpolar Grassland Formation, *Typha* spp. Herbaceous Vegetation Type (V.A.5.1) (Greenall 1996). The wooded groves at site H were classified as *Populus tremuloides* Tallgrass Prairie Wooded Herbaceous Vegetation Type (V.A.6.c).

#### 5.2.1.1 Vegetation Surveys

The number of species recorded in the June and August vegetation surveys is provided in Table 5. One hundred sixty-three species were inventoried during the study. A list of the species recorded is provided in Appendix I. The number of plant species was similar between regions, although there was considerable variability between the June and August surveys. There were 42 unique plant species recorded in the June survey and 47 unique plant species recorded in the August survey (Table 5). Abundance data for plant species recorded during the June and August surveys is provided in Appendix IIa and IIb, respectively.

	June vegetation survey		Early July flower counts		Mid-July flower counts		August vegetation survey		
	IL	SW MB	IL SW MB		IL	SW MB	IL	SW MB	
Total # species	87	88	43	46	46	52	61	71	
# Unique species	15	27	4	3	7	12	23	24	
Shared species	4	45.8%		39.1%		34.1%		36.1%	

Table 5. Summary of plant species recorded during surveys

The most abundant plant species observed in the Interlake research sites in June 2010 included "unknown grasses" (Table 6a). Many of the grasses were immature and could not be identified to species in the June 2010 survey. *Deschampsia caespitosa* (tufted hair grass) and *Eleocharis palustis* (creeping spike-rush) were also abundant in the Interlake. In southwestern Manitoba *Deschampsia caespitosa*, unknown sedges (*Carex*)

spp.) and unknown grasses were abundant in the June 2010 survey (Table 6a, Appendix IIIa). Graminoid species dominated in both regions and were the most commonly encountered species. Several abundant species were unique to the Interlake during the June 2010 survey including *Eleocharis palustris, Andropogon gerardii* and *Ranunculus cymbalaria* (seaside buttercup) while abundant unique species in the southwest included *Juncus balticus* (Baltic rush) and *Panicum virgatum* (switch grass) (Table 6a, Appendix IIIa).

Family	Scientific name	Interlake (% cover)	Family	Scientific name	SW MB (% cover)
Graminae	Unknown grass	19.69±3.13	Graminae	Deschampsia caespitosa	4.56±1.13
Graminae	Deschampsia caespitosa	8.96±2.14	Cyperaceae	Carex spp.	3.40±1.00
Cyperaceae	Eleocharis palustris	4.50±1.36	Graminae	Unknown grass	3.31±1.03
Graminae	Poa cusickii	2.88±1.29	Juncaceae	Juncus balticus	2.85±1.42
Rosaceae	Fragaria vesca	2.68±1.80	Plantaginaceae	Plantago eriopoda	2.80±0.95
Graminae	Andropogon gerardii	2.60±1.38	Graminae	Poa cusickii	2.19±0.94
Compositae	Sonchus arvensis*	2.60±0.57	Leguminosae	Medicago sativa*	1.98±1.39
Cyperaceae	Carex spp.	2.23±1.05	Compositae	Sonchus arvensis*	1.86±0.63
Graminae	Agropyron spp.	1.40±0.55	Compositae	Antennaria spp.	1.78±0.91
Ranunculaceae	Ranunculus cymbalaria	1.19±1.04	Graminae	Panicum virgatum	1.75±1.57

Table 6a. June 2010 vegetation survey, ten most abundant species (% cover per  $m^2$ , mean $\pm$ SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

During the August 2010 surveys, *Agrostis stolonifera* was the most commonly encountered species in the Interlake, followed by *Andropogon gerardii*, *Andropogon scoparius* and *Sonchus arvensis* (perennial sow-thistle) (Table 6b, Appendix IIIb). In the southwest, *Andropogon scoparius* dominated followed by *Agropyron repens* (quack grass) and *Sonchus arvensis* (Table 6b, Appendix IIIb)

Family	Scientific name	Interlake (% cover)	Family	Scientific name	SW MB (% cover)
Graminae	Agrostis stolonifera*	13.98±3.12	Graminae	Andropogon scoparius	19.50±3.76
Graminae	Andropogon gerardii	8.68±2.03	Graminae	Agropyron repens*	6.21±1.81
Graminae	Andropogon scoparius	6.38±2.06	Compositae	Sonchus arvensis*	6.04±1.57
Compositae	Sonchus arvensis*	5.98±1.26	Graminae	Andropogon gerardii	4.58±2.15
Graminae	Agropyron repens*	4.13±1.20	Graminae	Panicum virgatum	2.72±1.03
Graminae	Muhlenbergia racemosa	3.19±1.52	Graminae	Deschampsia caespitosa	2.08±0.91
Graminae	Deschampsia caespitosa	3.15±1.14	Graminae	Poa compressa*	2.02±1.02
Graminae	, Poa compressa*	3.04±1.09	Graminae	Unknown grass	1.67±1.17
Graminae	Poa cusickii	2.45±0.90	Graminae	Scolochloa festucacea	1.63±0.91
Graminae	Scolochloa festucacea	1.96±1.24	Compositae	Helianthus laetiflorus	1.33±0.89

Table 6b. August 2010 vegetation survey, ten most abundant species (% cover per  $m^2$ , mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

Zizia aptera (heart-leaved Alexanders), Aster ericoides or falcatus (heath aster), Cirsium flodmanii, Rudbeckia hirta, Solidago spathulata (mountain goldenrod) and Sonchus arvensis were found at all eight research sites in the vegetation surveys (Appendix IIa & IIb). Zygadenus gramineus (death camas), Rosa arkasana (low prairie rose) and Liatris ligulistylis (meadow blazingstar) were present at seven sites, while Agropyron repens, Andropogon gerardii, Andropogon scoparius, Panicum virgatum, Hypoxis hirsuta (yellow star-grass), Glycerrhiza lepidota (wild licorice), Petalostemon candidum, Polygala senega (Seneca root), Viola cucullata (northern bog violet), Galium boreale (northern bedstraw), Achillea millefolium, Helianthus nuttallii (tuberous-rooted sunflower), Solidago ptarmicoides (upland white goldenrod) and Solidago rigida (stiff goldenrod) were present at six research sites. Deschampsia caespitosa, Poa compressa (Canada blue grass), Poa cusickii (early blue grass), Lobelia spicata (spiked lobelia), Agoseris glauca, Antennaria spp. (pussytoes), Aster ericoides (many-flowered aster) and Aster laevis (smooth aster) were found in five research sites (Appendix IIa & IIb). Agropyron repens, Poa compressa and Sonchus arvensis are considered non-native in Manitoba prairies (Appendix I).

Plant species abundance was pooled by taxonomic groups and compared between regions (Table 7). Total cover of all species of plants was significantly greater in the Interlake in comparison to southwestern Manitoba in August 2010. Cyperaceae and Composites were more abundant in the Interlake in comparison to southwestern Manitoba, while plants categorized as Other Monocots were more abundant in southwestern Manitoba (Table 7).

Four currently documented Dakota Skipper larval food plant species were recorded in the June and August surveys (Table 8) and an additional ten species of plants considered to be possible larval food plants were also found in the surveys (Table 8). The mean percent cover of documented and possible larval food plant species identified in the literature were compared between regions (Table 9, Figure 6). *Andropogon gerardii* was significantly more abundant in the Interlake region, while *Andropogon scoparius* was significantly more abundant in the southwest. *Poa secunda* (Sandberg's blue grass), Unknown grasses, *Carex tetanica* (rigid sedge) and *Eleocharis palustris* were significantly more abundant in the Interlake, while *Stipa spartea* and *Carex* spp. were significantly more abundant in the southwest.

Plant diversity was compared between regions including species richness, evenness and dominance (Table 10). There was no significant difference in plant diversity indices between regions.

The ISA identified five species as significant indicators for the Interlake (Table 11). Five species were also identified as indicators for the southwest region. *Eleocharis palustris* was recorded only in the Interlake sites during surveys while *Stipa spartea* was recorded only in the southwest sites.

ISA analysis of sites within regions did not identify any plant species significantly associated with individual sites based on the Monte Carlo test. However, 19 species had high indicator values (>50%) for a particular site, suggesting some site affinity for particular species (Table 12).

Site ID	Field code		ecies over)		ninae over)	Cyperaceae (% cover)	Juncaceae (% cover)		onocots over)		dicots over)	Comp (% co	
		June	August	June	August	June	June	June	August	June	August	June	August
А	IL19	0.50±0.11	0.90±5.09	2.26±0.67	2.48±0.53	0.42±0.30	$0.00 \pm 0.00$	0.03±0.02	$0.00 \pm 0.00$	0.10±0.03	0.09±0.04	0.38±0.09	0.77±0.18
В	IL24	0.63±0.10	0.78±4.75	2.23±0.57	2.25±0.51	1.08±0.37	0.00±0.00	0.41±0.23	$0.00 \pm 0.00$	0.33±0.08	0.12±0.07	0.25±0.08	0.51±0.10
С	IL39	0.47±0.10	0.63±3.62	1.98±0.60	1.88±0.39	1.41±0.79	0.00±0.00	0.08±0.03	0.03±0.03	0.11±0.04	0.07±0.04	0.15±0.04	0.41±0.12
D	IL50	0.72±0.11	0.83±4.07	1.73±0.44	2.09±0.42	2.08±0.78	3.16±2.52	0.16±0.10	0.11±0.11	0.54±0.16	0.26±0.07	0.28±0.06	0.63±0.12
Mean	Inter- lake	0.58±0.05	0.79±4.43	2.05±0.29	2.18±0.23	1.25±0.30	0.79±0.64	0.17±0.06	0.03±0.03	0.27±0.05	0.13±0.03	0.27±0.03	0.58±0.07
Е	OK1	0.47±0.08	0.71±4.15	0.93±0.32	1.22±0.35	0.26±0.17	1.41±0.97	0.04±0.02	0.06±0.06	0.33±0.11	0.19±0.08	0.58±0.16	1.04±0.24
F	OK6	0.33±0.06	0.72±5.77	0.84±0.28	$2.35 \pm 0.64$	0.69±0.31	$0.00 \pm 0.00$	$0.10 \pm 0.04$	$0.00 \pm 0.00$	$0.18 \pm 0.06$	$0.15 \pm 0.07$	$0.32 \pm 0.09$	$0.19 {\pm} 0.07$
G	OK7	0.64±0.10	$0.62 \pm 4.45$	1.70±0.51	1.69±0.46	1.01±0.60	10.00±5.22	0.51±0.17	$0.00 \pm 0.00$	0.32±0.07	0.17±0.06	0.24±0.06	$0.40 \pm 0.16$
Н	OK14	0.39±0.05	0.66±3.88	1.11±0.28	1.84±0.41	0.43±0.24	0.00±0.00	0.55±0.14	0.08±0.05	0.23±0.05	0.20±0.06	0.22±0.05	0.36±0.10
Mean	SW MB	0.46±0.04	0.68±4.62	1.15±0.18	1.78±0.24	0.60±0.18	2.85±1.42	0.30±0.06	0.03±0.02	0.27±0.04	0.18±0.03	0.34±0.05	0.50±0.08
*Monn \	Z* P	-1.15 0.250	-2.85 0.004	-0.93 0.351	-1.45 0.147	-2.08 0.038	-1.20 0.229	-2.14 0.032	-0.80 0.425	-1.04 0.298	-0.85 0.398	-0.69 0.493	-3.90 <0.001

Table 7. Comparison of vegetative cover between Interlake and southwestern Manitoba sites in 2010 (% cover per m<sup>2</sup>, mean±SE)

\*Mann-Whitney U-test (P<0.05)

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Scientific name	Larval food plant record	Interlake (% cover)	SW MB (% cover)
Andropogon gerardii	Known larval food plant in Minnesota (Dana 1991).	8.68±2.03	4.58±2.15
Andropogon scoparius	Known larval food plant in Minnesota (Dana 1991) and North/South Dakota (Royer and Marrone 1992; Cochrane and Delphey 2002).	6.38±2.06	19.50±3.76
Bromus inermis*	Larval feeding in no-choice experiments in Minnesota (Dana 1991).	0.52±0.27	1.27±0.71
Panicum virgatum	Possible food plant as known to feed on <i>Panicum</i> wilcoxianum (Dichanthelium wilcoxianum) in Minnesota (Dana 1991).	1.06±0.59	2.72±1.03
Poa canbyi	Possible food plant as known to feed on <i>Poa pratense</i> in Minnesota (Dana 1991).	0.10±0.10	0.46±0.34
Poa compressa*	Possible food plant as known to feed on <i>Poa pratense</i> in Minnesota (Dana 1991).	3.04±1.09	2.02±1.02
Poa cusickii	Possible food plant as known to feed on <i>Poa pratense</i> in Minnesota (Dana 1991).	2.88±1.29	2.19±0.94
Poa secunda	Possible food plant as known to feed on <i>Poa pratense</i> in Minnesota (Dana 1991).	1.87±0.99	0.00±0.00
<i>Poa</i> spp.	Possible food plant as known to feed on <i>Poa pratense</i> in Minnesota (Dana 1991).	0.21±0.21	0.10±0.10
Stipa spartea	Occasional food plant in late instars in Minnesota (Dana 1991).	0.00±0.00	0.77±0.45
Unknown grass	Likely includes larval food plants such as Andropogon gerardii, Andropogon scoparius, Stipa spartea, Poa etc.	19.69±3.13	3.31±1.03
Carex aurea	Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila</i> ) in Minnesota (Dana 1991).	0.02±0.02	0.00±0.00
Carex parryana	Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila</i> ) in Minnesota (Dana 1991).	0.04±0.04	0.00±0.00
Carex siccata	Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila</i> ) in Minnesota (Dana 1991).	0.29±0.18	0.21±0.21
<i>Carex</i> spp.	Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila</i> ) in Minnesota (Dana 1991).	2.23±1.05	3.40±1.00
Carex tetanica	Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila</i> ) in Minnesota	0.43±0.20	0.00±0.00
Eleocharis palustris	(Dana 1991). Possible food plant as known to feed occasionally on <i>Carex pensylvanica digyna</i> ( <i>C. heliophila)</i> in Minnesota (Dana 1991).	4.50±1.36	0.00±0.00

Table 8. Documented and possible Dakota Skipper larval food plants found in the 2010 vegetation surveys (% cover per  $m^2$ , mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

Site ID	Field code	Andropogon gerardii	Andropogon scoparius	Bromus inermis*	Panicum virgatum	Poa canbyi	Poa compressa*	Poa cusickii	Poa secunda	Poa spp.
А	IL19	0.00±0.00	0.00±0.00	0.83±0.56	0.00±0.00	0.42 ±0.42	7.50 ±3.53	7.83 ±4.42	0.00±0.00	0.83 ±0.83
В	IL24	6.08±3.42	12.50±5.09	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.33 ±3.53	0.00±0.00
С	IL39	18.45±5.59	0.00±0.00	0.00±0.00	3.00 ±2.24	0.00±0.00	0.00±0.00	3.67 ±2.21	0.00±0.00	0.00±0.00
D	IL50	11.00±3.69	12.50±5.42	1.25±0.90	1.42 ±1.00	0.00±0.00	4.42 ±1.86	0.00±0.00	0.00±0.00	0.00±0.00
Mean	Interlake	8.68±2.03	6.38±2.06	0.52±0.27	1.06 ±0.59	0.10 ±0.10	3.04 ±1.09	2.88 ±1.29	1.87 ±0.99	0.21 ±0.21
Е	OK1	0.00±0.00	7.50±5.09	5.08±2.60	2.49 ±1.25	1.83 ±1.33	0.66 ±0.58	0.42 ±0.42	0.00±0.00	0.00±0.00
F	OK6	4.00±3.34	45.42±8.82	0.00±0.00	2.06 ±1.22	0.00±0.00	0.00±0.00	7.08 ±3.23	0.00±0.00	0.00±0.00
G	OK7	14.25±7.44	5.67±4.58	0.00±0.00	6.33 ±3.64	0.00±0.00	0.00±0.00	1.25 ±1.25	0.00±0.00	0.00±0.00
Н	OK14	0.08±0.08	19.42±4.98	0.00±0.00	0.00±0.00	0.00±0.00	7.42 ±3.70	0.00±0.00	0.00±0.00	0.42 ±0.42
Mean	SW MB	4.58±2.15	19.50±3.76	1.27±0.71	2.72 ±1.03	0.46 ±0.34	2.02 ±1.02	2.19 ±0.94	0.00±0.00	0.10 ±0.10
	Z**	-2.725	-2.749	-0.061	-1.828	-0.608	-0.659	-0.290	-2.308	0.015
	Р	0.006	0.006	0.951	0.068	0.543	0.510	0.772	0.021	0.988

Table 9. Comparison of documented and possible larval food plants in the Interlake and southwestern Manitoba sites in 2010 (% cover per  $m^2$ , mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

\*\*Mann-Whitney U-test (P<0.05)

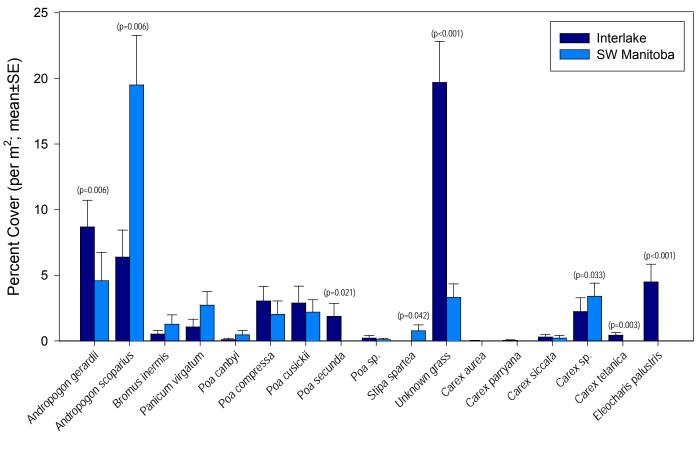
Site ID	Field code	Stipa spartea	Unknown grass	Carex aurea	Carex parryana	Carex siccata	Carex spp.	Carex tetanica	Eleocharis palustris	Estimated No. adults <sup>†</sup> (/hr)
А	IL19	0.00±0.00	25.83±8.39	0.08±0.08	0.00±0.00	0.00±0.00	0.08±0.08	0.74±0.66	1.66±1.66	0
В	IL24	0.00±0.00	16.25±4.61	0.00±0.00	0.00±0.00	1.17±0.68	1.42±0.65	0.16±0.11	3.75±1.85	2
С	IL39	0.00±0.00	32.08±5.66	0.00±0.00	0.17±0.17	0.00±0.00	4.92±3.32	0.00±0.00	3.40±3.32	0
D	IL50	0.00±0.00	4.58±2.17	0.00±0.00	0.00±0.00	0.00±0.00	2.50±2.50	0.83±0.44	9.16±3.36	1
Mean	Interlake	0.00±0.00	19.69±3.13	0.02±0.02	0.04±0.04	0.29±0.18	2.23±1.05	0.43±0.20	4.49±1.35	0.75
Е	OK1	0.00±0.00	2.67±1.13	0.00±0.00	0.00±0.00	0.00±0.00	1.58±0.96	0.00±0.00	0.00±0.00	0
F	OK6	1.00±0.64	7.25±2.97	0.00±0.00	0.00±0.00	0.83±0.83	3.33±1.52	0.00±0.00	0.00±0.00	1
G	OK7	2.67±1.67	1.25±1.25	0.00±0.00	0.00±0.00	0.00±0.00	6.08±3.33	0.00±0.00	0.00±0.00	2
Н	OK14	0.42±0.42	2.08±2.08	0.00±0.00	0.00±0.00	0.00±0.00	2.58±1.28	0.00±0.00	0.00±0.00	30
Mean	SW MB	0.77±0.45	3.31±1.03	0.00±0.00	0.00±0.00	0.21±0.21	3.40±1.00	0.00±0.00	0.00±0.00	8.25
	$Z^1$	-2.032	-4.922	-1.000	-1.000	-0.984	-2.128	-2.935	-3.844	
	Р	0.042	<0.001	0.317	0.317	0.325	0.033	0.003	<0.001	

Table 9. Comparison of documented and possible larval food plants in the Interlake and southwestern Manitoba sites in 2010 (% cover per  $m^2$ , mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

\*\*Mann-Whitney U-test (P<0.05)

<sup>†</sup>Estimated No. Adults (/hr) based on census in 2011 (see Section 6.2.1)



Larval Food Plant Species

Figure 6. Comparison between study regions of documented and possible larval food plant species (% cover per m<sup>2</sup>, mean) in 2010. Statistical significance between regions tested with Mann-Whitney U-test (P < 0.05)

Site ID	Field code	Shannon H	Shannon E	Simpson 1/D	Berger- Parker 1/d
А	IL19	2.77	0.55	9.45	4.43
В	IL24	2.95	0.58	12.46	5.80
С	IL39	2.65	0.52	8.11	3.50
D	IL50	3.51	0.69	26.06	11.95
Mean	Interlake	2.97	0.59	14.02	6.42
Е	OK1	3.31	0.65	22.02	7.01
F	OK6	2.49	0.49	4.93	2.28
G	OK7	3.25	0.64	20.16	8.12
Н	OK14	3.29	0.65	16.59	5.12
Mean	SW MB	3.09	0.61	15.92	5.63
	t*	-0.42	-0.42	-0.34	0.34
	Р	0.690	0.691	0.746	0.743

Table 10. Comparison of plant species diversity between the Interlake and southwestern Manitoba sites in 2010

\*t= *t*-test (*P*<0.05)

## Table 11. Indicator Species Analysis of plant species by region

Family	Scientific name	Indicator region	Indicator value	Standard deviation	P*
Polygalaceae	Polygala senega	SW MB	82.9	12.37	0.002
Compositae	Solidago rigida	Interlake	80.8	11.78	0.005
Graminae	Agrostis stolonifera**	Interlake	75.0	11.36	0.008
Plantaginaceae	Plantago eriopoda	SW MB	73.6	13.46	0.013
Equisetaceae	Equisetum spp.	SW MB	69.6	10.95	0.013
Cyperaceae	Eleocharis palustris <sup>†</sup>	Interlake	62.5	9.79	0.024
Santalaceae	Comandra umbellata	SW MB	62.5	11.11	0.024
Cyperaceae	Carex tetanica <sup>†</sup>	Interlake	62.5	10.82	0.024
Graminae	Stipa spartea <sup>†</sup>	SW MB	62.5	10.85	0.028
Salicaceae	Populus tremuloides	Interlake	62.5	10.99	0.029

\*Monte Carlo test (P<0.05)

\*\*Species are sometimes considered non-native in Manitoba prairies and aspen parkland <sup>†</sup>Possible or known larval food plant species

Family	Scientific name	Indicator site	Indicator value	Standard deviation	P*
Compositae	Achillea millefolium	OK14	63.9	13.53	0.052
Cyperaceae	Eleocharis palustris <sup>†</sup>	IL50	51.0	14.85	0.053
Graminae	Stipa spartea <sup>†</sup>	OK7	64.0	16.17	0.063
Graminae	Helictotrichon hookeri	OK14	100.0	15.40	0.064
Leguminosae	Melilotus alba <sup>‡</sup>	OK14	89.7	18.84	0.064
Amaryllidaceae	Hypoxis hirsuta	OK14	55.1	10.31	0.064
Compositae	Lactuca pulchella	OK6	93.8	17.12	0.064
Equisetaceae	Equisetum spp.	OK6	47.3	10.89	0.064
Graminae	Andropogon scoparius <sup>†</sup>	OK6	44.1	8.68	0.064
Leguminosae	Trifolium pratense**	IL19	100.0	13.80	0.065
Graminae	Muhlenbergia racemosa	IL50	100.0	15.96	0.066
Graminae	Sorghastrum nutans	IL50	100.0	14.78	0.066
Ranunculaceae	Ranunculus cymbalaria	IL50	98.4	16.98	0.066
Graminae	Poa secunda	IL24	100.0	16.58	0.068
Ericaceae	Arctostaphylos uva-ursi	IL24	98.2	19.03	0.068
Rosaceae	Potentilla anserina	IL24	56.4	12.76	0.068
Labiatae (Lamiaceae)	Stachys palustris	OK1	100.0	15.75	0.068
Compositae	Ambrosia psilostachya	OK1	91.1	18.01	0.068
Graminae	Bromus inermis** <sup>†</sup>	OK1	70.9	15.96	0.068
Santalaceae	Comandra umbellata	OK1	70.7	16.93	0.068
Compositae	Solidago rigida	IL39	54.1	12.77	0.070

Table 12.	Indicator	Species	Analysis	of plant	species	by sites
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\*Monte Carlo test (P<0.05)

\*\*Species are sometimes considered non-native in Manitoba prairies and aspen parkland

<sup>†</sup>Possible or known larval food plant species

<sup>‡</sup>Known adult nectar plant species

When plant species were examined by site associations with PCA (Figure 7), the first three axes explained 60.0% of the total variance (22.9%, 21.8% and 15.3%, for axes 1, 2 and 3 respectively). Axis 1 separated the sites within the two regions, where the southwest region sites are positively associated with Axis 1 while the Interlake sites are negatively associated with Axis 1.

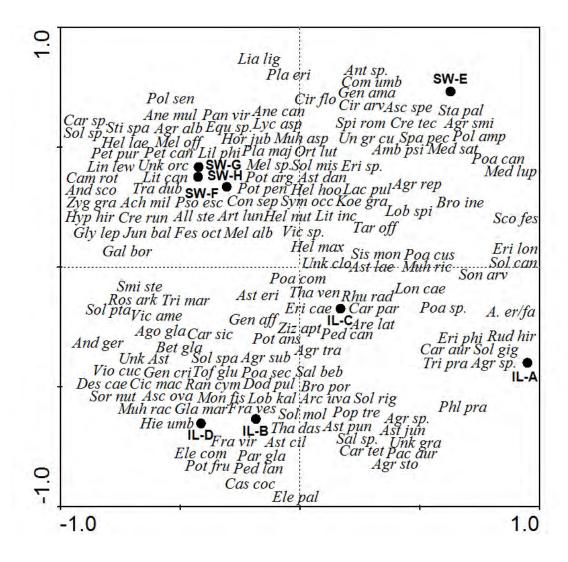


Figure 7. Principal Component Analysis of all plant species observed during vegetation surveys in 2010 showing the association with study sites (•). Species Codes: Ach mil = Achillea millefolium, Ago gla = Agoseris glauca, Agr alb = Agropyron albicans, Agr rep = Agropyron repens, Agr smi = Agropyron smithii, Agr sp. = Agropyron spp., Agr sto = Agrostis stolonifera, Agr sub = Agropyron subsecundum, Agr tra = Agropyron trachycaulum, All ste = Allium stellatum, Amb psi = Ambrosia psilostachya, And ger = Andropogon gerardii, And sco = Andropogon scoparius, Ane can = Anemone canadensis, Ane mul = Anemone multifida, Ant sp. = Antennaria spp., Arc uva = Arctostaphylos uvaursi, Are lat = Arenaria lateriflora, Art lun = Artemisia ludoviciana, Asc ova = Asclepias ovalifolia, Asc spe = Asclepias speciosa, Ast cil = Aster ciliolatus, Ast dan = Astragalus danicus, A. er/fa = Aster ericodes or A. falcatus, Ast eri = Aster ericoides, Ast jun = Aster junciformis, Ast lae = Aster laevis, Ast pun = Aster puncieus,

Bet gla = Betula glandulifera, Bro ine = Bromus inermis, Bro por = Bromus porteri, Cam rot = Campanula rotundifolia, Car aur = Carex aurea, Car par = Carex parryana,Car sic = Carex siccata, Car sp. = Carex spp., Car tet = Carex tetanica, Cas coc = Castilleja coccinea, Cic mac = Cicuta maculata, Cir arv = Cirsium arvense, Cir flo = Cirsium flodmanii, Com umb = Comandra umbellata, Con sep = Convolvulus sepium, Cre run = Crepis runcinata, Cre tec = Crepis tectorum, Des cae = Deschampsia caespitosa, Dod pul = Dodecatheon pulchellum, Ela com = Elaeagnus commutata, Ele pal = Eleocharis palustris, Equ sp. = Equisetum spp., Eri cae = Erigeron caespitosa, Eri lon = Erigeron lonchophyllus, Eri phi = Erigeron philadelphicus, Eri sp. = Erigeron spp., Fes oct = Festuca octoflora, Fra ves = Fragaria vesca, Fra vir = Fragaria virginiana, Gal bor = Galium boreale, Gen aff = Gentiana affinis, Gen ama = Gentiana amarella, Gen cri = Gentiana crinita, Gla mar = Glaux maritima, Gly lep = Glycyrrhizalepidota, Hel hoo = Helictotrichon hookeri, Hel lae = Helianthus laetiflorus, Hel max = Helianthus maximilianii, Hel nut = Helianthus nuttallii, Hie umb = Hieracium umbellatum, Hor jub = Hordeum jubatum, Hyp hir = Hypoxis hirsuta, Jun bal = Juncus balticus, Koe gra = Koeleria gracilis, Lac pul = Lactuca pulchella, Lia lig = Liatris ligulistylis, Lil phi = Lilium philadelphicum, Lin lew = Linum lewisii, Lit can = Lithospermum canescens, Lit inc = Lithospermum incisum, Lob kal = Lobelia kalmia, Lob spi = Lobelia spicata, Lon cae = Lonicera caerulea, Lyc asp = Lycopus asper, Med lup = Medicago lupulina, Med sat = Medicago sativa, Mel alb = Melilotus alba, Mel off = Melilotus officinalis, Mel sp. = Melilotus spp., Mon fis = Monarda fistulosa, Muh asp = Muhlenbergia asperifolia, Muh rac = Muhlenbergia racemosa, Muh ric = Muhlenbergia richardsonis, Ort lut = Orthocarpus luteus, Pac aur = Packera aurea, Pan vir = Panicum virgatum, Par gla = Parnassia glauca, Ped can = Pedicularis canadensis,Ped lan = Pedicularis lanceolata, Pet can = Petalostemon candidum, Pet pur = Petalostemon purpureus, Phl pra = Phleum pratense, Pla eri = Plantago eriopoda, Pla maj = Plantago major, Poa can = Poa canbyi, Poa com = Poa compressa, Poa cus = Poa cusickii, Poa sec = Poa secunda, Poa sp. = Poa spp., Pol amp = Polygonum amphibium, Pol sen = Polygala senega, Pop tre = Populus tremuloides, Pot ans = Potentilla anserina, Pot arg = Potentilla arguta, Pot fru = Potentilla fruticosa, Pot pen = Potentilla pensylvanica, Pso esc = Psoralea esculenta, Ran cym = Ranunculus cymbalaria, Rhu rad = Rhus radicans, Ros ark = Rosa arkansana, Rud hir = Rudbeckia hirta, Sal beb = Salix bebbiana, Sal sp. = Salix spp., Sco fes = Scolochloa festucacea, Sis mon = Sisyrinchium montanum, Smi ste = Smilacina stellata, Sol can = Solidago canadensis, Sol gig = Solidago gigantea, Sol mis = Solidago missouriensis, Sol mol = Solidago mollis, Sol pta = Solidago ptarmicoides, Sol rig = Solidago rigida, Sol sp. = Solidago spp., Sol spa = Solidago spathulata, Son arv = Sonchus arvensis, Sor nut = Sorghastrum nutans, Spa pec = Spartina pectinata, Spi rom = Spiranthes romanzoffiana, Sta pal = Stachys palustris, Sti spa = Stipa spartea, Sym occ = Symphoricarpos occidentalis, Tar off = Taraxacum officinale, Tha das = Thalictrum dasycarpum, Tha ven = Thalictrum venulosum, Tof glu = Tofieldia glutinosa, Tra dub = Tragopogon dubius, Tri mar = Triglochin maritima, Tri pra = Trifolium pratense, Un gr cul = Unknown grass cultivar spp., Unk Ast = Unknown Asteraceae, Unk clo = Unknown clover, Unk gra = Unknown grass, Unk orc = Unknown orchid, Vic ame = Vicia americana, Vic sp. = *Vicia* spp., *Vio* cuc = *Viola* cucullata, *Ziz* apt = *Zizia* aptera, and *Zyg* gra = *Zygadenus* gramineus

Species that were not larval food plants were removed from the PCA diagram (Figure 7) to examine the placement of documented or potential larval food plants (Figure 8). *Andropogon scoparius* and *Stipa spartea* are strongly associated with the southwest sites while *Andropogon gerardii* was associated with the Interlake. *Eleocharis palustris, Carex tetanica* and *Poa secunda* were strongly associated with the Interlake sites (Figure 8).

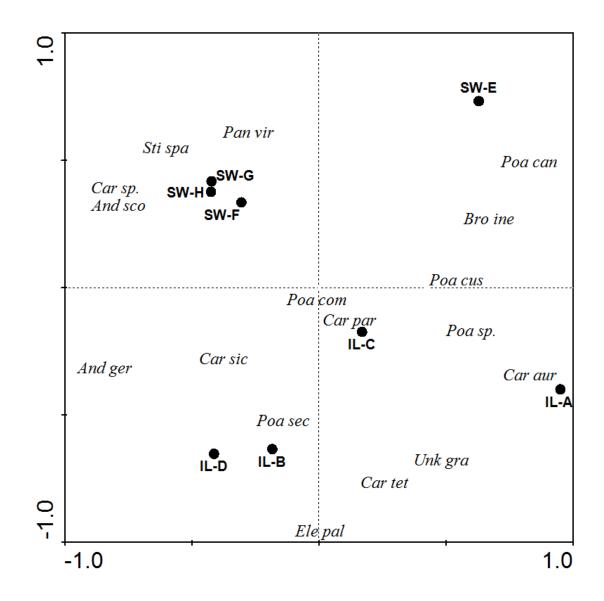


Figure 8. Principal Component Analysis of documented and possible larval food plant species in 2010 showing the association with study sites ( $\bullet$ ). See Figure 7 for species codes.

Plant species significantly associated with a region by the ISA were examined and all other species not found to be significantly associated with region in the ISA were removed from the PCA diagram (Figure 9). *Agrostis stolonifera, Carex tetanica, Eleocharis palustris, Populus tremuloides,* and *Solidago rigida* were negatively associated with the Interlake sites along Axis 1. *Equisetum* spp. (horsetail), *Stipa spartea, Polygala senega, Comandra umbellata* (bastard toadflax) and *Plantago eriopoda* (saline plantain) were positively associated with Axis 1 close to the southwest region sites (Figure 9).

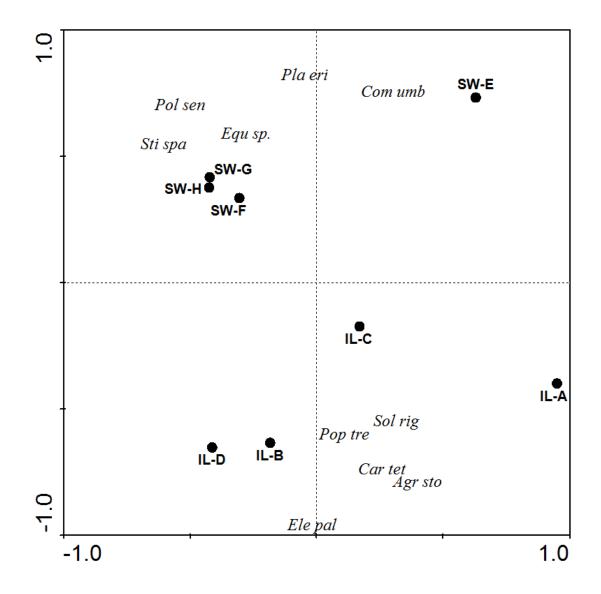


Figure 9. Principal Component Analysis of those plant species identified in the ISA by region as indicator species from the vegetation surveys in 2010, showing the association with study sites ( $\bullet$ ). See Figure 7 for species codes.

#### 5.2.1.2 Nectar Flower Surveys

As in the vegetation surveys, a similar number of species (43 to 52) occurred in the nectar flower surveys in each region (Table 5). There were seven unique flower species recorded in the first flower survey and 19 unique flower species recorded in the second flower survey, with 61 to 66 % of the species observed exclusively in either of the nectar flower surveys (Table 5). Abundance data for the flower species during the early and mid-July surveys is provided in Appendix IVa and IVb, respectively.

During the early July nectar flower surveys, *Rudbeckia hirta* was by far the most abundant species in the Interlake, whereas in the southwest *Crepis runcinata* (scapose hawk's-beard) dominated, and in both regions *Lobelia spicata* and *Campanula rotundifolia* were the next most abundant (Table 13a, Appendix Va). In the southwest, *Melilotus alba* (white sweet-clover) and *Petalostemon candidum* were also abundant.

Family	Scientific	Interlake	Family	Scientific	SW MB
	name	(# stems)		name	(# stems)
Compositae	Rudbeckia	857.25±308.47	Compositae	Crepis	381.50±144.98
	hirta			runcinata	
Lobeliaceae	Lobelia spicata	348.88±252.78	Lobeliaceae	Lobelia spicata	381.25±173.96
Campanulaceae	Campanula rotundifolia	133.00±61.45	Campanulaceae	Campanula rotundifolia	263.63±73.71
Compositae	Solidago spathulata	57.25±44.79	Leguminosae	Melilotus alba*	249.38±103.28
Compositae	Érigeron annuus	55.88±33.14	Leguminosae	Petalostemon candidum	231.38±74.33
Compositae	Crepis runcinata	41.00±29.15	Liliaceae	Zygadenus elegans	174.25±114.30
Leguminosae	Melilotus alba*	35.38±16.37	Leguminosae	Melilotus officinalis*	142.13±78.39
Liliaceae	Zygadenus gramineus	35.25±22.61	Compositae	Rudbeckia hirta	110.00±29.21
Liliaceae	Żygadenus elegans	35.13±13.42	Leguminosae	Petalostemon purpureus	63.50±53.39
Leguminosae	Petalostemon candidum	34.63±30.55	Rosaceae	Rosa arkansana	40.75±38.90

Table 13a.	Early July 2010 nectar plant survey, ten most abundant species (number of stems
per transec	$t [2500 \text{ m}^2], \text{mean}\pm\text{SE})$

\*Species considered non-native in Manitoba prairies and the aspen parkland

Similar to the early July survey, *Rudbeckia hirta* dominated the flower species in the Interlake, followed by *Parnassia glauca* (glaucous grass-of-parnassus), *Lobelia* 

*spicata* and *Lobelia kalmii* (Kalm's lobelia) (Table 13b, Appendix Vb). In southwest Manitoba the species distribution was more equitable with *Lobelia spicata* being most abundant followed more closely by *Melilotus alba*, *Petalostemon candidum*, *Crepis runcinata* and *Campanula rotundifolia*. Generally the most abundant species observed in either survey were the same species (for each region) (Table 13b, Appendix Vb).

Family	Scientific name	Interlake (# stems)	Family	Scientific name	SW MB (# stems)
Compositae	Rudbeckia	810.88±365.50	Lobeliaceae	Lobelia	512.13±172.51
	hirta			spicata	
Saxifragaceae	Parnassia qlauca	234.00±109.79	Leguminosae	Melilotus alba*	494.75±204.70
Lobeliaceae	Lobelia kalmii	188.50±160.36	Leguminosae	Petalostemon candidum	398.25±137.52
Lobeliaceae	Lobelia spicata	126.25±57.94	Compositae	Crepis runcinata	396.13±131.50
Compositae	Śolidago ptarmicoides	109.75±27.14	Campanulaceae	Campanula rotundifolia	341.88±84.34
Campanulaceae	Campanula rotundifolia	93.13±28.93	Compositae	Solidago ptarmicoides	320.50±100.61
Compositae	Solidago spathulata	91.25±29.58	Compositae	Rudbeckia hirta	246.75±93.10
Compositae	Á. ericodes or A. falcatus	89.50±58.24	Leguminosae	Petalostemon purpureus	171.38±70.41
Leguminosae	Petalostemon purpureus	63.38±28.33	Leguminosae	Medicago sativa*	151.5±138.17
Leguminosae	Petalostemon candidum	52.13±45.67	Compositae	Lactuca pulchella	92.00±88.17

Table 13b. Mid-July 2010 nectar plant survey, ten most abundant species (number of stems per transect [ $2500 \text{ m}^2$ ], mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

Campanula rotundifolia, Lobelia spicata, Cirsium flodmanii, Liatris ligulistylis, Rudbeckia hirta, Solidago ptarmicoides and Sonchus arvensis were found in all eight sites, while a further nine species were found in seven of the sites (Zygadenus elegans, Rosa arkansana, Melilotus alba, Petalostemon candidum, Petalostemon purpureus (purple prairie-clover), Achillea millefolium, Agoseris glauca, Erigeron annuus and Solidago spathulata) (Appendix IVa & IVb). Additionally, Zygadenus gramineus, Glycyrrhiza lepidopta, Medicago sativa, Aster eriocoides or falcatus and Crepis runcinata were recorded at six sites, and Allium stellatum (pink-flowered onion), *Melilotus officinalis, Helianthus maximillianii* (narrow-leaved sunflower) and *Solidago rigida* were found in five of the sites (Appendix IVa & IVb).

When flower species abundance was pooled by taxonomic groups and assessed by sites and regions (Table 14), there was no significant difference in the number of flower stems between the Interlake and southwest Manitoba for either flower survey. However, there were significantly more stems of the Monocot flower category in the Interlake versus the southwest during the mid-July survey. There were no significant differences between the two regions for any of the other taxonomic groups and survey dates (Table 14).

Thirty-four previously documented or potential nectar flower species were recorded at the study sites (Table 15). Dakota Skipper adults were observed in this study nectaring upon twelve species of flowering plants, of which six species are new records for North America: *Melilotus alba, Petalostemon purpureus, Oenothera biennis* (yellow evening-primrose), *Lobelia spicata, Crepis runcinata* and *Solidago ptarmicoides* (Table 15, Section 6.2.2). The mean number of stems of each nectar species was compared between regions (Table 16, Table 17, Figure 10, Figure 11). *Rudbeckia hirta* was more abundant in the Interlake, while *Melilotus alba, Petalostemon candidum, Petalostemon purpureus, Lobelia spicata* and *Crepis runcinata* were more abundant in the southwest region (Table 16). Additionally, there were significantly more flower stems of *Lilium philadelphicum, Melilotus officinalis, Asclepias speciosa, Campanula rotundifolia, Lactuca pulchella* (blue lettuce) and *Ratibida columnifera* in the southwest region (Table 17).

Site ID	Field	All flower species (# stems)		Monocots (# stems)		Other dicots (# stems)		Composites (# stems)	
U	code	Early July	Mid-July	Early July	Mid-July	Early July	Mid-July	Early July	Mid-July
А	IL19	0.0079±0.0046	0.0116±0.0065	0.0000±0.0000	0.0000±0.0000	0.0012±0.0005	0.0023±0.0012	0.0160±0.0102	0.0230±0.0142
В	IL24	0.0018±0.0006	0.0087±0.0034	0.0035±0.0021	0.0000±0.0000	0.0012±0.0005	0.0137±0.0069	0.0022±0.0012	0.0047±0.0017
С	IL39	0.0187±0.0075	0.0120±0.0048	0.0056±0.0036	0.0115±0.0078	0.0177±0.0102	0.0075±0.0030	0.0216±0.0126	0.0168±0.0100
D	IL50	0.0030±0.0008	0.0056±0.0019	0.0098±0.0063	0.0000±0.0000	0.0025±0.0007	0.0070±0.0036	0.0025±0.0015	0.0049±0.0017
Mean	Inter- lake	0.0078±0.0022	0.0095±0.0022	0.0047±0.0019	0.0028±0.0020	0.0057±0.0025	0.0077±0.0021	0.0106±0.0041	0.0123±0.0044
Е	OK1	0.0059±0.0019	0.0110±0.0042	0.0022±0.0020	0.0000±0.0000	0.0094±0.0039	0.0175±0.0085	0.0028±0.0011	0.0058±0.0022
F	OK6	0.0065±0.0021	0.0132±0.0038	0.0015±0.0014	0.0001±0.0001	0.0100±0.0039	0.0190±0.0069	0.0037±0.0020	0.0091±0.0043
G	OK7	0.0115±0.0039	0.0145±0.0042	0.0417±0.0318	0.0020±0.0010	0.0072±0.0025	0.0166±0.0065	0.0117±0.0068	0.0142±0.0061
Н	OK14	0.0168±0.0047	0.0265±0.0062	0.0034±0.0012	0.0023±0.0016	0.0279±0.0095	0.0340±0.0108	0.0072±0.0026	0.0221±0.0075
Mean	SW MB	0.0102±0.0017	0.0163±0.0023	0.0122±0.0081	0.0011±0.0005	0.0136±0.0028	0.0218±0.0041	0.0064±0.0019	0.0128±0.0027
	<i>Z</i> *	-1.11	-1.41	-0.37	-2.00	-0.98	-1.87	-0.48	-0.34
	P	0.267	0.159	0.714	0.045	0.326	0.061	0.631	0.732

Table 14. Comparison of flower survey species between Interlake and southwestern Manitoba sites in 2010 (number of stems per  $m^2$ , mean±SE)

\*Mann-Whitney U-test (P<0.05)

Family	Scientific name	Nectaring record	# Adults nectaring in 2010/11/12	Interlake (# stems)	SW MB (# stems)
Liliaceae	Lilium philadelphicum	Nectaring observed in Manitoba (Webster 2003; Webster 2007) and Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0004±0.0002
Liliaceae	Zygadenus elegans	Nectaring observed in Manitoba in 2012 and previously (Webster 2003; Webster 2007), and Minnesota (Swengel and Swengel 1999a)	1**	0.0070±0.0031	0.0361±0.0237
Liliaceae	Zygadenus gramineus	Possible nectar plant as known to feed on <i>Z. elegans</i> in Manitoba (Webster 2003; Webster 2007) and Minnesota (Swengel and Swengel 1999a)	0	0.0070±0.0047	0.0013±0.0006
Leguminosae	Astragalus canadensis	Possible nectar plant as known to feed on <i>A.</i> <i>crassicarpus</i> in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0014±0.0010
Leguminosae	Astragalus striatus	Nectaring observed in Minnesota (Dana 1991) and North Dakota (McCabe 1981)	0	0.0001±0.0001	0.0000±0.0000
Leguminosae	Medicago sativa*	Nectaring observed in Minnesota (Swengel and Swengel 1999a)	0	0.0058±0.0027	0.0380±0.0280
Leguminosae	Melilotus alba*	Nectaring newly observed in Manitoba by Rigney in 2010 and 2011; Nectaring was ONLY on short, <50cm tall plants	6**	0.0112±0.0038	0.1488±0.0460
Leguminosae	Melilotus officinalis*	Nectaring observed in Minnesota (Dana 1991)	0	0.0008±0.0007	0.0457±0.0186
Leguminosae	Petalostemon candidum	Nectaring observed in Manitoba by Rigney in 2010, 2011 and 2012, North/South Dakota (McCabe and Post 1977 or Royer and Marrone 1992 per Cochrane and Delphey 2002)	7**	0.0173±0.0106	0.1259±0.0314
Leguminosae	Petalostemon purpureus	Nectaring newly observed in Manitoba by Rigney in 2010	1**	0.0132±0.0063	0.0469±0.0179
Leguminosae	Trifolium pretense*	Nectaring observed in Minnesota (Swengel and Swengel 1999a)	0	0.0009±0.0007	0.0000±0.0000

Table 15. Documented, possible and new Dakota Skipper nectar plant species present in the 2010 flower surveys (number of stems per  $m^2$ , mean±SE)

Table 15. Documented, possible and new Dakota Skipper nectar plant species present in the 2010 flower surveys (number of
stems per m <sup>2</sup> , mean±SE)

Family	Scientific name	Nectaring record	# Adults nectaring in 2010/11/12	Interlake (# stems)	SW MB (# stems)
Onagraceae	Oenothera biennis*	Nectaring newly observed in Manitoba by Rigney in 2011, and possible nectar plant as known to feed on <i>O. serrulata</i> in North Dakota (McCabe 1981)	1**	0.0000±0.0000	0.0000±0.0000
Asclepiadaceae	Asclepias ovalifolia	Possible nectar plant as known to feed on <i>A.</i> speciosa and <i>A. syriaca</i> in Minnesota (Swengel and Swengel 1999a)	0	0.0001±0.0001	0.0000±0.0000
Asclepiadaceae	Asclepias speciosa	Nectaring observed in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0026±0.0016
Campanulaceae	Campanula rotundifolia	Nectaring observed in Manitoba (Webster 2003) and North Dakota (McCabe 1981)	0	0.0452±0.0132	0.1211±0.0220
Lobeliacea	Lobelia spicata	Nectaring newly observed in Manitoba by Rigney in 2011 and 2012	5**	0.0950±0.0514	0.1786±0.0478
Compositae	Achillea millefolium	Nectaring observed in Minnesota (Swengel and Swengel 1999a)	0	0.0039±0.0010	0.0216±0.0099
Compositae	Agoseris glauca	Nectaring observed in Manitoba by Rigney in 2011 and in Minnesota (Dana 1991)	1**	0.0014±0.0004	0.0025±0.0006
Compositae	Chrysopsis villosa	Nectaring observed on <i>Chrysopsis</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0037±0.0033
Compositae	Cirsium arvense*	Possible nectar plant as known to feed upon <i>C. flodmanii</i> and <i>Cirsium</i> spp. in Minnesota (Dana 1991; Swengel and Swengel 1999a)	0	0.0004±0.0004	0.0007±0.0004
Compositae	Cirsium flodmanii	Nectaring observed in Manitoba by Rigney 2011 and 2012 and nectaring observed in Minnesota (Dana 1991)	3**	0.0061±0.0030	0.0021±0.0007

Family	Scientific name	Nectaring record	# Adults nectaring in 2010/11/12	Interlake (# stems)	SW MB (# stems)
Compositae	Cirsium spp.	Possible nectar plant as known to feed upon <i>C. flodmanii</i> and <i>Cirsium</i> spp. in Minnesota (Dana 1991; Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0000±0.0000
Compositae	Crepis runcinata	Nectaring newly observed in Manitoba by Rigney in 2011 and 2012	2**	0.0139±0.0076	0.1555±0.0378
Compositae	Erigeron annuus	Nectaring observed in North Dakota (McCabe 1981) and Minnesota (Dana 1991)	0	0.0130±0.0068	0.0042±0.0025
Compositae	Erigeron asper	Possible nectar plant as known to feed on <i>E. annuus</i> in North Dakota (McCabe 1981) and Minnesota (Dana 1991), and on <i>Erigeron</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0000±0.0000
Compositae	Erigeron caespitosa	Possible nectar plant as known to feed on <i>E. annuus</i> in North Dakota (McCabe 1981) and Minnesota (Dana 1991), and on <i>Erigeron</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0000±0.0000
Compositae	Erigeron glabellus	Possible nectar plant as known to feed on <i>E. annuus</i> in North Dakota (McCabe 1981) and Minnesota (Dana 1991), and on <i>Erigeron</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0006±0.0004
Compositae	Erigeron Ionchophyllus	Possible nectar plant as known to feed on <i>E. annuus</i> in North Dakota (McCabe 1981) and Minnesota (Dana 1991), and on <i>Erigeron</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0065±0.0039	0.0000±0.0000

Table 15. Documented, possible and new Dakota Skipper nectar plant species present in the 2010 flower surveys (number of stems per  $m^2$ , mean±SE)

Table 15. Documented, possible and new Dakota Skipper nectar plant species present in the 2010 flower surveys (number of stems per  $m^2$ , mean±SE)

Family	Scientific name	Nectaring record	# Adults nectaring in 2010/11/12	Interlake (# stems)	SW MB (# stems)
Compositae	Erigeron philadelphicus	Possible nectar plant as known to feed on <i>E. annuus</i> in North Dakota (McCabe 1981) and Minnesota (Dana 1991), and on <i>Erigeron</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0025±0.0019	0.0000±0.0000
Compositae	Gaillardia aristata	Nectaring observed in Manitoba by Rigney in 2012 and in Minnesota (Swengel and Swengel 1999a)	6**	0.0000±0.0000	0.0012±0.0011
Compositae	Lactuca pulchella	Possible nectar plant as known to feed on <i>Lactuca</i> spp. in Minnesota (Swengel and Swengel 1999a)	0	0.0000±0.0000	0.0251±0.0184
Compositae	Ratibida columnifera	Nectaring observed in North Dakota (McCabe 1981)	0	0.0000±0.0000	0.0036±0.0020
Compositae	Rudbeckia hirta	Nectaring observed in Manitoba by Rigney in 2010, 2011 and 2012, and Webster (2003) plus in North Dakota (McCabe 1981) and Minnesota (Swengel and Swengel 1999a)	~110** & 2 <sup>†</sup>	0.3336±0.0924	0.0713±0.0201
Compositae	Solidago ptarmicoides	Nectaring newly observed in Manitoba by Rigney in 2010	2**	0.0235±0.0074	0.0691±0.0247

\*\*Observed nectaring in southwest Manitoba in 2010, 2011 or 2012 <sup>†</sup>Observed nectaring in the Interlake in 2010 or 2011

Site ID	Field code	Zygadenus elegans	Melilotus alba*	Petalostemon candidum	Petalostemon purpureus	Oenothera biennis*	Lobelia spicata	Agoseris glauca
А	IL19	$0.0000 \pm 0.0000$	0.0019±0.0010	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0478±0.0153	0.0000±0.0000
В	IL24	0.0051±0.0045	0.0060±0.0019	0.0001±0.0001	0.0005±0.0005	$0.0000 \pm 0.0000$	0.0052±0.0045	$0.0001 \pm 0.0001$
С	IL39	0.0161±0.0093	0.0211±0.0122	0.0648±0.0355	0.0274±0.0160	$0.0000 \pm 0.0000$	0.3038±0.1812	0.0034±0.0011
D	IL50	0.0069±0.0067	0.0160±0.0079	0.0045±0.0011	0.0250±0.0177	0.0000±0.0000	0.0233±0.0077	0.0024±0.0006
Mean	Interlake	0.0070±0.0031	0.0112±0.0038	0.0173±0.0106	0.0132±0.0063	0.0000±0.0000	0.0950±0.0514	0.0014±0.0004
Е	OK1	0.0063±0.0063	0.2198±0.1332	$0.0114 \pm 0.0066$	$0.0084 \pm 0.0058$	$0.0000 \pm 0.0000$	$0.1904 \pm 0.0745$	0.0027±0.0015
F	OK6	0.0045±0.0042	$0.0000 \pm 0.0000$	0.2090±0.0867	0.0330±0.0167	$0.0001 \pm 0.0001$	0.0708±0.0271	$0.0008 \pm 0.0005$
G	OK7	0.1279±0.0863	0.0617±0.0242	0.1478±0.0554	0.0108±0.0081	0.0001±0.0001	0.0333±0.0092	0.0017±0.0001
Н	OK14	0.0059±0.0031	0.3138±0.0587	0.1355±0.0472	0.1357±0.0494	0.0003±0.0003	0.4202±0.0945	0.0050±0.0017
Mean	SW MB	0.0361±0.0237	0.1488±0.0460	0.1259±0.0314	0.0469±0.0179	0.0001±0.0001	0.1786±0.0478	0.0025±0.0006
	$Z^{\dagger}$	-1.168	-2.231	-3.354	-2.432	-1.789	-2.205	-1.360
	Р	0.243	0.026	0.001	0.015	0.074	0.027	0.174

Table 16. Comparison of nectar plant species that Dakota Skipper were observed nectaring on in 2010, 2011 and 2012 (number of stems per  $m^2$ , mean $\pm$ SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland \*\*Estimated No. Adults (/hr) based on census in 2011 (see Section 6.2.1) † Mann-Whitney U-test (*P*<0.05)

Site ID	Field code	Cirsium flodmanii	Crepis runcinata	Gallardia aristata	Rudbeckia hirta	Solidago ptarmicoides	Estimated No. adults** (/hr)
А	IL19	0.0028±0.0016	0.0000±0.0000	$0.0000 \pm 0.0000$	0.6158±0.2114	0.0083±0.0025	0
В	IL24	0.0038±0.0011	0.0457±0.0252	$0.0000 \pm 0.0000$	$0.0460 \pm 0.0096$	0.0238±0.0153	2
С	IL39	0.0158±0.0119	$0.0100 \pm 0.0083$	$0.0000 \pm 0.0000$	0.6061±0.1519	0.0183±0.0105	0
D	IL50	0.0023±0.0018	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0666±0.0190	0.0436±0.0232	1
Mean	Interlake	0.0061±0.0030	0.0139±0.0076	0.0000±0.0000	0.3336±0.0924	0.0235±0.0074	0.75
Е	OK1	0.0038±0.0024	0.0296±0.0049	$0.0000 \pm 0.0000$	0.0680±0.0104	0.0358±0.0274	0
F	OK6	0.0009±0.0007	0.0758±0.0430	$0.0000 \pm 0.0000$	0.0032±0.0010	0.0413±0.0345	1
G	OK7	0.0026±0.0011	0.3624±0.0306	$0.0000 \pm 0.0000$	0.0498±0.0104	0.0692±0.0472	2
Н	OK14	0.0012±0.0006	0.1543±0.0642	0.0050±0.0047	0.1644±0.0573	0.1304±0.0788	30
Mean	SW MB	0.0021±0.0007	0.1555±0.0378	0.0012±0.0011	0.0713±0.0201	0.0691±0.0247	8.25
	$Z^{\dagger}$	-1.000	-3.963	-1.437	-2.337	-1.150	
	Р	0.317	<0.001	0.151	0.019	0.250	

Table 16. Comparison of nectar plant species that Dakota Skipper were observed nectaring on in 2010, 2011 and 2012 (number of stems per  $m^2$ , mean±SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland \*\*Estimated No. Adults (/hr) based on census in 2011 (see Section 6.2.1) † Mann-Whitney U-test (*P*<0.05)

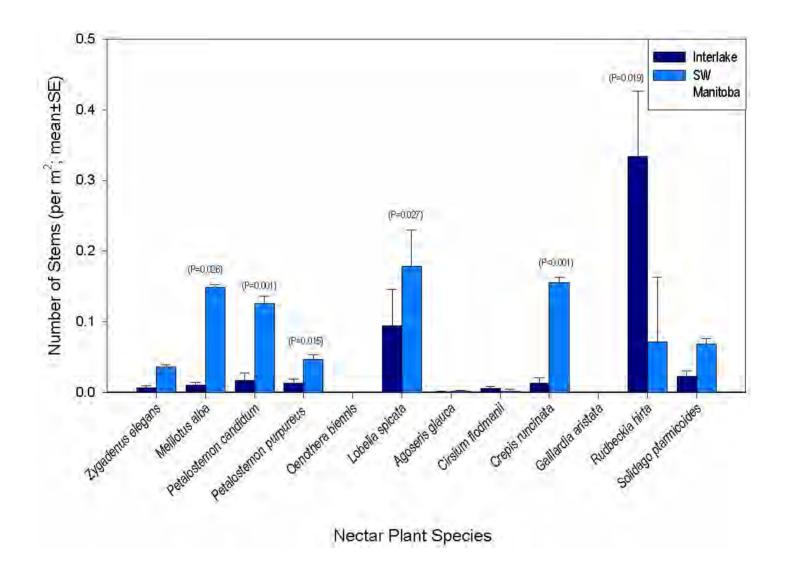


Figure 10. Comparison between study regions of nectar plant species (# stems/ per m<sup>2</sup>, mean) in 2010 flower surveys that Dakota Skipper were observed nectaring upon in 2010, 2011 and 2012. Statistical significance between regions tested with Mann-Whitney U-test (P < 0.05)

Site ID	Field code	Lilium philadelphicum	Zygadenus gramineus	Astragalus canadensis	Astragalus striatus	Medicago sativa*	Melilotus officinalis*	Trifolium pretense*	Asclepias ovalifolia
A	IL19	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0145±0.0081	0.0000±0.0000	0.0032±0.0026	0.0000±0.0000
В	IL24	$0.0000 \pm 0.0000$	0.0053±0.0047	0.0000±0.0000	$0.0000 \pm 0.0000$	0.0003±0.0003	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0000±0.0000
С	IL39	$0.0000 \pm 0.0000$	0.0009±0.0009	0.0000±0.0000	0.0005±0.0005	0.0082±0.0057	0.0034±0.0026	$0.0005 \pm 0.0005$	0.0000±0.0000
D	IL50	$0.0000 \pm 0.0000$	0.0220±0.0178	0.0000±0.0000	$0.0000 \pm 0.0000$	$0.0004 \pm 0.0004$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0005±0.0003
Mean	Inter- lake	0.0000±0.0000	0.0070±0.0047	0.0000±0.0000	0.0001±0.0001	0.0058±0.0027	0.0008±0.0007	0.0009±0.0007	0.0001±0.0001
Е	OK1	0.0000±0.0000	0.0001±0.0001	0.0000±0.0000	0.0000±0.0000	0.0104±0.0063	0.0592±0.0421	0.0000±0.0000	0.0000±0.0000
F	OK6	0.0002±0.0002	$0.0000 \pm 0.0000$	0.0000±0.0000	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0003±0.0001	$0.0000 \pm 0.0000$	0.0000±0.0000
G	OK7	0.0002±0.0002	0.0024±0.0018	0.0058±0.0037	$0.0000 \pm 0.0000$	0.1416±0.1042	0.0113±0.0069	$0.0000 \pm 0.0000$	0.0000±0.0000
Н	OK14	0.0015±0.0009	0.0030±0.0017	0.0000±0.0000	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.1121±0.0504	$0.0000 \pm 0.0000$	0.0000±0.0000
Mean	SW MB	0.0004±0.0002	0.0013±0.0006	0.0014±0.0010	0.0000±0.0000	0.0380±0.0280	0.0457±0.0186	0.0000±0.0000	0.0000±0.0000
	Z** P	-2.099 0.036	-0.044 0.956	-1.437 0.151	-1.000 0.317	-0.327 0.744	-3.990 <0.001	-1.788 0.074	-1.437 0.151

Table 17. Comparison of documented or possible nectar plant species from the flower surveys that Dakota Skipper were not observed feeding on in this study (number of stems per  $m^2$ , mean $\pm$ SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland

\*\*Mann-Whitney U-test (P<0.05)

Site ID	Field code	Asclepias speciosa	Campanula rotundifolia	Achillea millefolium	Chrysopsis villosa	Cirsium arvense*	Cirsium spp.	Erigeron annuus	Erigeron asper
А	IL19	0.0000±0.0000	0.0099±0.0069	0.0087±0.0027	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0354±0.0255	0.0001±0.0001
В	IL24	$0.0000 \pm 0.0000$	0.0491±0.0173	0.0016±0.0005	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$
С	IL39	0.0000±0.0000	0.1072±0.0336	0.0031±0.0011	$0.0000 \pm 0.0000$	0.0019±0.0015	$0.0000 \pm 0.0000$	0.0068±0.0057	$0.0000 \pm 0.0000$
D	IL50	0.0002±0.0002	0.0147±0.0057	0.0022±0.0015	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0001±0.0001	0.0101±0.0052	$0.0000 \pm 0.0000$
Mea n	Inter- lake	0.0000±0.0000	0.0452±0.0132	0.0039±0.0010	0.0000±0.0000	0.0004±0.0004	0.0000±0.0000	0.0130±0.0068	0.0000±0.0000
E	OK1	0.0082±0.0061	0.0583±0.0247	0.0010±0.0002	0.0000±0.0000	0.0029±0.0015	0.0000±0.0000	0.0007±0.0007	0.0000±0.0000
F	OK6	$0.0000 \pm 0.0000$	0.1723±0.0482	$0.0000 \pm 0.0000$	0.0150±0.0127	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0021±0.0012	$0.0000 \pm 0.0000$
G	OK7	0.0024±0.0013	$0.0648 \pm 0.0291$	$0.0066 \pm 0.0011$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0003 \pm 0.0003$	$0.0000 \pm 0.0000$
Н	OK14	$0.0000 \pm 0.0000$	0.1890±0.0335	0.0790±0.0227	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0137 \pm 0.0095$	$0.0000 \pm 0.0000$
Mea n	SW MB	0.0026±0.0016	0.1211±0.0220	0.0216±0.0099	0.0037±0.0033	0.0007±0.0004	0.0000±0.0000	0.0042±0.0025	0.0000±0.0000
	Z**	-2.215	-2.789	-0.208	-1.437	-0.447	-1.000	-1.511	-1.000
	Р	0.027	0.005	0.835	0.151	0.655	0.317	0.131	0.317

Table 17. Comparison of documented or possible nectar plant species from the flower surveys that Dakota Skipper were not observed feeding on in this study (number of stems per  $m^2$ , mean $\pm SE$ )

\*Species considered non-native in Manitoba prairies and the aspen parkland \*\* Mann-Whitney U-test (*P*<0.05)

Site ID	Field code	Erigeron caespitosa	Erigeron glabellus	Erigeron Ionchophyllus	Erigeron philadelphicus	Lactuca pulchella	Ratibida columnifera	Estimated No. Adults** (/hr)
А	IL19	0.0000±0.0000	0.0000±0.0000	0.0228±0.0131	0.0102±0.0069	0.0000±0.0000	$0.0000 \pm 0.0000$	0
В	IL24	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0032±0.0032	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	2
С	IL39	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0
D	IL50	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	1
Mean	Inter- lake	0.0000±0.0000	0.0000±0.0000	0.0065±0.0039	0.0025±0.0019	0.0000±0.0000	0.0000±0.0000	0.75
Е	OK1	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0003±0.0003	0.0000±0.0000	0.0001±0.0001	0
F	OK6	$0.0000 \pm 0.0000$	0.0007±0.0007	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0978±0.0669	0.0128±0.0065	2
G	OK7	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0018±0.0010	30
Н	OK14	$0.0000 \pm 0.0000$	0.0017±0.0017	$0.0000 \pm 0.0000$	$0.0000 \pm 0.0000$	0.0028±0.0015	$0.0000 \pm 0.0000$	1
Mean	SW MB	0.0000±0.0000	0.0006±0.0004	0.0000±0.0000	0.0000±0.0000	0.0251±0.0184	0.0036±0.0020	10.67
	Z <sup>†</sup> P	0.000 1.000	-1.437 0.151	-1.788 0.074	-1.461 0.144	-2.385 0.017	-2.918 0.004	

Table 17. Comparison of documented or possible nectar plant species from the flower surveys that Dakota Skipper were not observed feeding on in this study (number of stems per  $m^2$ , mean $\pm$ SE)

\*Species considered non-native in Manitoba prairies and the aspen parkland \*\*Estimated No. Adults (/hr) based on census in 2011 (see Section 6.2.1) † Mann-Whitney U-test (*P*<0.05)

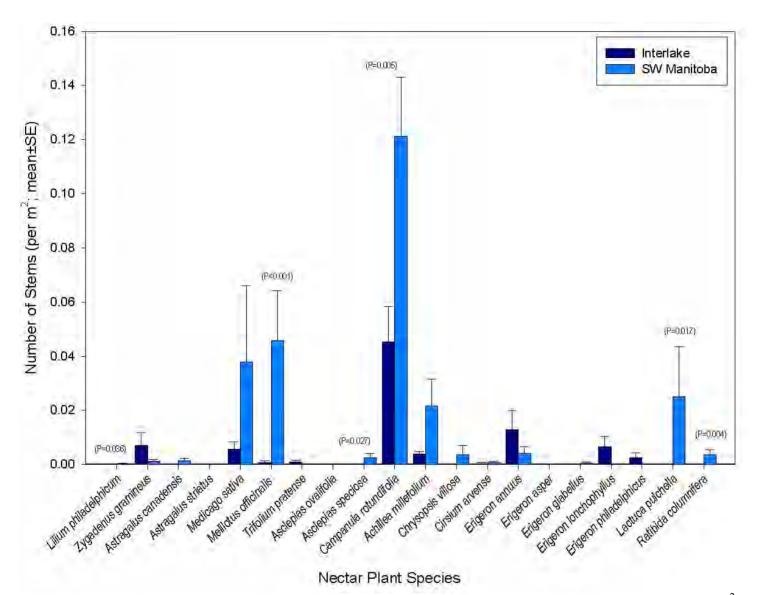


Figure 11. Comparison between study regions of documented or possible nectar plant species (# stems/ per m<sup>2</sup>, mean) in 2010 that Dakota Skipper were not observed feeding on in this study. Statistical significance between regions tested with Mann-Whitney U-test (P<0.05)

Flower diversity was compared between regions, including species richness, evenness or dominance (Table 18). There was no significant difference in flower diversity indices between regions.

Site ID	Sites	Shannon H	Shannon E	Simpson 1/D	Berger-Parker 1/d
A	IL19	1.32	0.29	2.00	1.43
В	IL24	2.16	0.48	5.73	3.13
С	IL39	1.93	0.43	3.95	2.29
D	IL50	2.55	0.57	7.96	3.82
Mean	Interlake	2.32	0.52	4.75	2.35
Е	OK1	2.21	0.49	5.93	3.49
F	OK6	2.40	0.53	7.84	4.28
G	OK7	2.34	0.52	6.71	3.25
Н	OK14	2.44	0.54	8.77	4.65
Mean	SW MB	2.75	0.61	7.31	6.70
	t*	-1.36	-1.30	-1.70	-2.04
	Р	0.222	0.240	0.141	0.088

Table 18. Comparison of flower species diversity between the Interlake and southwestern Manitoba sites in 2010 flower surveys

\*t = *t*-test (*P*<0.05)

Indicator Species Analysis identified five flowering plant species as significant indicators of the Interlake, while ten species were significant indicators of southwest Manitoba (Table 19). *Crepis runcinata, Melilotus officinalis, Ratibida columnifera, Petalostemon candidum, Campanula rotundifolia, Petalostemon purpureus* and *Rudbeckia hirta* are also known or probable Dakota Skipper nectar food plants species.

The ISA of sites within regions did not identify any flower species that were significantly associated with individual sites based on the Monte Carlo test. However, twelve species did have stronger indicator values (>50%) for a particular site (Table 20). Nine species in particular had indicator values greater then 75%, suggesting a strong but non-significant affinity to a particular site (Table 20).

Family	Scientific name	Indicator	Indicator	Standard	P*
<u> </u>		region	value	deviation	
Scrophulariaceae	Castilleja coccinea	Interlake	100	11.21	<0.001
Compositae	Aster ericoides or falcatus	Interlake	99.3	13.55	<0.001
Compositae	Crepis runcinata <sup>†</sup>	SW MB	91.8	11.77	0.002
Compositae	Solidago rigida	Interlake	84.9	12.14	0.003
Leguminosae	Melilotus officinalis** <sup>†</sup>	SW MB	85.9	13.56	0.005
Polygalaceae	Polygala senega	SW MB	75	11.91	0.007
Leguminosae	Glycyrrhiza lepidota	SW MB	80.6	12.06	0.011
Compositae	Ratibida columnifera <sup>†</sup>	SW MB	62.5	11.35	0.026
Compositae	Solidago missouriensis	SW MB	62.5	11.61	0.030
Leguminosae	Petalostemon candidum <sup>†</sup>	SW MB	76.9	11.3	0.033
Campanulaceae	Campanula rotundifolia <sup>†</sup>	SW MB	72.8	6.76	0.043
Compositae	Solidago spathulata	Interlake	81.5	11.11	0.047
Leguminosae	Petalostemon purpureus <sup>†</sup>	SW MB	78	11.44	0.048
Compositae	Solidago ptarmicoides <sup>†</sup>	SW MB	74.6	7.41	0.048
Compositae	Rudbeckia hirta <sup>†</sup>	Interlake	82.4	10.82	0.050

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I ahle 19	Indicator	Nnecies A	Analysis	of flower sur	vev snecies h	v region
1 4010 17.	malcator	Species 1	unary 515	of nower sur	vey species by	y region

\*Monte Carlo test (*P*<0.05)

\*\*Species are sometimes considered non-native in Manitoba prairies and aspen parkland <sup>†</sup>Possible or known adult nectar plant species

Family	Scientific name	Indicator	Indicator	Standard	P*
Tanny	Scientific Hame	site	value	deviation	F
Linaceae	Linum lewisii	OK7	100	13.38	0.065
Compositae	Helianthus laetiflorus	OK7	100	14.92	0.065
Liliacea	Zygadenus elegans**	OK7	74.1	13.25	0.065
Compositae	Crepis runcinata**	OK7	53.5	8.84	0.065
Labiatae	_abiatae Stachys palustris		100	15.91	0.066
Liliaceae	Allium stellatum	IL39	81	15.66	0.066
Compositae	Solidago spathulata	IL39	57.2	8.79	0.066
Scrophulariaceae	Orthocarpus luteus	OK6	95.7	18.68	0.067
Compositae	Ratibida columnifera**	OK6	87.1	19.74	0.067
Compositae	Gaillardia aristata**	OK14	100	13.2	0.069
Liliaceae	Lilium philadelphicum**	OK14	78.9	17.28	0.069
Compositae Achillea millefolium**		OK14	77.3	9.58	0.069

Table 20.	Indicator	Species	Analysis	of flower	survey	species	by sites
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\*Monte Carlo test (P<0.05)

\*\*Possible or known adult nectar plant species

All the flower species were examined by site associations with PCA (Figure 12). The first three axes explain 67.2% of the total variance (33.0%, 20.9% and 13.4% for axes 1, 2 and 3 respectively). Axis 2 separated the sites within the two regions, where the Interlake sites are positively associated with Axis 2 while the southwest region sites are negatively associated with Axis 2 (Figure 12).

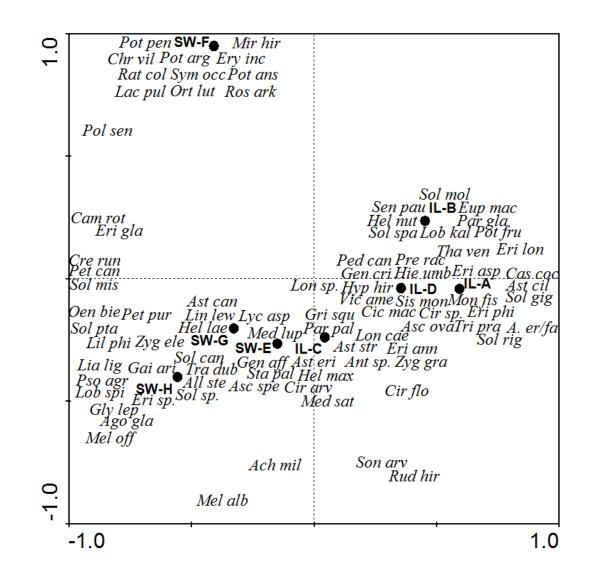


Figure 12. Principal Component Analysis of all plant species observed during the flower surveys in 2010 showing the association with study sites (•). Species codes: Ach mil = Achillea millefolium, Ago gla = Agoseris glauca, All ste = Allium stellatum, Asc ova = Asclepias ovalifolia, Asc spe = Asclepias speciosa, Ast can = Astragalus canadensis, Ast cil = Aster ciliolatus, A. er/fa = Aster ericodes or A. falcatus, Ast eri = Aster ericoides, Ast str = Astragalus striatus, Ant sp. = Antennaria spp., Cam rot = Campanula rotundifolia, Cas coc = Castilleja coccinea, Chr vil = Chrysopsis villosa, Cic mac = Cicuta maculata, Cir arv = Cirsium arvense, Cir flo = Cirsium flodmanii, Cir sp. = Cirsium spp., Cre run = Crepis runcinata, Eri ann = Erigeron annuus, Eri asp = Erigeron asper, Eri gla = Erigeron glabellus, Eri lon = Erigeron lonchophyllus, Eri phi = Erigeron philadelphicus, Eri sp. = Erigeron spp., Ery inc = Erysimum inconspicuum, Eup mac = Eupatorium maculatum, Gai ari = Gaillardia aristata, Gen aff = Gentiana affinis, Gen cri = Gentiana crinita, Gly lep = Glycyrrhiza lepidota, Gri squ = Grindelia squarrosa, Hel lae = Helianthus laetiflorus, Hel max = Helianthus maximilianii, Hel nut = Helianthus nuttallii, Hie umb = Hieracium umbellatum, Hyp hir = Hypoxis hirsuta,

Lac pul = Lactuca pulchella, Lia lig = Liatris ligulistylis, Lil phi = Lilium philadelphicum, Lin lew = Linum lewisii, Lob kal = Lobelia kalmia, Lob spi = Lobelia spicata, Lon cae = Lonicera caerulea, Lon sp. = Lonicera spp., Lyc asp = Lycopus asper, Med lup = Medicago lupulina, Med sat = Medicago sativa, Mel alb = Melilotus alba, *Mel off = Melilotus officinalis, Mir hir = Mirabilis hirsuta, Mon fis = Monarda fistulosa, Oen bie = Oenothera biennis, Ort lut = Orthocarpus luteus, Par gla = Parnassia glauca,* Par pal = Parnassia palustris, Ped can = Pedicularis canadensis, Pet can = Petalostemon candidum, Pet pur = Petalostemon purpureus, Pol sen = Polygala senega, Pot ans = Potentilla anserina, Pot arg = Potentilla arguta, Pot fru = Potentilla fruticosa, Pot pen = Potentilla pensylvanica, Pre rac = Prenanthes racemosa, Pso agr = Psoraleaagrophylla, Rat col = Ratibida columnifera, Ros ark = Rosa arkansana, Rud hir = Rudbeckia hirta, Sen pau = Senecio pauperculus, Sis mon = Sisyrinchium montanum, Sol can = Solidago canadensis, Sol gig = Solidago gigantea, Sol mis = Solidago missouriensis, Sol mol = Solidago mollis, Sol pta = Solidago ptarmicoides, Sol rig = Solidago rigida, Sol spa = Solidago spathulata, Sol sp. = Solidago spp., Son arv = Sonchus arvensis, Sta pal = Stachys palustris, Sym occ = Symphoricarpos occidentalis, Tha ven = Thalictrum venulosum, Tra dub = Tragopogon dubius, Tri pra = Trifolium pretense, Vic ame = Vicia americana, Zyg ele = Zygadenus elegans, and Zyg gra = Zygadenus gramineus

When flowering plant species not associated with Dakota Skipper adult feeding were removed from the PCA (Figure 12) the distribution of documented and potential nectar species showed that *Rudbeckia hirta* was associated with the Interlake sites, while *Lilium philadelphicum*, *Melilotus alba*, *Melilotus officinalis*, *Petalostemon candidum*, *Petalostemon purpureus*, *Ascelpias speciosa*, *Campanula rotundifolia*, *Lobelia spicata*, *Crepis runcinata*, *Lactuca pulchella* and *Ratibida columnifera* were strongly associated with the southwest region sites (Figure 13).

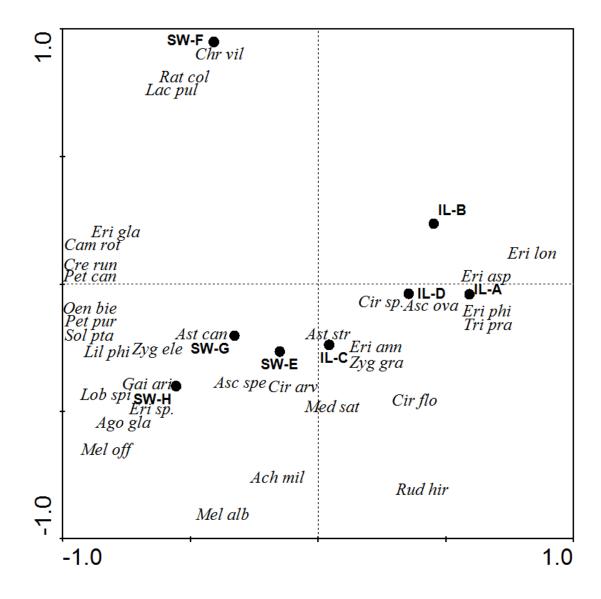


Figure 13. Principal Component Analysis of documented and potential adult nectar species in 2010 flower surveys showing the association to the study sites ( $\bullet$ ). See Figure 12 for species codes

Flowering plant species found to be indicators of region in the ISA were highlighted by site associations with PCA (Figure 14) and *Aster ericoides* or *falcatus*, *Castilleja coccinea* (scarlet paintbrush), *Rudbeckia hirta*, *Solidago rigida* and *Solidago spathulata* showed a positive association with the Interlake sites along Axis 1. Polygala *senega*, *Glycyrrhiza lepidota*, *Melilotus officinalis*, *Petalostemon candidum*, *Petalostemon purpureus*, *Campanula rotundifolia*, *Crepis runcinata*, *Ratibida columnifera*, *Solidago missouriensis* (low goldenrod) and *Solidago ptarmicoides* were negatively associated with Axis 1 and the southwestern Manitoba sites (Figure 14).

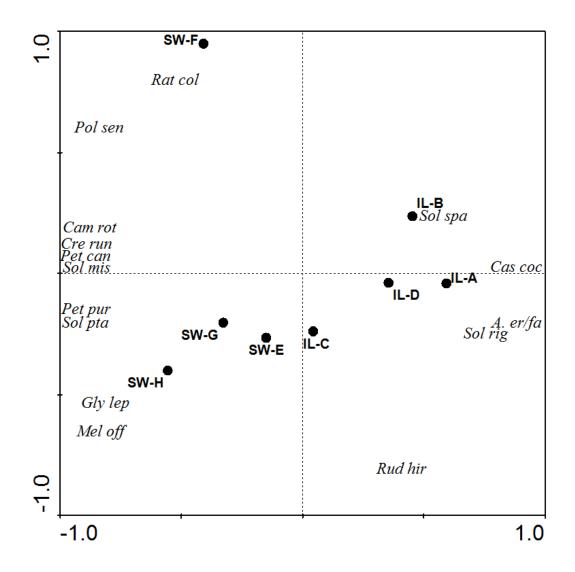


Figure 14. Principal Component Analysis of species identified in the ISA by region as significant from the 2010 flower surveys, showing the association with study sites ( $\bullet$ ). See Figure 12 for species codes

#### 5.2.2 Edaphic and Edaphic-Related Characterization

Most sites in the southwest contained wetlands and/or watercourses associated with tributaries of the Souris River. In the Interlake, most sites contained wetlands associated with the tributaries of Lake Manitoba. In 2010 sites received greater than normal precipitation throughout the summer and the soils were waterlogged by the end of the season (Environment Canada 2011). In spring and early summer 2011 there was widespread flooding throughout Manitoba, including at the study sites (Environment Canada 2011). Some standing water remained on sites throughout summer of 2011 (mainly sites B, F and G) but many of the sites dried and returned to more normal conditions by mid-June 2011. This enabled the soil sampling to be undertaken at the end of the 2011 season.

#### 5.2.2.1 Subsurface Geology and Soil Classification

The sites in the Interlake are underlain by Silurian Period deposits (Paleozoic Era) of the Interlake Group roughly 50 to 100 m deep (Province of Manitoba 1979). These are characterized by micritic, fosiliferous, stromatolitic and bistromal dolomite formations with several sandy or argillaceous marker beds. The sites in southwest Manitoba are underlain by Cretaceous Period formations (Mesozoic Era) that are 285 to 340 m deep. Specifically they are underlain by the Odanah Member of the Riding Mountain Formation, which is characterized by hard grey silaceous shale (Province of Manitoba 1979).

Soils at Interlake Sites A, B and D belong to the Black Chernozemic Greater Group, while those at Site C belong to the Gleysolic Greater Group. In the southwest, soils at Sites E and H are also Black Chernozomes, while the soils at Site F are Regosols and Glyesols at Site G. The specific soil series occurring at each study site are detailed in Table 21.

Site	Location within site	Soil series	Profile type	Parent material	Drainage	Topography	Stoniness	Salinity	Additional information
Site A	West portion, near Transect 1	Clarkleigh- Marsh Complex* (silty clay loam and mucky silty clay)	Calcareous Meadow, Saline Meadow & Calcareous Gleysol	Thin slightly lacustrine mantle over strongly calcareous till	Poor to very poor	Level with low ridge and swale micro- relief	Slightly to very stony	Usually partly saline	Clarkleigh soils occur in the more elevated areas with the Marsh soils in the lower wet areas.
Site A	East portion, near Transect 2	Isafold Series* (clay loam)	Rego Black	Strongly calcareous till	Moderately good to good	Level to irregular, gently sloping	Very stony	Not saline	
Site B	South-centre portion, near end of Transect 1	Marsh Complex* (mucky silty clay)	Calcareous Gleysol	Thin muck and silty deposits over strongly calcareous till	Very poor	Depressional	Slightly to moderately stony	Not saline	Marsh soils occur on land that has been covered with water until recent times and is frequently saturated for much of the year.
Site B	North-centre portion, near start of Transect 1	Lundar- Clarkleigh Complex* (clay loam and silty clay loam)	Gleyed Rego Black , Calcareous & Saline Meadow	Stongly calcareous till and thin silty deposits over till	Imperfect to poor	Irregular, very gently sloping (ridge and swale)	Moderately to very stony	Usually partly saline	Lundar soils occupy the low, narrow ridges and the Clarkleigh soils occur in the depressions in between.
Site B	Northeastern portion, near Transect 2	Clarkleigh Series* (silty clay loam)	Calcareous Meadow	4 to 15" of silty sediments over strongly calcareous till	Poor	Depressional to level	Moderately to very stony	Usually saline	

Table 21. Soil series present at the study sites

Site	Location within site	Soil series	Profile type	Parent material	Drainage	Topography	Stoniness	Salinity	Additional information
Site C	South-centre and north- centre portions, near Transects 1 and 2	Isafold- Lundar- Clarkleigh Complex* (clay loam and silty clay loam)	Rego Black, Gleyed Rego Black, Calcaerous & Saline Meadow	Stongly calcareous till and thin silty deposits over till	Good to poor	Irregular, gently sloping (ridge and swale)	Moderately to very stony	Usually partly saline	Isafold soils are along the NW to SE ridges, while the Lundar and Clarkleigh soils are in the low swales.
Site D	West-centre portion, near Transect 1	Clarkleigh Series* (silty clay loam)	Calcareous Meadow	4 to 15" of silty sediments over strongly calcareous till	Poor	Depressional to level	Moderately to very stony	Usually saline	
Site D	Central portion, near Transect 2	Lundar- Clarkleigh- Marsh Complex* (clay loam, silty clay loam and mucky silty clay)	Gleyed Rego Black , Calcareous & Saline Meadow, Calcareous Gleysol	Stongly calcareous till and thin silty deposits over till	Imperfect to poor	Irregular, very gently sloping	Slightly to very stony	Usually partly saline	Similar to the Lundar-Clarkleigh complex with depressions of poorly drained Marsh soils.
Site E	Southwest portion near Transect 1 and northwest portion near Transect 2	Souris Series** (loamy fine sand)	Gleyed Carbonated Rego Black	Weakly to moderately calcareous, loamy fine sand soils on sandy lacustrine deposits	Imperfect to poor	Level to depressional	Not stony	Slightly saline	
Site F	Southwest and north- central portions, near Transects 1 and 2	Souris-Oak Lake Complex <sup>†</sup> (loamy sand)	Gleyed Carbonated Rego Black, Carbonated Rego Humic Gleysol	Weakly to moderately calcareous, loamy sandy soils on sandy lacustrine deposits	Imperfect to poor	Level to depressional	Not stony	Slightly saline	Oak Lake soils occur in the more elevated areas with the Marsh soils in the lower wet areas.

Table 21. Soil series present at the study sites

Site	Location within site	Soil series	Profile type	Parent material	Drainage	Topography	Stoniness	Salinity	Additional information
Site G	Southeastern and northeastern portions, near Transects 1 and 2	Souris Series <sup>‡</sup> (loamy fine sand)	Gleyed Carbonated Rego Black	Weakly to moderately calcareous, loamy fine sand soils on sandy lacustrine deposits	Imperfect to poor	Level to depressional	Not stony	Slightly saline	
Site H	Central-east and southwestern portions, near Transects 1 and 2	Souris Series <sup>‡</sup> (loamy fine sand)	Gleyed Carbonated Rego Black	Weakly to moderately calcareous, loamy fine sand soils on sandy lacustrine deposits	Imperfect to poor	Level to depressional	Not stony	Slightly saline	

\*Pratt et al. 1961 \*\*Ellis and Schafer 1940 <sup>†</sup>Eilers et al. 1978 <sup>‡</sup>Ehrlich et al. 1956

## 5.2.2.2 Bare ground and Duff Layer

Generally the amount of bare ground represented 10 to 15% of the area within quadrats and the duff layer was 2 to 3 cm deep (Table 22). The amount of bare ground and depth of duff layer remained similar at sites throughout the summer season or declined slightly (Table 22). The duff layer in the Interlake was significantly deeper in comparison to the southwest region for the mean of the two surveys (Table 22).

Site	Field	Bare ground (% cover per m <sup>2</sup> , mean±SE)			Duff layer (cm, mean±SE)			
ID	Code	June	August	Mean	June	August	Mean	
		n = 95	n = 93	n = 188	n = 94	n = 93	n = 187	
А	IL19	19.58±4.67	7.67±0.96	13.63±2.64	1.70±0.36	1.15±0.12	1.43±0.19	
В	IL24	12.08±2.17	10.64±1.57	11.39±1.34	4.50±0.67	3.82±0.46	4.17±0.41	
С	IL39	10.92±2.18	13.64±1.89	12.22±1.45	1.70±0.21	1.90±0.29	1.80±0.18	
D	IL50	12.27±2.35	10.92±1.64	11.56±1.39	3.50±0.67	2.58±0.42	3.04±0.40	
Mean	Interlake	13.74±1.56	10.65±0.81	12.22±0.90	2.90±0.32	2.34±0.22	2.62±0.19	
Е	OK1	15.17±2.53	21.58±3.54	18.38±2.23	1.88±0.30	1.38±0.16	1.63±0.17	
F	OK6	20.83±3.84	14.08±2.70	17.46±2.40	2.15±0.30	1.65±0.19	1.90±0.18	
G	OK7	12.17±2.07	11.82±1.86	12.00±1.37	2.49±0.34	2.54±0.32	2.51±0.23	
Н	OK14	11.17±2.78	13.33±2.90	12.25±1.98	1.72±0.26	2.33±0.33	2.03±0.22	
Mean	SW Manitoba	14.83±1.50	15.28±1.49	15.05±1.05	2.06±0.15	1.96±0.14	2.01±0.10	
	<i>t</i> *	-0.74	-1.91	-1.81	1.92	0.97	2.06	
	Р	0.459	0.059	0.073	0.058	0.336	0.041	

Table 22. Summary of bare ground by site (% cover per  $m^2$ , mean±SE) and duff layer (cm, mean±SE)

\* *t*-test (*P*<0.05)

#### 5.2.2.3 Soil pH

The mean pH values recorded in the field were generally lower than those obtained in the laboratory (Table 23). In the field, the pH at sites ranged from a mean of 6.7 to 7.5, while in the laboratory the soils were found to range from pH 7.1 to 8.0. The laboratory results indicated that the soils are very slightly alkaline (pH 7.0 to 7.5) to

slightly alkaline (pH 7.5 to 8.0), thus rich in carbonates (Brady and Weil 2002; Scott 2008). The laboratory pH values exceed optimal levels for agricultural soils (pH 6.0 to 7.0) but are consistent with levels in semiarid grassland soils (Brady and Weil 2002; Scott 2008). The pH measured in the laboratory was significantly higher in the Interlake in comparison to the southwest region (Table 23).

Site ID	Field Code	University of Winnipeg lab soil pH (mean±SE)*	Field soil pH (mean±SE)**
		n = 13	n = 85
А	IL19	8.0±0.0	7.5±0.1
В	IL24	7.9±0.0	6.7±0.2
С	IL39	7.8±0.0	7.2±0.2
D	IL50	7.9±0.0	6.8±0.2
Mean	Interlake	7.9±0.0	7.0±0.1
Е	OK1	7.1±0.0	7.1±0.2
F	OK6	7.6±0.0	7.0±0.2
G	OK7	7.8±0.0	6.9±0.2
Н	OK14	7.4±0.3	7.0±0.1
Mean	SW Manitoba	7.4±0.2	7.0±0.1
	$t^{\dagger}$	-2.60	0.33
	Р	0.025	0.743

Table 23. Summary of pH values (mean±SE)

\*Measurements taken in the lab using the CaCl<sub>2</sub> method

\*\*Data collected in the field using a Kelway Soil pH and Moisture Meter

<sup>†</sup> *t*-test (*P*<0.05)

#### 5.2.2.4 Soil Moisture

In the field the soil moisture was found to range from 74 to 93% saturation (Table 24). In the University of Winnipeg laboratory the gravimetric moisture content ranged considerably from 17 to 61% moisture content, and ranged from 25 to 44% moisture content in samples sent to Exova Laboratories. The differences between the University of Winnipeg laboratory measurements and those from Exova Laboratories may be attributed to soil freshness whereby drying during transportion and different laboratory

handling techniques could have affected final values. None-the-less the overall trends in the moisture levels at each sites are generally consistent between the sites, with the exception of Site G where soil moisture was quite variable between measurement types. This variability for Site G may be a result of a particularly dry part of the soil subsample being tested at one laboratory, while in the other laboratory a more wet subsample may have been analyzed, although soil samples were blended as much as possible to avoid this from occurring.

Since the samples measured in the University of Winnipeg laboratory were fresher then those analyzed by Exova, further analysis of the gravimetric moisture content of soils will focus on those results from University of Winnipeg laboratory. There was a significant difference in the soil saturation measured in the field between the two regions, with the Interlake being more saturated (Table 24).

Site ID	Field Code	University of Winnipeg lab soil moisture (% gravimetric moisture, mean±SE)* n = 13	Exova lab soil moisture (% gravimetric moisture, mean±SE)* n = 13	Field soil moisture (% saturation, mean±SE)** n = 84
A	IL19	45.13±0.45	28.40±1.10	91.81±3.32
В	IL24	59.10±0.00	31.80±0.00	93.75±2.39
С	IL39	34.62±1.91	26.55±0.25	86.25±4.44
D	IL50	61.24±2.09	34.70±1.60	74.33±3.99
Mean	Interlake	48.72±4.53	30.16±1.41	86.42±2.08
Е	OK1	60.19±0.00	34.70±0.00	90.90±4.75
F	OK6	52.25±0.00	25.60±0.00	76.66±4.48
G	OK7	17.75±0.00	44.50±0.00	69.50±6.17
Н	OK14	51.04±3.37	32.93±1.29	76.81±3.94
Mean	SW Manitoba	47.22±6.26	33.93±2.54	79.78±2.59
	$t^{\dagger}$	-0.20	-1.349	2.08
	Р	0.846	0.205	0.041

Table 24. Summary of the soil moisture values (% gravimetric moisture content or % saturation, mean±SE)

\*Measurements taken in the lab using the CaCl<sub>2</sub> method

\*\*Data collected in the field using a Kelway Soil pH and Moisture Meter

<sup>†</sup> *t*-test (*P*<0.05)

# 5.2.2.5 Soil Compaction

Soil compaction varied at each of the depths and between the sites (Table 25). At greater depths it was more difficult to gather compaction values, especially at 40 cm, given the amount of gravel (stoniness) present in the soil (Table 21). Generally, compaction levels increased with the measurement depth. Compaction levels were generally low to moderate (<1375 kPA) at most sites, except sites A and C in the Interlake where levels sometimes exceeded 1375 kPA (moderate to high) (Table 25). The mean compaction and compaction at depths of 20, 30 and 40 cm was significantly greater in the Interlake region (Table 25).

## 5.2.2.6 Soil Bulk Density

The bulk density at research sites was within the range reported for uncultivated grasslands (Table 25) (Brady and Weil 2002; Brye et al. 2004). At Site F the bulk density value was slightly higher than expected for uncultivated grasslands and more in the range of what is seen in cultivated soils (Brady and Weil 2002). There was no significant difference between regions.

Site	Field		Field co	ompaction (kPA, m	ean±SE)		Bulk density
ID	Code	10 cm n = 86	20 cm n = 85	30 cm n = 75	40 cm n = 71	Mean n = 86	(kg/L, mean±SE) n = 13
A	IL19	879.08±78.50	1491.77±119.36	1643.96±68.71	1965.01±135.72	1334.30±83.29	1.09±0.01
В	IL24	571.69±53.41	795.76±63.57	1043.90±00.71	1217.55±90.32	898.71±52.03	0.96±0.00
С	IL39	850.35±68.20	1223.81±81.01	1581.09±103.89	1620.26±136.93	1310.36±79.70	1.09±0.03
D	IL50	680.85±58.20	939.41±100.87	1108.08±148.07	1098.23±130.17	907.80±69.06	0.98±0.05
Mean	Interlake	745.49±36.52	1104.62±59.29	1327.69±66.16	1433.19±79.75	1112.79±46.37	1.04±0.02
Е	OK1	794.46±114.13	824.23±94.69	922.95±94.56	810.13±60.87	837.95±82.27	1.04±0.00
F	OK6	990.16±124.33	1178.39±132.94	1189.34±141.80	1182.88±121.83	1165.08±123.95	1.30±0.00
G	OK7	706.71±61.50	741.18±86.52	795.76±87.48	884.82±126.71	782.14±74.51	0.78±0.00
Н	OK14	761.29±45.74	927.91±60.52	1065.81±81.83	1249.67±67.51	1001.18±54.54	1.03±0.02
Mean	SW MB	816.48±48.60	927.74±52.39	1006.26±53.94	1045.39±52.97	958.16±47.42	1.03±0.06
	t*	-1.38	2.14	3.70	3.82	2.31	0.09
	Р	0.172	0.035	<0.001	<0.001	0.023	0.931

Table 25. Summary of compaction (kilopascals [kPA], mean±SE) and apparent bulk density values (kg/L, mean±SE)

\**t*-test (*P*<0.05)

## 5.2.2.7 Soil Particle Size and Texture

Soils in the Interlake were classed as clay loams, while the soils in the southwest were classified as sandy loams as they were higher in sand and lower in clay and silt (Table 26). Site D was classed as a loam as it had less clay than the other sites in the Interlake. Site E was also classed as a loam, as it also had less clay like Site D (Table 26). There was significantly more sand in the soils in the southwest region and significantly more clay and silt in the Interlake region (Table 26). These differences in soil particle size support the average texture classification of the soils in each region (Table 26).

Site ID	Field Code	Sand (%, mean±SE)	Clay (%, mean±SE)	Silt (%, mean±SE)	Texture class
		n = 13	n = 13	n = 13	n = 13
А	IL19	38.50±4.50	27.45±2.35	34.05±2.15	Clay Loam
В	IL24	34.00±0.00	28.60±0.00	37.40±0.00	Clay Loam
С	IL39	30.00±1.00	30.85±1.55	39.15±0.55	Clay Loam
D	IL50	41.70±0.30	23.05±2.45	35.25±2.15	Loam
Mean	Interlake	36.34±2.15	27.32±1.47	36.32±1.07	Clay Loam
Е	OK1	37.40±0.00	22.30±0.00	40.30±0.00	Loam
F	OK6	62.00±0.00	12.90±0.00	25.10±0.00	Sandy Loam
G	OK7	69.00±0.00	9.80±0.00	21.20±0.00	Sandy Loam
Н	OK14	65.33±3.06	11.93±1.20	22.70±2.43	Sandy Loam
Mean	SW Manitoba	60.73±4.94	13.46±1.89	25.78±3.14	Sandy Loam
	t*	-4.588	5.866	3.384	N/A
*******	Р	0.001	<0.001	0.006	N/A

Table 26. Summary of soil particle size and texture class values

\*t-test (P<0.05)

# 5.2.2.8 Soil Organic Matter, Calcium, Magnesium and Sodium Cations

The levels of organic matter at research sites were elevated in comparison to those typically found in prairie soils or uncultivated lands (Brye et al. 2004; Kumaragamage et al. 2007; Espinoza et al. n.d.). The calcium levels exceed the levels typically preferred for production on agricultural lands (600 to 1000 mg/kg) or soils elsewhere on the Great Plains (approximately 500 mg/kg), however they were more similar to values found in

other soil studies in Manitoba (6400 to 18 800 mg/kg) (Kelling et al. 1999; Brye et al. 2004; Kumaragamage et al. 2007). The magnesium and sodium levels were also higher than suggested optimal levels (100 to 500 mg/kg for magnesium and >35 mg/kg for sodium) for agriculture (Kelling et al. 1999; Brye et al. 2004; Kumaragamage et al. 2007; Espinoza et al. n.d.). Levels of organic matter and available magnesium were highest in the Interlake region (Table 27).

Site ID	Field Code	Organic matter (% by weight, mean±SE) n = 13	Available calcium (mg/kg, mean±SE) n = 13	Available magnesium (mg/kg, mean±SE) n = 13	Available sodium (mg/kg, mean±SE) n = 13
А	IL19	11.05±0.95	3775.00±185.00	2390.00±230.00	80.00±50.00
В	IL24	12.70±0.00	3620.00±0.00	2540.00±0.00	130.00±0.00
С	IL39	13.80±0.20	4775.00±15.00	2075.00±25.00	185.00±85.00
D	IL50	12.35±1.05	4525.00±145.00	2120.00±80.00	54.50±25.50
Mean	Interlake	12.44±0.52	4252.85±198.89	2244.28±89.70	109.85±30.99
Е	OK1	7.40±0.00	5500.00±0.00	1800.00±0.00	390.00±0.00
F	OK6	7.20±0.00	3990.00±0.00	1090.00±0.00	29.00±0.00
G	OK7	8.40±0.00	3660.00±0.00	1280.00±0.00	29.00±0.00
Н	OK14	8.70±0.78	3293.33±143.33	1890.00±206.63	63.00±24.26
Mean	SW Manitoba	8.18±0.45	3838.33±357.31	1640.00±173.33	106.16±58.19
	ť*	6.14	1.05	3.24	0.64
	Р	<0.001	0.314	0.008	0.538

Table 27. Summary of soil organic matter, calcium, magnesium and sodium cation values

\**t*-test (*P*<0.05)

## 5.2.2.9 Temperature at the Soil Surface

In 2010, data was gathered over 22 weeks and in 2011 the measurement period was extended to 30 weeks (Figure 15, Appendix VIIa & b). At Site E the HOBO logger was shut off prematurely on September 21, 2011.

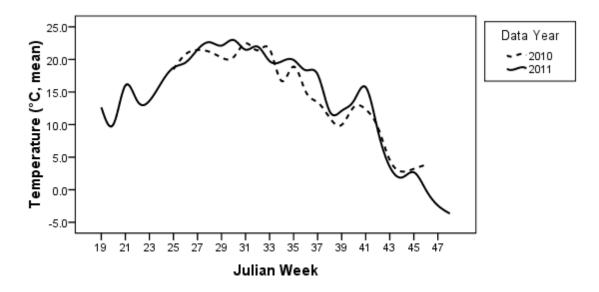


Figure 15. Comparison of air temperature (°C, mean) by Julian week in 2010 and 2011

During 2010 there were significant differences in weekly temperature between the Interlake and southwestern Manitoba regions. During Julian weeks 28, 35, 36 and 44 air temperature at the soil surface was significantly higher in the Interlake, whereas in weeks 26, 27, 29, 32 and 34 air temperatures were significantly higher in the southwest (Appendix VIIa). In 2011, air temperatures at the soil surface in the Interlake were significantly higher during Julian weeks 23, 25, 26, 30, 31, 32, 37, 41, 42, 46, 47, while during weeks 22, 44 and 48 the temperature was significantly higher in the southwestern Manitoba sites (Appendix VIIb). The overall mean temperature was also significantly higher in 2011 in the southwestern region (Appendix VIIb).

The mean temperature for the estimated "larval period" for both years ranged between was 17.74°C and 19.77°C (Table 28). The 2011 and mean of 2010/2011 air temperatures at the soil surface were significantly higher in the Interlake than the southwest region (Table 28).

Site ID	Field Code	2010 "larval period" temperature (°C; mean±SE)	2011 "larval period" temperature (°C; mean±SE)	2010 & 2011 "larval period" temperature (°C; mean±SE)
		n = 16128	n = 16046	n = 32174
Α	IL19	16.97±0.14	19.52±0.19	18.24±0.12
В	IL24	17.84±0.17	19.10±0.22	18.47±0.14
С	IL39	18.49±0.17	21.05±0.21	19.77±0.14
D	IL50	17.62±0.16	18.53±0.18	18.08±0.12
Mean	Interlake	17.73±0.08	19.55±0.10	18.64±0.06
Е	OK1	18.17±0.16	19.89±0.15	19.01±0.11
F	OK6	17.54±0.17	19.47±0.14	18.51±0.11
G	OK7	17.44±0.16	18.04±0.16	17.74±0.11
Н	OK14	17.49±0.16	18.56±0.13	18.03±0.11
	SW			
Mean	Manitoba	17.66±0.08	18.98±0.07	18.32±0.05
	<i>t</i> *	0.62	4.52	3.79
	Р	0.534	<0.001	<0.001

Table 28. Summary of the estimated "larval period" (Julian weeks 28 to 39) air temperature at the soil surface

\*t-test (P<0.05)

# 5.2.2.10 Relative Humidity at the Soil Surface

The relative humidity was approximately 85% at the soil surface throughout the 2010 and 2011 sampling period with periodic rises and drops through the season ranging from approximately 65 to 95% (Figure 16, Appendix VIIIa & b).

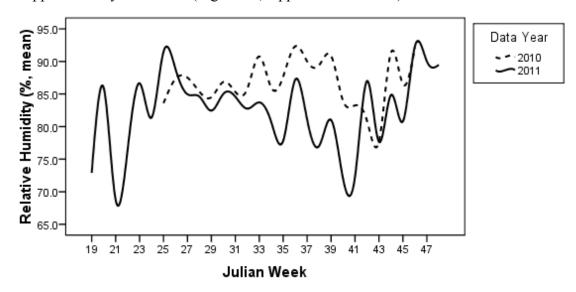


Figure 16. Comparison of relative humidity (%) by Julian week in 2010 and 2011

Almost every Julian week in 2010 and 2011 showed significant differences in the relative humidity between the regions, with the southwestern Manitoba sites mostly having higher humidity levels (Appendix VIIIa & b). The relative humidity was significantly higher in the southwest in 2010 for weeks 25, 28, 30 to 39, 44 to 46 (15 weeks total), and in 2011 for weeks 20, 25 to 48 (25 weeks total). In contrast weeks 27, 29, 40 and 42 in 2010 and weeks 19, 22 and 24 in 2011 were the only periods where the relative humidity was significantly higher in the Interlake (Appendix VIIIa & b). The mean overall relative humidity was also significantly higher in the southwest region in both 2010 and 2011.

The mean relative humidity of the 2010 and 2011 estimated "larval period" was 85.13%. When the relative humidity data was pooled it was found that the southwest region experience significantly higher humidity as compared to the Interlake for 2010, 2011 and the mean of both years (Table 29).

	Field	2010 "Larval Period"	2011 "Larval Period"	2010 & 2011 "Larval
Site ID	Code	relative humidity	relative humidity	Period" relative humidity
	0000	(%; mean±SE)	(%; mean±SE)	(%; mean±SE)
		n = 16128	n = 16046	n = 32174
А	IL19	90.26±0.17	76.72±0.40	83.49±0.24
В	IL24	82.62±0.29	74.22±0.45	78.42±0.28
С	IL39	84.50±0.30	72.94±0.47	78.72±0.29
D	IL50	87.00±0.21	82.44±0.30	84.72±0.18
Mean	Interlake	86.10±0.13	76.58±0.21	81.34±0.13
E	OK1	89.80±0.24	86.30±0.30	88.09±0.19
F	OK6	86.84±0.31	90.48±0.18	88.66±0.18
G	OK7	91.03±0.24	88.01±0.27	89.52±0.18
Н	OK14	91.19±0.27	87.71±0.25	89.45±0.19
	SW			
Mean	Manitoba	89.71±0.13	88.14±0.13	88.93±0.09
	<i>t</i> *	-19.41	-47.41	-48.06
	Р	<0.001	<0.001	<0.001

Table 29. Summary of the estimated "larval period" (Julian weeks 28 to 39) air relative humidity at the soil surface

\**t*-test (*P*<0.05)

# 5.2.2.11 RDA Analysis of Edaphic, Vegetation and Flower Surveys

In the RDA of edaphic data and plant species the first three axes explained 52.1% (19.7%, 17.2% and 15.1%, for axes 1, 2 and 3 respectively) of the variance observed (Figure 17). Axis 1 separated the Interlake and southwest region sites. Clay was found to have a significant (P = 0.046) relationship with the plant species and sites being positively associated with the Interlake sites (Figure 17).

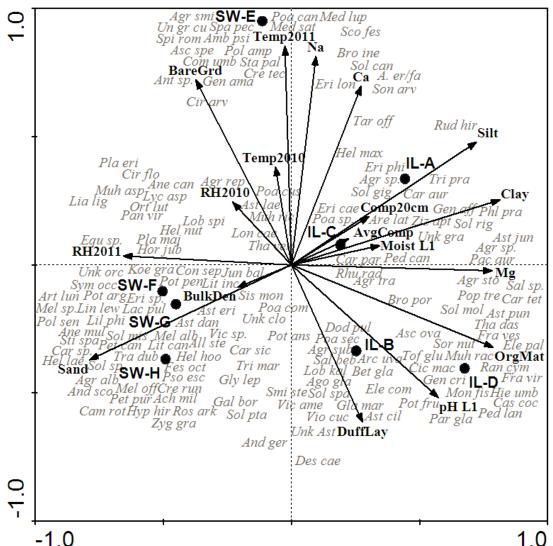


Figure 17. Redundancy Analysis of select edaphic parameter data from 2010 and/or 2011 and all plant species from 2010 showing the association to the study sites (•). Edaphic parameters: BareGrd = bare ground (% cover per  $m^2$ ); DuffLay = duff layer (cm); pH L1 = soil pH at University of Winnipeg laboratory; Moist L1 = soil moisture (%) at University of Winnipeg laboratory; Comp20cm = compaction at 20 cm depth (PSI); AvgComp = mean compaction of 10, 20, 30 and 40 cm depth (PSI); BulkDen = bulk density (kg/L); Sand = sand particle size (%); Clay = clay particle size (%); Silt = silt particle size (%); OrgMat = organic matter (% by weight); Ca = available calcium (mg/kg); Mg = available magnesium (mg/kg); Na = available sodium (mg/kg); Temp2010 = mean temperature (°C) of the air at the soil level in 2010; Temp2011 = mean temperature (°C) of the air at the soil level in 2011; RH2010 = mean relative humidity (%) of the air at the soil level in 2010; and RH2011 = mean relative humidity (%) of the air at the soil level in 2011. Species Codes: Ach mil = Achillea millefolium, Ago gla = Agoseris glauca, Agr alb = Agropyron albicans, Agr rep = Agropyron repens, Agr smi= Agropyron smithii, Agr sp. = Agropyron spp., Agr sto = Agrostis stolonifera, Agr sub = Agropyron subsecundum, Agr tra = Agropyron trachycaulum, All ste = Allium stellatum,

Amb psi = Ambrosia psilostachya, And ger = Andropogon gerardii, And sco = Andropogonscoparius, Ane can = Anemone canadensis, Ane mul = Anemone multifida, Ant sp. = Antennaria spp., Arc uva = Arctostaphylos uva-ursi, Are lat = Arenaria lateriflora, Art lun =Artemisia ludoviciana, Asc ova = Asclepias ovalifolia, Asc spe = Asclepias speciosa, Ast cil = Aster ciliolatus, Ast dan = Astragalus danicus, A. er/fa = Aster ericodes or A. falcatus, Ast eri = Aster ericoides, Ast jun = Aster junciformis, Ast lae = Aster laevis, Ast pun = Aster puncieus, Bet gla = Betula glandulifera, Bro ine = Bromus inermis, Bro por = Bromus porteri, Cam rot = Campanula rotundifolia, Car aur = Carex aurea, Car par = Carex parryana, Car sic = Carex siccata, Car sp. = Carex spp., Car tet = Carex tetanica, Cas coc = Castilleja coccinea, Cic mac = Cicuta maculata, Cir arv = Cirsium arvense, Cir flo = Cirsium flodmanii, Com umb = Comandra umbellata, Con sep = Convolvulus sepium, Cre run = Crepis runcinata, Cre tec = Crepis tectorum, Des cae = Deschampsia caespitosa, Dodpul = Dodecatheon pulchellum, Ela com = Elaeagnus commutata, Ele pal = Eleocharispalustris, Equ sp., = Equisetum spp., Eri cae = Erigeron caespitosa, Eri lon = Erigeron lonchophyllus, Eri phi = Erigeron philadelphicus, Eri sp. = Erigeron spp., Fes oct = Festuca octoflora, Fra ves = Fragaria vesca, Fra vir = Fragaria virginiana, Gal bor = Galium boreale, Gen aff = Gentiana affinis, Gen ama = Gentiana amarella, Gen cri = Gentiana crinita, Gla mar = Glaux maritima, Gly lep = Glycyrrhiza lepidota, Hel hoo = Helictotrichon hookeri, Hel lae = Helianthus laetiflorus, Hel max = Helianthus maximilianii, Hel nut = Helianthus nuttallii, Hie umb = Hieracium umbellatum, Hor jub = Hordeum jubatum, Hyp hir = Hypoxis hirsuta, Jun bal = Juncus balticus, Koe gra = Koeleria gracilis, Lac pul = Lactuca pulchella, Lia lig = Liatris ligulistylis, Lil phi = Lilium philadelphicum, Lin lew = Linum lewisii, Lit can = Lithospermum canescens, Lit inc = Lithospermum incisum, Lob kal = Lobelia kalmia, Lob spi = Lobelia spicata, Lon cae = Lonicera caerulea, Lyc asp = Lycopus asper, Med lup = Medicago lupulina, Med sat = Medicago sativa, Mel alb = Melilotus alba, Mel off = Melilotus officinalis, Mel sp. = Melilotus spp., Mon fis = Monarda fistulosa, Muh asp = Muhlenbergia asperifolia, Muh rac = Muhlenbergia racemosa, Muh ric = Muhlenbergia richardsonis, Ort lut = Orthocarpus luteus, Pac aur = Packera aurea, Pan vir = Panicum virgatum, Par gla = Parnassia glauca, Ped can = Pedicularis canadensis, Ped lan = Pedicularis lanceolata, Pet can = Petalostemon candidum, Pet pur = Petalostemon purpureus, Phl pra = Phleum pratense, Pla eri = Plantago eriopoda, Pla maj = Plantago major, Poa can = Poa canbyi, Poa com = Poa compressa, Poa cus = Poa cusickii, Poa sec = Poa secunda, Poa sp. = Poa spp., Pol amp = Polygonum amphibium, Pol sen = Polygala senega, Pop tre = Populus tremuloides, Pot ans = Potentilla anserina, Pot arg = Potentilla arguta, Pot fru = Potentilla fruticosa, Pot pen = Potentilla pensylvanica,  $Pso \ esc = Psoralea \ esculenta, Ran \ cym = Ranunculus \ cymbalaria,$ Rhu rad = Rhus radicans, Ros ark = Rosa arkansana, Rud hir = Rudbeckia hirta, Sal beb = Salix bebbiana, Sal sp. = Salix spp., Sco fes = Scolochloa festucacea, Sis mon = Sisyrinchium montanum, Smi ste = Smilacina stellata, Sol can = Solidago canadensis, Sol gig = Solidago gigantea, Sol mis = Solidago missouriensis, Sol mol = Solidago mollis, Sol pta = Solidago ptarmicoides, Sol rig = Solidago rigida, Sol sp. = Solidago spp., Sol spa = Solidago spathulata, Son arv = Sonchus arvensis, Sor nut = Sorghastrum nutans, Spa pec = Spartina pectinata, Spi rom = Spiranthes romanzoffiana, Sta pal = Stachys palustris, Sti spa = Stipa spartea, Sym occ = Symphoricarpos occidentalis, Tar off = Taraxacum officinale, Tha das = Thalictrum dasycarpum, Tha ven = Thalictrum venulosum, Tof glu = Tofieldia glutinosa, Tra dub = Tragopogon dubius, Tri mar = Triglochin maritima, Tri pra = Trifolium pratense, Un gr cul = Unknown grass cultivar spp., Unk Ast = Unknown Asteraceae,  $Unk \ clo =$  Unknown clover,  $Unk \ gra =$  Unknown grass,  $Unk \ orc =$  Unknown orchid, Vic ame = Vicia americana, Vic sp. = Vicia spp., Vio cuc = Viola cucullata, Ziz apt = Zizia aptera, and Zyg gra = Zygadenus gramineus

When documented and possible larval food plants were examined in a RDA with selected edaphic parameters and sites (Figure 18), the first three axes accounted for 69.4% of the total variation. Axis 1 explained 32.6% of the variation, Axis 2 accounted for 20.7% of the variance and Axis 3 16.1%. None of the physical variables were associated with the sites and the larval food plants.

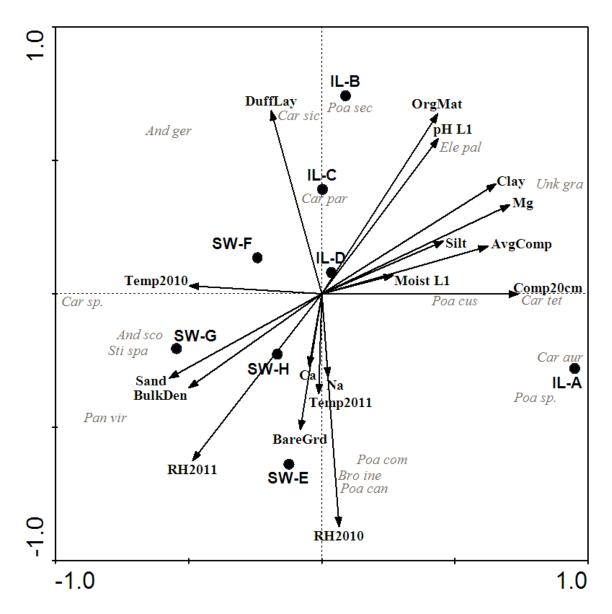


Figure 18. Redundancy Analysis of select edaphic parameter data from 2010 and/or 2011 and documented and possible larval food plant species data from 2010 showing the association to the study sites (•). See Figure 17 for edaphic and species codes

When edaphic variables were examined using RDA in conjunction with research sites and flower survey species (Figure 19), Axis 1, 2 and 3 explained 58.7% of the total variance (24.8%, 18.6% and 15.3% for axes 1, 2 and 3 respectively). Axis 1 separated the Interlake sites from those in the southwest (Figure 19). Magnesium was significantly negatively associated (P = 0.026) with the Interlake sites.

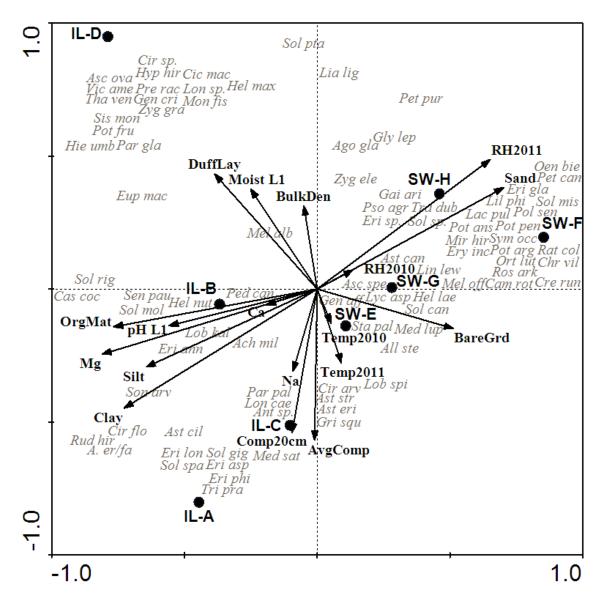


Figure 19. Redundancy Analysis of select edaphic parameter data from 2010 and/or 2011 and all flower survey species from 2010 showing the association to the study sites (•). See Figure 17 for edaphic codes. Species codes: Ach mil = Achillea millefolium, Ago gla = Agoseris glauca, All ste = Allium stellatum, Asc ova = Asclepias ovalifolia, Asc spe = Asclepias speciosa, Ast can = Astragalus canadensis, Ast cil = Aster ciliolatus, A. er/fa

= Aster ericodes or A. falcatus, Ast eri = Aster ericoides, Ast str = Astragalus striatus, Ant sp. = Antennaria spp., Cam rot = Campanula rotundifolia, Cas coc = Castilleja coccinea, Chr vil = Chrysopsis villosa, Cic mac = Cicuta maculata, Cir arv = Cirsium arvense, Cir flo = Cirsium flodmanii, Cir sp. = Cirsium spp., Cre run = Crepis runcinata, Eri ann = Erigeron annuus, Eri asp = Erigeron asper, Eri gla = Erigeron glabellus, Eri lon = Erigeron lonchophyllus, Eri phi = Erigeron philadelphicus, Eri sp.. = Erigeron spp., Ery inc = Erysimum inconspicuum, Eup mac = Eupatorium maculatum, Gai ari = Gaillardia aristata, Gen aff = Gentiana affinis, Gen cri = Gentiana crinita, Gly lep = Glycyrrhiza lepidota, Gri squ = Grindelia squarrosa, Hel lae = Helianthus laetiflorus, Hel max = Helianthus maximilianii, Hel nut = Helianthus nuttallii, Hie umb = Hieracium umbellatum, Hyp hir = Hypoxis hirsuta, Lac pul = Lactuca pulchella, Lia lig = Liatris ligulistylis, Lil phi = Lilium philadelphicum, Lin lew = Linum lewisii, Lob kal = Lobelia kalmia, Lob spi = Lobelia spicata, Lon cae = Lonicera caerulea, Lon sp. = Lonicera spp., Lyc asp = Lycopus asper, Med lup = Medicago lupulina, Med sat = Medicago sativa, Mel alb = Melilotus alba, Mel off = Melilotus officinalis, Mir hir = Mirabilis hirsuta, Mon fis = Monarda fistulosa, Oen bie = Oenothera biennis, Ort lut = Orthocarpus luteus, Par gla = Parnassia glauca, Par pal = Parnassia palustris, Ped can = Pedicularis canadensis, Pet can = Petalostemon candidum, Pet pur = Petalostemon purpureus, Pol sen = Polygala senega, Pot ans = Potentilla anserina, Pot arg = Potentilla arguta, Pot fru = Potentilla fruticosa, Pot pen = Potentilla pensylvanica, Pre rac = Prenanthes racemosa, Pso agr = Psoralea agrophylla, Rat col = Ratibida columnifera, Ros ark = Rosa arkansana, Rud hir = Rudbeckia hirta, Sen pau = Senecio pauperculus, Sis mon = Sisyrinchium montanum, Sol can = Solidago canadensis, Sol gig = Solidago gigantea, Sol mis = Solidago missouriensis, Sol mol = Solidago mollis, Sol pta = Solidago ptarmicoides, Sol rig = Solidago rigida, Sol spa = Solidago spathulata, Sol sp. = Solidago spp., Son arv = Sonchus arvensis, Sta pal = Stachys palustris, Sym occ = Symphoricarpos occidentalis, Tha ven = Thalictrum venulosum, Tra dub = Tragopogon dubius, Tri pra = Trifolium pretense, Vic ame = Vicia americana, Zyg ele = Zygadenus elegans, and Zyg gra = Zygadenus gramineus

When edaphic variables were evaluated using RDA with sites and documented or possible nectar flower species, (Figure 20), the first three axes explained 63.1% of the variance, with Axis 1 accounting for 29.2%, Axis 2 18.4% and Axis 3 15.5%. Clay was significantly (P = 0.020) related to sites and nectar flower species, being positively associated with the Interlake sites (Figure 20).

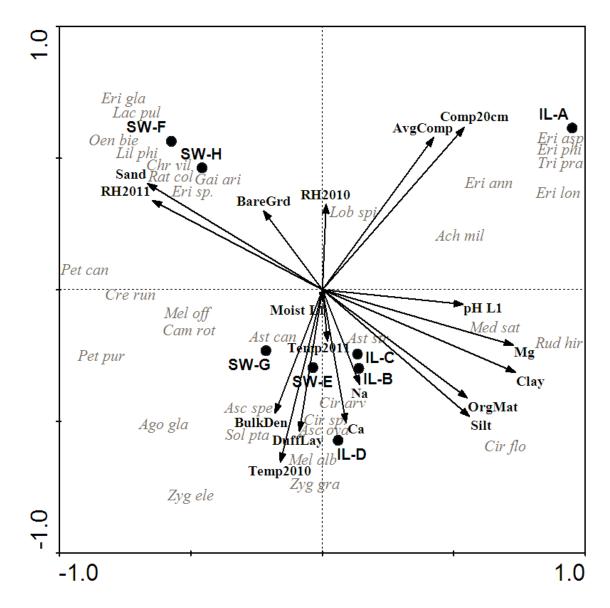


Figure 20. Redundancy Analysis of select edaphic parameter data from 2010 and/or 2011 and documented or possible nectar plant species in 2010 showing the association to the study sites ( $\bullet$ ). See Figure 17 for edaphic codes and Figure 19 for species codes

# 5.3 Discussion

### 5.3.1 Vegetation and Flower Surveys

There was considerable overlap in the plant species composition between the two regions for both the vegetation and flower surveys. Many of the most abundant species were observed in both regions. Grasses (Graminae) were the most commonly encountered group of species. Five species dominated in the June survey (*Deschampsia caespitosa*, *Poa cusickii*, Unknown grasses, *Carex* spp. and *Sonchus arvensis*). Immature *Andropogon gerardii* and other grass species were likely captured in the "Unknown grasses" category. In the August vegetation survey, seven species were abundant in both regions (*Agropyron repens*, *Andropogon gerardii*, *Andropogon scoparius*, *Deschampsia caespitosa*, *Poa compressa*, *Scolochloa festucacea* (spangletop) and *Sonchus arvensis*).

Similar trends were observed in the flower surveys where many of the most abundant species occured in both surveys. Seven species dominated in the early July flower survey (*Zygadenus elegans*, *Melilotus alba*, *Petalostemon candidum*, *Campanula rotundifolia*, *Lobelia spicata*, *Crepis runcinata* and *Rudbeckia hirta*). In the mid-July flower survey, six species were abundant in both regions (*Petalostemon candidum*, *Petalostemon purpureus*, *Campanula rotundifolia*, *Lobelia spicata*, *Rudbeckia hirta* and *Solidago ptarmicoides*). This co-occurance of dominant species in both regions for both types of surveys suggests that despite the distance between regions the overall vegetation composition at sites in both regions is quite similar.

There were significant differences in abundance of particular plant species between regions, where the ISA identified several significant indicator species for each of the regions. The PCA further demonstrated the fidelity of certain plant species to a particular region. Difference in plant species abundance between regions was also reflected in the Mann-Whitney U-test analysis.

There was little difference in plant species diversity between sites within regions indicating that sites were quite homogenous. Sites were selected on the basis of previous surveys for Dakota Skipper adults. The sites in this study represented those with the highest density of skippers in past surveys, thus if presence of certain larval food plants and adult nectar plants is linked to the presence of Dakota Skipper then it is not surprising that sites appear quite homogenous in respect to plant species diversity.

*Eleocharis palustris* was recorded within transects in the Interlake sites and was absent from transects in the southwest, but was found outside the transects at most of the sites in southwest Manitoba (especially site H). Conversely, *Stipa spartea* was recorded only in the southwest sites with the ISA analysis identifying this species as an indicator for the southwest region sites, *Stipa spartea* was also observed infrequently outside of transect areas in the Interlake sites B, C and D. *Stipa spartea* was difficult to detect during the vegetation surveys (it was immature and inconspicuous in June and past maturity, entering senescence in August). A species like *Stipa spartea* is easiest to detect in early July when the grass is tallest and seed heads are mature (Appendix X).

*Rudbeckia hirta* was the most abundant nectar flower species in the Interlake and also quite abundant in the southwest, although it was significantly more abundant in the Interlake where ISA also identified it as an indicator species (Appendix X). *Rudbeckia hirta* has been reported as a nectar source for adult Dakota Skippers in Canada (Webster 2003; Webster 2007) and it appears to be an essential component of the flowering species found in both the Interlake and southwest Manitoba sites.

*Lilium philadelphicum* was found along transects in the southwest Manitoba research sites during the vegetation and flower surveys, however, it was not recorded in the Interlake surveys. As with *Eleocharis palustris, Lilium philadelphicum* was observed sporadically in areas outside transects at several of the Interlake sites. This species tends to be found at very low densities but when in bloom they can be conspicuous due to their raised, bright orange flowers. Webster (2003) and Webster (2007) suggested this species could a useful indicator of Dakota Skipper habitat. However, this species can be difficult to detect at low densities or when not in bloom, so other species may be more appropriate indicators of habitat.

# 5.3.1.1 Vegetation Profile of Dakota Skipper Habitat

In Manitoba, sites supporting Dakota Skipper contain plant species required for shelter, and larval and adult feeding. Most sites contained extensive amounts of medium to tallgrass prairie, which has remained relatively intact over the last several decades. Haying and flooding are the most common disturbances.

Across Manitoba, Dakota Skipper habitat can be generally described as low, wetto-mesic tallgrass prairie (Cochrane and Delphey 2002; Webster 2003; Webster 2007). The research sites were generally characterized by the presence of several documented and possible larval food plants, including: *Andropogon gerardii, Andropogon scoparius* and *Poa cusickii*. Sites also contained many documented or newly recorded adult nectar plant species including: *Zygadenus elegans, Zygadenus gramineus, Petalostemon candidum, Petalostemon purpureus, Campanula rotundifolia, Lobelia spicata, Achillea millefolium, Agoseris glauca, Cirsium flodmanii, Crepis runcinata, Erigeron annuus, Rudbeckia hirta* and *Solidago ptarmicoides* (Appendix X). Finally, all sites contained other plant species commonly associated with the presence of Dakota Skipper including: *Deschampsia caespitosa, Panicum virgatum, Allium stellatum, Hypoxis hirsuta, Rosa arkansana, Glycyrrhiza lepidota, Polygala senega, Zizia aptera, Galium boreale, Aster ericodes or A. falcatus, Aster laevis, Helianthus maximilianii, Helianthus nuttallii, Liatris ligulistylis, Solidago rigida* and *Solidago spathulata* (Appendix X).

In this study *Rosa arkansana*, *Zizia aptera*, *Campanula rotundifolia*, *Lobelia spicata*, *Cirsium flodmanii*, *Liatris ligulistylis*, *Rudbeckia hirta*, *Solidago ptarmicoides* and *Solidago spathulata* were consistently found at all eight sites and should be regarded as indicators of the presence of Dakota Skipper habitat. These species are considered to be endemic to tallgrass prairies in Manitoba (Looman and Best 1981; Reaume 2009). The presence of *Stipa spartea* and *Eleocharis palustris* is also likely an important indicator of Dakota Skipper habitat (Appendix X). It may be prudent to consider *Lilium philadelphicum* and *Melilotus alba* as species that can be associated with Dakota Skipper sites in Manitoba as well.

When evaluating the quality of new Dakota Skipper habitat in Manitoba using plant species the survey period may be critical. For instance, if surveys for candidate Dakota Skipper sites are implemented in early summer investigators should search for *Lilium philadelphicum*, *Zygadenus elegans*, *Zygadenus gramineus* and *Zizia aptera* and for old stalks, flower heads and fruit of *Andropogon gerardii*, *Glycyrrhiza lepidota* and *Helianthus* species, which are often visible from the previous season. In late June, just before the emergence of Dakota Skipper adults, *Zygadenus* species are in full bloom, while *Campanula rotundifolia*, *Lobelia spicata*, *Cirsium flodmanii*, *Liatris ligulistylis* and *Rudbeckia hirta* are beginning to bloom (Appendix X). Most of the clover species (*Melilotus* and *Petalostemon* species) also come into bloom shortly after the start of the

flight period and are often quite conspicuous and distinguishable. *Solidago ptarmicoides* becomes prominent shortly after the start of the flight period (Appendix Z). Following the flight period, in late July and August, the tall grasses mature as do many of the late season composites, particularly the taller *Helianthus* species.

Many of the species identified in the ISA should be considered as important for determining potential habitat when evaluating new sites. In the Interlake, investigators should search for *Carex tetanica*, *Castilleja coccinea*, *Aster ericoides* or *falcatus*, *Rudbeckia hirta*, *Solidago rigida* and *Solidago spathulata*. In southwestern Manitoba potential sites should include *Equisetum* spp., *Comandra umbellata*, *Glycyrrhiza lepidota*, *Petalostemon candidum*, *Petalostemon purpureus*, *Polygala senega*, *Plantago eriopoda*, *Campanula rotundifolia*, *Crepis runcinata*, *Solidago missouriensis* and *Solidago ptarmicoides* (Appendix X).

#### 5.3.1.2 Comparison of Plant Surveys to Past Studies

Past surveys have reported Dakota Skipper habitat in Manitoba to be in "low, wetto-mesic tallgrass prairie" in "small (1.0 ha) to large (400 ha) openings among aspen and bur oak groves", which is consistent with what was found in the present study (Webster 2003; Webster 2007). Many of the dominant species reported previously were confirmed in this study, though *Lilium philadelphicum* was not as abundant as previously described (Webster 2003; Webster 2007). Webster (2003) and Webster (2007) previously reported that Sporobolus heterolepis was common at Dakota Skipper sites in Manitoba. This grass species has been reported as a larval food plant species in the United States (Dana 1991). However Sporobolus heterolepis was not recorded at any of the eight study sites, many of which were included in surveys by Webster (2003) and Webster (2007). It is unclear which of the sites visited by Webster had Sporobolus heterolepis (Webster 2003; Webster 2007). Sporobolus heterolepis has previously been recorded in southern Manitoba (Reaume 2009) but was not recorded in this study. Alternatively, it may have been misidentified in the past as several other grass species recorded in this study have a similar characteristics, such as Deschampsia caespitosa, Agrostis stolonifera and Andropogon gerardii (Best et al. 1977; Looman and Best 1981; Reaume 2009).

Webster (2007) suggested that *Lilium philadelphicum*, *Zygaendus elegans*, *Campanula rotundifolia* and *Rudbeckia hirta* are good indicator plants of Dakota Skipper

habitat in Manitoba. Although these species may be common in some sites with adult Dakota Skipper, they are sometimes difficult to observe or absent, so it is advisable to expand the list of indicator plant species to those listed in Section 5.3.1.1.

In the United States Dakota Skipper habitat is classified as either lower, wet prairies or upland, drier prairies (Cochrane and Delphey 2002). The composition of the lower, wet prairies is as described above by Webster (2003) and Webster (2007). The upland drier sites are dominated by bluestem (*Andropogon*) and needlegrass (*Stipa*) species as well as *Echinacea pallida* (pale purple coneflower), *Echinacea angustifolia* and *Gaillardia aristata* (Cochrane and Delphey 2002). Sites in southwestern Manitoba do have *Stipa spartea* at moderate densities and *Gaillardia aristata* at low densities, while Interlake sites have these two species at low densities. The *Echinacea* species are absent from Manitoba sites.

The findings in this study are consistent with the descriptions of the vegetation observed in other studies in Canada and the U.S. However, this study provided a more intensive quantitative assessment of the vegetation communities present in Dakota Skipper habitat than past studies.

#### 5.3.2 Edaphic and Edaphic-Related Characterization

#### 5.3.2.1 Evaluation of Individual Factors

Bare ground was present at sites in both regions, with approximately 10 to 15% of the soil surface exposed. The bare areas may heat faster during the day due to the reduced shading and the dark colour of the soil. Further, the soil may be more compacted and eroded by natural and anthropogenic forces. Increased bare ground may reduce the amount of habitat available to skipper larvae as they require plant shelter to decrease exposure to desiccation or predation.

The duff layer was approximately 2 to 3 cm deep, which likely reflects the land use at the sites. Most of the sites were subjected to annual or alternate year haying, meaning that the majority of the above ground vegetation was removed each fall and did not contribute to the duff layer; stubble and unharvested hay contributed the most to the duff layer. Correspondingly those sites less frequently hayed (Sites B and D in the Interlake) had a deeper duff layer. This may have accounted for significantly deeper measurements of duff layers in the Interlake region. The duff layer is known to help retain moisture and moderate temperatures at the soil surface, which is important to skipper larval survival (Dana 1991; Royer et al. 2008).

The pH of the soils at the site was relatively alkaline due, in part, to the presence of calcium carbonates causing the soils to be calcareous (Brady and Weil 2002; Scott 2008). High levels of calcium and magnesium may also increase the soil pH due to the increased presence of cations themselves (Brady and Weil 2002). Soils with a high pH were known to have adverse effects upon plant growth and are considered undesirable for agriculture (Brady and Weil 2002; Scott 2008). The pH of the soils in the Interlake was significantly higher than levels in the southwest. The pH values measured with a Kelway meter reported by Royer et al. (2008) at sites in North Dakota, South Dakota and Minnesota were generally lower then those found in the present study, although the measurements recorded with the Kelway meter more closely align with Royer's results (Table 30). Royer's closest research sites were in north-central North Dakota, which are approximately 150 km south of the southwestern Manitoba sites. Additional information on Royer's sites can be found in Appendix VI (Royer et al. 2008).

Site	State	Soil pH (mean)	Sand (%, mean)	Clay (%, mean)	Silt (%, mean)	Texture class	Bulk density (kg/L, mean)	"Larval Period" temp. (°C, mean)	"Larval Period" relative humidity (%, mean)
		n = 33	n = 33	n = 33	n = 33	n = 33	n = 33		
1) FP	Minnesota	6.2	53.3	8.3	38.3	Sandy Loam	0.86	19.14	82.01
2) HM	Minnesota	6.3	61.7	9.2	29.2	Sandy Loam	0.86	18.84	83.48
3) PC	Minnesota	6.6	60.8	7.7	31.5	Sandy Loam	0.91	20.53	81.16
4) MCC	North Dakota	6.4	65.6	6.9	27.5	Sandy Loam	1.04	17.93	82.90
5) SLS	North Dakota	6.7	61.0	9.0	30.0	Sandy Loam	1.14	18.04	82.29
6) SSS	North Dakota	6.4	74.7	11.7	14.0	Sandy Loam	1.28	None	None
7) SFP	South Dakota	6.5	56.7	5.8	37.5	Sandy Loam	0.78	19.03	80.79
8) KNP	South Dakota	6.7	56.2	4.8	38.9	Sandy Loam	0.96	19.57	75.46
9) CXL	South Dakota	6.4	61.5	3.7	34.8	Sandy Loam	0.92	19.93	81.72
Mean		6.5	61.3	7.5	31.3	Sandy Loam	0.97	19.00	81.37

Table 30. Summary of edaphic and edaphic-related results from sites in the United States (Royer et al. 2008)

Moisture levels at all sites in the present study were high. In the field, the soils were highly saturated (70 to 93% saturated) and University of Winnipeg laboratory analysis indicated that the soils had high gravimetric moisture content (mean of 48%). Considering the soils are sandy loams and clay loams, the amount of water held in the soils can be considered quite high, as these soils typically have a lower capacity to hold moisture (Brady and Weil 2002). Research sites contained many depressions with minimal elevation which resulted in poor drainage. Sites were often surrounded by adjacent wetlands and/or watercourses which either drained into the research sites or into nearby streams, ditches and depressions.

Percent soil saturation was significantly greater in the Interlake region, where sites are typically adjacent to more and larger wetland areas. Soil moisture is important to larval survival in that high moisture affects air humidity and helps to regulate temperatures at the soil level thereby reducing the chance of desiccation. However, excessively high moisture levels may promote toxic fungal growth or suffocate larva (McCabe 1981; Dana 1991), especially during periods of prolonged inundation. Royer et al. (2008) hypothesized that the presence of hydrofuge glands on the 7<sup>th</sup> and 8<sup>th</sup> abdominal segments may function to protect from inundation.

Soil compaction was generally low to moderate (<1375 kPA) at all the depths measured although occasionally the compaction was moderate (1375 to 2065 kPA) at Sites A and C. These results indicate that the soils have not been subject to significant compactive forces from haying operations, other vehicles, cattle grazing or other forces. Sites A and C may have been subject to the most intense agricultural activities (e.g. leveling, drainage alterations, movement of agricultural vehicles, tilling, etc.), which may account for the increased compaction. The mean compaction and the compaction at 20, 30 and 40 cm was also significantly higher in the Interlake, which can be attributed to the higher compaction present at Sites A and C. The soil series for the Interlake sites includes higher levels of gravel present in the soils, which is also known to increase soil compaction (Brady and Weil 2002). Higher soil compaction can adversely affect plant growth and reduce flora diversity (DeByle 1985; Dockrill et al. 2004), which in turn can affect the quality of Dakota Skipper habitat. Forces causing compaction (machinery,

cattle grazing, etc.) can also inadvertently kill larvae by either direct crushing or crushing of food and shelter plants.

The soil bulk density at the sites was within a range reported for other uncultivated grasslands (0.8 to 1.2 kg/L) (Brady and Weil 2002). There was no difference in the bulk density between regions. Bulk density is an indicator of soil compaction and is also influenced by the soil texture, particle size and organic matter which affects the arrangement of the soil particles (Brady and Weil 2002). Soils high in sand will have a higher bulk density, while soils high in clay will have a low bulk density. The bulk density at the sites was quite low considering the high percentage of sand present in some sites but perhaps higher levels of organic matter partly reduced the bulk density. The bulk density results also reflect the moderate compaction values and indicate that generally the sites have not been subject to significant compactive forces from cattle grazing, tilling or agricultural vehicles in the past. The bulk density at Site F was slightly higher than other sites suggesting that the soils here have been more compacted by natural or anthropogenic forces. Royer et al. (2008) also reported similar bulk density values found in the present study at sites in the United States (Table 30).

The particle composition and texture of the Interlake sites are similar with three of the sites having a clay loam texture while Site D soils were a loam texture due to the slightly lower levels of silt. In the southwest, Sites F, G and H also had similar soil composition and were considered to be Sandy Loams. However Site E was more similar to Site D, because it had much lower levels of sand and much higher levels of clay and silt. The cause for these differences in particle composition is most likely related to local variation in glacial deposits (Fulton 1984). The significant differences found in the levels of sand and clay present between the two regions also explains the differences in soil texture seen between the two regions overall, and is also likely a result of differences in glacial deposits (Fulton 1984). In the United States, Royer et al. (2008) reported sites being generally sandy loams which were most similar to the sites in southwestern Manitoba in the present study (Table 30). The levels of sand were similar, though the levels of clay appear slightly lower while the levels of silt were slightly higher.

The organic matter levels in the present study were very high, beyond what is typically desired for agricultural soils (Brye et al. 2004; Espinoza et al. n.d.). However,

these high levels are typical of other studies in southern Manitoba (Kumaragamage et al. 2007). High organic matter levels found in the present study indicate that decomposition at these sites is slow, which is likely to due to higher soil saturation and cooler temperatures associated with higher latitudes (Brady and Weil 2002). In particular, the Interlake sites had significantly higher organic matter and soil saturation levels compared to those in the southwest region which may be because the Interlake is at a higher latitude.

The amounts of calcium, magnesium and sodium present in research sites was also quite high and well above levels reported for agricultural fields in the Great Plains (Kelling et al. 1999; Brye et al. 2004; Espinoza et al. n.d.). Calcium levels were within ranges reported elsewhere in Manitoba suggesting that these values are not atypical for the province (Kumaragamage et al. 2007). The magnesium levels were significantly higher in the Interlake as compared to the southwest. There was no significant difference in the calcium and sodium levels between the two regions. The sodium levels at Site F and G were much lower than the other sites in the southwest but within the range found on some agricultural lands and prairies (Malley et al. 2002; Brye et al. 2004; Espinoza et al. n.d.). Furthermore, soil sodium levels at Site E were nearly twice that found for the other sites. Calcium is an important nutrient in plants and is essential for plant cell walls and cell permeability (Brady and Weil 2002). High levels of calcium in soils can reduce drainage and lead to poor soil aeration. Magnesium is an important nutrient for plant growth but high levels can impede drainage, cause soil crusting and reduce root growth (Brady and Weil 2002). High levels of sodium can be toxic to plants and reduce soil permeability. High cation levels are likely related to the parent material present (Brady and Weil 2002). Since calcium, magnesium and sodium are cations, they can influence soil pH thus these factors may also explain in part why the research site soils had high moisture contents, poor drainage, and have not been seeded with agricultural crops.

The air temperature at the soil surface was significantly warmer in 2010 in the Interlake for 18% of the weeks and in southwestern Manitoba for 23% of the weeks. In 2011, the temperature was significantly higher in the Interlake for 37% of the weeks and in the southwest for 10% of the weeks. This indicated that despite fluctuating temperatures on a week-by-week basis there was some consistency in temperature

between the regions, for over 50% of the weeks there was no difference between the The mean temperature in 2011 was significantly higher in southwestern regions. Manitoba, although the weekly temperature was significantly higher four times as often in the Interlake. This shows that there was considerable variability in the temperature when evaluated by weeks versus the entire season. The depth of the duff layer may influence the air temperature at the soil level as HOBO loggers were placed on the soil within the duff layer, which could moderate or insulate temperatures. The duff layer was significantly thicker in the Interlake which may explain some of the higher temperatures observed as the duff layer may insulate against night time cooling. For the "larval period" the mean air temperature at the soil surface was significantly higher in the Interlake in 2011 and when the two years were combined. The mean temperature at the soil surface at sites in the United States during the "larval period" was 19°C (Table 30) which was approximately 0.5°C higher than soil surface temperatures present at sites in the present study (Royer et al. 2008). The lower temperature at sites in Manitoba may be attributed to the higher latitude as research sites in Manitoba are 150 to 675 km further north than sites in the United States (Appendix VI).

The weekly relative humidity at the soil surface over the study period ranged from 65 to 95%, with values remaining near 85% for much of the sampling period. The relative humidity was significantly higher in the southwest for 68% of the weeks in 2010 and 83% of the weeks in 2011. It was higher in the Interlake for only 18% of the weekly sampling period in 2010 and 10% of the time in 2011. The mean relative humidity for the entire season was also significantly higher in the southwest for 2010 and 2011. During the "larval period" the relative humidity was significantly higher at research sites in southwestern Manitoba. Royer et al. (2008) reported that in the United States in 2000, the mean relative humidity at research sites was 81% (Table 30), while in comparison the mean relative humidity was 86% in 2010 and 85% in 2011 at the sites in Manitoba (Royer et al. 2008). Royer et al. (2008) hypothesized that temperature and relative humidity at the soil surface may be very important to Dakota Skipper larval and pupal survival. Temperature and relative humidity at the soil surface may influence the risk of desiccation, suffocation or fungal growth during the sensitive larval or pupal stages (Royer et al. 2008).

Royer et al. (2008) measured the air temperature at the soil surface in their study from July 5 to September 23, 2000 which he called the "larval period" (Julian weeks 28 to 39). While it is recognized that the actual period when Dakota Skipper larvae go from egg to winter diapause (1st to 4th or 5th instars) may vary depending on geographic location, weather or other factors, Julian weeks 28 to 39 are a reasonable estimate of the time frame for the egg to diapause stage in the United States and Canada, based on the information available.

The RDA showed associations between the physical variables (edaphic factors) and vegetation or flower species, explaining considerable amounts of variation (58 to 74% on the first three axes). Percent clay content in soil was significantly greater in the Interlake sites as well as magnesium cations.

## 5.3.2.2 Edaphic and Edaphic-Related Profile of Dakota Skipper Habitat

The null hypothesis that Dakota Skipper study sites share similar edaphic and edaphic-related characters is both supported and contradicted in the results from the present study. As mentioned above some edaphic variables differed significantly between regions. The thickness of the duff layer, soil compaction, soil particle size and texture, organic matter, magnesium, and temperature and relative humidity showed regional differences while percent bare ground, pH, soil moisture, soil calcium, sodium and bulk density were not different between regions.

In Manitoba, Dakota Skipper sites can be broadly characterized by:

- 10 to 15% bare ground;
- duff layer 1.5 to 4 cm deep;
- soil pH of 7.0 to 8.0 (with the 0.01 M CaCl<sub>2</sub> method);
- average gravimetric moisture content of 45 to 50%;
- soil compaction at 10 cm of 570 to 990 kPA;
- soil calcium content of approximately 3000 to 5500 mg/kg;
- soil magnesium content of approximately 1000 to 2500 mg/kg;
- soil sodium content greater than 30 mg/kg;
- bulk density ranging from 0.75 to 1.30 kg/L;
- mean air temperature at the soil surface of 18°C from Julian week 28 to 39; and,

• mean relative humidity at the soil surface of 85% from Julian week 28 to 39.

The factors that are most distinctive between regions are summarized in Table 31.

Factor	Interlake region	Southwest Manitoba region
Duff layer	• ~1.5 to 4.1 cm deep	<ul> <li>~1.6 to 2.5 cm deep</li> </ul>
Soil pH	• ~7.8 to 8.0	• ~7.1 to 7.8
Soil compaction	<ul> <li>~795 to 1495 kPA at 20 cm depth</li> <li>~1010 to 1645 kPA at 30 cm depth</li> <li>~1095 to 1970 kPA at 40 cm depth</li> <li>~895 to 1335 kPA for the mean of 10 to 40 cm depth</li> </ul>	<ul> <li>~740 to 1180 kPA at 20 cm depth</li> <li>~795 to 1190 kPA at 30 cm depth</li> <li>~810 to 1250 kPA at 40 cm depth</li> <li>~780 to 1170 kPA for the mean of 10 to 40 cm depth</li> </ul>
Soil particles & texture	<ul> <li>~30 to 42% sand</li> <li>~23 to 30% clay</li> <li>Clay Loams (or occasionally Loams)</li> </ul>	<ul> <li>~35 to 70% sand</li> <li>~9 to 23% clay</li> <li>Sandy Loams (or occasionally Loams)</li> </ul>
Soil organic matter	<ul> <li>~11 to 14% by weight</li> </ul>	• ~7 to 9% by weight

Table 31. Edaphic factors associated with the Interlake or southwestern Manitoba sites

# 5.3.2.3 Site Characterization in Other Studies

Royer et al. (2008) hypothesized that edaphic features, temperature and local humidity during the larval and pupal stages may be an important limiting factor in Dakota Skipper populations. Royer et al. (2008) described two habitat substrate types that characterized Dakota Skipper sites. Type A habitats had: low relief (<1 m), nearly saturated soils (88 to 95% saturation) at greater depths, higher bulk density (>1.0 kg/L), and soils were relatively sandy and free of gravel to at least 60 cm depth. Type A habitats were associated with glacial lake margins, and portions of the habitat had a high water table and were subject to periodic flooding in the spring. Type A habitats tended to be found at the western portion of the Dakota Skipper's range in the United States where Royer et al. (2008) believed that humidity may be an important regulating factor for larvae in an arid climate.

Type B habitats tended to have: higher relief (>1 m) associated with a more gravelly landscape, more variable soil moisture (60 to 90% saturation), lower bulk density

(<1.0 kg/L) with soils more compacted at all depths due to the higher amount of gravel present, and higher soil temperatures (Royer et al. 2008). Type B habitats were found more in the central and eastern portion of the Dakota Skipper's United States range. Royer et al. (2008) emphasized that the most important distinction between Type A and Type B habitat was the topographic relief with Type A habitat being relatively flat and Type B habitat having more rolling hills.

The sites in present study do not distinctly fall into either the Type A or Type B habitats. For certain factors the sites readily fall into one type, while for other factors they fall into the other type of habitat. Generally the sites in southwestern Manitoba tended to align more with Type B habitat while sites in the Interlake appeared to be an amalgamation of Type A and B habitats. However, when one considers relief alone, sites in both regions of this study distinctly align with Type A habitat. This suggests that in terms of "typing" the habitat, sites in Manitoba may not readily be classified according to Royer et al. (2008). Sites in Manitoba are perhaps best categorized in the manner detailed in Section 5.3.2.2.

# 6.0 DAKOTA SKIPPER LIFE HISTORY

This section addresses objectives 3 to 6:

- Objective 3: Determine the adult flight period of Dakota Skipper in Manitoba;
- Objective 4: Estimate the population size at study sites based on adult counts;
- Objective 5: Contribute additional life history data of Manitoba Dakota Skippers based on incidental observations of adults (i.e. nectaring, predation, egg laying, etc.); and,
- Objective 6: Amplify a partial region of the mitochondrial cytochrome *c* oxidase I gene (COI) from live Dakota Skipper DNA isolated from a single mid-leg sampled in a non-lethal manner.

## 6.1 Methods

# 6.1.1 Flight Period and Density

Adult surveys were conducted throughout the flight period from late June to late July in 2010, 2011 and 2012. Surveys were attempted following a modified version of the Pollard-Yates transect method (Pollard and Yates 1993). However, given the low densities of Dakota Skipper, a wander and seek approach was implemented instead. The per person effort time for each survey was used to help standardize survey results between sites. All adult surveys were carried out in or near suitable Dakota Skipper habitat within the limits of the property parcels in which the study sites were located. Adult movement was monitored in an effort to reduce the double-counting of individuals however this was difficult to do. To further reduce double-counting, patches of habitat within a site were not revisited during a survey. All butterfly species found in the survey were enumerated and individuals were caught by net when necessary to confirm identification. The number and sex of all Dakota Skippers were recorded and voucher specimens were collected for species verification. Permits were obtained from Manitoba Conservation to handle and collect vouchers of Dakota Skipper.

Surveys were conducted within -4.5 and +4.0 hours from solar noon (approximately 0900 hrs to 1730 hrs in June and July). To prevent potential bias in the data as a result of temperature, cloud cover and wind speed, surveys were conducted during the following conditions when possible: temperatures above 17°C with mostly

sunny, clear skies, or temperatures above 25°C irrespective of sun and cloud cover, and an average wind speed no higher than Beaufort 5 (fresh breeze, 17 to 22 km/hr).

The survey results were pooled and tabulated for each site. An index of the population size and the time of peak emergence were determined for each site using the software program INCA (Insect Count Analyzer 2002), when sufficient data was gathered. The adult counts and population estimates were compared to past surveys at the sites to discern trends in the population. All other butterfly species observed during surveys were also recorded and identified to species. Butterflies were identified using Klassen et al. (1989) and Layberry et al. (1998), and nomenclature follows Layberry et al. (1998). Where feasible, particular associations of other butterfly species with Dakota Skipper sites were recorded.

#### 6.1.2 Behaviour

During the course of the adult and flower surveys, observations of adult activity were documented including: nectaring, movement behaviour, predation, interspecific interactions, attempted mating and ovipositing. Life history observations on the egg and larval stages were also made. Behaviour was recorded and photo documented to the extent possible. Video recording was attempted but generally failed to record valuable information as skippers are too active and small to properly record, and the windy conditions at the sites made the skippers hard to follow and cause vegetation to move around too much.

#### 6.1.3 Genetic Analysis

During the 2010 and 2011 Dakota Skipper flight period, a single leg was collected from live individuals for genetic analysis. One mid-thoracic leg was removed from each individual (only from adult males and worn females). If both mid-thoracic legs were absent, no leg was removed. If a fore or hind leg was missing, then the mid leg on that side was not removed, so as to not affect the butterfly's ability to stand, perch, balance and climb. A leg was only removed when an adult possessed five or six legs.

Once an adult was netted, the wings were held closed through the netting. The butterfly was then inverted through the net so that the butterfly was held with legs pointing vertically. One leg was removed with sterilized tweezers at the joint immediately above the femur. Legs samples were typically 7 to 10 mm in length. The leg was placed in a sterile microcentrifuge tube filled with 97% ethanol. Gloves were worn at all times while handling the butterflies and samples. Standard sample collection information was recorded, as well as the sex, wing wear and any other observations about the individual. The precise location of the collection was recorded using a GPS. Legs were also harvested (prior to pinning) from whole voucher specimens that were collected at the sites during the course of the study.

DNA extraction, amplification, purification and sequencing of the mitochondrial cytochrome c oxidase I gene (COI) was attempted to test the success of obtaining sufficient genetic material from a single, live harvested mid leg. The COI gene is highly conservative and codes for a protein important in the electron transport chain (Hillis et al. 1996). This gene was selected because it is used as a standard species identifier ("barcode") employed by the BarCode of Life (Hebert 2009) and is frequently used in molecular research to examine intraspecific variation (Hebert et al. 2004; Joyce and Pullin 2004; Roe and Sperling 2007).

A DNeasy® Tissue Kit (Qiagen, Ileia S.L., Madrid, Spain) was used for DNA extraction following the purification of total DNA from animal tissues (Spin-Column) protocol. Each leg was ground up in a 1.5 ml microcentrifuge tube using a manual Kontes Pellet Pestle Grinder in 40  $\mu$ l of the ATL buffer. The remaining 140  $\mu$ l of ATL buffer was then added to proceed with the protocol. DNA was eluted by adding 100  $\mu$ l of AE buffer to the spin column followed by centrifugation at 8000 rpm for 1 minute. An additional 100  $\mu$ l of AE buffer was then quantified on a nanospectrophotometer with a 50 lid (2mm) using 1  $\mu$ l of sample.

Amplification of partial COI sequences was performed following the methods of Hebert et al. (2004), using the primer pair designed for Lepidoptera LEP-F1 (5'-ATTCA ACCAATCATAAAGATAT-3') and LEP-R1 (5'-TAAACTTCTGGATGTCCAAAAA-3') to generate a 648-bp fragment. The LEP-F1 forward primer was also paired with LEP-R2 (5'-CTTATATTATTTATTCGTGGGAAAGC-3') to generate a 350-bp product as this pairing is often effective when the first pairing does not work, particularly in older specimens (Hebert et al. 2004). The approaches taken by Roe and Sperling (2007) and

Vila et al. (2009) were also considered. Three modifications to the Hebert et al. (2004) methods were made. In the PCR mix 200  $\mu$ M of dNTPs was used (instead of 20  $\mu$ M of dNTPs), and 500 pmol of each primer was used (instead of 5 pmol) so that ultimately 0.5  $\mu$ l of 100 pmol/ $\mu$ l primer could be added to each sample. In most instances 5.0  $\mu$ l of genomic DNA (10 to 50 ng/ml) was used as template. The PCR protocol on the thermocycler consisted of one cycle of 1 minute at 94°C, followed by 6 cycles of 1 minute at 94°C, 1 minute 30 seconds at 45°C and 1 minute 15 seconds at 72°C, then followed by 36 cycles of 1 minute at 94°C, 1 minute at 72°C. PCR products were fractionated on a 1% w/v agarose gel containing 0.05  $\mu$ g/ml ethidium bromide and visualized with UV light.

For purification of PCR products, a low melting point 1% w/v agarose gel was prepared and 25 µl of each PCR product sample underwent agarose gel electrophoresis until the products were halfway down the gel. The DNA was visualized under UV light and each sample of DNA was excised from the gel and placed in a 1.5 ml microcentrifuge tube. Purification was performed per the methodologies provided in the S.N.A.P. ™ Gel Purification Kit (Invitrogen Corporation, Carlsbad, California). Samples were then prepared for sequencing following the Sanger Sequencing Sample Submission Guidelines and sent to The Centre for Applied Genomics (TCAG, Sick Kids Hospital, University of Toronto, Ontario) for sequencing. For each individual sample, multiple runs and concentrations were submitted to generate the best consensus for each sample of an individual. PCR products were sequenced on both strands using the same primers used for the original amplification.

Sequences and chromatograms were reviewed for misreads and potential polymorphisms using the Lasergene software package (DNASTAR Inc. 2006). All the sequences generated for an individual were aligned together to create a single consensus file. The consensus file for each individual sampled were then imported into MEGA (Tamura et al. 2007), aligned and trimmed. Any variations in the sequence were re-examined for misreads and potential polymorphisms.

Three Dakota Skipper sequences (2 males and 1 female) from specimens collected by R. Royer in McHenry County, North Dakota in 2007 were obtained from the BarCode of Life Database (BOLD) (Ratnasingham and Hebert 2007). The samples from North Dakota were aligned with the sequences obtained in this study.

Every effort was made to ensure that legs were only taken from Dakota Skipper and not from other similar skipper species flying at the same time. Long Dash Skipper (Polites mystic W.H. Edwards) and Tawny-edged Skipper (Polites themistocles Latreille) fly at the same time, are abundant at the sites and can be confused for Dakota Skipper at times. COI sequences for 11 Polites mystic and six Polites themistocles samples were also obtained from the BarCode of Life Database (Ratnasingham and Hebert 2007). These sequences were also aligned and compared to the sequences obtained in this study to verify that all the individuals sampled were actually Dakota Skipper. DNA sequences were compared to sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) to determine which sequences were similar to Dakota Skipper. Genetic similarity of the Dakota Skipper sequences was also analyzed by constructing a bootstrapped neighbour-joining tree including COI sequences from P. mystic and P. themistocles. The neighbour-joining tree was constructed using the MEGA phylogenetics software package (Tamura et al. 2007). Confidence levels for major nodes on the tree were calculated based on 500 bootstrap replicates of the sequence alignment. In MEGA, the nucleotide differences were examined further and the variable sites present in the Dakota Skipper sequences from this study were exported in table format.

## 6.2 Results

#### 6.2.1 Flight Period and Density

In 2010 and 2011 sites were surveyed several days immediately prior to adult emergence and until the last adults were observed. It proved difficult to detect adults at the start and end of the flight period because adult density was low and active adults are difficult to observe. In 2010 Dakota Skipper adults were observed flying from 11 to 21 July at Sites F and H (Appendix IX). All the observations of adults in 2010 were at sites in southwestern Manitoba, with the first adult observed at Site F (1 $\bigcirc$ ) and the last adult observed at Site H (1 $\bigcirc$ ). Five males and six females were observed in good body condition at Site H on 12 July indicating that the 2010 emergence of adults likely started several days prior (approximately two to four days prior). The last adult observed was a very worn female on 21 July. Thus the 2010 flight period was approximately 13 to 15 days in duration.

Adults were observed from 7 to 21 July, 2011 in both the Interlake and southwestern Manitoba (Sites B, D, F, G and H). On 7 July one freshly emerged adult male was observed at Site D suggesting the flight period in the Interlake commenced one or two days prior (Appendix IX). Also on 7 July eight worn males and six females in good condition were observed at Site H suggesting that the flight period in the southwest had commenced approximately two to four days prior. The last adult, a very worn female, was observed on 21 July at Site H. The 2011 flight period was therefore approximately 17 to 19 days in duration.

In 2012, efforts were directed more towards detecting the start of the flight period and gathering behavioural data. Sites were not visited as frequently as in previous years. Adults were observed in 2012 from 26 June to 6 July in both the Interlake and southwest Manitoba sites (Sites B, D, F and H) (Appendix IX). On 26 June a very fresh male was observed at Site H suggesting that the flight period in the southwest had started one or two days prior. On 4 July a male in good condition was observed at Site B, while at Site D five males and one female in good condition were observed, which suggests that the flight period in the Interlake had commenced one to three days prior. The last adults were observed at Site H on 6 July and consisted of approximately 50 female and male skippers (predominantly females) with moderate to severe wing wear. Site H was revisited on 9 July and no adult Dakota Skippers were observed, indicating that the flight period was probably over at this point which suggests that the flight period was approximately 13 to 16 days in duration.

A summary of the adult counts for each year is provided in Table 32. Dakota Skipper was detected at five (Sites B, D, F, G and H) of the eight sites where they were previously reported. At Sites B, D, F and G generally one to six adults were detected at the site each year, whereas a range of 20 to 170 adults were detected at Site H over the three survey years (Table 32). Each site was sampled at least twice in 2010 and 2011 during ideal, favourable conditions to confirm absence/presence and in most instances each site was sampled multiple times per season. At Site H, density surveys were implemented during the peak of the flight period. During the surveys approximately 50%

Site	Field	Total number of adults observed							Adults density (mean no./hr)		
ID code		Webster 2002	Morden/ Westwood 2005	Webster 2007	Rigney 2010	Rigney 2011	Rigney 2012	2012 2011 2012 2	adults 2002-2012		
А	IL19	24	NA	NA	0	0	0	0	0	6.00	
В	IL24	12	3	NA	0	<b>2</b> ්	<b>1</b> ♂	~ 2/hr	~1/hr	3.60	
С	IL39	43	NA	NA	0	0	0	0	0	10.75	
D	IL50	NA	21	NA	0	1♂ & 1♀	5♂ & 1♀	~ 1/hr	~6/hr	7.25	
	Interlake							~ 1.5/hr	~1.75	6.90	
Е	OK1	NA	NA	13	0	0	0	0	0	3.25	
F	OK6	NA	NA	11	<b>1</b> ♀	<b>1</b> ♀	<b>1</b> ♀	~ 1/hr	~1/hr	3.50	
G	OK7	NA	NA	11	0	1♀ & 1 sex?	0	~ 2/hr	0	3.25	
Н	OK14	21	NA	273	7♂ <b>&amp; 12</b> ♀	>70♂ & >100♀	17 <i></i> ∂ & 15♀	~ 30/hr	~33/hr	103.00	
	SW Manitoba							~ 8.25/hr	~8.5/hr	28.25	

Table 32. Summary of past and recent adult counts and estimated densities at study sites

of the parcel was surveyed (and about 80% of the habitat) with each patch of Dakota Skipper habitat visited once. Since the remaining sites (B, D, F and G) had few Dakota Skipper adults, density estimates were based on the highest count of individuals observed per hour during a survey.

The INCA software generally requires a minimum of five counts which are preferably evenly spaced in time and include surveys prior to the start and after the end of the flight period (zero counts). Counts used in INCA should only include observations done during favorable weather conditions. Thus INCA could only be applied to the counts from Site H in 2011, as there were insufficient observations at the other sites and at Site H in 2010 and 2012.

In 2011 at Site H, INCA estimated the index of the population size (N) to be 277.128 individuals based on seven sampling dates. The standard deviation (s.d. = 167.999) and coefficient of variation (c.v. = 0.606) values are relatively high, indicating that the population size estimate is quite variable. The count from 13 July 2011 of 5 individuals was removed from the model, as the count was made during unfavourable weather conditions. The 2011 surveys at Site H encompassed approximately 80% of the suitable habitat available, thus the population estimate at the site in 2011 could range up to 332 individuals. The time of peak emergence (mu) was 4.762 days (s.d. = 1.060, c.v. = 0.223). There is no goodness-of-fit test for the INCA model, although the index of population size had a large coefficient of variance value (>0.250). Given the high coefficient of variance values and the low number of counts, the results of INCA should be viewed with caution and regarded as coarse estimates.

In 2010, three Dakota Skipper male and four female voucher specimens were collected (Appendix IX). One of the female vouchers was from Site F, while the rest were from Site H. One male and four females from Site H were also live sampled for tissue (single mid-leg) for genetic analysis. The male sampled for genetic analysis had significant wear at the time of sampling and was later kept as a voucher specimen, hence it was included in the genetic and whole voucher sample counts. In 2011, two male and three female vouchers were collected (Appendix IX). All the vouchers were again from Site H, with the exception of one female voucher which was collected at Site G. Tissue was sampled for molecular analysis from four male and four female in 2011 from Site H.

In 2012, no adult voucher or genetic samples were taken, with the exception of one male, which had been captured by a crab spider and was already dead (Appendix IX). Voucher specimens will be deposited at the Wallis Roughley Museum of Entomology, University of Manitoba and at the University of Winnipeg, Manitoba).

Thirty-nine other species of butterflies were observed within the study sites (Table Site D had the highest species diversity with 30 species of butterfly observed, 33). followed by Site B (27 species), Site H (20 species) and Site G (19 species). Site E had the lowest diversity with nine species of butterflies, followed by Site F (13 species) and Site A (16 species) (Table 33). Polites mystic, Great Spangled Fritillary (Speyeria cybele Fabricius), Common Wood-nymph (Cercyonis pegala Fabricius) and Monarch (Danaus *plexippus* Linnaeus) were the most commonly encountered species and were found at all eight sites. The most abundant species was *Cercyonis pegala*, followed by European Skipper (Thymelicus lineola Ochsenheimer). The least abundant or least frequently encountered species of butterflies included: Delaware Skipper (Anatrytone logan W.H. Edwards), Pink-edged Sulphur (Colias interior Scudder), Spring Azure (Celastrina ladon Cramer), Summer Azure (Celastrina neglecta W.H. Edwards), Silvery Blue (Glaucopsyche lygdamus Doubleday), Melissa Blue (Lycaeides melissa W.H. Edwards), Callippe Fritillary (Speveria callippe Boisduval), Gorgone Checkerspot (Chlosyne gorgone Hübner), Harris's Checkerspot (Chlosyne harrisii Scudder), Grey Comma (Polygonia progne Cramer), Mourning Cloak (Nymphalis antiopa Linnaeus), American Lady (Vanessa virginiensis Drury) and Red Admiral (Vanessa atalanta Linnaeus) (Table 33).

Family	Scientific name	Common name	Site A	Site B	Site C	Site D	Site E	Site F	Site G	Site H	No. sites present	No. adults seen
Hesperiidae	Epargyreus clarus	Silver-spotted Skipper	0	1	1	1	0	0	0	0	3	4
Hesperiidae	Oarisma garita	Garita Skipperling	0	1	0	1	0	0	0	0	2	4
Hesperiidae	Thymelicus lineola	European Skipper	2	3	2	3	1	1	0	2	7	1068
Hesperiidae	Hesperia dacotae	Dakota Skipper	0	2	0	2	0	3	1	3	5	268
Hesperiidae	Polites peckius	Peck's Skipper	1	2	1	2	0	0	0	2	5	43
Hesperiidae	Polites themistocles	Tawny-edged Skipper	0	1	0	2	0	2	2	2	5	59
Hesperiidae	Polites mystic	Long Dash Skipper	1	3	1	3	2	1	3	2	8	359
Hesperiidae	Anatrytone logan	Delaware Skipper	0	0	0	0	0	0	1	1	2	2
Hesperiidae	Euphyes vestris	Dun Skipper	0	1	1	2	0	0	0	0	3	16
Papilionidae	Papilio polyxenes	Black Swallowtail	0	0	0	1	0	0	1	1	3	7
Pieridae	Pontia occidentalis	Western White	0	0	0	0	0	2	1	0	2	6
Pieridae	Pieris rapae	Cabbage White	1	0	0	1	1	1	2	2	6	83
Pieridae	Colias philodice	Clouded Sulphur	0	1	1	1	1	2	1	1	6	98
Pieridae	Colias eurytheme	Orange Sulphur	1	1	1	0	1	2	1	1	7	189
Pieridae	Colias interior	Pink-edged Sulphur	0	0	0	1	0	0	0	0	1	1
Lycaenidae	Lycaena dorcas	Dorcas Copper	0	2	0	1	0	0	0	0	2	7
Lycaenidae	Lycaena helloides	Purplish Copper	1	1	0	1	0	0	0	0	3	4
Lycaenidae	Satyrium acadicum	Acadian Hairstreak	0	1	1	1	0	0	0	0	3	5
Lycaenidae	Celastrina ladon	Spring Azure	0	0	0	1	0	0	0	0	1	1
Lycaenidae	Celastrina neglecta	Summer Azure	0	1	0	1	0	0	0	0	2	2
Lycaenidae	Glaucopsyche lygdamus	Silvery Blue	0	0	0	0	0	0	1	0	1	2
Lycaenidae	Lycaeides melissa	Melissa Blue	0	0	0	0	0	1	0	0	1	1
Nymphalidae	Speyeria cybele	Great Spangled Fritillary	1	2	2	1	1	1	1	1	8	154
Nymphalidae	Speyeria aphrodite	Aphrodite Fritillary	1	1	1	1	0	1	1	2	6	128
Nymphalidae	Speyeria callippe	Callippe Fritillary	0	0	0	0	0	0	0	1	1	10
Nymphalidae	Boloria selene	Silver-bordered Fritillary	0	1	0	2	0	0	1	0	3	12

Table 33. Summary of all butterfly species observed at the research sites from 2010 to 2012

Family	Scientific name	Common name	Site A	Site B	Site C	Site D	Site E	Site F	Site G	Site H	No. sites present	No. adults seen
Nymphalidae	Boloria bellona	Meadow Fritillary	1	1	2	2	0	0	1	1	6	48
Nymphalidae	Chlosyne gorgone	Gorgone Checkerspot	0	1	0	0	0	0	0	0	1	1
Nymphalidae	Chlosyne harrisii	Harris's Checkerspot	0	0	0	1	0	0	0	0	1	3
Nymphalidae	Phyciodes cocyta	Northern Crescent	1	2	2	1	0	0	1	1	6	223
Nymphalidae	Polygonia progne	Grey Comma	0	0	0	1	0	0	0	0	1	1
Nymphalidae	Nymphalis antiopa	Mourning Cloak	0	1	0	0	0	0	0	0	1	1
Nymphalidae	Vanessa virginiensis	American Lady	1	0	0	0	0	0	0	0	1	1
Nymphalidae	Vanessa atalanta	Red Admiral	0	0	0	0	1	0	0	0	1	1
Nymphalidae	Limenitis arthemis	White Admiral	1	1	0	1	0	0	0	1	4	4
Nymphalidae	Limenitis archippus	Viceroy	0	2	1	1	0	0	1	1	5	7
Nymphalidae	Satyrodes eurydice	Eyed Brown	1	2	2	2	0	0	1	1	6	14
Nymphalidae	Coenonympha tullia	Common Ringlet	1	2	2	1	0	0	0	0	4	221
Nymphalidae	Cercyonis pegala	Common Wood- nymph	1	1	1	2	1	2	1	2	8	3238
Nymphalidae	Danaus plexippus	Monarch	1	1	1	1	1	2	1	2	8	80
	Total numb	er of species	16	27	17	30	9	13	19	20		

Table 33. Summary of all butterfly species observed at the research sites from 2010 to 2012

A pattern in the timing of the emergence of other skipper species present at the sites was observed (Appendix IX). Based on three years of qualitative observations skipper emergence order for those species occurring from early June onward was approximately: *Thymelicus lineola, Polites mystic, Polites themistocles*, Peck's Skipper (*Polites peckius* W. Kirby), Dakota Skipper, Silver-spotted Skipper (*Epargyresu clarus* Cramer) and Dun Skipper (*Euphyes vestries* Boisduval). Not all these species were necessarily found at all the sites but most were present at the sites with Dakota Skipper. *Thymelicus lineola* and *Polites mystic* emerged one to two weeks prior to Dakota Skipper, while *Polites themistocles* appeared about two to seven days prior to Dakota Skipper. *Polites peckius* emerged several days prior to or at same time as Dakota Skipper. *Epargyresu clarus* emerged at or immediately after the start of the Dakota Skipper flight period, while the flight period of the *Euphyes vestries* started just as the Dakota Skipper flight period ended.

*Cercyonis pegala* emerged approximately one to two weeks prior to the Dakota Skipper flight period, with the *Cercyonis pegala* being quite abundant and conspicuous at the start of the Dakota Skipper flight period (Appendix IX). In regards to plant phenology, *Stipa spartea* seeds become mature one to three days after the start of the Dakota Skipper flight period. When mature and ready for dispersal, the *Stipa spartea* seeds ripen, become loose and tend to stick to clothes and insect nets as they are barbed. The seed dispersal stage is quite noticeable and could also serve as an indicator of the start of the flight period.

## 6.2.2 Behaviour

Between 2010 and 2012 Dakota Skipper were observed nectaring upon twelve species of flower, with the majority of feeding observations occurring in southwestern Manitoba on *Rudbeckia hirta* (Table 15 & 34, Appendix IX). When adults nectared or perched upon *Melilotus alba*, they consistently selected plants that were under 50 cm tall, and were never observed on larger (>70 cm tall) and more bushy plants (Appendix IX, Appendix X).

Family	Scientific name	No. a	New record?	
i anny		Interlake	SW Manitoba	
Liliaceae	Zygadenus elegans	0	<b>1</b> ♀	
Leguminosae	Melilotus alba	0	1♂, 1♀ & 5sex?	Yes
Leguminosae	Petalostemon candidum	0	1♂& 6♀	
Leguminosae	Petalostemon purpureus	0	<b>1</b> ♀	Yes
Onagraceae	Oenothera biennis	0	<b>1</b> ♀	Yes
Lobeliaceae	Lobelia spicata	0	1♂, 3♀ & 1sex?	Yes
Compositae	Agoseris glauca	0	1♀	
Compositae	Cirsium flodmanii	0	1♂ & 2♀	
Compositae	Crepis runcinata	0	1♂ & 1♀	Yes
Compositae	Gaillardia aristata	0	<b>2</b> ð <b>&amp; 4</b> ♀	
Compositae	Rudbeckia hirta	<b>2</b> ්	4♂, 3♀ & ~110sex?	
Compositae	Solidago ptarmicoides	0	1∂ & 1♀	Yes

Table 34. Summary of flower species Dakota Skipper were observed nectaring upon, 2010 to 2012

Dakota Skipper was not observed nectaring upon *Lilium philadelphicum*, *Zygadenus gramineus*, *Medicago sativa*, *Melilotus officinalis*, *Asclepias speciosa*, *Campanula rotundifolia*, *Achillea millefolium* and *Erigeron annuus* despite being common at many of the sites and reported previously as nectar plants (McCabe 1981; Dana 1991; Swengel and Swengel 1999a; Webster 2003; Webster 2007). In 2012 at Site F, cattle were pastured on site early in the season and there were virtually no flowers of any species present.

In regards to movement behaviour, on several occasions adult Dakota Skippers were observed flying at least 8 m vertically when disturbed by observers or when pursuing or being pursued by other Hesperiids. Also, Dakota Skippers were observed on several occasions flying a distance of approximately 150 m in a single flight, again when disturbed. Adults were most active and visible during the first quarter to the halfway point of the flight period, during midday on hot (~30°C), very humid days with low wind speeds and particularly when there were threats of rain and summer storms. Just before downpours adults were observed flying in pursuit of one another and nectaring in significant numbers.

In the Interlake where the prairie occurs in long corridors amongst the aspen forest, skippers were observed frequenting the edges of the prairie but did not enter into the adjacent aspen forest. This suggests that the adults move between patches using open, connecting corridors and not flying through the aspen stands, even when the flight distance between patches would be shorter by flying through the aspen. In the Interlake and southwest regions adult females were often seen flying from upland prairie patches of flowers and grasses to adjacent, slightly lower areas where mats of sedges were present. In these lower sedge areas the females would sometimes fly slightly above plants, while at other times they would crawl down into the sedges. Numerous attempts were made to determine if females were ovipositing in the sedge areas but no clear observations were made (Appendix IX).

Evidence of predation was observed on two occasions. A dead male Dakota Skipper was found in the grasp of a female Northern Crab Spider (*Misumenops asperatus* Hentz, Araneae, Thomisidae) perched on a blanket flower (Appendix IX). The dead skipper and spider were photographed and taken as vouchers to authenticate the record (Appendix X). The species and gender of the spider was determined by Mr. David Wade, Insect Control Branch, City of Winnipeg. On another occasion, while observing a female Dakota Skipper in grasses perhaps preparing to oviposite, a robberfly (Asilidae) attempted to grasp the skipper repeatedly but the fly was unsuccessful in capturing its prey and the female Dakota Skipper escaped (Appendix IX). This particular female skipper was observed subsequently but was never observed depositing eggs. After this observation of Asilidae predation, efforts were made to find more robberflies in the vicinity of the observation, and a similar appearing robber fly was found in that location the next morning. The fly was identified as a Machimus paropus Walker, Diptera, Asilidae (Appendix X). It is likely that this species was the same one observed attacking the female Dakota Skipper on the previous day as this species is known to feed on Lepidoptera.

There were numerous instances of interspecific interactions between adult Dakota Skippers and other butterfly species. Most often these interactions were with other Hesperidae species. During the early flight period male Dakota Skippers were observed pursuing other Hesperidae flying in proximity to the area being used by Dakota Skipper males (Appendix IX). Sometimes, males chased other species of skipper away from the immediate area, while at other times they would attempt to copulate with other skipper species including *Polites mystic*, *Polites themistocles, Polites peckius* and *Thymelicus lineola*. On several occasions male Dakota Skippers were observed aggressively chasing

and attempting to copulate with female *Polites themistocles*, including walking down a blades of grass toward females while vibrating their wings and attempting to make contact (Appendix IX). In each instance, female *Polites themistocles* appeared to reject male Dakota Skipper activity.

Frequently during the early and middle portion of the flight period interactions between male and female Dakota Skippers were observed (Appendix IX). Often females were approached by males who flew to them directly from perches or were already flying in the immediate vicinity of females. These males would fly around females as if to ascertain whether she was receptive or had already mated. When the female flew away the males would often abandon pursuit of the female (Appendix IX). During these episodes the female's flight trajectory was generally linear, while the males flew directly to the female and then around her.

In other instances, males would approach or pursue females, with females subsequently landing on blades of grass and males landing nearby. Males were observed walking down blades of grass to within 1 to 10 cm of females. Sometimes females would take flight before males made contact, and with males in pursuit the entire process would be repeated. In some instances this behavior ended when females and males flew off in different directions (Appendix IX). On certain occasions males would vibrate their wings in close proximity to females and if females did not fly away, move closer to the females who in turn also vibrated their wings (Appendix X). Males were also observed arching the end of their abdomen to contact the female genitalia and attempting to mate. Attempts at copulation were observed on two occasions and were very brief (approximately 2 seconds in duration) (Appendix IX). Following copulation attempts the male immediately flew away from the female. During these courting and copulation attempts, Dakota Skippers were not observed to feed upon flowers even when perched upon nectar plants.

During the mid to later portion of the flight period intensive observations of females were made in order to observe ovipositing. On numerous occasions females were seen walking down into grasses and moving their abdomen as if to probe the plants or in preparation to deposit an egg, but no eggs were deposited (Appendix IX, Appendix X). Frequently, females were observed flying into the slightly lower, lusher areas of

sedges immediately adjacent to the slightly higher, prairie areas (Appendix IX). Sedge patches were generally composed of *Eleocharis palustris*.

In two instances females were observed ovipositing eggs. In the first instance, a female deposited two eggs onto a blade of *Poa cusickii* (Appendix X). Within approximately a 30 cm radius of the bluegrass there were clumps of *Stipa spartea*, clumps of other *Poa* species, several clumps of *Agropyron* species and a few clumps of *Andropogon scoparius*. In the second instance another female laid one egg on an unknown species of *Poa*, however identification of the adjacent graminoid species was not possible on this occasion (Appendix IX).

The blade of grass with the two eggs was collected for further documentation, photography and potential rearing of larva (Appendix X). The eggs were slightly greater than 1 mm in diameter and hemispherical (Appendix IX). The eggs were laid immediately adjacent to one another in the depression of the upper surface of the blade of grass. Eggs were solid creamy white with a slightly darker area on the dorsal surface. Larvae emerged from the eggs nine or ten days after deposition and were photo documented. The first instar larvae were 3.5 to 4.0 mm in length (Appendix IX, Appendix X). The head capsule resembled a typically oversized Hesperiid larval head being amber to rust brown in colour with some pitting and hairs. There was also an amber to rust brown coloured prothoracic shield (Appendix X). The body was yellow-creamy white with some darker internal areas (gut) visible and scattered hairs. Larvae constructed a shelter within a couple of days after emergence from blades of grass and silk and occupied these shelters during the day time. An attempt to rear larvae was made but failed within two weeks of their emergence.

Efforts were also made to find larvae in the field in August and September 2010 and 2011, during soil and vegetation surveys. Clumps of *Andropogon* species were intensively checked for larvae or their shelters but no Dakota Skipper larvae were found.

### 6.2.3 Genetic Analysis

In 2010 a leg was taken from five live Dakota Skipper individuals, while in 2011 a leg was taken from seven live individuals (these are referred to as genetic samples) (Table 35). An effort was made to collect genetic samples from each sex and at different

stages of wear (age). Given the restrictions on collecting samples of Dakota Skipper, the permitted number of whole voucher specimens (voucher samples) was collected at each site initially, and once this quota was filled, leg samples were collected. At least one leg and any other body parts that broke off prior to pinning individuals in the laboratory were also collected from the 13 voucher specimens collected (Table 35). The majority of adults were observed at Site H and as a result most of the samples were obtained from that site. Leg samples were obtained from five males and seven females at Site H, plus five male and six female whole voucher samples were collected (Table 35). One unintended specimen also became a voucher sample (OK 14 Genetic Sample #2 & Voucher #6) as the male was very worn and weak, such that it would have probably died within a day, so following leg removal it was decided to keep the whole individual. One female voucher was also taken from Site F and from Site G (Table 35).

Nine of the samples collected were used for molecular analysis, sampling from as many sites as possible, both sexes, years and types of sample sources (live legs and dead vouchers) (Table 35). The remaining 16 samples have not been processed as they were not required to demonstrate the success of the methodology, and are therefore available for future molecular work. One leg from each of the following samples was processed: OK6V1, OK7V1, OK14V6, OK14V11, OK14V12, OK14G1, OK14G4, OK14G8 and OK14G11, where "V" denotes Voucher samples and "G" denotes leg samples (Figure 21). Following testing and revisions to the methodology, COI partial sequences were successfully amplified from all nine samples. The results for each PCR were visualized in an agarose gel and are shown in Figure 21.

Collection date	Site	Sample #	Sample type*	Sex	Body condition	Sequenced
July 11, 2010	Site F	OK6 Voucher #1	Voucher	F	good condition	Yes
July 12, 2010	Site H	OK14 Voucher #1	Voucher	F	good condition	No
July 12, 2010	Site H	OK14 Voucher #2	Voucher	Μ	good condition	No
July 12, 2010	Site H	OK14 Genetic Sample #1	Genetic	F	good condition	Yes
July 12, 2010	Site H	OK14 Voucher #3	Voucher	Μ	good condition	No
July 18, 2010	Site H	OK14 Voucher #4	Voucher	F	slightly worn	No
July 20, 2010	Site H	OK14 Voucher #5	Voucher	F	worn	No
July 20, 2010	Site H	OK14 Genetic Sample #2 & Voucher #6	Genetic & Voucher Sample	Μ	very worn	Yes
July 20, 2010	Site H	OK14 Genetic Sample #3	Genetic	F	not recorded	No
July 20, 2010	Site H	OK14 Genetic Sample #4	Genetic	F	very worn	Yes
July 21, 2010	Site H	OK14 Genetic Sample #7	Genetic	F	very worn	No
July 7, 2011	Site H	OK14 Voucher #10	Voucher	F	good condition	No
July 7, 2011	Site H	OK14 Voucher #7	Voucher	Μ	worn	No
July 7, 2011	Site H	OK14 Voucher #8	Voucher	F	good condition	No
July 10, 2011	Site G	OK7 Voucher #1	Voucher	F	good condition	Yes
July 13, 2011	Site H	OK14 Genetic Sample #8	Genetic	М	not recorded	Yes
July 13, 2011	Site H	OK14 Genetic Sample #9	Genetic	М	not recorded	No
July 13, 2011	Site H	OK14 Genetic Sample #10	Genetic	М	not recorded	No
July 13, 2011	Site H	OK14 Genetic Sample #11	Genetic	М	not recorded	Yes
July 16, 2011	Site H	OK14 Genetic Sample #12	Genetic	F	moderately worn	Yes
July 16, 2011	Site H	OK14 Genetic Sample #13	Genetic	F	moderately worn	No
July 16, 2011	Site H	OK14 Voucher #11	Voucher	М	moderately worn	Yes
July 17, 2011	Site H	OK14 Genetic Sample #14	Genetic	F	moderately worn	No
July 18, 2011	Site H	OK14 Voucher #12	Voucher	F	very worn	Yes

Table 35. List of Dakota Skipper voucher and genetic samples collected

\*Genetic samples are a single mid-leg live sampled in the field. Voucher samples are a leg and other body parts obtained from whole voucher specimens.

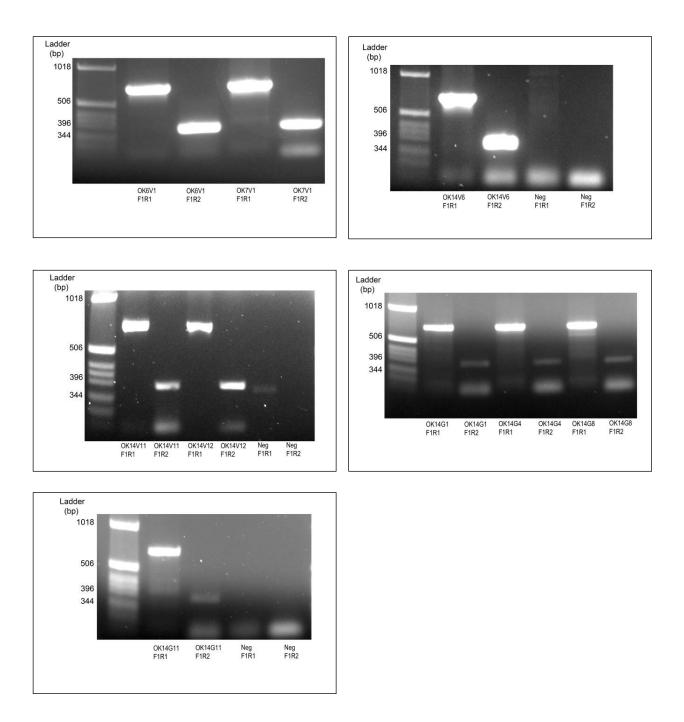


Figure 21. Photo of gels used to visualize the PCR product for each sample processed. Photo confirms that the PCR products using the F1/R1 primer combination were approximately 648 bp in length, while those using the F1/R2 primer combination are approximately 350 bp in length

At least four sequences were obtained for each sample, one for each primer in each primer pairing (i.e. F1 and R1 in FI/R1 pairing and F1 and R2 in FI/R2 paring). In some cases, diluted and concentrated samples were also submitted to adhere to sequencing protocol requirements. The samples processed with the F1/R1 primer pair were approximately 648 bp, while the F1/R2 primer pair products were approximately 350 bp. All the PCR product sequences generated for each individual specimen were aligned together to produce a sequence contig and a consensus sequence file of approximately 648 bp. When aligned, all nine of the Dakota Skipper sequences from this study and samples from Dakota Skipper collected in North Dakota showed high sequence identity to each other (Figure 22). The nine Dakota Skipper sequences shared less sequence identity with the Polites mystic and Polites themistocles sequences and included variable sites, confirming that neither of these species was accidentally sampled. The BLAST search of GenBank (Altschul et al. 1990) of the COI sequence for Dakota Skipper retrieved 17 taxa with highly similar ( $\geq 93\%$  maximum identity) sequences to Dakota Skipper (Table 36). All the taxa retrieved from the BLAST search had an expectation value of 0.0 indicating that there was a highly significant correlation in the alignments between Dakota Skipper samples from the present study and the ones retrieved in the BLAST. All taxa with significant alignments were from the same subfamily as Dakota Skipper, where taxanomic information was available. The Dakota Skipper sequence from sample OK14G1 was deposited into GenBank (Accession: JX679242.1).

Family & Subfamily of Lepidoptera	Таха	No. records	E value*	Max. identity**
Hesperiidae, Hesperiinae	Hesperia comma	7	0.0	96%
Hesperiidae, Hesperiinae	Wallengrenia egerement	2	0.0	94% & 95%
Hesperiidae, (No Subfamily given)	"Hesperidae gen."	2	0.0	93% & 94%
Hesperiidae, Hesperiinae	Polites themistocles	1	0.0	94%
Hesperiidae, Hesperiinae	Atalopedes campestris	2	0.0	94%
(No Family given)	"Lepidoptera spp."	2	0.0	93% & 94%
Hesperiidae, Hesperiinae	Polites peckius	2	0.0	93% & 94%
Hesperiidae, Hesperiinae	Hylephila phyleus	1	0.0	94%
Hesperiidae, Hesperiinae	Conga chydaea	25	0.0	94%
Hesperiidae, Hesperiinae	Poanes zabulon	5	0.0	94%
Hesperiidae, Hesperiinae	Ochlodes venata	1	0.0	93%
Hesperiidae, Hesperiinae	Poanes hobomok	1	0.0	93%
Hesperiidae, Hesperiinae	Ochlodes sylvanus	1	0.0	93%
Hesperiidae, Hesperiinae	"Nyctelius spp. nyctelius"	20	0.0	93%
Hesperiidae, Hesperiinae	<i>Ćynea</i> spp.	23	0.0	93%
Hesperiidae, Hesperiinae	Vacerra spp.	4	0.0	93%
Hesperiidae, Hesperiinae	Neoxeniades spp.	1	0.0	93%

Table 36. List of COI sequences from the BLAST in GenBank producing significant alignments

\*E value - The Expectation value represents the number of different alignments with scores equivalent to or better than S that is expected to occur in a database search by chance. The lower the E value, the more significant the score and the alignment (as defined by GenBank).

\*\*Max. identity – Identity is the extent to which two (nucleotide or amino acid) sequences have the same residues at the same positions in an alignment, often expressed as a percentage (as defined by GenBank).

	<del>.</del>	6	8 8	64 64
H. dacotae - OK6V1	AACTTTATA	ATTTTATTTT	GGTATTTGAGC	тддтататтаддаастто
H. dacotae - OK7V1	AACTTTATA		GGTATTTGAGC	т д д т ат ат т а д д а а с т т с
H. dacotae - OK14G1				ΤGGTATATTAGGAACTTO
H. dacotae - OK14G4	AACTTTATA			ΤGGTATATTAGGAACTTO
H. dacotae - OK14G8	AACTTTATA			Τ G G T A T A T T A G G A A C T T C
H. dacotae - OK14G11	AACTTTATA			Τ G G T A T A T T A G G A A C T T G
H. dacotae - OK14V6E1	AACTIATA			
H. dacotae - OK14V11				T G G T A T A T T A G G A A C T T C
H. dacotae - OK14V12	AACTTTATA			ΤGGTATATTAGGAACTTO
	AACTTTATA			TGGTATATTAGGAACTTO
H. dacotae - LEPRR009-08				TGGTATATTAGGAACTTO
P. themistocles - 08BBLEP-00005	AACTTTATA			AGGAATATTAGGAACTTO
P. mystic - 08BBLEP-01313	AACTTTATA	ATTTTATTTT	GGTATTTGAGC	AGGAATATTAGGAACTTO
	_	_	_	
H. dacotae - OK6V1	8 T T T A A G T T T	<b>8</b>		8 8 8 TAATCCTGGATCTTTAAT
H. dacotae - OK7V1				ТААТССТО БАТСТТТААТ
H. dacotae - OK14G1				
H. dacotae - OK14G1				ТААТССТОВАТСТТТААТ
				ТААТССТОВАТСТТТААТ
H. dacotae - OK14G8				ТААТССТОВАТСТТТААТ
H. dacotae - OK14G11				TAATCCTGGATCTTTAAT
H. dacotae - OK14V6E1				ТААТ ССТ G G АТ С Т Т Т А АТ
H. dacotae - OK14V11	TTTAAGTTI	АТТААТТССА	ACAGAATTAGG	таат сстобатстттаат
H. dacotae - OK14V12	ТТТААСТТІ	АТТААТТССА	ACAGAATTAGG	ТААТ ССТ G G A T C T T T A A T
H. dacotae - LEPRR009-08	TTTAAGTTI	АТТААТТССА	ACAGAATTAGG	ТААТ ССТ G G A T C T T T A A T
P. themistocles - 08BBLEP-00005	ATTAAGTTI	АТТААТТССА	ACAGAATTAGG	ТААТ ССТ G G A T C T T T A A T
P. mystic - 08BBLEP-01313	TTTAAGTTI	АТТААТТССА	ACAGAATTAGG	ТАА <mark>С</mark> ССТООАТСТТТААТ
H depetere OVENI	<u>8</u>	9	8	<b>4</b> 9 <b>3</b> 3
H. dacotae - OK6V1 H. dacotae - OK6V1	TGGAGATGA		AACACTATTGT	TACAGCTCATGCTTTCAT
H. dacotae - OK7V1	T G G A G A T G A T G G A G A T G A	Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т	A A C A C T A T T G T T A A C A C T A T T G T T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1	T G G A G A T G A T G G A G A T G A T G G A G A T G A	<del>,</del> А Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4	T G G A G A T G A T G G A G A T G A T G G A G A T G A T G G A G A T G A	<u>+</u> А Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т А Т С А А А Т Т Т А Т	A A C A C T A T T G T <sup>-</sup> A A C A C T A T T G T <sup>-</sup> A A C A C T A T T G T <sup>-</sup> A A C A C T A T T G T <sup>-</sup>	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8	T G G A G A T G A T G G A G A T G A	<u>+</u> A T C A A A T T T A T A T C A A A T T T A T A T C A A A T T T A T A T C A A A T T T A T A T C A A A T T T A T	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11	T G G A G A T G A T G G A G A T G A	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1	T G G A G A T G A T G G A G A T G A	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11	T G G A G A T G A T G G A G A T G A		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V12	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T         T       G       G       A       A       T		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - UEPRR009-08 P. themistocles - 089BLEP-00005	T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T       G       A       T	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G       A         T       G       G       A       A       T       G       A       A       T       G	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - UEPRR009-08 P. themistocles - 089BLEP-00005	T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G       A         T       G       G       A       A       T       G       A       A       T       G	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T	T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T         T A C A G C T C A T G C T T T C A T
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313	T G G A G A T G A T G G A G A T G A	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C T A T T G T A A T A C T A T T G T	TACAGCTCATGCTTTCAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTAT         TACAGCTCATGCTTTAT
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       G       A       T       G         T       G       G       G       A       T       G       A         T       G       G       G       A       T       G         T	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T	A       C       A       C       A       C       A       T       T       A       A       T       C       A       T       T       A       T       A       A       T       A       A       T       A       A       T       T       A       T       A       A       T       A       A       A       A       A       A       A       A       A       A       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313 H. dacotae - OK6V1 H. dacotae - OK7V1	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       G       A       T       G         T       G       G       G       A       T       G       A         T       G       G       G       A       T       G         T	АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ АТСАААТТТАТ	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T	TACAGCTCATGCTTTCAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTAT         TACAGCTCATGCTTTAT
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       G       A       T       G         T       G       G       G       A       T       G       A         T       G       G       G       A       T       G         T	A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A         A       T       C       A       A       T       T       A       T       A         A       T       C       A       A       T       T       A       T       T       A       T       T       A       T       T       T       T       A       T       T       T       A       T       T       T       T       T       T       T       T       T       T       T       T	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       C       A       T       T       A       A       T       C       A       T       T       A       T       A       A       T       A       A       T       A       A       T       T       A       T       A       A       T       A       A       A       A       A       A       A       A       A       A       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313 H. dacotae - OK6V1 H. dacotae - OK7V1	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G       A         T       G       G       A       A       T       G       A       T       G         T       G       G       A       G       A       T       A       A       T       T       A         T       A       T       A       T <td>Image: constraint of the second se</td> <td>A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T</td> <td>TACAGCTCATGCTTTCAT         TACAGCTCATGCTTTCAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCATGCTTTAT         AACAGCTCATGCATGCATA         AATTGGA         GATTTGGA</td>	Image: constraint of the second se	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T	TACAGCTCATGCTTTCAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCTTTTAT         TACAGCTCATGCATGCTTTAT         AACAGCTCATGCATGCATA         AATTGGA         GATTTGGA
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H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313 H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G1	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       A       T       A       T       T       T       T         T       A       T       A       T	Image: Constraint of the text of the text of te	A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       C       A       A       T       C       A       C       A       A       A       C       C       C       C       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OKBBLEP-00005 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313 H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G       A         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G       A       A       T       G         T       G       G       A       G       A       T       T       A       T       A       T       A       T       A		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       C       A       A       C       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK6V1 H. dacotae - OK6V1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G1	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       G       A         T       G       G       A       G       A         T       G       G       A       G       A         T       G       G       A       G       A       G         T       G       G       G       G       A       G       A         T       G       G       G       G       A       T       A         T       A       T       A       T       T       T		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       C       A       T       C       A       A       A       C       C       C       C       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK6V1 H. dacotae - OK6V1 H. dacotae - OK6V1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14G1	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       G       A       T       G         T       G       G       A       G       A       T       G       A         T       G       G       A       G       A       A       T       G       A       A       T       G       A       A       T       A       T       A       A       T       T       A       A       A       T       T       A<		A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A C A C T A T T G T A A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T A T A C C A A T T A T	TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTCAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCTTTTAT TACAGCTCATGCATA AATTGGAGGATTTGGAAA AATTGGAGGATTTGGAAA AATTGGAGGATTTGGAAA AATTGGAGGATTTGGAAA
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G8 H. dacotae - OK14VEE1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK6V1 H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14VE1 H. dacotae - OK14VE1 H. dacotae - OK14VE1 H. dacotae - OK14VE1 H. dacotae - OK14VE1	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G       A         T       G       G       A       G       A       T       A         T       G       G       A       G       A       T       A       A       T       T       A         T       A       T       A       T       T       T       T       T       T       T       T       T       T       T			A       C       A       C       A       T       T       A       T       T       A       T       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14G11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK8V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G8 H. dacotae - OK14G1 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12	T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       G         T       G       G       A       A       T       A         T       G       G       A       A       T       A         T       A       T       A       T       T       T       A         T       A       T       A       T       T       T       A       T       A       T       A       T       A       T       A       T       A       T       A		A A C A C T A T T G T A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       C       A       A       C       A
H. dacotae - OKTV1 H. dacotae - OK14G1 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14G8 H. dacotae - OK14VEE1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - OK6V1 H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12	T       G       A       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       G       G       A       T       G         T       A       T       A       T       T       A         T       A       T       A       T       T       T       T         T       A       T       A       T       T       T       T       T       T       T       T       T       T       T       T <t< td=""><td></td><td>A A C A C T A T T G T A T A C C A A T T A T A T A C C A A T T A T</td><td>A       C       A       C       A       T       T       A       T       T       A       T       A</td></t<>		A A C A C T A T T G T A T A C C A A T T A T A T A C C A A T T A T	A       C       A       C       A       T       T       A       T       T       A       T       A

Figure 22a. Alignment of nine *Hesperia dacotae* samples sequenced in this study ("OK6", "OK7" and "OK14") plus sequences of specimens from North Dakota ("LEPRR") and sequences from *Polites mystic* and *Polites themistocles*. Lighter grey shading denotes intraspecific variation in the sequences, while darker grey shading denotes interspecific polymorphisms ("-" = no sequence generated beyond this point)

	8	9	R	8 8
H. dacotae - 0K6V1	TTGATTAGT	ACCTCTAAT	ATTAGGAGCTC	CTGATATAGCTTTYCCACG
H. dacotae - OK7V1				CTGATATAGCTTTYCCACG
H. dacotae - OK14G1			ATTAGGAGCTC	
H. dacotae - OK14G4				CTGATATAGCTTTTCCACG
H. dacotae - OK14G8				
H. dacotae - OK14G11			ATTAGGAGCTC	
H. dacotae - OK14V6E1			ATTAGGAGCTC	
H. dacotae - OK14V0E1			ATTAGGAGCTC	
H. dacotae - OK14V12			ATTAGGAGCTC	
			ATTAGGAGCTC	
H. dacotae - LEPRR009-08			ATTAGGAGCTC	
P. themistocles - 08BBLEP-00005				CTGATATAGCTTTCCCTCG
P. mystic - 08BBLEP-01313	TTGATTAGT	ACCATTAAT	ACTAGGAGCTC	CTGATATAGCTTT C C C T C G
		-		
H. dacotae - OK6V1	ΜΑΤΑΑΑΤΑΑ			K         K <thk< th="">         K         K         K</thk<>
H. dacotae - OK7V1				CTCCTTCATTATTATTATT
H. dacotae - OK14G1				
H. dacotae - OK14G4				
H. dacotae - OK14G4	WATAAATAA			
H. dacotae - OK14G8 H. dacotae - OK14G11			TTGAATATTAC	
			TTGAATATTAC	
H. dacotae - OK14V6E1				CTCCTTCATTATTATTATT
H. dacotae - OK14V11		TATAAGATT		
H. dacotae - OK14V12				CTCCTTCATTATTATTATT
H. dacotae - LEPRR009-08				СТССТТСАТТАТТАТТАТТ
P. themistocles - 08BBLEP-00005			TTGAATATTAC	CTCCTTCACTAACATTATT
P. mystic - 08BBLEP-01313	ATAAATAA	TATAAGATT	ΤΤ G Α Α Τ Α Τ Τ Α C	CACCTTCACTAACATTGTT
7. mysec - OODDEEF-01010				
	58	88	310	38 38 38
H. dacotae - OK6V1	<b>8</b> A A T T T C A G G	<b>8</b> A A G A A T T G T	<b>£</b> A G A A A A T G G T G	SE S
H. dacotae - OK6V1 H. dacotae - OK7V1	8 A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	<b>,                                    </b>	8         10         10 <th10< th=""> <th10< th=""> <th< td=""></th<></th10<></th10<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1	<b>8</b> A A T T T C A G G A A T T T C A G G A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T A A G A A T T G T	g A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         8         7         8         7         7         7         7         7         7         7         7         7         7         7 <th7< th=""> <th7< th=""> <th7< th=""> <th7< th=""></th7<></th7<></th7<></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4	8 A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T A A G A A T T G T A A G A A T T G T	E A G A A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         8         7         8         7         7         7         7         7         7         7         7         7         7         7 <th7< th=""></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8	<b>8</b> A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	<b>2</b> A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         8         7         8         7         7         7         7         7         7         7         7         7         7         7 <th7< th=""> <th7< th=""> <th7< th=""> <th7< th=""></th7<></th7<></th7<></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11	<b>8</b> A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	<b>2</b> A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         7 <th7< th=""> <th7< th=""> <th7< th=""> <th7< th=""></th7<></th7<></th7<></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1	8 A A T T T C A G G A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	<b>2</b> A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         7 <th7< th=""> <th7< th=""> <th7< th=""> <th7< th=""></th7<></th7<></th7<></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11	8           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G	8         7         7 <th7< th=""></th7<>
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14V611 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12	8           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	<b>S</b> A G A A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G A G A A A A T G G T G	8         7         8         7          7         7         7
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	8           A A T T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A T G G T G           A G A A A A T G G T G	8         7         8         7          7         7         7
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK1V61 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 089BLEP-00005	8           A A T T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G	8         8         8         8           C T G G A A C A G G T T G A A C T G T         G A A C T G T         G A A C T G T         G A A C T G T           C T G G A A C A G G T T G A A C T G T         C A G G T T G A A C T G T         G A A C A G G T T G A A C T G T         G T G G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G A A C A G G T T G A A C T G T         G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G T G T G T G T
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	8           A A T T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G	8         7         8         7          7         7         7
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK1V61 H. dacotae - OK14G1 H. dacotae - OK14G8 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 089BLEP-00005	8           A A T T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G	8         8         8         8           C T G G A A C A G G T T G A A C T G T         G A A C T G T         G A A C T G T         G A A C T G T           C T G G A A C A G G T T G A A C T G T         C A G G T T G A A C T G T         G A A C A G G T T G A A C T G T         G T G G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G A A C A G G T T G A A C T G T         G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G A A C A G G T T G A A C T G T           C T G G A A C A G G T T G A A C T G T         G T G T         G T G T G T G T
H. dacotae - OK6V1 H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313	8           A A T T T T C A G G           A A T T T C A G G	<b>8</b> A A G A A T T G T A A G A A T T G T	E           A G A A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A A T G G T G           A G A A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G           A G A A A A T G G T G	8       8       8       8       8       8         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G T G G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G T         C T G G A A C A G G T T G A A C T G T       G T A C A G G T T G A A C T G T       G T         C T G G A A C A G G T T G A A C T G T       G T G T       G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G T G T       G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G T G T       G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G T       G A A C A G G T T G A A C A G T         C T G G A A C A G G T T G A A C A G T       G T       G T         C A G G A A C A G G T T G A A C A G T       G T       G A A C A G G T T G A A C A G T         C A G G A A C A G G T T G A A C A G T       G T       G T         C A G G A A C A G G T T G A A C A G T       G T       G T         C A G G A A C A G G T T G A A C A G T       G T       G T         C A G G A A C A G G T T G A A C A G T       G T       G T         C A G G A A C A G G
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    G       G         A       A       T       T       C       A       G       G         A       A       T       T       C       C       C       C       C         T       T       A       T       C       C       C	88         A A G A A T T G T         C T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C         T T A T C T T C	B         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       G         A       G       A       A       A       T       G       G       T       C         A       G       A       A       A       T <td>8       8       8       8         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G T G T G G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G T       G T G T         C T G G A A C A G G T T G A A C T G T       G T G T G A A C A G G T T G A A C T G T       G T       G T G T G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G T       G T G T G T G A A C A G G T T G A A C T G T         C T G G A A C A G G A T C A G G T T G A A C T G T       G T       G A A C A G G T T G A A C T G T       G T         C T G G A A C A G G A T C C T C T G T T G A       G T G T G A A C A G T G T T G A A C A G T       G T       G T         C A G G A A C A G G A T C C T C T G T T G A       G T G T T G A A G G A T C C T C T G T T G A       G A T C A A G G A T C C T C T G T T G A         A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T G T T G A       A T C A A G G A T C C T C T G T T G A         A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T C T G</td>	8       8       8       8         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G T G T G G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C T G T       G A A C T G T       G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G T       G T G T         C T G G A A C A G G T T G A A C T G T       G T G T G A A C A G G T T G A A C T G T       G T       G T G T G A A C A G G T T G A A C T G T         C T G G A A C A G G T T G A A C T G T       G A A C A G G T T G A A C T G T       G T       G T G T G T G A A C A G G T T G A A C T G T         C T G G A A C A G G A T C A G G T T G A A C T G T       G T       G A A C A G G T T G A A C T G T       G T         C T G G A A C A G G A T C C T C T G T T G A       G T G T G A A C A G T G T T G A A C A G T       G T       G T         C A G G A A C A G G A T C C T C T G T T G A       G T G T T G A A G G A T C C T C T G T T G A       G A T C A A G G A T C C T C T G T T G A         A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T G T T G A       A T C A A G G A T C C T C T G T T G A         A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T C T G T T G A       A T C A A G G A T C C T C T C T G

Figure 22b. Alignment of nine *Hesperia dacotae* samples sequenced in this study ("OK6", "OK7" and "OK14") plus sequences of specimens from North Dakota ("LEPRR") and sequences from *Polites mystic* and *Polites themistocles*. Lighter grey shading denotes intraspecific variation in the sequences, while darker grey shading denotes interspecific polymorphisms ("Y" = "T" or "C" nucleotide while "W" = "A" or "T" nucleotide)

	8	8	2	8	8 8
H. dacotae - OK6V1		TTTTCTCTTCA	TTTAGCTGGAA	TTTCATCTAT	CTTAGG
H. dacotae - OK7V1	ТТТААСААТТ		ТТТАССТССАА		TTTAGG
H. dacotae - OK14G1	ТТТААСААТТ		ТТТАССТССАА		TTTAGG
H. dacotae - OK14G4	ТТТААСААТТ	TTTTCTCTTCA		TTTCATCTAT	TTTAGG
H. dacotae - OK14G8	ТТТААСААТТ		ТТТАБСТББАА		TTTAGG
H. dacotae - OK14G11	ТТТААСААТТ		ТТТАССТССАА		TTTAGG
H. dacotae - OK14V6E1		TTTTCTCTTCA			TTTAGG
H. dacotae - OK14V11	ТТТААСААТТ			TTTCATCTAT	TTTAGG
H. dacotae - 0K14V12	ТТТААСААТТ		ТТТАБСТББАА		TTTAGG
H. dacotae - LEPRR009-08	ТТТААСААТТ		ТТТАССТССАА		TTTAGG
P. themistocles - 08BBLEP-00005	TTTAGCAATT		ТТТА БСТ Б Б А А		TTTAGG
P. mystic - 08BBLEP-01313	TTTAGCAATT		ГТТА G C C G G A A		TTTAGG
	4	20	09	02	8
H. dacotae - OK6V1	AGCTATTAAT	TTTATTACAAC		TACGAATTAA	AAATTT
H. dacotae - OK7V1	А G C T A T T A A T	TTTATTACAAC		ТАСБААТТАА	AAATTT
H. dacotae - OK14G1	А G C T A T T A A T	TTTATTACAAC		ТАСБААТТАА	AAATTT
H. dacotae - OK14G4	А G C T A T T A A T	TTTATTACAAC		ТАСБААТТАА	AAATTT
H. dacotae - OK14G8		TTTATTACAAC			
H. dacotae - OK14G11		TTTATTACAAC			
H. dacotae - OK14V6E1		TTTATTACAAC			
H. dacotae - OK14V11	А G C T A T T A A T	TTTATTACAAC		ТАСБААТТАА	AAATTT
H. dacotae - OK14V12	А СТАТТААТ	TTTATTACAAC	ААТТАТТААТА	ТАСБААТТАА	AAATTT
H. dacotae - LEPRR009-08	А G C T A T T A A T	TTTATTACAAC		ТАСБААТТАА	AAATTT
P. themistocles - 08BBLEP-00005	А G C T A T T A A T	TTTATTACAAC	ааттаттаата	ТАСБААТТАА	АААТТТ
P. mystic - 08BBLEP-01313	AGCTATTAAT	TTTATTACAAC	ААТТАТТААТА	ТАСБААТТАА	AAATTT
	8	89	510	20	23
H. dacotae - OK6V1	ATCTTTGAT	: <b>8</b> сааатассттт,	S ATTTGTTTGAT	<b>8</b> 8 ⊂ T G T A G G A A T	80 T A C A G C
H. dacotae - OK6V1 H. dacotae - OK7V1		Сааатассттт, Сааатассттт,			
	АТСТТТТБАТ	САААТАССТТТ	АТТТ G Т Т G АТ		ТАСАБС
H. dacotae - OK7V1	А Т С Т Т Т Т G А Т А Т С Т Т Т Т G А Т	САААТАССТТТ	аттт G T Т Т G А Т А Т Т Т G T Т T G А Т	СТ G T A G G A A T	Т А С А G С Т А С А G С
H. dacotae - OK7V1 H. dacotae - OK14G1	A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T	С А А А Т А С С Т Т Т / С А А А Т А С С Т Т Т /	A T T T G T T T G A T A T T T G T T T G A T A T T T G T T G A T	С Т G Т А G G A A T С Т G Т А G G A A T	T A C A G C T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4	A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8	A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11	A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T	C A A A T A C C T T T 7 C A A A T A C C T T T 7 C A A A T A C C T T T 7 C A A A T A C C T T T 7 C A A A T A C C T T T 7 C A A A T A C C T T T 7	A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1	A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T A T C T T T T G A T	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11	A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G T T T G A T A T T T G G T T T G A T A T T T G G T T T G A T A T T T G G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 080BLEP-00005	A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T         A T C T T T T G A T	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G T T T G A T A T T T G G T T T G A T A T T T G G T T T G A T A T T T G G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G T T T G A T A T T T G G T T T G A T A T T T G G T T T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G G T       T T G T T       T G A T         A T T T G G T       T T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       A T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. themistocles - 080BLEP-00005	A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G G T       T T G T T       T G A T         A T T T G G T       T T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       A T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPRR009-08 P. therristocles - 08BBLEP-00005 P. mystic - 08BBLEP-01313	A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A         A       T       C       T       T       T       G       A	C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A C A A A T A C C T T T A	A T T T G G T       T T G T T       T G A T         A T T T G G T       T T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       T G A T         A T T T G G T       A T G A T	C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T C T G T A G G A A T	T A C A G C T A C A G C
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Figure 22c. Alignment of nine *Hesperia dacotae* samples sequenced in this study ("OK6", "OK7" and "OK14") plus sequences of specimens from North Dakota ("LEPRR") and sequences from *Polites mystic* and *Polites themistocles*. Lighter grey shading denotes intraspecific variation in the sequences, while darker grey shading denotes interspecific polymorphisms

	280	230	009	610	620
H. dacotae - 0K6V1	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	
H. dacotae - OK7V1	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	гттттсбатс	CAGCAGG
H. dacotae - OK14G1	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	TTTTCGATC.	CAGCAGG
H. dacotae - OK14G4	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	CAGCAGG
H. dacotae - OK14G8	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	TTTTCGATC.	CAGCAGG
H. dacotae - OK14G11	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	CAGCAGG
H. dacotae - OK14V6E1	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	CAGCAGG
H. dacotae - OK14V11	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	CAGCAGG
H. dacotae - OK14V12	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	ГТТТТСБАТС	CAGCAGG
H. dacotae - LEPRR009-08	ATTACTTACT	GATCGAAATTTA	ААТАСТТСТІ	гттттсбатс	CAGCAGG
P. themistocles - 08BBLEP-00005	АТТАСТТАСА	GATCGAAATTTA	ААТАСТТСТТ	гттттбатс	CAGCTGG
P. mystic - 08BBLEP-01313	АТТАСТТАСА	GATCGAAATTTA	ААТАСТТСАТ	гттттбасс	CAGCAGG
	630	640	650	658	
H. dacotae - OK6V1	AGGAGGAGAT	C C A A T T	<u>8</u> 	82	
H. dacotae - OK7V1		C C A A T T	<u>ç</u>	89 99 	
H. dacotae - OK7V1 H. dacotae - OK14G1	AGGAGGAGAT	ССААТТ ССААТ	88 	<b>88</b>   T T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4	A G G A G G A G A T A G G A G G A G A T	С С А А Т Т С С А А Т С С А А Т Т Т Т А Т А Т		  F T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8	A G G A G G A G A T A G G A G G G A G A T A G G A G G G A G A T A G G A G G A G A T	С С А А Т Т С С А А Т С С А А Т Т Т Т А Т А Т		 F T T F T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11	A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G	С С А А Т Т С С А А Т С С А А Т Т Т Т А Т А Т С С А А Т Т Т Т А Т А Т	С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т	  - T T T T T T T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1	A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G	C C A A T T	С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т	 - T T T T T T T T T T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V6E1 H. dacotae - OK14V11	A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       A       G       G       G       G       G       G       G       G       G       G       G       G       G	C C A A T T	Г С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т С А А С А С Т Т А Т Г С А А С А С Т Т А Т	  - T T - T T - T T - T T - T T - T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12	A       G       G       A       G       G       A       G       G	C C A A T T	C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T	  - T T - T T - T T - T T - T T - T T - T T	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V12 H. dacotae - OK14V12 H. dacotae - LEPRR009-08	A       G       G       A       G       G       A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       A       G       G	C C A A T T	C A A C A C T T A T C A A C A C A C T T A T C A A C A C A C T T A T C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T C A A C A C T T A T	  - T T - T T   	
H. dacotae - OK7V1 H. dacotae - OK14G1 H. dacotae - OK14G4 H. dacotae - OK14G8 H. dacotae - OK14G11 H. dacotae - OK14V11 H. dacotae - OK14V11 H. dacotae - OK14V12	A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         A         G         G         G         G         G         G         G         G         G         G         G	C C A A T T		  - T T - T T   	

Figure 22d. Alignment of nine *Hesperia dacotae* samples sequenced in this study ("OK6", "OK7" and "OK14") plus sequences of specimens from North Dakota ("LEPRR") and sequences from *Polites mystic* and *Polites themistocles*. Lighter grey shading denotes intraspecific variation in the sequences, while darker grey shading denotes interspecific polymorphisms ("-" = no sequence generated beyond this point)

The results of the neighbour-joining tree are provided in Figure 23. All the samples of Dakota Skipper clustered together within a clade with a robust 99% bootstrap value. The samples from North Dakota formed a subclade with 59% boostrap value within the larger cluster. However one sample from Site H ("OK14V12") also clustered in the North Dakota subclade. All the *Polites mystic* and *Polites themistocles* samples cluster in a separate clade with 99% bootstrap support (Figure 23).

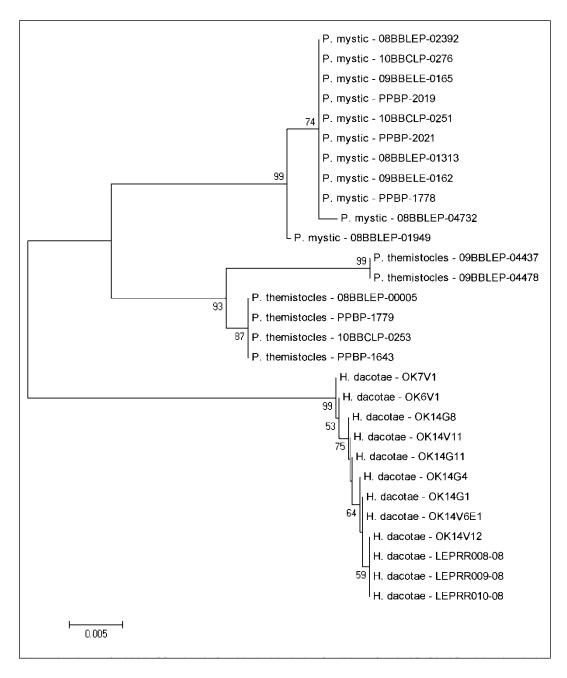


Figure 23. Bootstrapped neighbour-joining tree of COI sequences from *Hesperia dacotae* from Manitoba ("OK6", "OK7" and "OK14") and North Dakota ("LEPRR"), and *Polites mystic* and *Polites themistocles* from across North America. Bootstrap values are based on the percentage of 500 pseudoreplicate datasets are given at each node of the tree

When the Dakota Skipper sequences generated in this study were compared to each other and those from North Dakota it was found that there were three variable sites present (Table 37). At site 235 of all Dakota Skipper sequences, all the specimens from Manitoba exhibited either a cytidine ("C") or a thymidine ("T") nucleotide (coded as "Y") while the specimens from North Dakota all had a thymidine nucleotide in this region. Similarly, at site 241 all the Manitoba Dakota Skipper specimens exhibited either a adenosine ("A") or a thymidine ("T") nucleotide (coded as "W") while all the specimens from North Dakota had a thymidine nucleotide (Table 37). When the individual chromatographs for each run of each specimen were reviewed, two trends became apparent. In the sequences using the F1/R1 primer combination the nucleotides were always thymidine at site 235 and site 241, while in the F1/R2 primer combination the nucleotides were always cytidine at site 235 and adenosine at site 241.

Specimen sequence	Specimon origin	Site along sequence*		
Specimen sequence	Specimen origin	235	241	427
H. dacotae - OK6V1	Southwest Manitoba	Y	W	С
<i>H. dacotae</i> - OK7V1	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14G1	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14G4	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14G8	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14G11	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14V6E1	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14V11	Southwest Manitoba	Y	W	Т
<i>H. dacotae</i> - OK14V12	Southwest Manitoba	Т	Т	Т
<i>H. dacotae</i> - LEPRR009-08	North Dakota	Т	Т	Т
<i>H. dacotae</i> - LEPRR010-08	North Dakota	Т	Т	Т
H. dacotae - LEPRR008-08	North Dakota	Т	Т	Т

Table 37. Summary of nucleotides present at polymorphic sites in Dakota Skipper COI sequences from Manitoba and North Dakota

\*Y = cytidine ("C") or thymidine ("T") nucleotide; W = adenosine ("A") or thymidine ("T") nucleotide

In the instance of the F1/R2 primer combination, the R2 sequence generated was not long enough to reach to site 235 and site 241. In other words, only the F1 sequence in this combination generated the cytidine and adenosine nucleotides at these sites respectively. The R2 primer did not reach these sites as it is a shorter sequence (350 bp) that generates sequences in the "reverse" direction. In the F1/R1 primer combination both the F1 and R1 primers are sequenced here as the sequence is a longer strand.

In the OK6V1 specimen a third variable site was identified at site 427 where a cytidine nucleotide was present, while all the other Dakota Skipper specimens had a thymidine nucleotide (Table 37). The OK6V1 sequencing appears to be a good, strong read generated from two strands (the F1 and R1 primers) and there is no apparent reason for this variation.

#### 6.3 Discussion

#### 6.3.1 Flight Period and Density

Based on surveys in 2010, 2011 and 2012, the flight period was approximately 13 to 19 days in duration. Estimating the duration of the flight period was made more difficult given challenges in detecting adults at the start and the end of the flight period when adult densities are at their lowest. The start date of the flight period also varies considerably. There was 11 days difference between the start of the flight period between 2011 and 2012, and 15 days difference between 2010 and 2012 but only four days difference between the 2010 and 2011 flight period. The timing of the start of the flight period is governed by the temperature in spring and early summer (Bink and Bik 2009; Koda and Nakamura 2012). For the three sample years, 2012 had the warmest spring and summer which accounts for the earlier flight period (Environment Canada 2011). The date of the start of the flight period may be different in the Interlake in comparison to southwestern Manitoba. It appears adults emerge a few days earlier in the southwest but there are insufficient observations at the start of the flight period to confirm long term annual differences. Overall the flight period results are consistent with past findings (Section 2.3.2), however the flight period duration does not likely exceed three weeks in Canada as has been reported elsewhere (COSEWIC 2003; Environment Canada 2007).

The first males observed in this study emerged approximately 2 to 4 days prior to the first females. In the early portions of the flight period, males were found before females, or the body condition of males was more worn than females. Some later emerging males (emerging at the same time as females) do occur, but the majority of males emerge before the females start to emerge. Due to earlier emergence or a shorter adult male life span mostly females are observed at the end of the flight period. The asynchronous flight period observations in this study support the trends observed by Dana (1991), Swengel and Swengel (1999a) and Cochrane and Delphey (2002), and are contrary to the statement by McCabe (1981) that both sexes fly at the same time.

The density of Dakota Skippers at the research sites appeared to be in decline when the current counts (2010 to 2012) are compared to those from past surveys (2002, 2005 and 2007). No skippers were found at Sites A, C and E despite relatively high counts at those sites in past surveys. Given the intensive surveys done in 2010 to 2012 and high degree of skill of the surveyors, it seems likely that Dakota Skipper may now absent from these sites. If Dakota Skipper persist at such low densities they remain extremely difficult to detect. Alternatively, the past surveys were located elsewhere in the sites or were inaccurate. At the remaining five sites (B, D, F, G and H) the population counts from 2010 to 2012 are well below those previously observed. Generally past counts at the sites were based on a single visit to the site once or twice during the flight period (Webster 2003; Webster 2007), where as the current population counts involved multiple site visits over the duration of the flight period. In this study the density of Dakota Skipper at Site H were approximately 5 to 33 times higher than observed at other sites, which initially suggest that the population at this site is more robust. However, this is likely not the case. Site H has one of the largest areas of suitable habitat and when one considers the densities of skippers across the site, they are still quite low. Despite the having the most individuals of all the sites sampled, the number of Dakota Skippers within Site H can still be considered relatively low given the extensive amount of habitat available. Likely in the past Dakota Skippers did occur at much higher densities at Site H. The cause(s) for population declines at all the sites is unclear. The declines could be attributed to changes or decline in habitat and/or direct mortality due to anthropogenic or natural factors. It is clear that recent counts indicate that populations at the sites surveyed are likely in decline and a cause for concern, because if the current trend continues they may be lost from four of the remaining sites (B, D, F and G) in the near future and perhaps even from Site H.

It remains a significant challenge to visit sites with sufficient frequency and intensity during the flight period. The sites are widely separated, the skippers are difficult to detect and the flight period is very short. The INCA model indicated that there were up to 332 individuals present at Site H in 2011, though this estimate is quite

coarse. Webster estimated the density at Site H to be 1000 individuals in 2002 and 2000 individuals in 2007, but cautioned that this was a very rough population estimate (COSEWIC 2003; Webster 2007). Webster estimated the population at sites based on the following calculation: population density = (mean number of adults per hectare in the 0.5hectare sections counted) x (estimated proportion of drier prairie habitat) x (estimated size of prairie) (COSEWIC 2003). Webster's estimates in 2002 and 2007 were based on one or two visits to the site in each year (COSEWIC 2003; Webster 2007). Thus comparison between population estimates done by INCA and Webster should be viewed with caution as they employ different methods and the survey intensity differs. Also Dakota Skipper are not uniformly distributed across their suitable habitat, rather they are aggregated in small patches within their habitat. The population estimates done with INCA better reflect these patchy agregations of adults, while Webster's approach does not. However, both surveys factor in the number of individuals observed and the amount of suitable habitat. None-the-less, the population appears to have declined considerably from past estimates. The peak of Dakota Skipper emergence was estimated to be 4.762 days in duration. This estimate appears to be consistent with field observations, as the peak of female emergence coincides with the males still on the wing.

It has been reported previously that the Interlake region has the largest population of Dakota Skippers in Canada while the southwest Manitoba region has a smaller population (Environment Canada 2007; Webster 2007). The sites selected for this study had among the highest counts of Dakota Skipper previously reported (Webster 2003; Morden 2006; Webster 2007). Examination of the counts from this study and past studies (Table 32) indicates that the populations in the southwest are actually as high or higher then those found in the Interlake.

The majority of other butterfly species observed at the sites were widespread, generalist species and/or relatively common species. Garita skipperling (*Oarisma garita* Reakirt) is a prairie specialist and four adults were found at Sites B and D in the Interlake (Klassen et al. 1989; Layberry et al. 1998). Populations of *Epargyreus clarus* fluctuate greatly between years, ranging from moderately common to scarce in Manitoba (Klassen et al. 1989). *Epargyreus clarus* is normally restricted to scrubby areas and openings near woodlands and four adults were seen at three sites (B, C and D) in the Interlake (Klassen

et al. 1989; Layberry et al. 1998). *Speyeria callippe* is found in southwest Manitoba and is restricted to sandy prairies, with ten adults found at Site H (Klassen et al. 1989; Layberry et al. 1998). *Anatrytone logan* is also a less common butterfly found in prairies, marshes and fields, and two adults were seen at Sites G and H in the southwest (Klassen et al. 1989; Layberry et al. 1998). None of the species of butterflies observed were considered unusual for the research sites and cannot be considered as specific habitat specialists to degree that Dakota Skipper is. The high number of butterfly species observed at most of the sites is probably attributed to the presence of relatively undisturbed prairie, wetland and forested habitats with an abundance of nectar flowers and larval food plants.

When observed in the field, worn specimens of *Polites mystic* and *Polites themistocles* can be confused with worn Dakota Skipper specimens. Distinguishing these species in fresh specimens is relatively easy but care needs to be taken when identifying worn specimens. It may be possible that some of the earlier counts for Dakota Skipper may have included misidentifications and, given that there are not necessarily vouchers available to examine, it is difficult to compare densities between different surveys. In future, additional voucher specimens should be taken when allowed under permits, and ample photos should be taken of the enumerated skippers from both the dorsal and ventral perspective. The skippers should be handled as little as possible to reduce erosion of markings. In particular, the identity of each individual skipper counted should be visually confirmed in the field when doing intensive site counts as misidentifications most likely occur when doing rapid survey counts.

The timing and order of various skipper species emergence was found to be a simple and easy indicator of Dakota Skipper emergence. When attempting to detect the start of the Dakota Skipper flight period within suitable habitat, expect skippers to emerge in the following order: *Thymelicus lineola, Polites mystic, Polites themistocles, Polites peckius,* Dakota Skipper, *Epargyreus clarus* and *Euphyes vestris. Polites peckius* will emerge immediately before and at the same time as Dakota Skipper while *Euphyes vestris* emerge near the end of the Dakota Skipper flight period. However, not all these species necessarily occur at all Dakota Skipper sites. The start of the Dakota Skipper flight period also appears to coincide with the peak of the flight period of the *Cercyonis* 

*pegala*, and with the maturation of *Stipa spartea* seeds. The inflorescence of *Stipa spartea* are relatively tall and prominent, and a few days prior to the Dakota Skipper flight period the seeds are very conspicuous with a darkened inflorescence (Appendix Z). At the start of the Dakota Skipper flight period the fruits will start to be released when brushed, clinging especially to butterfly nets and clothing. When present at sites, *Stipa spartea* and its fruits are quite visible to surveyors.

During the peak of the Dakota Skipper flight period the ratio of some skipper species may be an indirect indicator of quality or extent of the Dakota Skipper habitat. In certain research sites the core areas of high quality habitat (in particular in the southwest research sites) had a ratio that was approximately 20 Dakota Skippers: 3 *Polites mystic*: 1.5 *Polites themistocles*: 1.5 *Polites peckius*: and 0.5 *Thymelicus lineola*. Where the *Polites species* and *Thymelicus lineola* are abundant during the Dakota Skipper flight period, Dakota Skippers are less likely to occur, which may be linked to the quality of the habitat present or competition.

#### 6.3.2 Behaviour

Dakota Skipper in Manitoba nectar predominately upon black-eyed Susan (Appendix X). They were also observed feeding upon eleven other species of flower including six species which were new records for the skipper (Appendix X). The six new nectar plant species were: *Melilotus alba*, *Petalostemon purpureus*, *Oenothera biennis*, *Lobelia spicata*, *Crepis runcinata* and *Solidago ptarmicoides* (Appendix X). Compared to records from the United States, Dakota Skipper in Manitoba appear to be more dependent upon *Rudbeckia hirta* and also appear to utilize other flowering plant species (McCabe 1981; Dana 1991; Swengel and Swengel 1999a). This is likely a reflection of the differences in floral composition between habitats in Manitoba and the United States, which are associated with climatic and ecotype differences. Observations of Dakota Skipper nectar feeding on shorter specimens of *Melilotus alba* is not surprising as the Dakota Skipper generally flies low to the ground and selects lower plants to perch and feed upon. This desire to remain closer to the ground may be a predator avoidance strategy or a strategy to avoid heavy winds, which requires additional energy expenditure and may increase dessication. The selection of shorter specimens of *Melilotus alba* 

(Appendix X) may also demonstrate that the skipper is opportunistically using the species and is not dependent upon it as it typically grows much larger and is non-native (Looman and Best 1981; Reaume 2009).

Dakota Skipper has been reported to nectar upon *Lilium philadelphicum*, *Zygadenus gramineus, Medicago sativa, Melilotus officinalis, Asclepias speciosa, Campanula rotundifolia, Achillea millefolium* and *Erigeron annuus*, but avoided nectaring upon these plants in the present study. Due to the abundance of these plant species there was ample opportunity to nectar on these species and while skippers sometimes perched on these, none were observed nectaring This is particularly relevant to *Lilium philadelphicum* and *Campanula rotundifolia*, which have been reported as nectar plants in Canada (Webster 2003; Webster 2007). These observations were likely inaccurate or atypical of the species. None-the-less both these plant species can be considered indicators of Dakota Skipper habitat (Section 5.3.1.1).

The intensive browsing by cattle at Site F is of concern given that in 2012, virtually all the flower heads were absent at the site during the flight period (Appendix X). The cattle appear to have been pastured at the site in early summer of 2012 and consumed all the nectar flower buds. The result was that there were no nectar sources present at the site during the flight period. The site previously resembled a "wildflower garden" due to the abundance and diversity of flowers during the flight period in 2010 and 2011. The results of the grazing in 2012 were in stark contrast to the previous survey years and skippers observed at the site in 2012 would have to obtained adequate nectar for hydration and energy from elsewhere.

Very little is known about distances Dakota Skipper fly within habitats or their dispersal ability between habitats. They are thought to have a low dispersal ability and typically do not wander very far from their emergence location (Dana 1991; Royer and Marrone 1992; Cochrane and Delphey 2002). During the course of this study, Dakota Skippers were generally observed to fly low to the ground and have a fidelity to a particular patch of flowers and grasses within a site. On occasion they would fly up to 8 m high and a distance of 150 m but these instances were generally only when pursuing another skipper or evading a predator. Considering this, all the elements Dakota Skipper would require for survival (larval food and shelter plants, adult nectar, perching and

ovipositioning plants, etc.) would have to be in proximity to each other, probably within a few hundred meter radius, which is a relatively small space from a landscape perspective. Co-occurrence of all the critical elements Dakota Skipper require may explain their spacing within sites and their absence from sites that superficially appear appropriate. Their site fidelity and unwillingness to cross unsuitable habitat support the notion that they have a low dispersal ability which is consistent with past observations (Environment Canada 2007). Dispersal to adjacent sites or patches within sites is dependent upon suitable open connecting corridors, which are often not present. The lack of suitable corridors is likely a limiting factor in Dakota Skipper populations and a significant threat in terms of isolation of populations between habitats.

The reason for females flying into the lower sedge areas remains unclear. Perhaps the females are ovipositing in those areas, as there are reports of larvae feeding upon *Carex pensylvanica digyna* in the United States. Patches frequented by females were largely composed of the sedge *Eleocharis palustris*. It is unknown whether larvae would feed upon *Eleocharis palustris* but this remains a possibility. Another possibility is that the females are frequenting these sedge patches as they are generally cooler, more humid and sheltered areas. The females may seek shelter to avoid desiccation or consume water for hydration. They may even perhaps lay their eggs here to prevent them from desiccating, and larvae simply feed on nearby grasses instead of these sedges.

There are no known records of predation upon Dakota Skipper reported in Canada. In the United States crab spiders and robberflies have been reported to feed upon Dakota Skipper (Environment Canada 2007). It is unclear whether the predators were identified to species in the United States and the current predation records may be the first instances that the identity of the predators was identified to species. Crab spiders and robberflies are likely among the main predators of Dakota Skippers in Manitoba as they are common at the sites and have ample opportunity to catch Dakota Skippers. Crab spiders are ambush predators upon flowers and are frequently found on the flowers that skippers frequent. Robberflies patrol during the day for suitable prey to capture, and are commonly observed within the Dakota Skipper habitat during the flight period.

Dakota Skipper frequently interact with other skipper species. Males were often seen chasing individuals of another species perhaps due to mating competition or as a potential mate. In some instances the males would even attempt to mate with the female from another species. These interactions are of interest because they demonstrate the degree to which males will maintain a home range and seek mates, and also their ability to detect conspecifics versus other skipper species. Furthermore, these interspecific interactions occur quite frequently. Care should be taken when observing and enumerating these aerial skipper interactions to affirm the species of skippers involved to prevent inaccurate reporting.

Mating attempts of Dakota Skipper resembled those previously described; scramble competition polygyny in which males spend most of their time engaged in mate-seeking behavior (McCabe 1981; Dana 1991). The observations reported during this study of male and female behavior immediately before copulation attempts appear to not have been documented elsewhere. Specifically, when the males and females were perched on blades of grass males would gently vibrate their wings. Then sometimes the female would also gently vibrate her wings in response to the male. In all those instances, the male would attempt copulation with the female. This behavioral display could be a courtship display or to test female receptivity, and indicates that a visual, olfactory or vibration cue may be involved in mating.

Female Dakota Skipper often appeared as though they were attempting to oviposit but refrained from doing so. It is unclear but perhaps the females were probing to determine the suitability of the plant as a larval food plant or perhaps she had eggs remaining but was unable to lay an egg at that moment. However, Dana (1991) reported that females did not seem to evaluate the suitability of plants as larval food plants as they would frequently lay eggs on unsuitable plants or on plants adjacent to the plant they were perched upon. How and where females select oviposition points remains unclear, as does the behavior attempting to deposit eggs but not depositing any. The ovipositing motions observed in this study are consistent with findings reported elsewhere (MacNeill 1964; Dana 1991). These authors observed ovipositing upon a potential larval food plant (*Poa cusickii* and unknown *Poa* species), and there were several other known (*Andropogon scoparius* and *Stipa spartea*) and probable food plants (*Poa cusickii* and unknown *Poa* species) in close proximity. Generally the appearance of eggs and first instar larvae are consistent with findings reported elsewhere (MacNeill 1964; McCabe 1981; Dana 1991). This may be the first time the larval incubation period has been reported in the literature, which was nine to ten days. Furthermore, the appearance of the newly emerged first instar larvae in this study seems to differ from the appearance of larva described elsewhere. The one to two day old first instar larvae had an amber to rust brown head capsule and prothoracic shield, and their bodies were yellow-creamy white. Elsewhere the larvae have been described as having a black head and prothoracic shield, and light brown or flesh coloured bodies (MacNeill 1964; McCabe 1981). These past observations may have been of more mature, later instar larvae where chitin had hardened and body colouring reflected host plant feeding. The shelters created by the first instar larvae were not photographed. When the opportunity exists, larval shelters should be photographed to enable other researchers to recognize and locate shelters. Additional studies on the larval stages should also be undertaken.

#### 6.3.3 Genetic Analysis

In regards to Objective 6, sufficient and good quality DNA can be extracted from a single mid-leg taken from live Dakota Skipper. There was no apparent difference in the quality of the DNA obtained from dead voucher specimens and live specimens, demonstrating that live harvested tissues collected in semi-sterile field conditions are equivalent to conventional pinned or live insect tissue sources. Capture and harvest of legs from Dakota Skipper was minimally invasive. Adults live-sampled often released a leg when grasped with tweezers and very rarely was pressure needed to remove a leg. The ease with which Dakota Skipper release a leg is likely a predator avoidance strategy and often Dakota skipper are seen missing one or several legs. Sampling of legs as a tissue source for molecular analysis is probably less invasive and less harmful to Dakota Skipper than wing clippings. It is often very difficult to handle Dakota Skipper adults as they tend to flap their wings hard resulting in wing damage if the wings are not carefully restrained. Clipping a small portion of the wing may result in wing damage beyond the area cut as the wings are difficult to restrain for such sampling. Additionally in Manitoba, Dakota Skipper sites can be very windy, making it difficult to restrain wings and hold clipped material. Legs are very easy to grasp and hold with tweezers (they do not catch the wind so readily). Additionally, positioning Dakota Skippers for leg sampling is very straight-forward and non-injurious. While in the net, the wings are grasped and held together through the net (with gloved hands). The net is then inverted so that they butterfly is held legs upwards and a leg can be removed.

The COI region analyzed in this study was successfully amplified by PCR and sequenced. The primer design and PCR conditions followed the protocols of Hebert et al. (2004) with slight modifications. The Dakota Skipper COI sequences from this study show high sequence identity to COI sequences of Dakota Skipper individuals collected elsewhere in North Dakota. There was very little variation in the sequences between the samples collected in southwest Manitoba and central North Dakota. This lack of divergence in DNA sequence between Dakota Skippers in southwest Manitoba and central North Dakota is expected because COI is a relatively conservative region that evolves slowly and exhibits little intraspecific variation (Hillis et al. 1996).

It appears that there is a sequence polymorphism present at sites 235, 241 and 427 in the Dakota Skipper samples sequenced. Closer comparison of the sequence contigs for each sample revealed that the variance at sites 235 and 241 are associated with the F1 primer in the F1/R2 primer pairing and is likely somehow associated with the primer pairing or the sequencing of that primer pairing. The primers used in the primer parings may be detecting two different haplotypes present and if this is this case, then this would Heteroplasmy, the presence of two different be an instance of heteroplasmy. mitochondrial haplotypes in an individual is caused by a single nucleotide substitution Heteroplasmy has been reported to occur in the COI gene of insects mutation. (Magnacca and Danforth 2006; Paduan and Ribolla 2008). When investigating Aedes *aegypti* (the mosquito known to spread yellow feaver and dengue fever) in Brazil, Paduan and Ribolla (2008) defined seven different haplotypes in the species which they attributed to heteroplasmy. They found that they had two distinct mitochrondrial gene sequences present in one individual. They suggested that the heteroplasmy could be due to one of two factors: "mutation within the mtDNA within the cells or paternal leakage of mitochondrial DNA" (Paduan and Ribolla 2008). Typically in sexual reproduction mitochondrial DNA is only inherited from the maternal line and there is no paternal

contribution. "Paternal leakage" of mitochondria is the concept that through unknown mechanisms mitochondrial DNA from the father is passed on to offspring(Paduan and Ribolla 2008). While studying the biogeography of Hawaiian *Hylaeus* bees, Magnacca and Danforth (2006) found ten species to have a high amount of polymorphism in sequences from the COI and COII region. They cloned the sequences from those regions and found support for the presence of at least two mtDNA haplotypes which they attribute to heteroplasmy (Magnacca and Danforth 2006). In both studies, the researchers remarked that heteroplasmy in the mtDNA of insects was surprising as it has been under reported and both recommend further investigation (Magnacca and Danforth 2006; Paduan and Ribolla 2008). Further investigation to determine what the cause of the variation observed here in Dakota Skipper is beyond the scope of this study, and is recommended particularly since it initially appears to not be present in those specimens sampled from North Dakota.

# 7.0 OVERALL DISCUSSION

This section addresses objective 7: examine potential correlations between management history, site character profile and population size.

## 7.1 Links Between Adult Density, Flora, Edaphic Factors and Land Use

Comparison between the adult density, floral diversity, edaphic factors and land management showed some general trends and relationships between these variables. At all the research sites, the density of Dakota Skipper adults appears to be in decline when compared to past surveys. The declining population density at sites may be affected by the structure and composition of the plant communities, soil and soil-related conditions, land use and various anthropogenic impacts at the sites.

At Site A, Dakota Skipper surveys indicated a decline from 12 individuals in 2002 to none in 2010 to 2012. The habitat at this site appears to be degraded based on limited site descriptions made in 2002 and very little suitable habitat remains. Tilling in 2006 and 2007, along with the proliferation of Agrostis stolonifera likely explains the loss of habitat and disappearance of Dakota Skipper from the site. Site A had the lowest diversity of flora (46 species of plants) and butterflies (16 species) observed among the Interlake sites and also had relatively high levels of compaction compared to the other sites. Increased compaction may have affected soil structure and flora present. Generally there was a lack of key larval food plants common to Dakota Skipper habitat such as Andropogon gerardii, Andropogon scoparius or Stipa spartea. Almost all of the important nectar plant species were absent or at low densities in Site A, with the exception of Rudbeckia hirta which was very abundant. Rudbeckia hirta is a prairie species but also a species that thrives in recently disturbed areas, which may explain its abundance at Site A. Site A also had high levels of several non-native, invasive species such as Medicago sativa, Melilotus alba and Bromus inermis. Given these unfavourable attributes at the Site A, it's not surprising that Dakota Skipper was absent from this site. One small patch (approximately 1.0 ha) of relatively undisturbed prairie exists in the southeast corner of Site A (away from the transect areas) and may still be suitable habitat, but Dakota Skipper was not observed in this patch and the area may be too small to support them.

Site C has also experienced a dramatic decline in the Dakota Skipper population from 43 individuals in 2002 to none in 2010 to 2012. Contrary to observations of habitat degradation at Site A, the decline at Site C is less easy to explain. The land use history is favourable with a long period of fall having and no apparent instances of tilling, burning or other agricultural practices that could lower the habitat quality of this site. The number of species of plants (50 species) and butterflies (17 species) in Site C was marginally lower compared to Sites B and D. Among the other butterfly species observed in Site C there were only four less common species, which is quite low compared to the other sites in the Interlake positive for Dakota Skipper (Site B had 10 less common butterfly species and Site D had 12 species). The low count of less common butterfly species may be a subtle indication of poorer quality prairie habitat in parts of Site C, or that perhaps the entire parcel is generally lacking in habitat diversity and structure. The soil compaction levels were higher compared to Sites B and D which may be a reflection of different soil characteristics or perhaps long-standing use of farm machinery on the property. Unlike Site A where Dakota Skipper was absent, Site C contained many of the key larval and adult food plants in moderate to high densities. Andropogon gerardii was quite abundant at Site C, and although Andropogon scoparius and Stipa spartea were not observed along the transects, they were present at low densities outside of the transect areas. Rudbeckia hirta was very abundant at Site C and there were several other important nectar species at moderate to high densities in the transect areas. Several non-native species were present at moderate (Trifolium pratense and Cirsium arvense, Canada thistle) or low densities (Medicago sativa, Melilotus alba and *Melilotus officinalis*). There was a *Medicago sativa* field in the north portion of the parcel, which appeared to be the source for the colonization of Site C by non-native plants. Perhaps the non-native species have degraded the habitat. Otherwise there is no apparent cause for the absence of Dakota Skipper during this study.

In the Interlake Site B and Site D appear to be most similar from a vegetation perspective and while supporting Dakota Skipper, both sites are experiencing similar population declines. At Site B twelve Dakota Skippers were observed in 2002, which declined to three individuals in 2005, two individuals in 2011 and one individual in 2012. In Site D twenty-one individuals were observed in 2005, two were seen in 2011 and six in

2012. Both sites appear to have relatively high quality Dakota Skipper habitat. The sites are haved irregularly with annual having during favourable years and no having during years that are too wet for having or when the hay yield is too low. The sites had not been haved for several years prior to the vegetation surveys in this study. The result was a deeper duff layer that was much higher than observed elsewhere and *Populus tremuloides* succession into the prairie communities was quite prominent. The effects of the increased duff layer upon the larval stages are unknown but the deeper duff may have inhibited growth of nectar flowers and likely increased the fire fuel load. A large fire burned most of Site D in fall 2012. Populus tremuloides succession results in a direct loss of habitat, diminishing suitable habitat for larval and adult Dakota Skipper. Prior to the recent fire at Site D, the area being lost to ongoing Populus tremuloides succession was relatively large. There were moderate to high levels of key larval food plants at both sites. Rudbeckia hirta densities were low to moderate, and the other nectar species occurred mostly at low or moderate densities. Perhaps Dakota Skipper at these sites are limited by the availability of preferred nectar sources. The main invasive plant species of concern found at other sites where generally absent or at low densities in Sites B and D. Causes of Dakota Skipper decline at Sites B and D are unclear but maybe due to a decline in habitat quality particularly from encroaching aspen forest, insufficient having frequency and lack of sufficient nectar sources. The impact of the recent fire upon the Dakota Skipper population at Site D remains unclear. While the fire and fire abatement measures may have caused larval mortality and disturbed key larval or adult plants, the fire may have also killed much of the Populus tremuloides and other woody plants that were encroaching into the prairie and removed much of the duff layer. The fire may ultimately improve the habitat at Site D and if Dakota Skipper has survived, the population may increase.

In the southwest, no adults were observed at Site E. In 2007, 13 adults were observed at Site E. Based on the absence of skipper found in this study the decline has been quite abrupt. Site E is divided among two parcels; a very small patch of prairie (approximately 0.56 ha) immediately north of Menteith School Road (Transect 1), and a second small patch of prairie (approximately 1.14 ha) south of the road (Transect 2). The decline at this site is probably associated with a loss and degradation of habitat. In

addition, the site is quite small in size and isolated from other suitable patches or parcels habitat in the general vicinity. The controled burn in 2007 on the southern parcel may have caused Dakota skipper mortality in this parcel. This site had the highest amounts of bare ground among all the sites with the least vegetation cover, particularly grasses. Site E was quite open and surrounded by cultivated croplands and wetlands. The remaining habitat is very uniform and lacking in structure, woody vegetation and relief, with the exception of a small, cattail marsh along the eastern limits. Only nine other species of butterflies were observed at this site, which is much lower than observed elsewhere and suggests there is a lack of suitable, diverse habitat present. There may be insufficient larval food sources, as there was no Andropogon gerardii or Stipa spartea present, and only moderate levels of Andropogon scoparius. There were moderate levels of *Rudbeckia hirta* and low to moderate levels of other adult nectar source plants. There were also moderate to high levels of many invasive, non-native species (Bromus inermis, Medicago sativa, Melilotus alba, Melilotus officinalis and Cirsium arvense). There are several large patches of non-native species within and adjacent to Site E, including a cultivated field of *Medicago sativa* adjacent to the northern parcel.

In 2007 eleven Dakota Skippers were observed at each of Site F and Site G. Since then only one adult was observed each year in 2010, 2011 and 2012 at Site F. At Site G two adults were observed there in 2011 and none in 2010 or 2012. Both sites appear to have relatively high quality habitat with moderate floral and butterfly species diversity compared to other sites. Site F had moderate levels of *Andropogon gerardii* and *Stipa spartea* and very high levels of *Andropogon scoparius*, while Site G had high levels of *Andropogon gerardii* and *Stipa spartea* and very high levels of *Rudbeckia hirta* but moderate to high levels of the *Petalostemon candidium* and *Petalostemon purpureus*, and moderate to high levels of the key larval food plants, but the levels of adult nectar sources may not be sufficient given that Dakota Skipper prefer *Rudbeckia hirta* over other flowers. Only one invasive, non-native species, *Melilotus officinalis*, was present at Site F, in low densities. At Site G, *Medicago sativa* was present in patches at high densities, while *Melilotus alba* densities were moderate and *Melilotus officinalis* densities were low. Both Site F and G

were subject to regular fall haying when conditions permitted. Considerable portions of both sites have experienced abnormal flooding in 2011 which persisted well after adjacent parcels had drained. At Site F the prolonged flooding and drainage impediments resulted in loss of a large area of habitat previously suitable for Dakota Skipper. At Site G, the flooding was associated with Plum Creek and resulted in inundation or alteration of a large area of habitat in proximity to the creek. The prolonged inundation of water likely caused larval mortality and a mortality of suitable nectar plants and larval food plants. Approximately 25% of suitable habitat at each of these sites remained free of flooding in 2011.

The decline in Dakota Skipper at Sites F and G is potentially due to insufficient levels of *Rudbeckia hirta*, a decline in the quality of the habitat associated with invasive plants and the loss of habitat associated with flooding. There may be other impacts to the sites which are not apparent. The pasturing of cattle at Site F in early summer 2012 also appears to have significantly degraded the remaining habitat at the site and eliminated the flower buds of nectar plants in 2012. Nectar is vital to adults as a hydration and energy source to prevent desiccation, and provide resources for egg laying and mating. Thus removal of nectar plants at this site would potentially force existing skipper adults to seek nectar elsewhere. Cattle grazing also caused soil rutting and compaction, removed larval food plants (grasses) and likely trampled larvae. The extent of the impact of cattle pasturing at Site F upon Dakota Skipper populations and the habitat at the site remains unclear at present.

Site H has the highest populations of Dakota Skipper among all sites but nonethe-less the population appears to be on the decline. In 2003, 21 individuals were found at the site during a brief visit, while in a more intensive survey in 2007, 273 individuals were observed. During the present study 19 individuals were observed at the site in 2010, at least 170 individuals were observed in 2011 and 32 individuals were observed in 2012. Caution should be taken when assessing adult counts in 2010 and 2012 as less visits to Site H were made to this site than in 2011. Also in 2010 the surveyors were less skilled at locating Dakota Skippers. Despite less visits in 2010 and 2012, the population has declined by approximately 60%, assuming 2007 and 2011 were the most representative counts. The cause for the decline at this site remains unclear. The site has been subject to regular fall haying, and spring flooding levels that do not seem to be out of the ordinary compared to historic flooding levels. The variety of butterflies and flora at the site were as high as or higher than other sites. The edaphic and edaphic-related parameters were generally comparable to the other sites. There were high levels of the larval and adult food plants with high densities of *Andropogon scoparius* and *Rudbeckia hirta*, moderate to high densities of the other key nectar plants, moderate densities of *Stipa spartea* and low densities of *Andropogon gerardii*. Perhaps Site H has the highest population of Dakota Skippers because it has the widest assortment of larval and adult food plants, with high densities of certain key food and nectar plants. There were high levels of the invasive *Melilotus alba* and *Melilotus officinalis* which indicate some habitat degradation, but these patches are quite localized at the site. Otherwise, there were no other evident impacts from the data gathered in this study, and the cause for the decline of Dakota Skippers at Site H remains unclear.

In conclusion, populations of Dakota Skipper at all the sites appear to be on the decline, although there are no clearly identifiable causes this decline could be attributed to a variety of factors Sites A and E, appear to have degraded to the point that they may be unsuitable for Dakota Skipper. The decline in Sites A and E are probably related to a loss of key larval or adult food plants, and/or an abundance of non-native, invasive species. The perceived population declines may also be attributed to a difference in density estimations. Dakota Skipper adults are not uniformly distributed across their habitat and instead are aggregated in small patches, which past population estimates did not factor into their estimations.

Results of the vegetation assessment indicate a correlation with the presence of certain key plants and Dakota Skipper. Sites found to support Dakota Skipper had large amounts of either *Andropogon gerardii* or *Andropogon scoparius* and both species had to be present at the site, as was *Stipa spartea*. The abundance of *Rudbeckia hirta* did not seem to correlate with Dakota Skipper abundance but the sites that were positive for Dakota Skipper also had moderate to high levels of several of the following nectar species: *Rudbeckia hirta*, *Petalostemon candidium*, *Petalostemon purpureus*, *Campanula rotundifolia*, *Lobelia spicata*, *Agoseris glauca*, *Cirsium flodmanii*, *Crepis runcinata* and *Solidago ptarmicoides*. There may be a requirement for a particular grouping of larval

and adult food plants at sufficient densities. In some sites, prolonged flooding has reduced the amount of available habitat area as have other anthropogenic forces (fire, recreational vehicle damage and cattle grazing, conversion to agricultural crops, etc.). Like many species of insects perhaps Dakota Skipper populations in Manitoba experience cyclic expansions and contractions, and may always be generally low or very local in nature (Klassen et al. 1989; Layberry et al. 1998). Given the extremely low number of skippers observed in this study, the question can be asked – when are levels too low to maintain viable populations?

#### 7.2 Towards Identification of Dakota Skipper Critical Habitat

As noted in Section 2.2, Critical Habitat is defined under SARA as "the habitat that is necessary for the survival or recovery of a listed wildlife species" (Government of Canada 2002). With Dakota Skipper, this would be the habitat that it requires for survival and all the elements within that habitat that it requires for feeding, shelter, growth, reproduction and dispersal.

Broadly, Dakota Skipper critical habitat in Manitoba consists of tallgrass prairie or medium to tallgrass prairies, which have been subject to minimal disturbance. At present, extant Dakota Skipper sites can be found in the Interlake region and southwest Manitoba. The prairie communities examined in this study with and without Dakota Skipper range from high quality sites subject to only light disturbance to sites that appear to be degraded beyond the parameters required for a natural prairie ecosystem in Manitoba. Fall haying appears to be the best management regime currently in use to maintain Dakota Skipper habitat. Controlled burns are inadvisable as current practices appear to not adequately consider impacts to Dakota Skipper, too much habitat may be burned to permit recolonization, and accidental burns have been known to burn areas left intentionally unburned (Swengel 1996; Swengel 1998; Morden 2006).

Dakota Skipper sites in Manitoba generally consist of higher, dryer prairies adjacent to lower areas with sedges. The majority of the following species are present at sites and generally characterize the sites in Manitoba: Andropogon gerardii, Andropogon scoparius, Deschampsia caespitosa, Panicum virgatum, Poa cusickii, Stipa spartea, Eleocharis palustris, Lilim philadelphicum, Allium stellatum, Zygadenus elegans, Zygadenus gramineus, Hypoxis hirsuta, Rosa arkansana, Glycyrrhiza lepidota, Petalostemon candidum, Petalostemon purpureus, Polygala senega, Zizia aptera, Galium boreale, Campanula rotundifolia, Lobelia spicata, Achillea millefolium, Agoseris glauca, Aster ericodes or A. falcatus, Aster laevis, Cirsium flodmanii, Crepis runcinata, Erigeron annuus, Helianthus maximilianii, Helianthus nuttallii, Liatris ligulistylis, Rudbeckia hirta, Solidago ptarmicoides, Solidago rigida and Solidago spathulata.

The previously mentioned species are all native species and many are endemic to tallgrass prairie. Given that the sites are prairies, grass species dominate in Dakota Skipper habitat. Sedges, such as *Eleocharis palustris* are also a prominent group of plants present and important at sites. Dakota Skipper spend their entire life cycle in prairie habitat and adults are believed to be reluctant to venture far outside suitable habitat. Thus all the elements that the species requires for survival must be present at the sites that the adults occupy.

Reproductive adults require large enough areas of suitable prairie habitat to allow for mate seeking and oviposition. They also require prominent grasses, flowers and other plants for perching, to rest and watch for potential mates and predators. During courtship and copulation, stalks of grasses and other plants are required for perching and interaction. Following copulation, adult females are presumed to require nectar as an energy source for egg development. Females then lay their eggs on suitable larval food plants, or on other plant species in proximity to suitable larval food plants. Female and male Dakota Skipper adults also nectar on particular flower species throughout the flight period for hydration and as a general energy source.

When larvae hatch from eggs attached to a blade of grass they drop to the ground and must be able to find the appropriate food plant and build a shelter at the base of a bunch grass. Larvae are nocturnal and leave their shelters at night to clip off a piece of food plant and return to their shelter to feed. Dakota Skippers overwinter in the larval stage, where they build a shelter just below the soil surface in a clump of bunch grass, waterproofing these shelters with a waxy hydrofuge substance. Thus Dakota Skipper critical habitat must include those plant species required for feeding, shelter, perching and mating.

In Manitoba, *Rudbeckia hirta* is the main nectar source for Dakota Skipper and probably of vital importance to Dakota Skipper survival. Other documented or possible

nectar species utilized present at sites this study included: Lilium philadelphicum, Zygadenus elegans, Astragalus striatus, Medicago sativa, Melilotus alba, Melilotus officinalis, Petalostemon candidum, Petalostemon purpureus, Trifolium pratense, Oenothera biennis, Asclepias speciosa, Campanula rotundifolia, Lobelia spicata, Achillea millefolium, Agoseris glauca, Cirsium flodmanii, Crepis runcinata, Erigeron annuus, Gaillardia aristata, Ratibida columnifera and Solidago ptarmicoides. Zygadenus gramineus, Astragalus canadensis (Canada milk-vetch), Asclepias ovalifolia (dwarf milkweed), Chrysopsis villosa (hairy goldenrod), Cirsium arvense, Erigeron asper (rough fleabane), Erigeron caespitosa (tufted fleabane), Erigeron glabellus (smooth fleabane), Erigeron lonchophyllus (hirsute fleabane), Erigeron philadelphicus (Philadelphia fleabane) and Lactuca pulchella.

Previously documented or potential larval food plant species present at sites in this study included: Andropogon gerardii, Andropogon scoparius, Bromus inermis and Stipa spartea. Panicum virgatum, Poa canbyi (Canby blue grass), Poa compressa, Poa cusickii, Poa secunda, Carex aurea (golden sedge), Carex parryana (Parry's sedge), Carex siccata (hay sedge), Carex tetanica and Eleocharis palustris. All of these larval food plant species may serve as shelters for larvae, as would other bunchgrass species in Dakota Skipper habitat. It should be noted that this description of Dakota Skipper habitat is based upon eight sites in Manitoba and does not include habitat in Saskatchewan. The habitat in Saskatchewan differs in vegetation composition and relief (COSEWIC 2003; Environment Canada 2007); further studies are required to determine Dakota Skipper Critical Habitat in Saskatchewan.

Several other plant or animal species-at-risk were observed at the study sites, in prairie patches and near to Dakota Skipper habitat. Those species included:

- small white lady's-slipper (*Cypripedium candidum* Muhl. ex Willd. ) which was present at a site in the Interlake and is ranked as Endangered federally and provincially (Manitoba Conservation n.d.; NatureServe n.d.);
- Sprague's Pipit (*Anthus spraguell* Audubon) which was confirmed breeding (fledged young) at a site in southwest Manitoba and is ranked as Threatened federally and provincially (COSEWIC 2010a; NatureServe n.d.);

- Bobolink (*Dolichonyx oryzivorus* Linnaeus) which was probably breeding (on territory) at Sites B and G, possibly breeding (singing) at Site C, and is ranked as Threatened federally (COSEWIC 2010b);
- Barn Swallow (*Hirundo rustica* Linnaeus) which was confirmed breeding at Site C (adult carrying fecal sac) and Site G (adult entering nest site), probably breeding at Site A and B (on territory) and is ranked as Threatened federally (COSEWIC 2011); and,
- Monarch (*Danaus plexippus* Linnaeus) which was present at all eight sites and which is ranked as Special Concern federally (COSEWIC 2010c).

The *Cypripedium candidum* and *Anthus spraguell* are dependent upon high quality prairie habitat much like the Dakota Skipper (COSEWIC 2010a; Manitoba Conservation n.d.). Site locations for *Cypripedium candidum* and *Anthus spraguell* available on request. *Dolichonyx oryzivorus, Hirundo rustica* and *Danaus plexippus* are widespread species that breed and/or feed in prairie habitats; these species are generally not considered prairie specialists (COSEWIC 2010b; COSEWIC 2010c; COSEWIC 2011). However, the fact that these species occur at the sites, may confirm that they meet many of the criteria required for high quality prairie habitat. Sites were not intensively surveyed for species-at-risk, rather the above mentioned species were incidentally noted at the sites. There is a high potential that other species-at-risk occur at the sites as well, and additional surveys are suggested.

## 7.3 Future Study Design Improvements and Research Directions

There are several aspects to this project that could be improved upon or expanded. In addition to mid-June and mid-August vegetation surveys, the vegetation surveys could also be done in mid-July in order to capture plant species that mature in mid-summer such as *Stipa spartea*. *Stipa spartea* is small in June and absent by mid-August. Furthermore, during the June surveys there could also be more rigorous identification of the immature grasses so that fewer taxa were placed into the "Unknown Grasses" category. In July and August the sedges and rushes could also be included in the vegetation surveys, not just in June, as many of the species at the sites do continue to grow in late summer. Additional skill at identifying graminoids would be an asset as this is a very challenging group to identify, particularly when plants are immature or lacking inflorescences.

It may be a benefit to expand the width of the vegetation transects to encompass more of the lower, sedge areas that often form a 'ridge' immediately adjacent to upland prairie habitat since skippers were seen flying into these areas. It remains unclear how or why Dakota Skipper use these areas, so an inventory of the flora in sedge habitat would be beneficial. Furthermore, additional observations of adult behaviour would be beneficial to better determine the adults behaviour in sedge areas.

Measurements of the small-scale relief within sites may be a worthwhile exercise to visualize these subtle ridges between the upper prairie areas and the lower sedge areas. If the position and movement of adult Dakota Skippers were tracked within a site, geographic information systems (GIS) could be used to look at the spatial relationships between the patches of prairie habitat and lower sedge areas.

The location of adults could also be potentially related to the land uses and impacts within the site. For instance, adult location relative to the limits of the annual haying and aspen encroachment, areas intensively grazed by cattle or burned areas may explain habitat usage. The location and movement of adults could be evaluated relative to particular patches of nectar flowers or larval food plants on site. GIS analysis may reveal trends in how the females utilize particular areas of larval food plants for oviposition. Further studies on the relationship between oviposition, larval food plants and larval location within sites are needed. Dakota Skipper spend most of its life as a larva and yet we know little about this life stage. In that regard, further studies evaluating which species of graminoids larvae feed upon in Canada are also needed. Are the larvae also feeding upon the sedge and blue grass species at the sites? How diverse is their diet? Are they very selective and feeding on only one grass species or are they opportunistic and feeding on multiple species? The composition of the flora at sites in Manitoba differs from habitat in the United States, so results from larval feeding studies in the United States may not be fully applicable in Canada.

Given the percieved differences in flora at sites in Manitoba and the United States, it would be worthwhile to perform an in-depth comparison between the overall vegetation, nectar flowers and larval food plants in each of these jurisdictions. This could provide a better understanding of the variation in habitat sub-types across the range of Dakota Skipper. It would also be worthwhile to further compare the results of this study with the flora at the Tall Grass Prairie Preserve (TGPP) in Manitoba, as Dakota Skipper were found here until recently (COSEWIC 2003). A comparison of edaphic and edaphic-related features between existing sites and the TGPP may also be beneficial in understanding why Dakota Skipper is absent from southeastern Manitoba.

Since the majority of Dakota Skipper sites in Manitoba are in proximity to waterbodies or watercourses and flooding occurs at many sites, it may be worthwhile to further study the surface and subsurface hydrological conditions at the sites. Site hydrology appears to have an important impact upon Dakota Skipper and their habitat. In many instances the poor drainage or periodic flooding make these parcels poor candidates for cultivation and crop production, leaving them as prairie. However, the inundation also causes a direct loss of habitat and/or soil saturation which may affect the growth of key vegetation or result in larval mortality. Sufficient humidity and moisture is also required at sites to ensure the survival of key plants, and prevent larval and adult desiccation. Dakota Skipper most likely require a certain combination of hydrological, edaphic, temperature and humidity conditions to survive.

Further assessment of the extent of the impact of fire at Site D and cattle grazing at Site F should be undertaken. The physical limits of disturbance should be measured. Additionally, intensive, regular counts of the adult population should be undertaken to determine long term populations levels, identify possible decline or growth, and impact of future disturbances. Changes in vegetation composition should be monitored as well.

In order to better gauge adult densities, adult counts at all the sites should be done more frequently and regularly. More skilled personnel are needed for such an undertakings and this is the only way that accurate population estimates can be made across Dakota Skipper range in Canada.

## 7.4 Land Management, Stewardship and Impact Reduction Recommendations

At present fall having appears to be the best land use regime to maintain the tallgrass prairie habitat in Manitoba, which Dakota Skipper requires for survival. Annual having commencing in late August should be continued at sites and occur at minimum every two to five years depending upon the site. In the U.S. short term low density cattle

grazing and small scale rotational sprint or fall burning have been found to be compatible with Dakota skipper survival in some extant sites. Generally Canadian sites which have experienced wildfire often result in burning the full extend of available habitat. This is further compounded by the fact that there are no nearby sites with Dakota skipper that may repopulate these burned sites. Therefore at this time fire is not recommended as a management option in Manitoba sites. Fire officials should be informed in advance of sites that contain Dakota Skipper to minimize disturbance caused by their fire abatement measures. The impacts of cattle grazing upon Dakota skipper at sites in Canada have not been explored and caution should be taken when grazing cattle at Dakota skipper sites.

Excessive flooding and water impoundment at sites remains a concern in terms of lost habitat. Road repairs in 2011 immediately north of Site E likely impeded the natural drainage of water off site and have resulted in the expansion of the depressed wet area into a large wetland. If this site is to continue to support Dakota Skipper natural drainage needs to be restored to the area. The excessive flooding and subsequent ponding of water at Site G also remains a concern. A review of the actual cause of this flooding should be undertaken if possible to ascertain what, if any, measures can be taken in the future to reduce the loss of habitat.

The owners of property with Dakota Skippers should be supplied with management recommendations and guidance on how to preserve their populations of Dakota Skipper. If possible, the owners should be invited to enter into stewardship agreements or other programs to conserve Dakota Skipper and their habitat. This is particularly important at Site H, which appears to have the highest population of Dakota Skipper in Canada and potentially the best remaining habitat.

# 8.0 CONCLUSIONS

- Land management history at the study sites was generally similar with most sites being used for hay production. Hay is harvested in late summer which appears to be conducive to Dakota Skipper larval survival. Haying at Sites B and D has been infrequent over the past eight years and aspen encroachment is gradually decreasing available remaining Dakota Skipper habitat. Site A was previously cultivated, cattle grazing occurred at Site F in early summer 2012, and a wild fire occurred at Site D in fall of 2012. Recreational vehicles at Site B caused habitat disturbance, and excessive water impoundment at Sites F and G resulted in a loss of habitat over a large area.
- The floral and edaphic features at the study sites were generally similar, though some 2. regional and site differences were found. Dakota Skipper habitat can be characterized by a particular suite of indicator plants, and the presence of several larval food plants and adult nectar source plants. The research sites in this study were generally characterized by the presence of several documented and potential larval food plants, including: Andropogon gerardii, Andropogon scoparius, Poa cusickii and Eleocharis palustris. Sites also contained many documented or newly recorded adult nectar plant species including: Zygadenus elegans, Petalostemon candidum, Petalostemon purpureus, Campanula rotundifolia, Lobelia spicata, Achillea millefolium, Agoseris glauca, Cirsium flodmanii, Crepis runcinata, Erigeron annuus, Rudbeckia hirta and Solidago ptarmicoides. Finally all sites contained other plant species commonly associated with the presence of Dakota Skipper including: Deschampsia caespitosa, Panicum virgatum, Allium stellatum, Hypoxis hirsuta, Rosa arkansana, Glycyrrhiza lepidota, Polygala senega, Zizia aptera, Galium boreale, Aster ericodes or A. falcatus, Aster laevis, Helianthus maximilianii, Helianthus nuttallii, Liatris ligulistylis, Solidago rigida and Solidago spathulata.
- 3. Specific soil and soil-related conditions which broadly characterize Dakota Skipper habitat include:
  - 10 to 15% bare ground;
  - duff layer 1.5 to 4.0 cm deep;

- soil pH of 7.0 to 8.0 (with the 0.01 M CaCl<sub>2</sub> method);
- average gravimetric moisture content of 45 to 50%;
- soil compaction at 10 cm of 550 to 1000 kPA;
- bulk density ranging from 0.75 to 1.30 kg/L;
- soil calcium content of approximately 3000 to 5500 mg/kg;
- soil magnesium content of approximately 1000 to 2500 mg/kg;
- soil sodium content greater than 30 mg/kg;
- mean air temperature at the soil surface of 18°C from Julian week 28 to 39, and
- mean relative humidity at the soil surface of 85% from Julian week 28 to 39.
- 4. While there is some overlap in the vegetation composition within Dakota Skipper habitat in Manitoba and habitat in the United States, there are also different species of larval host plants and nectar plants present in Manitoba study sites. Adult Dakota Skipper in Manitoba nectar largely on *Rudbeckia hirta* and occasionally on other species. Several new nectar records were documented in this study. The skippers are preferentially nectaring upon different species and at different rates then observed in the United States.
- 5. The average Dakota Skipper adult flight period in 2010, 2011 and 2012 ranged from 13 to 19 days with males emerging prior to females. A mechanism to detect the start of the Dakota Skipper flight period was proposed using the timing of emergence of other more common skipper species that share these sites. Densities of adult Dakota Skipper was estimated for each site. Dakota Skipper adults were absent from several sites which had previously contained skippers, and the largest adult population (Site H) was estimated to be approximately 332 individuals in 2011. Dakota Skipper populations at all the sites appear to have dramatically declined.
- 6. The COI region was successfully amplified and sequenced demonstrating that removal of single Dakota Skipper mid-leg is an effective way to non-lethally sample tissue for molecular analysis. Two sequence polymorphisms were identified and found to be associated with the primer combinations used. The ultimate cause of this polymorphism remains unknown.

- The majority of the following species are present at sites and generally characterize Dakota Skipper critical habitat:
  - Andropogon gerardii;
  - Andropogon scoparius;
  - Deschampsia caespitosa;
  - Panicum virgatum;
  - Poa cusickii;
  - Stipa spartea;
  - Eleocharis palustris;
  - *Lilim philadelphicum*;
  - Allium stellatum;
  - Zygadenus elegans;
  - Zygadenus gramineus;
  - Hypoxis hirsuta;
  - Rosa arkansana;
  - *Glycyrrhiza lepidota*;
  - Petalostemon candidum;
  - Petalostemon purpureus;
  - Polygala senega;
  - Zizia aptera;

- Galium boreale;
- Campanula rotundifolia;
- Lobelia spicata;
- Achillea millefolium;
- Agoseris glauca;
- Aster ericodes or A. falcatus;
- Aster laevis;
- Cirsium flodmanii;
- Crepis runcinata;
- Erigeron annuus;
- Helianthus maximilianii;
- Helianthus nuttallii;
- Liatris ligulistylis;
- Rudbeckia hirta;
- Solidago ptarmicoides;
- Solidago rigida; and,
- Solidago spathulata.

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## APPENDICES

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Equisetaceae Juncaginaceae Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae)	Equisetum spp. Triglochin maritima Agropyron albicans Agropyron repens Agropyron smithii	L. Scribn. & Smith (L.) Beauv. Rydb.	<i>Pascopyrum smithii</i> (Rydb.) A. Löve	horsetail spp. seaside arrow-grass awned northern wheatgrass quack grass, couch grass western wheatgrass	х
Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae)	Agropyron spp. Agropyron subsecundum Agropyron trachycaulum Agrostideae spp. Agrostis stolonifera	(Link) Hitchc. (Link) Malte L.	<i>A. alba</i> var. stolonifera (L.) Sm.; <i>A. palustris</i> Huds.	unknown wheatgrass awned wheatgrass slender wheatgrass unknown Agrostidae redtop	х
Graminae (Poaceae) Graminae (Poaceae)	Andropogon gerardii Andropogon scoparius	Vitman Michx.	Schizachyrium scoparium (Michx.) Nash.	big bluestem little bluestem	
Graminae (Poaceae) Graminae (Poaceae)	Bromus inermis Bromus porteri	Leyss. Nash	<i>B. anomalus</i> auct. non Rupr. ex Fourn.	smooth brome nodding brome	Х
Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae)	Deschampsia caespitosa Festuca octoflora Helictotrichon hookeri Hordeum jubatum Koeleria gracilis	(L.) Beauv. Walt. (Scribn.) Henr. L. Pers.	<i>K. cristata</i> (L.) Pers.; <i>K. macrantha</i> (Ledeb.) J.A. Schultes	tufted hair grass six-weeks fescue Hooker's oat grass wild barley, foxtail barley June grass	
Graminae (Poaceae)	Muhlenbergia asperifolia	(Nees & Mey.) Parodi		scratch grass	
Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae) Graminae (Poaceae)	Muhlenbergia racemosa Muhlenbergia richardsonis Panicum virgatum Phleum pratense Poa canbyi Poa compressa Poa cusickii	(Michx.) BSP. (Trin.) Rydb. L. L. (Scribn.) Piper L. Vasey		marsh muhly mat muhly switch grass Timothy Canby blue grass Canada blue grass early blue grass	x x

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Graminae (Poaceae)	Poa secunda	Presl		Sandberg's blue grass	
Graminae (Poaceae)	Poa spp.			blue grass	
Graminae (Poaceae)	Scolochloa festucacea	(Willd.) Link		spangletop	
Graminae (Poaceae)	Sorghastrum nutans	(L.) Nash		Indian grass	
Graminae (Poaceae)	Spartina pectinata	Link Trin.	Hesperostipa spartea	prairie cord grass	
Graminae (Poaceae)	Stipa spartea	11111.	(Trin.) Barkworth	porcupine grass	
Graminae (Poaceae)	Unknown grass			unknown grasses	
Graminae (Poaceae)	Unknown grass (cultivar?)			unknown grass (cultivar?)	
Cyperaceae	Carex aurea	Nutt.		golden sedge	
Cyperaceae	Carex parryana	Dewey		Parry's sedge	
Cyperaceae	Carex siccata	Dewey	C. foenea Willd.	hay sedge	
Cyperaceae	Carex spp.	Cable		unknown sedges	
Cyperaceae Cyperaceae	Carex tetanica Eleocharis palustris	Schk. (L.) R. & S.	<i>E. smallii</i> Britt.	rigid sedge creeping spike-rush	
Juncaceae	Juncus balticus	Willd.	<i>J. arcticus</i> var. <i>balticus</i>	Baltic rush	
Juncaceae	Juneus Balleus	wind.	(Willd.) Trautv.	Datte fush	
Liliaceae	Allium stellatum	Fraser		pink-flowered onion	
Liliaceae	Lilium philadelphicum	L.	L. umbellatum Pursh	wood lily, tiger lily	
Liliaceae	Smilacina stellata	(L.) Desf.	<i>Maianthemum stellatum</i> (L.) Link	star-flowered solomon's-seal	
Liliaceae	Tofieldia glutinosa	(Michx.) Pers.		sticky asphodel	
Liliaceae	Zygadenus elegans	Pursh	<i>Anticlea elegans</i> (Pursh) Rydb.	smooth camas	
Liliaceae	Zygadenus gramineus	Rydb.	Toxicoscordion	death camas	
Lindoodo	Zygadonao grammouo	rtydd.	gramineum Rydb.		
Amaryllidaceae	Hypoxis hirsuta	(L.) Covillle		yellow star-grass	
Iridaceae	Sisyrinchium montanum	Greene		common blue-eyed grass	
Orchidaceae	Spiranthes romanzoffiana	Cham.		hooded lady's tresses	
Orchidaceae	Unknown orchid	N.C. I		unknown orchid	
Salicaceae	Populus tremuloides	Michx.		aspen poplar, trembling aspen	
Salicaceae Salicaceae	Salix bebbiana	Sarg.		beaked willow unknown willow	
Betulaceae	Salix spp. Betula glandulifera	(Regel) Butler	<i>B. pimula</i> var.	swamp birch, dwarf birch	
Detulaceae	Detula glandulliera	(Regel) Duller	glandulifera Regal; B. glandulosa var. glandulifera Regal	Swamp birch, uwan birch	
Santalaceae	Comandra umbellata	(L.) Nutt.	<i>C. pallida</i> A. DC;	bastard toadflax	
		( )	<i>C.richardsiana</i> Fern.		

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Polygonaceae	Polygonum amphibium	L.	<i>P. coccineum</i> (Willd.) Muhl.; <i>Persicaria</i> <i>amphibia</i> (L.) S.F. Gray	swamp persicaria, water smartweed	
Nyctaginaceae	Mirabilis hirsuta	(Pursh) MacM.	Oxybaphus hirsutus (Pursh) Sweet.	hairy umbrellawort	
Caryophyllaceae	Arenaria lateriflora	L.	Moehringia lateriflora (L.) Fenzl.	blunt-leaved sandwort	
Ranunculaceae	Anemone canadensis	L.		Canada anemone	
Ranunculaceae	Anemone multifida	Poir.		cut-leaved anemone	
Ranunculaceae	Ranunculus cymbalaria	Pursh		seaside buttercup	
Ranunculaceae	Thalictrum dasycarpum	Fishch. & Lall.		tall meadow-rue	
Ranunculaceae	Thalictrum venulosum	Trel.	T. confine Fern.	veiny meadow-rue	
Crucifera (Brassicaceae)	Erysimum inconspicuum	(S. Wats.) MacM.	<i>E. parviflorum</i> Nutt.	small flowered prairie-rocket	
Saxifragaceae	Parnassia glauca	Raf.	P. americana Muhl.	glaucous grass-of-parnassus	
Saxifragaceae	Parnassia palustris	L.		northern grass-of-parnassus	
Rosaceae	Fragaria vesca	L.	<i>F. americana</i> (Porter) Britt.	American wild strawberry	
Rosaceae	Fragaria virginiana	Dcne.	<i>F. glauca</i> (S. Wats.) Rydb.	smooth wild strawberry	
Rosaceae	Potentilla anserina	L.	<i>Argentian anserina</i> (L.) Rydb.	silverweed	
Rosaceae	Potentilla arguta	Pursh	<i>Drymocallis agrimonioides</i> (Pursh) Rydb.	white cinquefoil	
Rosaceae	Potentilla fruticosa	L.	P. floribunda Pursh; Dasiphora floribunda (Pursh) Kartesz	shrubby cinquefoil	
Rosaceae	Potentilla pensylvanica	L.		prairie cinquefoil	
Rosaceae	Rosa arkansana	Porter		low prairie rose	
Leguminosae (Fabaceae)	Astragalus canadensis	L.		Canadian milk-vetch	
Leguminosae (Fabaceae)	Astragalus danicus	Retz.	A. goniatus Nutt.; A. hypoglottis Richardson; A. agrestis Dougl.	purple milk-vetch	
Leguminosae (Fabaceae)	Astragalus striatus	Nutt.	A. adsurgens Hook.	ascending purple milk-vetch	
Leguminosae (Fabaceae)	Glycyrrhiza lepidota	(Nutt.) Pursh		wild licorice	
Leguminosae (Fabaceae)	Medicago lupulina	L.		black medick	Х
Leguminosae (Fabaceae)	Medicago sativa	L.	M. falcata L.	alfalfa	Х
Leguminosae (Fabaceae)	Melilotus alba	Medic.		white sweet-clover	Х

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Leguminosae (Fabaceae)	Melilotus officinalis	(L.) Pall.		yellow sweet-clover	Х
Leguminosae (Fabaceae)	Melilotus spp.			sweet-clover spp.	Х
Leguminosae (Fabaceae)	Petalostemon candidum	(Willd.) Michx.	Dalea candida Michx.	white prairie-clover	
Leguminosae (Fabaceae)	Petalostemon purpureus	(Vent.) Rydb.	<i>Dalea purpurea</i> Vent.	purple prairie-clover	
Leguminosae (Fabaceae)	<i>Psoralea</i> argophylla	Pursh	<i>Pediomelum argophyllum</i> (Pursh) J. Grimes; <i>Psoralidium agrophyllum</i> (Pursh) Rydb.	silverleaf psoralea	
Leguminosae (Fabaceae)	Psoralea esculenta	Pursh	Pediomelum esculentum (Pursh) Rydb.	indian breadroot	
Leguminosae (Fabaceae)	Trifolium pratense	L.		red clover	Х
Leguminosae (Fabaceae)	Unknown clover			unknown clover	
Leguminosae (Fabaceae)	Vicia americana	Mulh.		American vetch	
Leguminosae (Fabaceae)	<i>Vicia</i> spp.			vetch spp.	
Linaceae	Linum lewisii	Pursh	<i>L. perenne</i> var. <i>lewisii</i> (Pursh) Eat. & J. Wright	Lewis wild flax	
Polygalaceae	Polygala senega	L.		Seneca snakeroot	
Anacardiaceae	Rhus radicans	L.	Toxicodendron rydbergii (Small) Greene.	poison-ivy	
Violaceae	Viola cucullata	Ait.	V. nephrophylla Greene.	northern bog violet	
Elaeagnaceae	Elaeagnus commutata	Bernh.		silverberry, wolf-willow	
Onagraceae	Oenothera biennis	L.		yellow evening primrose	Х
Umbelliferae (Apiaceae)	Cicuta maculata	L.	<i>C. occidentalis</i> Greene; <i>C. douglasii</i> (DC) Coult. & Rose	water-hemlock	
Umbelliferae (Apiaceae)	Zizia aptera	(Gray) Fern.	Z. cordata (Walt.) Koch.	heart-leaved Alexanders	
Ericaceae	Arctostaphylos uva-ursi	(L.) Spreng.		bearberry	
Primulaceae	Dodecatheon pulchellum	(Raf.) Merr.	<i>Primula pauciflora var. pauciflora</i> (Greene) A.R. Mast & Reveal	saline shootingstar	
Primulaceae	Glaux maritima	L.		sea-milkwort	
Gentianaceae	Gentiana affinis	Griseb.	Pneumonanthe affinis (Griseb.) Weber; Dasystephana affinis (Griseb.) Rydb.; <i>G.</i> interrupta Greene	oblong-leaved gentian	
Gentianaceae	Gentiana amarella	L.	<i>Gentianella amarella</i> (L.) Boerner	northern gentian	

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Gentianaceae	Gentiana crinita	Froel.	Gentianopsis virgata (Raf.) Holub; Anthopogon crinitus (Froel.) Raf.	fringed gentian	
Asclepiadaceae	Asclepias ovalifolia	Dcne.	. ,	dwarf milkweed	
Asclepiadaceae	Asclepias speciosa	Torr.		showy milkweed	
Convolvulaceae	Convolvulus sepium	L.	<i>Calystegia sepium</i> (L.) R. Br.	hedge bindweed	Х
Boraginaceae	Lithospermum canescens	(Michx.) Lehm.		hoary puccoon	
Boraginaceae	Lithospermum incisum	Lehm.	L. angustifolium Michx.	narrow-leaved puccoon	
Labiatae (Lamiaceae)	Lycopus asper	Greene		western water-horehound	
Labiatae (Lamiaceae)	Monarda fistulosa	L.	<i>M. methaefolia</i> Graham	wild bergamot	
Labiatae (Lamiaceae)	Stachys palustris	L.	<i>S. pilosa</i> Nutt.	marsh hedge-nettle	
Scrophulariaceae	Castilleja coccinea	(L.) Spreng.		scarlet paintbrush (yellow form)	
Scrophulariaceae	Orthocarpus luteus	Nutt.		owl's-clover	
Scrophulariaceae	Pedicularis canadensis	L.		common lousewort, Canada lousewort	
Scrophulariaceae	Pedicularis lanceolata	Michx.		swamp lousewort	
Scrophulariaceae	Penstemon gracilis	Nutt.		lilac-flowered beardtongue	
Plantaginaceae	Plantago eriopoda	Torr.		saline plantain	
Plantaginaceae	Plantago major	L.		common plantain	Х
Rubiaceae	Galium boreale	L.	G. septentrionale R. & S.	northern bedstraw	
Caprifoliacieae	Lonicera caerulea	L.	<i>L. villosa</i> (Michx.) J.A. Schultes	blue fly honeysuckle	
Caprifoliacieae	Lonicera spp.			honeysuckle spp.	
Caprifoliaceae	Symphoricarpos occidentalis	Hook.		western snowberry; buckbush	
Campanulaceae	Campanula rotundifolia	L.		harebell	
Lobeliaceae	Lobelia kalmii	L.	<i>L. strictiflora</i> (Rydb.) Lunell.	Kalm's lobelia	
Lobeliaceae	Lobelia spicata	Lam.		spiked lobelia	
Compositae (Asteraceae)	Achillea millefolium	L.	A. lanulosa Nutt.	yarrow, milfoil	
Compositae (Asteraceae)	Agoseris glauca	(Pursh) Raf.		false dandelion, prairie dandelion	
Compositae (Asteraceae)	Ambrosia psilostachya	DC.		perennial ragweed	
Compositae (Asteraceae)	Antennaria spp.			everlasting spp., pussytoes spp.	
Compositae (Asteraceae)	Artemisia Iudoviciana	Nutt.		prairie sage	
Compositae (Asteraceae)	Aster ciliolatus	Lindl.	<i>Symphotrichium ciliolatum</i> (Lindl.) A. & D. Löve; <i>A. lindleyanus</i> Torr. & Gray	Lindley's aster	

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Compositae (Asteraceae) Compositae (Asteraceae)	A. ericodes or A. falcatus Aster ericoides	L.	Symphotrichium ericoides (L.) Nesom; A. pansus (Blake) Cronquist; A. multiflorus Ait.; A. adsurgens Greene.	"heath aster" many-flowered aster, white heath aster	
Compositae (Asteraceae)	Aster falcatus	Lindl.	Symphotrichium falcatum (Lindl.) Nesom; A. commutatus (Torr. & Gray) A. Gray	white prairie aster	
Compositae (Asteraceae)	Aster junciformis	Rydb.	Symphotrichium boreale (Torr. & Gray) A. & D. Löve	rush aster	
Compositae (Asteraceae)	Aster laevis	L.	Symphotrichium laeve (L.) A. & D. Löve	smooth aster	
Compositae (Asteraceae)	Aster puniceus	L.	Symphotrichium puniceum (L.) A. & D. Löve	purple-stemmed aster	
Compositae (Asteraceae)	Chrysopsis villosa	(Pursh) Nutt.	Heterotheca villosa (Pursh) Shinners	hairy golden-aster	
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Cirsium arvense Cirsium flodmanii Cirsium spp.	(L.) Scop. (Rydb.) Arthur		Canada thistle Flodman's thistle thistle spp.	Х
Compositae (Asteraceae)	Crepis runcinata	(James) Torr. & Gray		scapose hawk's-beard	
Compositae (Asteraceae) Compositae (Asteraceae)	Crepis tectorum Erigeron annuus	L. (L.) Pers.	<i>E. strigosus</i> Mulh.	narrow-leaved hawk's-beard whitetop, rough fleabane, daisy fleabane	Х
Compositae (Asteraceae) Compositae (Asteraceae)	Erigeron asper Erigeron caespitosus	Nutt. Nutt.		rough fleabane tufted fleabane	
Compositae (Asteraceae) Compositae (Asteraceae)	Erigeron glabellus Erigeron lonchophyllus	Nutt. Hook.	<i>Trimorpha lonchophylla</i> (Hook.) Nesom.	smooth fleabane hirsute fleabane	
Compositae (Asteraceae) Compositae (Asteraceae)	Erigeron philadelphicus Erigeron spp.	L.	. ,	Philadelphia fleabane fleabane spp.	
Compositae (Asteraceae) Compositae (Asteraceae)	Eupatorium maculatum Gaillardia aristata	L. Pursh		spotted Joe-pye weed great-flowered gaillardia, blanket flower	

Family	Scientific Name	Authority	Synonyms*	Common Name	Non- native*
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Grindelia squarrosa Helianthus laetiflorus Helianthus maximilianii	(Pursh) Dunal Pers. Schrad.	<i>G. perennis</i> A. Nels. <i>H. pauciflorus</i> Nutt.	gumweed beautiful sunflower narrow-leaved sunflower	
Compositae (Asteraceae) Compositae (Asteraceae)	Helianthus nuttallii Hieracium umbellatum	Torr. & Gray L.	H. canadense Michx.	tuberous-rooted sunflower Canada hawkweed, narrow- leaved hawkweed	
Compositae (Asteraceae) Compositae (Asteraceae)	Lactuca pulchella Liatris ligulistylis	(Pursh) DC (A. Nels.) K. Schum.	L. tatarica (L.) C.A. Mey.	blue lettuce meadow blazingstar	
Compositae (Asteraceae) Compositae (Asteraceae)	Packera aurea Prenanthes racemosa	(L.) A. & D. Löve Michx.	Senecio aureus L. Nabalus racemosus (Michx.) DC	golden ragwort glaucous white lettuce	
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Ratibida columnifera Rudbeckia hirta Senecio pauperculus Solidago canadensis	(Nutt.) Woot. & Standl. L. Michx. L.	Lepachy columnifera (Nutt.) Rydb. S. canadensis L. or S. altissima L., S. canadensis L. var. gilvocanescens Rydb.	long-headed coneflower, prairie coneflower black-eyed susan balsam groundsel Canada goldenrod, graceful goldenrod	
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Solidago gigantea Solidago missouriensis Solidago mollis Solidago ptarmicoides Solidago rigida	Ait. Nutt. Bartl. (Nees) Boiv. L.	S. serotina Ait. S. glaberrima Martens Oligoneuron album (Nutt.) Nesom; Aster ptarmicoides (Nees) Torr. & Gray Oligoneuron rigidum (L.)	late goldenrod low goldenrod velvety goldenrod upland white goldenrod, prairie goldenrod, upland white aster stiff goldenrod	
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Solidago spp. Solidago spathulata Sonchus arvensis	DC. L.	Small Solidago simplex Kunth spp. simplex S. uliginosus Bieb.	goldenrod spp. mountain goldenrod perennial sow-thistle	Х
Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae) Compositae (Asteraceae)	Taraxacum officinale Tragopogon dubius Unknown Asteraceae	Weber Scop.	o. unginosus dieb.	dandelion yellow goat's-beard unknown Asteraceae	X X X

\*Sources: Looman and Best 1981; Reaume 2009; Integrated Taxonomic Information System (ITIS) 2012; USDA NRCS 2012; NatureServe n.d.

						Region	& Site ('	% cover)				
Family	Scientific Name			Interlake					W Manito			- Total
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	
Equisetaceae	Equisetum spp.	0.00	0.08	0.00	0.00	0.02	0.16	0.49	0.31	0.00	0.24	0.13
Juncaginaceae	Triglochin maritima	0.00	2.92	0.00	0.00	0.73	0.15	0.00	1.83	0.42	0.60	0.66
Graminae	Agropyron repens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.58	0.00	1.15	0.57
Graminae	Agropyron smithii	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	1.67	0.83
Graminae	Agropyron spp.	3.33	0.00	0.00	2.25	1.40	0.00	0.00	0.00	0.00	0.00	0.70
Graminae	Agropyron subsecundum	0.00	0.42	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.05
Graminae	Andropogon gerardii	0.00	0.00	0.00	10.42	2.60	0.00	0.00	0.83	3.08	0.98	1.79
Graminae	Bromus inermis	0.83	0.00	0.00	1.25	0.52	5.08	0.00	0.00	0.00	1.27	0.89
Graminae	Deschampsia caespitosa	0.00	23.50	0.00	12.33	8.96	0.00	0.00	8.75	9.50	4.56	6.76
Graminae	Helictotrichon hookeri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.48	0.24
Graminae	Koeleria gracilis	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.21	0.10
Graminae	Muhlenbergia richardsonis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.17	0.08
Graminae	Panicum virgatum	0.00	0.00	0.00	0.42	0.10	0.17	0.00	6.25	0.58	1.75	0.93
Graminae	Phleum pratense	1.67	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.21
Graminae	Poa canbyi	0.42	0.00	0.00	0.00	0.10	1.83	0.00	0.00	0.00	0.46	0.28
Graminae	Poa compressa	0.00	0.00	0.00	0.00	0.00	0.08	0.00	5.17	1.33	1.64	0.82
Graminae	Poa cusickii	7.83	0.00	3.67	0.00	2.88	0.42	7.08	1.25	0.00	2.19	2.53
Graminae	Poa spp.	0.83	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.42	0.10	0.16
Graminae	Stipa spartea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.42	0.77	0.39
Graminae	Unknown grass	25.83	16.25	32.08	4.58	19.69	2.67	7.25	1.25	2.08	3.31	11.50
Cyperaceae	Carex aurea	0.08	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01
Cyperaceae	Carex parryana	0.00	0.00	0.17	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.02
Cyperaceae	Carex siccata	0.00	1.17	0.00	0.00	0.29	0.00	0.83	0.00	0.00	0.21	0.25
Cyperaceae	Carex spp.	0.08	1.42	4.92	2.50	2.23	1.58	3.33	6.08	2.58	3.40	2.81
Cyperaceae	Carex tetanica	0.74	0.16	0.00	0.83	0.43	0.00	0.00	0.00	0.00	0.00	0.22
Cyperaceae	Eleocharis palustris	1.67	3.75	3.41	9.17	4.50	0.00	0.00	0.00	0.00	0.00	2.25
Juncaceae	Juncus balticus	0.00	0.00	0.00	3.17	0.79	1.42	0.00	10.00	0.00	2.85	1.82
Liliaceae	Lilium philadelphicum	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.16	0.00	0.08	0.04
Liliaceae	Smilacina stellata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.08	0.10	0.05
Liliaceae	Tofieldia glutinosa	0.00	0.00	0.00	0.83	0.21	0.00	0.00	0.00	0.00	0.00	0.10
Liliaceae	Zygadenus gramineus	0.00	0.08	0.08	0.40	0.14	0.08	0.08	0.58	0.67	0.35	0.24
Amaryllidaceae	Hypoxis hirsuta	0.00	0.63	0.53	0.23	0.35	0.00	0.16	1.23	3.42	1.20	0.78
Iridaceae	Sisyrinchium montanum	0.30	0.00	0.15	0.00	0.11	0.00	0.00	0.00	0.41	0.10	0.11
Orchidaceae	Unknown orchid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.06	0.03
Salicaceae	Populus tremuloides	0.00	0.00	0.57	0.49	0.31	0.00	0.00	0.00	0.00	0.00	0.15
Salicaceae	Salix bebbiana	0.00	0.75	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.09
Salicaceae	Salix spp.	0.00	0.00	0.00	0.58	0.19	0.00	0.00	0.00	0.00	0.00	0.05
Betulaceae	Betula glandulifera	0.42	0.00	0.00	0.38	0.29	0.00	0.00	0.00	0.00	0.00	0.15
Santalaceae	Comandra umbellata	0.00	0.00	0.00	0.42	0.00	1.00	0.00	0.00	0.00	0.00	0.03

**Appendix IIa.** June 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

**Appendix IIa.** June 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

						Region	& Site (					
Family	Scientific Name			Interlake					W Manito			Tota
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	
Caryophyllaceae	Arenaria lateriflora	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01
Ranunculaceae	Anemone canadensis	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.08	0.00	0.06	0.03
Ranunculaceae	Anemone multifida	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.24	0.17	0.25	0.12
Ranunculaceae	Ranunculus cymbalaria	0.00	0.00	0.00	4.74	1.19	0.00	0.00	0.08	0.00	0.02	0.60
Ranunculaceae	Thalictrum dasycarpum	0.24	0.00	0.00	1.33	0.39	0.00	0.00	0.00	0.00	0.00	0.20
Ranunculaceae	Thalictrum venulosum	0.00	0.00	0.42	0.00	0.10	0.00	0.00	0.08	0.00	0.02	0.06
Saxifragaceae	Parnassia glauca	0.00	3.50	0.00	0.83	1.08	0.00	0.00	0.00	0.00	0.00	0.54
Rosaceae	Fragaria vesca	0.66	0.00	0.00	10.08	2.68	0.00	0.00	0.00	0.08	0.02	1.35
Rosaceae	Fragaria virginiana	0.00	0.25	0.83	2.75	0.96	0.00	0.00	0.00	0.00	0.00	0.48
Rosaceae	Potentilla anserina	0.92	2.99	0.00	0.00	0.98	0.00	0.00	2.67	0.08	0.69	0.83
Rosaceae	Potentilla fruticosa	0.00	1.25	0.00	0.83	0.52	0.00	0.00	0.00	0.00	0.00	0.26
Rosaceae	Potentilla pensylvanica	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.04	0.02
Rosaceae	Rosa arkansana	0.17	1.17	0.08	0.67	0.52	0.00	2.58	0.58	0.42	0.90	0.71
Leguminosae	Astragalus danicus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.08	0.04
Leguminosae	Glycyrrhiza lepidota	0.00	0.00	0.08	0.25	0.08	0.58	0.08	1.25	0.00	0.48	0.28
Leguminosae	Medicago lupulina	0.58	0.00	0.00	0.00	0.15	2.92	0.00	0.00	0.00	0.73	0.44
Leguminosae	Medicago sativa	0.00	0.00	1.67	0.00	0.42	7.92	0.00	0.00	0.00	1.98	1.20
Leguminosae	Melilotus alba	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.49	0.87	0.44
Leguminosae	Melilotus officinalis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.31	0.16
Leguminosae	Melilotus spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.10	0.05
Leguminosae	Petalostemon candidum	0.00	0.00	0.08	0.08	0.04	0.00	2.17	0.00	0.33	0.63	0.33
Leguminosae	Petalostemon purpureus	0.00	0.00	0.00	0.25	0.06	0.00	0.00	0.33	0.23	0.14	0.10
Leguminosae	Psoralea esculenta	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01
Leguminosae	Trifolium pratense	0.17	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.02
Leguminosae	Unknown clover	0.41	0.00	0.23	0.08	0.18	0.00	0.00	0.08	0.66	0.18	0.18
_equminosae	Vicia americana	0.00	0.00	0.00	0.08	0.02	0.00	0.00	0.00	0.08	0.02	0.02
Leguminosae	Vicia spp.	0.15	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.33	0.08	0.06
Linaceae	Linum lewisii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.06	0.0
Polygalaceae	Polygala senega	0.00	0.08	0.00	0.32	0.10	0.58	2.74	1.08	2.57	1.74	0.92
Violaceae	Viola cucullata	0.00	2.25	0.08	0.66	0.79	0.00	0.00	1.24	0.23	0.37	0.58
Elaeagnaceae	Elaeagnus commutata	0.00	0.58	0.08	0.25	0.23	0.00	0.00	0.17	0.00	0.04	0.13
Umbelliferae	Cicuta maculata	0.00	0.00	0.00	1.17	0.20	0.00	0.00	0.00	0.00	0.00	0.15
Umbelliferae	Zizia aptera	0.00	0.32	0.88	1.48	0.23	0.83	0.00	0.33	0.00	0.50	0.64
Gentianaceae	Gentiana affinis	0.00	0.02	0.00	0.17	0.04	0.00	0.42	0.00	0.40	0.00	0.02
Ericaceae	Arctostaphylos uva-ursi	0.00	2.50	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.02
Primulaceae	Dodecatheon pulchellum	0.00	1.42	0.08	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.32
	Glaux maritima	0.00	0.15	0.00	0.00	0.35					0.00	0.04
Primulaceae		0.00	0.15				0.00 0.00	0.00 0.00	0.08 0.00	0.00 0.00	0.02	0.04
Asclepiadaceae	Asclepias ovalifolia			0.00	1.25	0.31						-
Asclepiadaceae	Asclepias speciosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.02	0.0

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Appendix IIa.	June 2010 vegetation	i survey plant speci	les observed by sit	es and regions (% cover	per m, mean)

				late de l		Region	& Site (	% cover)				
Family	Scientific Name	IL19	IL24	Interlake IL39	e IL50	Total	OK1	OK6	W Manite OK7	OK14	Total	- Tota
Convolvulaceae	Convolvulus sepium	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.10	0.05
Boraginaceae	Lithospermum canescens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.15	0.07
Boraginaceae	Lithospermum incisum	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.02	0.01
Labiatae	Lycopus asper	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.02	0.01
Scrophulariaceae	Castilleja coccinea	0.08	0.25	0.00	0.33	0.16	0.00	0.00	0.00	0.00	0.00	0.08
Scrophulariaceae	Pedicularis canadensis	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.0
Scrophulariaceae	Pedicularis lanceolata	0.00	0.42	0.00	0.17	0.15	0.00	0.00	0.00	0.00	0.00	0.0
Plantaginaceae	Plantago eriopoda	0.00	0.00	0.23	0.00	0.06	4.33	0.00	5.83	1.08	2.81	1.4
Plantaginaceae	Plantago major	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.75	0.00	0.44	0.2
Rubiaceae	Galium boreale	0.00	0.16	0.58	0.58	0.33	0.00	0.00	0.74	0.83	0.39	0.3
Caprifoliacieae	Lonicera caerulea	0.83	0.00	0.08	0.00	0.23	0.00	0.83	0.00	0.00	0.21	0.2
Campanulaceae	Campanula rotundifolia	0.00	0.00	0.00	0.08	0.02	0.00	0.00	0.15	0.00	0.04	0.0
Compositae	Achillea millefolium	0.08	0.08	0.08	0.08	0.08	0.00	0.00	0.23	0.97	0.30	0.1
Compositae	Agoseris glauca	0.00	0.00	0.08	1.00	0.27	0.24	0.00	0.00	0.83	0.27	0.2
Compositae	Ambrosia psilostachya	0.00	0.00	0.15	0.00	0.04	0.39	0.08	0.00	0.00	0.12	0.0
Compositae	Antennaria spp.	0.00	0.00	1.42	0.00	0.35	5.00	0.15	1.33	0.66	1.78	1.0
Compositae	Artemisia ludoviciana	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.10	0.0
Compositae	Aster ciliolatus	0.42	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.0
Compositae	Aster ericoides	0.66	0.90	0.00	0.08	0.41	0.00	2.91	0.17	0.00	0.77	0.5
Compositae	Aster laevis	0.17	0.67	0.00	0.00	0.21	0.42	0.00	0.08	0.08	0.14	0.1
Compositae	Aster puniceus	0.00	0.00	0.00	1.40	0.35	0.00	0.00	0.08	0.00	0.02	0.1
Compositae	Cirsium arvense	0.00	0.00	0.00	0.00	0.00	1.58	0.00	0.00	0.58	0.54	0.2
Compositae	Cirsium flodmanii	0.33	0.08	0.66	0.24	0.33	0.67	1.08	0.25	0.42	0.60	0.4
Compositae	Crepis runcinata	0.00	0.58	0.00	0.24	0.21	0.00	1.16	1.99	0.00	0.79	0.5
Compositae	Crepis tectorum	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.02	0.0
Compositae	Erigeron caespitosus	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.0
Compositae	Erigeron philadelphicus	0.48	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.0
Compositae	Helianthus laetiflorus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.10	0.0
Compositae	Helianthus maximilianii	0.00	0.00	0.00	0.00	0.00	0.08	0.42	0.00	0.00	0.12	0.0
Compositae	Helianthus nuttallii	0.00	2.23	0.00	0.25	0.62	1.92	0.00	0.58	0.17	0.67	0.6
Compositae	Hieracium umbellatum	0.00	0.08	0.08	0.42	0.14	0.00	0.00	0.00	0.00	0.00	0.0
Compositae	Lactuca pulchella	0.00	0.00	0.00	0.00	0.00	0.00	1.83	0.00	0.00	0.46	0.2
Compositae	Liatris ligulistylis	0.00	0.17	0.00	0.00	0.04	0.67	0.00	0.49	1.08	0.56	0.3
Compositae	Packera aurea	0.42	0.24	0.00	0.15	0.20	0.00	0.00	0.00	0.00	0.00	0.1
Compositae	Rudbeckia hirta	2.58	0.08	0.65	0.49	0.95	0.00	0.17	0.25	0.40	0.20	0.5
Compositae	Solidago missouriensis	0.17	0.00	0.00	0.00	0.04	0.00	0.58	0.00	0.17	0.19	0.1
Compositae	Solidago rigida	0.48	0.00	0.24	1.08	0.45	0.42	0.00	0.00	0.00	0.10	0.2
Compositae	Solidago spathulata	0.49	0.25	0.48	0.00	0.30	0.83	0.83	0.00	0.00	0.41	0.3
Compositae	Sonchus arvensis	5.15	2.08	0.72	2.49	2.61	4.82	0.08	1.89	0.65	1.86	2.2

			Region & Site (% cover)									
Family	Scientific Name			Interlake	;			S	W Manit	oba		Total
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	Total
Compositae	Taraxacum officinale	0.00	0.00	0.00	0.49	0.12	0.33	0.00	0.00	0.00	0.08	0.10
Compositae	Tragopogon dubius	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.02	0.01
Compositae	Unknown Asteraceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.04	0.02

**Appendix IIa.** June 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

		Region & Site (% cover)											
Family	Scientific Name			Interlake					W Manito			Total	
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total		
Graminae	Agropyron albicans	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.00	2.67	1.15	0.58	
Graminae	Agropyron repens	13.83	1.08	0.00	1.25	4.13	5.24	0.00	11.33	8.25	6.21	5.18	
Graminae	Agropyron trachycaulum	0.00	0.33	1.18	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.18	
Graminae	Agrostideae spp.	1.25	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.16	
Graminae	Agrostis stolonifera	31.25	21.08	0.00	2.42	13.98	0.00	0.00	0.00	0.00	0.00	6.92	
Graminae	Andropogon gerardii	0.00	6.08	18.45	11.00	8.68	0.00	4.00	14.25	0.08	4.58	6.61	
Graminae	Andropogon scoparius	0.00	12.50	0.00	12.50	6.38	7.50	45.42	5.67	19.42	19.50	13.01	
Graminae	Bromus porteri	0.00	0.50	1.08	0.17	0.42	0.00	0.00	0.00	0.00	0.00	0.21	
Graminae	Deschampsia caespitosa	0.00	5.83	7.09	0.00	3.15	0.00	0.00	3.17	5.17	2.08	2.61	
Graminae	Festuca octoflora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.25	0.13	
Graminae	Hordeum jubatum	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.41	0.00	0.12	0.06	
Graminae	Muhlenbergia asperifolia	0.00	0.00	0.00	0.00	0.00	0.25	0.57	0.00	0.00	0.20	0.10	
Graminae	Muhlenbergia racemosa	0.00	0.00	0.00	12.50	3.19	0.00	0.00	0.00	0.00	0.00	1.58	
Graminae	Muhlenbergia richardsonis	1.25	1.50	0.00	0.00	0.70	0.98	0.00	0.00	1.66	0.66	0.68	
Graminae	Panicum virgatum	0.00	0.00	3.00	1.42	1.06	2.49	2.06	6.33	0.00	2.72	1.90	
Graminae	Phleum pratense	2.67	0.00	1.82	0.83	1.32	0.00	0.00	0.00	0.00	0.00	0.65	
Graminae	Poa compressa	7.50	0.00	0.00	4.42	3.04	0.66	0.00	0.00	7.42	2.02	2.53	
Graminae	Poa cusickii	0.00	0.00	10.45	0.00	2.45	0.00	3.83	0.17	0.00	1.00	1.71	
Graminae	Poa secunda	0.00	7.33	0.00	0.00	1.87	0.00	0.00	0.00	0.00	0.00	0.93	
Graminae	Scolochloa festucacea	4.17	0.00	3.82	0.00	1.96	6.50	0.00	0.00	0.00	1.63	1.79	
Graminae	Sorghastrum nutans	0.00	0.00	0.00	5.67	1.45	0.00	0.00	0.00	0.00	0.00	0.72	
Graminae	Spartina pectinata	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.06	0.03	
Graminae	Stipa spartea	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.24	0.31	0.16	
Graminae	Unknown grass	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.25	0.13	
Graminae	Unknown grass (cultivar?)	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	1.67	0.84	
Liliaceae	Allium stellatum	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.25	0.06	0.04	
Liliaceae	Smilacina stellata	0.00	0.00	0.00	0.33	0.09	0.00	0.00	0.00	0.00	0.00	0.04	
Orchidaceae	Spiranthes romanzoffiana	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.04	0.02	
Salicaceae	Salix spp.	0.25	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.03	
Betulaceae	Betula glandulifera	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.04	0.02	
Santalaceae	Comandra umbellata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.04	0.02	
Polygonaceae	Polygonum amphibium	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00	0.00	0.15	0.07	
Ranunculaceae	Anemone canadensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.08	0.04	
Saxifragaceae	Parnassia glauca	0.00	0.25	0.00	3.74	1.02	0.00	0.00	0.00	0.00	0.00	0.50	
Rosaceae	Potentilla anserina	0.33	1.92	0.00	0.00	0.57	0.00	0.00	1.42	0.00	0.35	0.46	
Rosaceae	Potentilla arguta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.04	0.02	
Rosaceae	Rosa arkansana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.10	0.05	
Leguminosae	Glycyrrhiza lepidota	0.00	0.00	0.00	4.00	1.02	0.42	1.67	1.08	1.50	1.17	1.09	
	, , , , , , , , , , , , , , , , , , ,	2.00											

**Appendix IIb.** August 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

		Region & Site (% cover)											
Family	Scientific Name			Interlake	;			S	W Manit	oba		Total	
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total		
Leguminosae	Medicago lupulina	0.83	0.08	0.00	0.00	0.23	0.42	0.00	0.00	0.00	0.10	0.17	
Leguminosae	Medicago sativa	0.17	0.00	0.00	0.00	0.04	4.33	0.00	0.00	0.00	1.08	0.57	
Leguminosae	Melilotus alba	0.40	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.05	
Leguminosae	Melilotus officinalis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.83	0.27	0.14	
Leguminosae	Petalostemon candidum	0.00	0.00	2.09	0.33	0.57	0.08	2.33	0.91	1.25	1.14	0.86	
Leguminosae	Petalostemon purpureus	0.00	0.00	0.27	0.17	0.11	0.00	0.00	1.33	1.74	0.77	0.44	
Leguminosae	Psoralea esculenta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.15	0.07	
Leguminosae	Trifolium pratense	1.25	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.16	
Leguminosae	<i>Vicia</i> spp.	0.08	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	
Polygalaceae	Polygala senega	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.02	0.01	
Anacardiaceae	Rhus radicans	0.00	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	
Umbelliferae	Cicuta maculata	0.00	0.00	0.00	0.83	0.21	0.00	0.00	0.00	0.00	0.00	0.11	
Gentianaceae	Gentiana affinis	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.02	0.01	
Gentianaceae	Gentiana amarella	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.17	0.00	0.12	0.06	
Gentianaceae	Gentiana crinita	0.00	0.00	0.00	0.16	0.04	0.00	0.00	0.00	0.00	0.00	0.02	
Ericaceae	Arctostaphylos uva-ursi	0.00	2.08	0.00	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.26	
Asclepiadaceae	Asclepias ovalifolia	0.00	0.00	0.00	0.33	0.09	0.00	0.00	0.00	0.00	0.00	0.04	
Asclepiadaceae	Asclepias speciosa	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.08	0.04	
Labiatae	Lycopus asper	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.04	0.02	
Labiatae	Monarda fistulosa	0.00	0.00	0.00	0.15	0.04	0.00	0.00	0.00	0.00	0.00	0.02	
Labiatae	Stachys palustris	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.17	0.08	
Scrophulariaceae	Orthocarpus luteus	0.00	0.00	0.00	0.00	0.00	0.08	0.17	0.00	0.00	0.06	0.03	
Scrophulariaceae	Pedicularis lanceolata	0.00	0.33	0.00	0.67	0.26	0.00	0.00	0.00	0.00	0.00	0.13	
Plantaginaceae	Plantago eriopoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.42	0.21	
Plantaginaceae	Plantago major	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.06	0.03	
Rubiaceae	Galium boreale	0.00	0.08	0.00	0.00	0.02	0.08	0.00	0.42	0.00	0.12	0.07	
Caprifoliaceae	Symphoricarpos occidentalis	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.00	0.42	0.21	
Campanulaceae	Campanula rotundifolia	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.08	0.04	0.02	
Lobeliaceae	Lobelia kalmii	0.00	0.08	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	
Lobeliaceae	Lobelia spicata	0.15	0.00	0.16	0.00	0.08	0.08	0.08	0.00	0.23	0.09	0.09	
Compositae	A. ericodes or A. falcatus	4.24	2.33	0.35	0.74	1.95	4.25	0.08	0.33	0.41	1.27	1.60	
Compositae	Agoseris glauca	0.00	0.25	0.00	0.33	0.15	0.00	0.00	0.00	0.83	0.21	0.18	
Compositae	Ambrosia psilostachya	0.00	0.00	0.00	0.00	0.00	2.42	0.00	0.00	0.00	0.60	0.31	
Compositae	Artemisia ludoviciana	0.00	0.00	0.00	0.00	0.00	0.00	0.92	0.00	0.00	0.23	0.12	
Compositae	Aster ciliolatus	0.00	0.92	0.00	0.49	0.36	0.00	0.00	0.00	0.58	0.15	0.25	
Compositae	Aster junciformis	0.75	0.00	0.18	0.58	0.38	0.00	0.00	0.00	0.00	0.00	0.19	
Compositae	Aster laevis	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.25	0.10	0.05	
Compositae	Aster puniceus	0.58	0.00	0.00	0.58	0.30	0.00	0.00	0.00	0.00	0.00	0.15	
Compositae	Cirsium arvense	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

**Appendix IIb.** August 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

						Region	& Site (	% cover	)			
Family	Scientific Name			Interlake	;			S	W Manit	oba		Total
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	Total
Compositae	Crepis runcinata	0.00	0.58	0.00	0.00	0.15	0.00	0.00	0.17	0.00	0.04	0.09
Compositae	Erigeron lonchophyllus	0.58	0.00	0.00	0.00	0.15	0.25	0.00	0.08	0.00	0.08	0.11
Compositae	Erigeron spp.	0.08	0.00	0.00	0.00	0.02	0.08	0.00	0.00	0.42	0.13	0.07
Compositae	Helianthus laetiflorus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.17	2.17	1.33	0.67
Compositae	Helianthus maximilianii	0.00	0.00	0.00	3.75	0.96	3.50	0.00	0.00	0.00	0.88	0.92
Compositae	Helianthus nuttallii	0.00	2.08	0.00	0.58	0.68	0.00	2.75	0.50	0.00	0.81	0.75
Compositae	Hieracium umbellatum	0.00	0.00	0.00	0.58	0.15	0.00	0.00	0.00	0.00	0.00	0.07
Compositae	Lactuca pulchella	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.00	0.17	0.23	0.11
Compositae	Liatris ligulistylis	0.00	0.17	0.17	0.17	0.13	1.08	0.33	0.00	0.58	0.49	0.31
Compositae	Rudbeckia hirta	1.16	0.00	1.17	1.00	0.83	0.92	0.00	0.00	0.08	0.25	0.53
Compositae	Solidago canadensis	2.08	0.00	0.00	0.00	0.53	0.83	0.00	0.00	0.00	0.21	0.37
Compositae	Solidago gigantea	0.58	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.07
Compositae	Solidago missouriensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.17	0.08
Compositae	Solidago mollis	0.50	0.00	1.09	1.42	0.74	0.00	0.42	0.00	0.00	0.10	0.42
Compositae	Solidago ptarmicoides	0.00	0.33	0.08	0.15	0.14	0.08	0.00	0.24	0.25	0.14	0.14
Compositae	Solidago rigida	1.08	0.50	4.55	1.42	1.83	0.00	0.00	0.25	0.00	0.06	0.94
Compositae	Solidago spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	1.08	0.40	0.20
Compositae	Solidago spathulata	0.41	3.42	0.64	1.92	1.61	0.91	0.00	0.17	2.17	0.81	1.21
Compositae	Sonchus arvensis	10.83	4.00	4.18	4.75	5.98	16.17	0.08	6.67	1.24	6.04	6.01
Compositae	Taraxacum officinale	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.17	0.08
Compositae	Unknown Asteraceae	0.08	0.58	0.00	0.42	0.27	0.00	0.00	0.50	0.00	0.13	0.20

**Appendix IIb.** August 2010 vegetation survey plant species observed by sites and regions (% cover per m<sup>2</sup>, mean)

Family	Scientific Name	Interlake (% cover)	Family	Scientific Name	SW MB (% cover)
Graminae	Unknown grass	19.69±3.13	Graminae	Deschampsia caespitosa	4.56±1.13
Graminae	Deschampsia caespitosa	8.96±2.14	Cyperaceae	Carex spp.	3.40±1.00
Cyperaceae	Eleocharis palustris	4.50±1.36	Graminae	Unknown grass	3.31±1.03
Graminae	Poa cusickii	2.88±1.29	Juncaceae	Juncus balticus	2.85±1.42
Rosaceae	Fragaria vesca	2.68±1.80	Plantaginaceae	Plantago eriopoda	2.80±0.95
Graminae	Andropogon gerardii	2.60±1.38	Graminae	Poa cusickii	2.19±0.94
Compositae	Sonchus arvensis	2.60±1.00	Leguminosae	Medicago sativa	1.98±1.39
Cyperaceae	Carex spp.	2.23±1.05	Compositae	Sonchus arvensis	1.86±0.63
Graminae	Agropyron spp.	1.40±0.55	Compositae	Antennaria spp.	1.78±0.91
Ranunculaceae	Ranunculus cymbalaria	1.19±1.04	Graminae	Panicum virgatum	1.75±1.57
Saxifragaceae	Parnassia glauca	1.08±0.52	Polygalaceae	Polygala senega	1.74±0.57
Rosaceae	Potentilla anserina	0.98±0.53	Graminae	Agropyron smithii	$1.67 \pm 1.20$
Rosaceae	Fragaria virginiana	0.96±0.71	Graminae	Poa compressa	1.64±0.97
Compositae	Rudbeckia hirta	0.95±0.37	Graminae	Bromus inermis	1.27±0.71
Juncaceae	Juncus balticus	0.79±0.64	Amaryllidaceae	Hypoxis hirsuta	1.20±0.29
Violaceae	Viola cucullata	0.79±0.33	Graminae	Agropyron repens	1.15±1.04
Umbelliferae	Zizia aptera	0.77±0.23	Graminae	Andropogon gerardii	$0.98\pm0.52$
Juncaginaceae	Triglochin maritima	0.73±0.52	Rosaceae	Rosa arkansana	0.90±0.46
Ericaceae	Arctostaphylos uva-ursi	0.65±0.62	Leguminosae	Melilotus alba	0.87±0.41
Compositae	Helianthus nuttallii	0.62±0.43	Compositae	Crepis runcinata	0.79±0.29
Rosaceae	Potentilla fruticosa	0.52±0.45	Graminae	Stipa spartea	0.77±0.45
Graminae	Bromus inermis	0.52±0.00	Compositae	Aster ericoides	0.77±0.35
Rosaceae	Rosa arkansana	0.52±0.27	Leguminosae	Medicago lupulina	0.73±0.52
Compositae	Solidago rigida	0.45±0.20	Rosaceae	Potentilla anserina	$0.69\pm0.30$
Cyperaceae	Carex tetanica	0.43±0.20	Compositae	Helianthus nuttallii	0.66±0.44
Graminae	Phleum pratense	0.42±0.42	Leguminosae	Petalostemon candidum	0.63±0.43
Leguminosae	Medicago sativa	0.42±0.42	Juncaginaceae	Triglochin maritima	0.60±0.40
Compositae	Aster ericoides	0.41±0.15	Compositae	Cirsium flodmanii	0.60±0.24
Ranunculaceae	Thalictrum dasycarpum	0.39±0.31	Compositae	Liatris ligulistylis	0.56±0.24
Primulaceae	Dodecatheon pulchellum	0.35±0.25	Compositae	Cirsium arvense	0.54±0.33
Compositae	Antennaria spp.	0.35±0.19	Umbelliferae	Zizia aptera	0.51±0.18
Compositae	Aster puniceus	0.35±0.11	Graminae	Helictotrichon hookeri	0.48±0.32
Amaryllidaceae	Hypoxis hirsuta	0.35±0.07	Leguminosae	Glycyrrhiza lepidota	0.48±0.27
Rubiaceae	Galium boreale	0.33±0.15	Graminae	Poa canbyi	0.46±0.34
Compositae	Cirsium flodmanii	0.33±0.10	Compositae	Lactuca pulchella	0.46±0.33
Asclepiadaceae	Asclepias ovalifolia	0.31±0.31	Plantaginaceae	Plantago major	0.44±0.33
Salicaceae	Populus tremuloides	0.31±0.14	Compositae	Solidago spathulata	0.41±0.25
Compositae	Solidago spathulata	0.30±0.10	Rubiaceae	Galium boreale	0.39±0.21
Cyperaceae	Carex siccata	0.29±0.18	Violaceae	Viola cucullata	0.37±0.22
Salicaceae	Salix spp.	0.29±0.10	Liliaceae	Zygadenus gramineus	0.35±0.16
Cullouddu	Guin opp.	0.2010.17	Lindocae		0.00±0.10

**Appendix IIIa.** June 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (% cover)	Family	Scientific Name	SW MB (% cover)
Umbelliferae	Cicuta maculata	0.29±0.17	Santalaceae	Comandra umbellata	0.35±0.15
Compositae	Agoseris glauca	0.29±0.17 0.27±0.13	Leguminosae	Melilotus officinalis	$0.35\pm0.15$ 0.31±0.31
Caprifoliacieae	Lonicera caerulea	0.23±0.21	Compositae	Achillea millefolium	$0.30\pm0.31$
•	Elaeagnus commutata	0.23±0.21	Compositae	Agoseris glauca	0.27±0.13
Elaeagnaceae Graminae	_ 0	$0.23\pm0.10$ $0.21\pm0.21$	Ranunculaceae	Agosens giauca Anemone multifida	0.25±0.16
Liliaceae	Poa spp. Tofieldia glutinosa	0.21±0.21	Equisetaceae	Equisetum spp.	0.24±0.07
		0.21±0.21 0.21±0.15	Graminae		0.24±0.07 0.21±0.21
Compositae	Crepis runcinata	0.21±0.15 0.21±0.12		Koeleria gracilis	0.21±0.21
Compositae	Aster laevis Packera aurea	$0.21\pm0.12$ $0.20\pm0.11$	Cyperaceae	Carex siccata Lonicera caerulea	0.21±0.21 0.21±0.17
Compositae			Caprifoliacieae		
Salicaceae	Salix bebbiana	0.19±0.13	Compositae	Rudbeckia hirta	0.20±0.08
Leguminosae	Unknown clover	0.18±0.06	Compositae	Solidago missouriensis	0.19±0.15
Scrophulariaceae	Castilleja coccinea	0.16±0.09	Leguminosae	Unknown clover	0.18±0.10
Leguminosae	Medicago lupulina	0.15±0.15	Graminae	Muhlenbergia richardsonis	0.17±0.17
Scrophulariaceae	Pedicularis lanceolata	0.15±0.11	Boraginaceae	Lithospermum canescens	0.15±0.15
Compositae	Hieracium umbellatum	0.14±0.08	Compositae	Aster laevis	0.14±0.11
Liliaceae	Zygadenus gramineus	0.14±0.06	Leguminosae	Petalostemon purpureus	0.14±0.07
Compositae	Taraxacum officinale	0.12±0.11	Compositae	Helianthus maximilianii	0.12±0.11
Compositae	Erigeron philadelphicus	0.12±0.07	Compositae	Ambrosia psilostachya	0.12±0.05
Iridaceae	Sisyrinchium montanum	0.11±0.04	Graminae	Poa spp.	0.10±0.10
Graminae	Agropyron subsecundum	0.10±0.10	Leguminosae	Melilotus spp.	0.10±0.10
Graminae	Panicum virgatum	0.10±0.10	Convolvulaceae	Convolvulus sepium	0.10±0.10
Graminae	Poa canbyi	0.10±0.10	Compositae	Artemisia Iudoviciana	0.10±0.10
Ranunculaceae	Thalictrum venulosum	0.10±0.10	Compositae	Solidago rigida	0.10±0.10
Compositae	Aster ciliolatus	0.10±0.10	Iridaceae	Sisyrinchium montanum	0.10±0.07
Betulaceae	Betula glandulifera	0.10±0.07	Liliaceae	Smilacina stellata	0.10±0.05
Polygalaceae	Polygala senega	0.10±0.05	Compositae	Helianthus laetiflorus	0.10±0.10
Leguminosae	Glycyrrhiza lepidota	0.08±0.06	Leguminosae	Astragalus danicus	0.08±0.08
Compositae	Achillea millefolium	0.08±0.04	Leguminosae	<i>Vicia</i> spp.	0.08±0.06
Leguminosae	Petalostemon purpureus	0.06±0.05	Compositae	Taraxacum officinale	0.08±0.06
Primulaceae	Glaux maritima	0.06±0.03	Liliaceae	Lilium philadelphicum	0.08±0.05
Plantaginaceae	Plantago eriopoda	0.06±0.03	Orchidaceae	Unknown orchid	0.06±0.06
Cyperaceae	Carex parryana	0.04±0.04	Linaceae	Linum lewisii	0.06±0.06
Leguminosae	Trifolium pratense	0.04±0.04	Ranunculaceae	Anemone canadensis	0.06±0.05
Gentianaceae	Gentiana affinis	0.04±0.04	Elaeagnaceae	Elaeagnus commutata	0.04±0.04
Compositae	Liatris ligulistylis	0.04±0.04	Rosaceae	Potentilla pensylvanica	0.04±0.03
Compositae	Solidago missouriensis	0.04±0.04	Campanulaceae	Campanula rotundifolia	0.04±0.03
Leguminosae	Petalostemon candidum	0.04±0.03	Compositae	Unknown Asteraceae	0.04±0.03
Leguminosae	Vicia spp.	0.04±0.03	Ranunculaceae	Ranunculus cymbalaria	0.02±0.02
Compositae	Ambrosia psilostachya	0.04±0.03	Ranunculaceae	Thalictrum venulosum	0.02±0.02
Equisetaceae	Equisetum spp.	0.02±0.02	Rosaceae	Fragaria vesca	0.02±0.02
			-	<b>.</b>	

**Appendix IIIa.** June 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake	Family	Scientific Name	SW MB
		(% cover)	-		(% cover
Cyperaceae	Carex aurea	0.02±0.02	Leguminosae	Vicia americana	0.02±0.0
Caryophyllaceae	Arenaria lateriflora	0.02±0.02	Primulaceae	Glaux maritima	0.02±0.0
Leguminosae	Psoralea esculenta	0.02±0.02	Asclepiadaceae	Asclepias speciosa	0.02±0.0
Leguminosae	Vicia americana	0.02±0.02	Boraginaceae	Lithospermum incisum	0.02±0.0
Scrophulariaceae	Pedicularis canadensis	0.02±0.02	Labiatae	Lycopus asper	0.02±0.0
Campanulaceae	Campanula rotundifolia	0.02±0.02	Compositae	Aster puniceus	0.02±0.0
Compositae	<i>Erigeron</i> caespitosus	0.02±0.02	Compositae	Crepis tectorum	0.02±0.0
Graminae	Agropyron repens	0.00±0.00	Compositae	Tragopogon dubius	0.02±0.0
Graminae	Agropyron smithii	0.00±0.00	Graminae	Agropyron spp.	0.00±0.0
Graminae	Helictotrichon hookeri	0.00±0.00	Graminae	Agropyron subsecundum	0.00±0.0
Graminae	Koeleria gracilis	0.00±0.00	Graminae	Phleum pratense	0.00±0.0
Graminae	inae Muhlenbergia richardsonis 0.00±0.00 Cyperaceae		Carex aurea	0.00±0.0	
Graminae	•		Carex parryana	0.00±0.0	
Graminae			Carex tetanica	0.00±0.0	
Liliaceae	Lilium philadelphicum	0.00±0.00	Cyperaceae	Eleocharis palustris	0.00±0.0
Liliaceae	Smilacina stellata	0.00±0.00	Liliaceae	Tofieldia glutinosa	0.00±0.0
Orchidaceae	Unknown orchid	0.00±0.00	Salicaceae	Populus tremuloides	0.00±0.0
Santalaceae	Comandra umbellata	0.00±0.00	Salicaceae	Salix bebbiana	0.00±0.0
Ranunculaceae	Anemone canadensis	0.00±0.00	Salicaceae	Salix spp.	0.00±0.0
Ranunculaceae	Anemone multifida	0.00±0.00	Betulaceae	Betula glandulifera	0.00±0.0
Rosaceae	Potentilla pensylvanica	0.00±0.00	Caryophyllaceae	Arenaria lateriflora	0.00±0.0
Leguminosae	Astragalus danicus	0.00±0.00	Ranunculaceae	Thalictrum dasycarpum	0.00±0.0
Leguminosae	Melilotus alba	0.00±0.00	Saxifragaceae	Parnassia glauca	0.00±0.0
Leguminosae	Melilotus officinalis	0.00±0.00	Rosaceae	Fragaria virginiana	0.00±0.0
Leguminosae	Melilotus spp.	$0.00 \pm 0.00$	Rosaceae	Potentilla fruticosa	0.00±0.0
Linaceae	Linum lewisii	0.00±0.00	Leguminosae	Psoralea esculenta	0.00±0.0
Asclepiadaceae	Asclepias speciosa	0.00±0.00	Leguminosae	Trifolium pratense	0.00±0.0
Convolvulaceae	Convolvulus sepium	0.00±0.00	Umbelliferae	Cicuta maculata	0.00±0.0
Boraginaceae	Lithospermum canescens	0.00±0.00	Gentianaceae	Gentiana affinis	0.00±0.0
Boraginaceae	Lithospermum incisum	0.00±0.00	Ericaceae	Arctostaphylos uva-ursi	0.00±0.0
Labiatae	Lycopus asper	0.00±0.00	Primulaceae	Dodecatheon pulchellum	0.00±0.0
Plantaginaceae	Plantago major	0.00±0.00	Asclepiadaceae	Asclepias ovalifolia	0.00±0.0
Compositae	Artemisia ludoviciana	0.00±0.00	Scrophulariaceae	Castilleja coccinea	0.00±0.0
Compositae	Cirsium arvense	0.00±0.00	Scrophulariaceae	Pedicularis canadensis	0.00±0.0
Compositae	Crepis tectorum	0.00±0.00	Scrophulariaceae	Pedicularis lanceolata	0.00±0.0
Compositae	Helianthus laetiflorus	0.00±0.00	Compositae	Aster ciliolatus	0.00±0.0
Compositae	Helianthus maximilianii	0.00±0.00	Compositae	Erigeron caespitosus	0.00±0.0
Compositae		$0.00\pm0.00$ $0.00\pm0.00$	Compositae	Erigeron philadelphicus	0.00±0.0
	Lactuca pulchella	$0.00\pm0.00$ $0.00\pm0.00$		Hieracium umbellatum	0.00±0.0
Compositae	<i>Tragopogon dubius</i> Unknown Asteraceae	0.00±0.00 0.00±0.00	Compositae	Packera aurea	0.00±0.0 0.00±0.0
Compositae	UTIKITUWIT ASTELACEAE	Compositae	r achera aurea	0.00±0.0	

**Appendix IIIa.** June 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

Family	Scientific Name Interlake (% cover) F		Family	Scientific Name	SW MB (% cover)
Graminae	Agrostis stolonifera	13.98±3.12	Graminae	Andropogon scoparius	$19.50\pm3.76$
Graminae	Andropogon gerardii	8.68±2.03	Graminae	Agropyron repens	6.21±1.81
Graminae	Andropogon scoparius	6.38±2.06	Compositae	Sonchus arvensis	6.04±1.57
Compositae	Sonchus arvensis	5.98±1.26	Graminae	Andropogon gerardii	4.58±2.15
Graminae	Agropyron repens	4.13±1.20	Graminae	Panicum virgatum	2.72±1.03
Graminae	Muhlenbergia racemosa	3.19±1.52	Graminae	Deschampsia caespitosa	2.08±0.91
Graminae	Deschampsia caespitosa	3.15±1.14	Graminae	Poa compressa	2.02±1.02
Graminae	Poa compressa	3.04±1.09	Graminae	Unknown grass (cultivar?)	1.67±1.17
Graminae	Poa cusickii	2.45±0.90	Graminae	Scolochloa festucacea	$1.63 \pm 0.91$
Graminae	Scolochloa festucacea	1.96±1.24	Compositae	Helianthus laetiflorus	1.33±0.89
Compositae	A. ericodes or A. falcatus	1.95±0.47	Compositae	A. ericodes or A. falcatus	1.27±0.69
Graminae	Poa secunda	1.87±0.99	Leguminosae	Glycyrrhiza lepidota	1.17±0.53
Compositae	Solidago rigida	1.83±0.60	Graminae	Agropyron albicans	1.15±0.54
Compositae	Solidago spathulata	1.61±0.43	Leguminosae	Petalostemon candidum	1.14±0.39
Graminae	Sorghastrum nutans	1.45±0.64	Leguminosae	Medicago sativa	1.08±0.73
Graminae	Phleum pratense	1.32±0.67	Graminae	Poa cusickii	1.00±0.49
Graminae	Panicum virgatum	1.06±0.59	Compositae	Helianthus maximilianii	0.88±0.60
Leguminosae	Glycyrrhiza lepidota	1.02±0.51	Compositae	Helianthus nuttallii	0.81±0.48
Saxifragaceae	Parnassia glauca	1.02±0.48	Compositae	Solidago spathulata	0.81±0.43
Compositae	Helianthus maximilianii	0.96±0.49	Leguminosae	Petalostemon purpureus	0.77±0.39
Compositae	Rudbeckia hirta	0.83±0.24	Graminae	Muhlenbergia richardsonis	0.66±0.33
Compositae	Solidago mollis	0.74±0.25	Compositae	Ambrosia psilostachya	0.60±0.27
Graminae	Muhlenbergia richardsonis	0.70±0.38	Compositae	Liatris ligulistylis	0.49±0.16
Compositae	Helianthus nuttallii	0.68±0.30	Plantaginaceae	Plantago eriopoda	0.42±0.42
Rosaceae	Potentilla anserina	0.57±0.43	Caprifoliaceae	Symphoricarpos occidentalis	0.42±0.42
Leguminosae	Petalostemon candidum	0.57±0.35	Compositae	Solidago spp.	0.40±0.19
Ericaceae	Arctostaphylos uva-ursi	0.53±0.53	Rosaceae	Potentilla anserina	0.35±0.27
Compositae	Solidago canadensis	0.53±0.53	Graminae	Stipa spartea	0.31±0.17
Graminae	Bromus porteri	0.42±0.24	Leguminosae	Melilotus officinalis	0.27±0.22
Compositae	Aster junciformis	0.38±0.21	Graminae	Festuca octoflora	0.25±0.25
Graminae	Agropyron trachycaulum	0.36±0.23	Graminae	Unknown grass	0.25±0.18
Compositae	Aster ciliolatus	0.36±0.19	Compositae	Rudbeckia hirta	0.25±0.18
Graminae	Agrostideae spp.	0.32±0.32	Compositae	Artemisia ludoviciana	0.23±0.21
Leguminosae	Trifolium pratense	0.32±0.32	Compositae	Lactuca pulchella	0.23±0.15
Compositae	Aster puniceus	0.30±0.17	Compositae	Solidago canadensis	0.21±0.21
Compositae	Unknown Asteraceae	0.27±0.17	Compositae	Agoseris glauca	0.21±0.17
Scrophulariaceae	Pedicularis lanceolata	0.26±0.13	Graminae	Muhlenbergia asperifolia	0.20±0.11
Leguminosae	Medicago lupulina	0.23±0.21	Compositae	Solidago missouriensis	0.17±0.17
Umbelliferae	Cicuta maculata	0.21±0.17	Compositae	Taraxacum officinale	0.17±0.17
Compositae	Crepis runcinata	0.15±0.15	Labiatae	Stachys palustris	0.17±0.13

**Appendix IIIb.** August 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

CompositaeSolidago gigantea0.15±0.15Polygonaceae </th <th>Family</th> <th>Scientific Name</th> <th>Interlake (% cover)</th> <th>Family</th> <th>Scientific Name</th> <th>SW MB (% cover)</th>	Family	Scientific Name	Interlake (% cover)	Family	Scientific Name	SW MB (% cover)
CompositaeErigerion Ionchophyllus0.15±0.13CompositaeAster ciliolatus0.15±0.13CompositaeAgosenis glauca0.15±0.03LeguminosaePsoralea esculenta0.15±0.01CompositaeSolidago ptarmicoides0.14±0.07CompositaeUnknown Asteraceae0.13±0.01CompositaeLiatris liguilistylis0.13±0.06CompositaeUnknown Asteraceae0.13±0.07LeguminosaePetalostemon purpureus0.11±0.07RubiaceaeGalium boreale0.12±0.02LeguminosaeMelliotus alba0.10±0.05GerminaeHordeum jubatum0.12±0.02LiaceaeAsclepiadoceaeAsclepias ovalifolia0.09±0.09GraminaeHordeum jubatum0.12±0.02LobeliaceaeLobeliaceae1.0b±0.05GerminaeHordeum jubatum0.12±0.02LaguminosaeMedicago sativa0.04±0.03CompositaeSolidago inpulina0.10±0.10SaticaceaeGaritian crinita0.04±0.03LobeliaceaeLobeliaceae0.09±0.09LabiataeMonarda fistulosa0.04±0.03LobeliaceaeAster laevis0.10±0.07GernitanaceaeGernitana crinita0.04±0.03LobeliaceaeAbelias spicata0.99±0.09LabiataeMonarda fistulosa0.02±0.02AsclepiadaceaeAster laevis0.08±0.06LabiataeMonarda fistulosa0.04±0.03RanunculaceaePartina pecinata0.06±0.06CompositaeSilacapa0.02±0.02ScrophulariaceaePolatiaa0.06±0.06Compositae	Compositae	Solidago gigantea	/	Polvgonaceae	Polygonum amphibium	0.15±0.15
CompositaeHieracium umbellatum0.15±0.13LeguminosaePsoralea esculenta0.15±0.13CompositaeSolidago ptarmicoides0.14±0.06CompositaeSolidago ptarmicoides0.14±0.07CompositaeLiatris liguilistylis0.13±0.06CompositaeErigeron spp.0.13±0.01LeguminosaePetalostemon purpureus0.11±0.07RubiaceaeGalium boreale0.12±0.01LeguminosaeMelilotus alba0.09±0.06GentianaceaeGalium boreale0.12±0.06LilaceaeSmilacina stellata0.09±0.06RosaceaeGontiana amarella0.12±0.06LobeliaceaeLobelia spicata0.08±0.04LeguminosaeMedicago hupulina0.10±0.05SalicaceaeSalix spp.0.06±0.06CompositaeAster laevis0.10±0.10LeguminosaeMedicago sativa0.04±0.04CompositaeAster laevis0.10±0.01LeguminosaeMedicago sativa0.04±0.03RanunculaceaeAnemone canadensis0.08±0.04LabiataeMonarda fistulosa0.04±0.03RanunculaceaeAstelpias speciosa0.08±0.06LeguminosaeVicia spp.0.02±0.02GraminaeSpecitat0.06±0.06CompositaeErigeron nochophylius0.08±0.02GraminaeSpecitai0.06±0.06LeguminosaeVicia spp.0.02±0.02CompositaeSpecitai0.06±0.06LeguminosaeVicia spp.0.02±0.02CompositaeSpecitai0.06±0.06CompositaeErigeron spp.0.02±0.02Comp	•		0.15±0.13	,0		0.15±0.15
CompositaeAgoseris glauca0.15±0.08CompositaeSolidago ptarmicoides0.14±0.01CompositaeLiatris iguilstytis0.13±0.06CompositaeUnknown Asteraceae0.13±0.07LeguminosaePetalostemon purpureus0.11±0.07RubiaceaeGalium boreale0.12±0.01LeguminosaeMelilotus alba0.10±0.05GerntianaceaeGalium boreale0.12±0.01LiliaceaeSmilacina stellata0.09±0.09GraminaeHordeum jubatum0.12±0.02LaguninosaeLobelia spicata0.08±0.04LeguminosaeMedicago lupulina0.10±0.10LeguninosaeMedicago sativa0.04±0.04CompositaeSolidago mollis0.10±0.10LeguninosaeMedicago sativa0.04±0.04CompositaeSolidago mollis0.10±0.10LabiataeMonarda fistulosa0.04±0.04LobeliaceaeLobelia spicata0.08±0.08LiliaceaeAlium stellatum0.02±0.02CompositaeSolidago rigida0.08±0.08LiliaceaeAlium stellatum0.02±0.02CompositaeSolidago rigida0.08±0.08LiliaceaeAlium stellatum0.02±0.02GraminaeSpartina pectinata0.08±0.08LibiaceaeLobelia ceaeCompositaeSolidago rigida0.08±0.08LibiaceaeLobelia ceaeCompositaeSolidago rigida0.08±0.08LibiaceaeAlium stellatum0.02±0.02CompositaeSolidago rigida0.08±0.08CompositaeFigeron spp.0.02±0.02CompositaeSolidag	Compositae		0.15±0.13	Leguminosae	Psoralea esculenta	0.15±0.10
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SantalaceaeComandra umbellata0.00±0.00GraminaeAgropyron trachycaulum0.00±0.00PolygonaceaePolygonum amphibium0.00±0.00GraminaeAgrostideae spp.0.00±0.00RanunculaceaeAnemone canadensis0.00±0.00GraminaeAgrostis stolonifera0.00±0.00RosaceaePotentilla arguta0.00±0.00GraminaeBromus porteri0.00±0.00RosaceaeRosa arkansana0.00±0.00GraminaeMuhlenbergia racemosa0.00±0.00LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Orchidaceae	Spiranthes romanzoffiana	0.00±0.00	Polygalaceae	Polygala senega	0.02±0.02
PolygonaceaePolygonum amphibium0.00±0.00GraminaeAgrostideae spp.0.00±0.00RanunculaceaeAnemone canadensis0.00±0.00GraminaeAgrostis stolonifera0.00±0.00RosaceaePotentilla arguta0.00±0.00GraminaeBromus porteri0.00±0.00RosaceaeRosa arkansana0.00±0.00GraminaeMuhlenbergia racemosa0.00±0.00LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Betulaceae	Betula glandulifera	0.00±0.00	Gentianaceae	Gentiana affinis	0.02±0.02
RanunculaceaeAnemone canadensis0.00±0.00GraminaeAgrostis stolonifera0.00±0.00RosaceaePotentilla arguta0.00±0.00GraminaeBromus porteri0.00±0.00RosaceaeRosa arkansana0.00±0.00GraminaeMuhlenbergia racemosa0.00±0.00LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Santalaceae	Comandra umbellata	0.00±0.00	Graminae	Agropyron trachycaulum	0.00±0.00
RosaceaePotentilla arguta0.00±0.00GraminaeBromus porteri0.00±0.00RosaceaeRosa arkansana0.00±0.00GraminaeMuhlenbergia racemosa0.00±0.00LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Polygonaceae	Polygonum amphibium	0.00±0.00	Graminae	Agrostideae spp.	0.00±0.00
RosaceaeRosa arkansana0.00±0.00GraminaeMuhlenbergia racemosa0.00±0.00LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Ranunculaceae	Anemone canadensis	0.00±0.00	Graminae	Agrostis stolonifera	0.00±0.00
LeguminosaeMelilotus officinalis0.00±0.00GraminaePhleum pratense0.00±0.00LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Rosaceae	Potentilla arguta	0.00±0.00	Graminae		0.00±0.00
LeguminosaePsoralea esculenta0.00±0.00GraminaePoa secunda0.00±0.00PolygalaceaePolygala senega0.00±0.00GraminaeSorghastrum nutans0.00±0.00	Rosaceae	Rosa arkansana	0.00±0.00	Graminae		0.00±0.00
Polygalaceae         Polygala senega         0.00±0.00         Graminae         Sorghastrum nutans         0.00±0.00	Leguminosae	Melilotus officinalis	0.00±0.00	Graminae	Phleum pratense	0.00±0.00
	Leguminosae	Psoralea esculenta	0.00±0.00	Graminae	Poa secunda	0.00±0.00
	Polygalaceae	Polygala senega		Graminae	Sorghastrum nutans	0.00±0.00
Gentianaceae Gentiana attinis 0.00±0.00 Liliaceae Smilacina stellata 0.00±0.00	Gentianaceae	Gentiana affinis	0.00±0.00	Liliaceae	Smilacina stellata	0.00±0.00

**Appendix IIIb.** August 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (% cover)	Family	Scientific Name	SW MB (% cover)
Gentianaceae	Gentiana amarella	0.00±0.00	Salicaceae	Salix spp.	0.00±0.00
Asclepiadaceae	Asclepias speciosa	0.00±0.00	Saxifragaceae	Parnassia glauca	0.00±0.00
Labiatae	Lycopus asper	0.00±0.00	Leguminosae	Melilotus alba	0.00±0.00
Labiatae	Stachys palustris	0.00±0.00	Leguminosae	Trifolium pratense	0.00±0.00
Scrophulariaceae	Orthocarpus luteus	0.00±0.00	Leguminosae	Vicia spp.	0.00±0.00
Plantaginaceae	Plantago eriopoda	0.00±0.00	Anacardiaceae	Rhus radicans	0.00±0.00
Plantaginaceae	Plantago major	0.00±0.00	Umbelliferae	Cicuta maculata	0.00±0.00
Caprifoliaceae	Symphoricarpos occidentalis	0.00±0.00	Gentianaceae	Gentiana crinita	0.00±0.00
Campanulaceae	Campanula rotundifolia	0.00±0.00	Ericaceae	Arctostaphylos uva-ursi	0.00±0.00
Compositae	Ambrosia psilostachya	0.00±0.00	Asclepiadaceae	Asclepias ovalifolia	0.00±0.00
Compositae	Artemisia ludoviciana	0.00±0.00	Labiatae	Monarda fistulosa	0.00±0.00
Compositae	Aster laevis	0.00±0.00	Scrophulariaceae	Pedicularis lanceolata	0.00±0.00
Compositae	Cirsium arvense	0.00±0.00	Lobeliaceae	Lobelia kalmii	0.00±0.00
Compositae	Helianthus laetiflorus	0.00±0.00	Compositae	Aster junciformis	0.00±0.00
Compositae	Lactuca pulchella	0.00±0.00	Compositae	Aster puniceus	0.00±0.00
Compositae	Solidago missouriensis	0.00±0.00	Compositae	Cirsium arvense	0.00±0.00
Compositae	Solidago spp.	0.00±0.00	Compositae	Hieracium umbellatum	0.00±0.00
Compositae	Taraxacum officinale	0.00±0.00	Compositae	Solidago gigantea	0.00±0.00

**Appendix IIIb.** August 2010 vegetation survey plant species ranked (% cover per m<sup>2</sup>, mean±SE)

						Region	& Site (#	stems)				
Family	Scientifc Name			Interlake				S	W Manito	ba		Total
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	TOLAI
Liliaceae	Allium stellatum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0004	0.0004
Liliaceae	Lilium philadelphicum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0008	0.0060	0.0076	0.0076
Liliaceae	Zygadenus elegans	0.0000	0.0204	0.0644	0.0276	0.1124	0.0252	0.0180	0.4916	0.0228	0.5576	0.6700
Liliaceae	Zygadenus gramineus	0.0000	0.0212	0.0036	0.0880	0.1128	0.0004	0.0000	0.0080	0.0120	0.0204	0.1332
Amaryllidaceae	Hypoxis hirsuta	0.0000	0.0000	0.0000	0.0004	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Iridaceae	Sisyrinchium montanum	0.0000	0.0008	0.0000	0.0020	0.0028	0.0008	0.0000	0.0000	0.0000	0.0008	0.0036
Nyctaginaceae	Mirabilis hirsuta	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ranunculaceae	Thalictrum venulosum	0.0000	0.0004	0.0000	0.0012	0.0016	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016
Crucifera	Erysimum inconspicuum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0004	0.0004
Saxifragaceae	Parnassia glauca	0.0000	0.0040	0.0000	0.0400	0.0440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0440
Saxifragaceae	Parnassia palustris	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Rosaceae	Potentilla anserina	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0060	0.0060
Rosaceae	Potentilla arguta	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0216	0.0004	0.0000	0.0220	0.0220
Rosaceae	Potentilla fruticosa	0.0000	0.0020	0.0000	0.0156	0.0176	0.0000	0.0000	0.0000	0.0000	0.0000	0.0176
Rosaceae	Potentilla pensylvanica	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Rosaceae	Rosa arkansana	0.0008	0.0048	0.0008	0.0000	0.0064	0.0004	0.1252	0.0040	0.0008	0.1304	0.1368
Leguminosae	Astragalus canadensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0156	0.0000	0.0156	0.0156
Leguminosae	Astragalus striatus	0.0000	0.0000	0.0020	0.0000	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020
Leguminosae	Glycyrrhiza lepidota	0.0000	0.0000	0.0016	0.0084	0.0100	0.0052	0.0020	0.0204	0.0164	0.0440	0.0540
Leguminosae	Medicago lupulina	0.0000	0.0000	0.0000	0.0000	0.0000	0.0188	0.0000	0.0000	0.0000	0.0188	0.0188
Leguminosae	Medicago sativa	0.0216	0.0000	0.0056	0.0000	0.0272	0.0160	0.0000	0.1072	0.0000	0.1232	0.1504
Leguminosae	Melilotus alba	0.0060	0.0164	0.0612	0.0296	0.1132	0.2040	0.0000	0.0728	0.5212	0.7980	0.9112
Leguminosae	Melilotus officinalis	0.0000	0.0000	0.0112	0.0000	0.0112	0.1816	0.0008	0.0308	0.2416	0.4548	0.4660
Leguminosae	Petalostemon candidum	0.0000	0.0000	0.1008	0.0100	0.1108	0.0248	0.2996	0.1912	0.2248	0.7404	0.8512
Leguminosae	Petalostemon purpureus	0.0000	0.0000	0.0068	0.0020	0.0088	0.0016	0.0208	0.0016	0.1792	0.2032	0.2120
Leguminosae	Psoralea argophylla	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Leguminosae	Trifolium pratense	0.0016	0.0000	0.0020	0.0000	0.0036	0.0000	0.0000	0.0000	0.0000	0.0000	0.0036
Leguminosae	Vicia americana	0.0000	0.0000	0.0000	0.0004	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Linaceae	Linum lewisii	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0284	0.0000	0.0284	0.0284
Polygalaceae	Polygala senega	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0036	0.0016	0.0000	0.0112	0.0112
Onagraceae	Oenothera biennis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Umbelliferae	Cicuta maculata	0.0000	0.0000	0.0000	0.0160	0.0160	0.0000	0.0000	0.0000	0.0000	0.0000	0.0160
Gentianaceae	Gentiana affinis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Gentianaceae	Gentiana crinita	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Asclepiadaceae	Asclepias ovalifolia	0.0000	0.0000	0.0000	0.0020	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020
Asclepiadaceae	Asclepias speciosa	0.0000	0.0000	0.0000	0.0008	0.0008	0.0260	0.0000	0.0024	0.0000	0.0284	0.0292
Labiatae	Lycopus asper	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Labiatae	Monarda fistulosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Labiatae	Stachys palustris	0.0000	0.0000	0.0000	0.0000	0.0000	0.0036	0.0000	0.0000	0.0000	0.0036	0.0036

**Appendix IVa**. Early July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

						Region	& Site (#					
Family	Scientifc Name			Interlake				S	W Manitol	ba		Total
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	
Scrophulariaceae	Castilleja coccinea	0.0032	0.0108	0.0072	0.0140	0.0352	0.0000	0.0000	0.0000	0.0000	0.0000	0.0352
Scrophulariaceae	Orthocarpus luteus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0092	0.0000	0.0000	0.0092	0.0092
Scrophulariaceae	Pedicularis canadensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Scrophulariaceae	Penstemon gracilis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Caprifoliacieae	Lonicera caerulea	0.0000	0.0000	0.0024	0.0000	0.0024	0.0000	0.0000	0.0000	0.0000	0.0000	0.002
Caprifoliacieae	<i>Lonicera</i> spp.	0.0000	0.0000	0.0000	0.0004	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Caprifoliaceae	Symphoricarpos occidentalis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Campanulaceae	Campanula rotundifolia	0.0328	0.0540	0.3256	0.0132	0.4256	0.0836	0.2948	0.1000	0.3652	0.8436	1.269
Lobeliaceae	Lobelia kalmii	0.0000	0.0164	0.0000	0.0000	0.0164	0.0000	0.0000	0.0000	0.0000	0.0000	0.016
Lobeliaceae	Lobelia spicata	0.0456	0.0000	1.0020	0.0688	1.1164	0.2432	0.0772	0.0476	0.8520	1.2200	2.336
Compositae	A. ericodes or A. falcatus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Achillea millefolium	0.0268	0.0040	0.0100	0.0076	0.0484	0.0012	0.0000	0.0108	0.0944	0.1064	0.154
Compositae	Agoseris glauca	0.0000	0.0000	0.0104	0.0032	0.0136	0.0064	0.0024	0.0036	0.0120	0.0244	0.038
Compositae	Antennaria spp.	0.0000	0.0000	0.0976	0.0000	0.0976	0.0000	0.0000	0.0000	0.0000	0.0000	0.097
Compositae	Aster ciliolatus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Aster ericoides	0.0000	0.0000	0.0064	0.0000	0.0064	0.0008	0.0000	0.0000	0.0000	0.0008	0.007
Compositae	Chrysopsis villosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0072	0.0000	0.0000	0.0072	0.007
Compositae	Cirsium arvense	0.0000	0.0000	0.0012	0.0000	0.0012	0.0052	0.0000	0.0000	0.0000	0.0052	0.006
Compositae	Cirsium flodmanii	0.0000	0.0040	0.0080	0.0016	0.0136	0.0012	0.0004	0.0020	0.0000	0.0036	0.017
Compositae	Cirsium spp.	0.0000	0.0000	0.0000	0.0004	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Crepis runcinata	0.0000	0.0964	0.0348	0.0000	0.1312	0.0736	0.1336	0.7964	0.2172	1.2208	1.352
Compositae	Erigeron annuus	0.1272	0.0000	0.0256	0.0260	0.1788	0.0028	0.0036	0.0012	0.0000	0.0076	0.186
Compositae	Erigeron asper	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Erigeron glabellus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Erigeron lonchophyllus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Erigeron philadelphicus	0.0316	0.0000	0.0000	0.0000	0.0316	0.0012	0.0000	0.0000	0.0000	0.0012	0.032
Compositae	Erigeron spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0452	0.0452	0.045
Compositae	Eupatorium maculatum	0.0000	0.0004	0.0000	0.0004	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Gaillardia aristata	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0192	0.019
Compositae	Grindelia squarrosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Helianthus laetiflorus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Helianthus maximilianii	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Helianthus nuttallii	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0004	0.000
Compositae	Hieracium umbellatum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
Compositae	Lactuca pulchella	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1076	0.0000	0.0004	0.1080	0.108
Compositae	Liatris ligulistylis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0052	0.0052	0.005
Compositae	Prenanthes racemosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.000
Compositae	Ratibida columnifera	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0144	0.0000	0.0000	0.0144	0.000
Compositae	Rudbeckia hirta	1.1008	0.0716	1.4088	0.1620	2.7432	0.1088	0.0048	0.0792	0.1592	0.3520	3.095

**Appendix IVa**. Early July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

			Region & Site (# stems)											
Family	Scientifc Name		Interlake					S	W Manitol	ba		Total		
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	Total		
Compositae	Senecio pauperculus	0.0000	0.0024	0.0000	0.0000	0.0024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0024		
Compositae	Solidago canadensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
Compositae	Solidago gigantea	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
Compositae	Solidago missouriensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0300	0.0000	0.0300	0.0300		
Compositae	Solidago mollis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
Compositae	Solidago ptarmicoides	0.0124	0.0052	0.0000	0.0072	0.0248	0.0176	0.0172	0.0296	0.0168	0.0812	0.1060		
Compositae	Solidago rigida	0.0012	0.0004	0.0000	0.0000	0.0016	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016		
Compositae	Solidago spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0252	0.0252	0.0252		
Compositae	Solidago spathulata	0.0164	0.0004	0.1664	0.0000	0.1832	0.0100	0.0156	0.0000	0.0000	0.0256	0.2088		
Compositae	Sonchus arvensis	0.0016	0.0008	0.0020	0.0036	0.0080	0.0064	0.0000	0.0084	0.0000	0.0148	0.0228		
Compositae	Tragopogon dubius	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0012	0.0012	0.0012		

**Appendix IVa**. Early July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

		Region & Site (# stems)										
Family	Scientific Name		Interlake SW Manitoba						- Total			
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	TOLAI
Liliaceae	Allium stellatum	0.0000	0.0000	0.1380	0.0004	0.1384	0.0000	0.0016	0.0028	0.0272	0.0316	0.1700
Liliaceae	Lilium philadelphicum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Liliaceae	Zygadenus elegans	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0200	0.0008	0.0208	0.0208
Liliaceae	Zygadenus gramineus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016	0.0000	0.0016	0.0016
Amaryllidaceae	Hypoxis hirsuta	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Iridaceae	Sisyrinchium montanum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Nyctaginaceae	Mirabilis hirsuta	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0044	0.0000	0.0000	0.0044	0.0044
Ranunculaceae	Thalictrum venulosum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Crucifera	Erysimum inconspicuum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0004	0.0004
Saxifragaceae	Parnassia glauca	0.0000	0.3788	0.0000	0.3700	0.7488	0.0000	0.0000	0.0000	0.0000	0.0000	0.7488
Saxifragaceae	Parnassia palustris	0.0000	0.0000	0.0004	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Rosaceae	Potentilla anserina	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Rosaceae	Potentilla arguta	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0132	0.0000	0.0000	0.0136	0.0136
Rosaceae	Potentilla fruticosa	0.0000	0.0152	0.0000	0.0160	0.0312	0.0000	0.0000	0.0000	0.0000	0.0000	0.0312
Rosaceae	Potentilla pensylvanica	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0136	0.0000	0.0004	0.0140	0.0140
Rosaceae	Rosa arkansana	0.0000	0.0008	0.0000	0.0000	0.0008	0.0000	0.0860	0.0192	0.0000	0.1052	0.1060
Leguminosae	Astragalus canadensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0076	0.0000	0.0076	0.0076
Leguminosae	Astragalus striatus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Leguminosae	Glycyrrhiza lepidota	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0388	0.0280	0.0728	0.0728
Leguminosae	Medicago lupulina	0.0000	0.0000	0.0000	0.0000	0.0000	0.0024	0.0000	0.0000	0.0000	0.0024	0.0024
Leguminosae	Medicago sativa	0.0364	0.0012	0.0272	0.0016	0.0664	0.0256	0.0000	0.4592	0.0000	0.4848	0.5512
Leguminosae	Melilotus alba	0.0016	0.0076	0.0232	0.0344	0.0668	0.6752	0.0000	0.1740	0.7340	1.5832	1.6500
Leguminosae	Melilotus officinalis	0.0000	0.0000	0.0024	0.0000	0.0024	0.0552	0.0004	0.0144	0.2068	0.2768	0.2792
Leguminosae	Petalostemon candidum	0.0000	0.0004	0.1584	0.0080	0.1668	0.0208	0.5364	0.4000	0.3172	1.2744	1.4412
Leguminosae	Petalostemon purpureus	0.0000	0.0020	0.1028	0.0980	0.2028	0.0320	0.1112	0.0416	0.3636	0.5484	0.7512
Leguminosae	Psoralea argophylla	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0456	0.0456	0.0456
Leguminosae	Trifolium pratense	0.0112	0.0000	0.0000	0.0000	0.0112	0.0000	0.0000	0.0000	0.0000	0.0000	0.0112
Leguminosae	Vicia americana	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Linaceae	Linum lewisii	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0092	0.0000	0.0092	0.0092
Polygalaceae	Polygala senega	0.0000	0.0000	0.0000	0.0000	0.0000	0.0040	0.1720	0.0000	0.0152	0.1912	0.1912
Onagraceae	Oenothera biennis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0004	0.0012	0.0020	0.0020
Umbelliferae	Cicuta maculata	0.0000	0.0000	0.0000	0.0036	0.0036	0.0000	0.0000	0.0000	0.0000	0.0000	0.0036
Gentianaceae	Gentiana affinis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0004	0.0004
Gentianaceae	Gentiana crinita	0.0000	0.0000	0.0000	0.0036	0.0036	0.0000	0.0000	0.0000	0.0000	0.0000	0.0036
Asclepiadaceae	Asclepias ovalifolia	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Asclepiadaceae	Asclepias speciosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0068	0.0000	0.0072	0.0000	0.0140	0.0140
Labiatae	Lycopus asper	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0036	0.0000	0.0036	0.0036
Labiatae	Monarda fistulosa	0.0000	0.0000	0.0000	0.0008	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008
Labiatae	Stachys palustris	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000	0.0104	0.0104

**Appendix IVb.** Mid-July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

Scrophulariaceae         Orthocárpus luteurs         0.0000         0.0012         0.0000 </th <th></th> <th></th> <th></th> <th colspan="8">Region &amp; Site (# stems)</th> <th></th>				Region & Site (# stems)									
Introl         Intro         Intro         Intro <th>Family</th> <th>Scientific Name</th> <th colspan="3"></th> <th></th> <th></th> <th>SI</th> <th>N Manito</th> <th>ba</th> <th></th> <th>Tatal</th>	Family	Scientific Name						SI	N Manito	ba		Tatal	
Scrophulariaceae         Orthocárpus luteus         0.0000         0.0012         0.0000         0.0012         0.0000         0.0010         0.0008         0.0000         0.0008         0.0000         0.0008         0.0000         0.0008         0.0000         0.0008         0.0000         0.0008         0.0000 <td></td> <td></td> <td>IL19</td> <td>IL24</td> <td>IL39</td> <td>IL50</td> <td>Total</td> <td>OK1</td> <td>OK6</td> <td>OK7</td> <td>OK14</td> <td>Total</td> <td>TOLAI</td>			IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	TOLAI
Scrophulariaceae         Pedicularis canadensis         0.0000         0.0088         0.0000         0.0088         0.0000         0.0088         0.0000         0.000	Scrophulariaceae	Castilleja coccinea	0.0028	0.0124	0.0048	0.0028	0.0228	0.0000	0.0000	0.0000	0.0000	0.0000	0.0228
Scrophulariaceae         Pensterion gracilitis         0.0000	Scrophulariaceae	Orthocarpus luteus	0.0000	0.0000	0.0012	0.0000	0.0012	0.0028	0.0808	0.0000	0.0000	0.0836	0.0848
Capitoliacieae         Lonicera carulea         0.0000	Scrophulariaceae	Pedicularis canadensis	0.0000	0.0088	0.0000	0.0000	0.0088	0.0000	0.0000	0.0088	0.0000	0.0088	0.0176
Capirifoliacieae         Lonicera spp.         0.0000	Scrophulariaceae	Penstemon gracilis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Capinfoliaceae         Symphonicarpos occidentalis         0.0000         0.	Caprifoliacieae	Lonicera caerulea	0.0000	0.0000	0.0052	0.0000	0.0052	0.0000	0.0000	0.0000	0.0000	0.0000	0.0052
Caprifoliaceae         Symphoricarpos occidentalis         0.0000         0.	Caprifoliacieae	Lonicera spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Lobeliaceae         Lobelia kalmii         0.0000         0.5936         0.0092         0.0004         0.6032         0.0000	Caprifoliaceae		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0168	0.0000	0.0000	0.0168	0.0168
Lobeliaceae         Lobelia spicata         0.1456         0.0208         0.2132         0.0244         0.4040         0.5184         0.2060         0.0856         0.8288         1.6388         2.0428           Compositae         A. ericodes or A. falcatus         0.2396         0.0364         0.0114         0.0004         0.0000         0.0016         0.0000         0.0010         0.0024         0.0224         0.2440         0.2844         0.0004         0.0002         0.0004         0.0004         0.0002         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0004         0.0000 <t< td=""><td>Campanulaceae</td><td>Campanula rotundifolia</td><td>0.0068</td><td>0.1424</td><td>0.1032</td><td>0.0456</td><td>0.2980</td><td>0.1496</td><td>0.3944</td><td>0.1592</td><td>0.3908</td><td>1.0940</td><td>1.3920</td></t<>	Campanulaceae	Campanula rotundifolia	0.0068	0.1424	0.1032	0.0456	0.2980	0.1496	0.3944	0.1592	0.3908	1.0940	1.3920
Compositae         A. ericodes or A. falcatus         0.2396         0.0056         0.0304         0.0108         0.2864         0.0004         0.0000         0.0016         0.0020         0.2884           Compositae         Achillea millefolium         0.0080         0.0024         0.0012         0.0104         0.0028         0.0000         0.0166         0.2216         0.2400         0.2540           Compositae         Antennaria spp.         0.0000 <td>Lobeliaceae</td> <td>Lobelia kalmii</td> <td>0.0000</td> <td>0.5936</td> <td>0.0092</td> <td>0.0004</td> <td>0.6032</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.6032</td>	Lobeliaceae	Lobelia kalmii	0.0000	0.5936	0.0092	0.0004	0.6032	0.0000	0.0000	0.0000	0.0000	0.0000	0.6032
Compositae         Achillea millefolium         0.0080         0.0024         0.0012         0.0140         0.0028         0.0000         0.0156         0.2216         0.2400         0.2540           Compositae         Agoseris glauca         0.0000         0.000	Lobeliaceae	Lobelia spicata	0.1456	0.0208	0.2132	0.0244	0.4040	0.5184	0.2060	0.0856	0.8288	1.6388	2.0428
Compositae         Agoseris glauca         0.0000         0.0034         0.0032         0.0044         0.0038         0.0032         0.0080         0.0144         0.0264           Compositae         Anternaria spp.         0.0000 <td>Compositae</td> <td>A. ericodes or A. falcatus</td> <td>0.2396</td> <td>0.0056</td> <td>0.0304</td> <td>0.0108</td> <td>0.2864</td> <td>0.0004</td> <td>0.0000</td> <td>0.0016</td> <td>0.0000</td> <td>0.0020</td> <td>0.2884</td>	Compositae	A. ericodes or A. falcatus	0.2396	0.0056	0.0304	0.0108	0.2864	0.0004	0.0000	0.0016	0.0000	0.0020	0.2884
Compositae         Antennaria spp.         0.0000	Compositae	Achillea millefolium	0.0080	0.0024	0.0024	0.0012	0.0140	0.0028	0.0000	0.0156	0.2216	0.2400	0.2540
Compositae         Antennaria spp.         0.0000	Compositae	Agoseris glauca	0.0000	0.0004	0.0032	0.0064	0.0100	0.0044	0.0008	0.0032	0.0080	0.0164	0.0264
Compositae         Aster ericoides         0.0000	Compositae		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Compositae         Chrysopsis villosa         0.0000	Compositae	Aster ciliolatus	0.0040	0.0004	0.0000	0.0004	0.0048	0.0000	0.0000	0.0000	0.0000	0.0000	0.0048
Compositae         Cirsium arvense         0.0000         0.0064         0.0004         0.0064         0.0000         0.0004         0.0001         0.0000         0.0004         0.0001         0.0000         0.0004         0.0001         0.0000         0.0004         0.0001         0.0000         0.0001         0.0001         0.0000	Compositae	Aster ericoides	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Compositae         Cirsium arvense         0.0000         0.0064         0.0004         0.0064         0.0000         0.0000         0.0004         0.0002           Compositae         Cirsium flodmanii         0.0112         0.0112         0.01552         0.0076         0.0852         0.0140         0.0032         0.0004         0.0004         0.0112           Compositae         Cirsium spp.         0.0000         0.	Compositae	Chrysopsis villosa	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0528	0.0000	0.0000	0.0528	0.0528
Compositae         Cirsium spp.         0.0000         <	Compositae		0.0000	0.0000	0.0064	0.0000	0.0064	0.0064	0.0000	0.0000	0.0000	0.0064	0.0128
Compositae         Crepis runcinata         0.0000         0.0864         0.0052         0.0000         0.0916         0.0448         0.1696         0.6532         0.4000         1.2676         1.3592           Compositae         Erigeron annuus         0.0144         0.0000         0.0016         0.0144         0.0304         0.0000         0.0048         0.0000         0.0048         0.0000 <td>Compositae</td> <td>Cirsium flodmanii</td> <td>0.0112</td> <td>0.0112</td> <td>0.0552</td> <td>0.0076</td> <td>0.0852</td> <td>0.0140</td> <td>0.0032</td> <td>0.0084</td> <td>0.0048</td> <td>0.0304</td> <td>0.1156</td>	Compositae	Cirsium flodmanii	0.0112	0.0112	0.0552	0.0076	0.0852	0.0140	0.0032	0.0084	0.0048	0.0304	0.1156
Compositae         Crepis runcinata         0.0000         0.0864         0.0052         0.0000         0.0448         0.1696         0.6532         0.4000         1.2676         1.3592           Compositae         Erigeron annuus         0.0144         0.0000         0.0016         0.0144         0.0304         0.0000         0.0048         0.0000         0.0004         0.0000         0.0004         0.0000 <td>Compositae</td> <td>Cirsium spp.</td> <td>0.0000</td>	Compositae	Cirsium spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Compositae         Erigeron asper         0.0004         0.0000         0.0000         0.0004         0.0000	Compositae	Crepis runcinata	0.0000	0.0864	0.0052	0.0000	0.0916	0.0448	0.1696	0.6532	0.4000	1.2676	1.3592
Compositae         Erigeron glabellus         0.0000	Compositae	Erigeron annuus	0.0144	0.0000	0.0016	0.0144	0.0304	0.0000	0.0048	0.0000	0.0548	0.0596	0.0900
Compositae         Erigeron lonchophyllus         0.0912         0.0128         0.0000         0.1040         0.0000	Compositae	Erigeron asper	0.0004	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Compositae         Erigeron philadelphicus         0.0092         0.0000         0.0002         0.0000 <td>Compositae</td> <td>Erigeron glabellus</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0028</td> <td>0.0000</td> <td>0.0068</td> <td>0.0096</td> <td>0.0096</td>	Compositae	Erigeron glabellus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0028	0.0000	0.0068	0.0096	0.0096
Compositae         Erigeron spp.         0.0000         0.0000         0.0000         0.0000         0.0000         0.0016         0.0000         0.0000         0.0016         0.0000         0.0000         0.0016         0.0000	Compositae		0.0912	0.0128	0.0000	0.0000	0.1040	0.0000	0.0000	0.0000	0.0000	0.0000	0.1040
Compositae         Eupatorium maculatum         0.0000         0.0004         0.0000         0.0004         0.0000         0.0004         0.0000	Compositae	Erigeron philadelphicus	0.0092	0.0000	0.0000	0.0000	0.0092	0.0000	0.0000	0.0000	0.0000	0.0000	0.0092
Compositae         Gaillardia aristata         0.0000	Compositae	Erigeron spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016	0.0000	0.0000	0.0000	0.0016	0.0016
Compositae         Grindelia squarrosa         0.0000         0.0016         0.0000         0.0016         0.0000         0.0000         0.0016         0.0000	Compositae	Eupatorium maculatum	0.0000	0.0004	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Compositae         Helianthus laetiflorus         0.0000	Compositae	Gaillardia aristata	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0008	0.0008
Compositae         Helianthus maximilianii         0.0008         0.0000         0.0144         0.0152         0.0032         0.0008         0.0000         0.0252           Compositae         Helianthus nuttallii         0.0008         0.0000         0.0144         0.0152         0.0032         0.0008         0.0000         0.0252           Compositae         Helianthus nuttallii         0.0000         0.0076         0.0000 </td <td>Compositae</td> <td>Grindelia squarrosa</td> <td>0.0000</td> <td>0.0000</td> <td>0.0016</td> <td>0.0000</td> <td>0.0016</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0000</td> <td>0.0016</td>	Compositae	Grindelia squarrosa	0.0000	0.0000	0.0016	0.0000	0.0016	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016
Compositae         Helianthus nuttallii         0.0000         0.0076         0.0000         0.0076         0.0000         0.0076         0.0000	Compositae	Helianthus laetiflorus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0076	0.0000	0.0076	0.0076
Compositae         Hieracium umbellatum         0.0000         0.0012         0.0008         0.0024         0.0044         0.0000         0.0000         0.0004         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.0000         0.0000         0.0044         0.00000         0.0000         0.0000	Compositae	Helianthus maximilianii	0.0008	0.0000	0.0000	0.0144	0.0152	0.0032	0.0008	0.0000	0.0060	0.0100	0.0252
Compositae         Lactuca pulchella         0.0000	Compositae	Helianthus nuttallii	0.0000	0.0076	0.0000	0.0000	0.0076	0.0000	0.0000	0.0000	0.0000	0.0000	0.0076
	Compositae	Hieracium umbellatum	0.0000	0.0012	0.0008	0.0024	0.0044	0.0000	0.0000	0.0000	0.0000	0.0000	0.0044
Compositor Listria liquilistulia 0.0008 0.0004 0.0016 0.0124 0.0153 0.0016 0.0028 0.0026 0.0253 0.0476 0.0028	Compositae	Lactuca pulchella	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2836	0.0000	0.0108	0.2944	0.2944
Compositae Liatris liguiistylis 0.0008 0.0004 0.0016 0.0124 0.0152 0.0016 0.0028 0.0080 0.0352 0.0476 0.0628	Compositae	Liatris ligulistylis	0.0008	0.0004	0.0016	0.0124	0.0152	0.0016	0.0028	0.0080	0.0352	0.0476	0.0628
Compositae Prenanthes racemosa 0.0000 0.0000 0.0000 0.0040 0.0040 0.0000 0.0000 0.0000 0.0000 0.0000 0.0040	Compositae	Prenanthes racemosa	0.0000	0.0000	0.0000	0.0040	0.0040	0.0000	0.0000	0.0000	0.0000	0.0000	0.0040
Compositae Ratibida columnifera 0.0000 0.0000 0.0000 0.0000 0.0004 0.0368 0.0072 0.0000 0.0444 0.0444	Compositae	Ratibida columnifera	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0368	0.0072	0.0000	0.0444	0.0444
Compositae Rudbeckia hirta 1.3624 0.1124 1.0156 0.1044 2.5948 0.1632 0.0080 0.1200 0.4984 0.7896 3.3844	Compositae	Rudbeckia hirta	1.3624	0.1124	1.0156	0.1044	2.5948	0.1632	0.0080	0.1200	0.4984	0.7896	3.3844

**Appendix IVb.** Mid-July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

		Region & Site (# stems)										
Family	Scientific Name		Interlake				SW Manitoba				Tatal	
		IL19	IL24	IL39	IL50	Total	OK1	OK6	OK7	OK14	Total	Total
Compositae	Senecio pauperculus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Compositae	Solidago canadensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0028	0.0000	0.0036	0.0036
Compositae	Solidago gigantea	0.0008	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008
Compositae	Solidago missouriensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0140	0.0000	0.0516	0.0656	0.0656
Compositae	Solidago mollis	0.0000	0.0008	0.0000	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008
Compositae	Solidago ptarmicoides	0.0208	0.0900	0.0732	0.1672	0.3512	0.1256	0.1480	0.2472	0.5048	1.0256	1.3768
Compositae	Solidago rigida	0.0208	0.0000	0.0040	0.0252	0.0500	0.0000	0.0000	0.0000	0.0016	0.0016	0.0516
Compositae	Solidago spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Compositae	Solidago spathulata	0.0600	0.0476	0.1672	0.0172	0.2920	0.0112	0.0252	0.0460	0.0000	0.0824	0.3744
Compositae	Sonchus arvensis	0.0436	0.0092	0.0152	0.0152	0.0832	0.1024	0.0004	0.0492	0.0072	0.1592	0.2424
Compositae	Tragopogon dubius	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

**Appendix IVb.** Mid-July 2010 flower survey species observed by sites and regions (number of stem per m<sup>2</sup>, mean)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Compositae	Rudbeckia hirta	0.3429±0.1233	Compositae	Crepis runcinata	0.1526±0.0579
Lobeliaceae	Lobelia spicata	0.1395±0.1011	Lobeliaceae	Lobelia spicata	0.1525±0.0695
Campanulaceae	Campanula rotundifolia	0.0532±0.0245	Campanulaceae	Campanula rotundifolia	0.1055±0.0294
Compositae	Solidago spathulata	0.0229±0.0179	Leguminosae	Melilotus alba	0.0998±0.0413
Compositae	Erigeron annuus	0.0224±0.0132	Leguminosae	Petalostemon candidum	0.0926±0.0297
Compositae	Crepis runcinata	0.0164±0.0116	Liliaceae	Zygadenus elegans	0.0697±0.0457
Leguminosae	Melilotus alba	0.0142±0.0065	Leguminosae	Melilotus officinalis	0.0568±0.0313
Liliaceae	Zygadenus gramineus	0.0141±0.0090	Compositae	Rudbeckia hirta	0.0440±0.0116
Liliaceae	Zygadenus elegans	0.0140±0.0053	Leguminosae	Petalostemon purpureus	0.0254±0.0213
Leguminosae	Petalostemon candidum	0.0138±0.0122	Rosaceae	Rosa arkansana	0.0163±0.0155
Compositae	Antennaria spp.	0.0122±0.0122	Leguminosae	Medicago sativa	0.0154±0.0128
Compositae	Achillea millefolium	0.0060±0.0017	Compositae	Lactuca pulchella	0.0135±0.0134
Saxifragaceae	Parnassia glauca	0.0055±0.0047	Compositae	Achillea millefolium	0.0133±0.0076
Scrophulariaceae	Castilleja coccinea	0.0044±0.0011	Compositae	Solidago ptarmicoides	0.0102±0.0038
Compositae	Erigeron philadelphicus	0.0040±0.0037	Compositae	<i>Erigeron</i> spp.	0.0056±0.0037
Leguminosae	Medicago sativa	0.0034±0.0025	Leguminosae	Glycyrrhiza lepidota	0.0055±0.0025
Compositae	Solidago ptarmicoides	0.0031±0.0013	Compositae	Solidago missouriensis	0.0038±0.0037
Rosaceae	Potentilla fruticosa	0.0022±0.0012	Asclepiadaceae	Asclepias speciosa	0.0036±0.0032
Lobeliaceae	Lobelia kalmii	0.0020±0.0020	Linaceae	Linum lewisii	0.0036±0.0031
Umbelliferae	Cicuta maculata	0.0020±0.0017	Compositae	Solidago spp.	0.0032±0.0031
Compositae	Agoseris glauca	0.0017±0.0008	Compositae	Solidago spathulata	0.0032±0.0019
Compositae	Cirsium flodmanii	0.0017±0.0007	Compositae	Agoseris glauca	0.0030±0.0010
Leguminosae	Melilotus officinalis	0.0014±0.0014	Rosaceae	Potentilla arguta	0.0028±0.0026
Leguminosae	Glycyrrhiza lepidota	0.0012±0.0010	Liliaceae	Zygadenus gramineus	0.0026±0.0012
Leguminosae	Petalostemon purpureus	0.0011±0.0007	Compositae	Gaillardia aristata	0.0024±0.0024
Compositae	Sonchus arvensis	0.0010±0.0003	Leguminosae	Medicago lupulina	0.0024±0.0023
Compositae	Aster ericoides	0.0008±0.0008	Leguminosae	Astragalus canadensis	0.0019±0.0019
Rosaceae	Rosa arkansana	0.0008±0.0005	Compositae	Ratibida columnifera	0.0018±0.0016
Leguminosae	Trifolium pratense	0.0004±0.0003	Compositae	Sonchus arvensis	0.0018±0.0010
Iridaceae	Sisyrinchium montanum	0.0004±0.0002	Polygalaceae	Polygala senega	0.0014±0.0008
Caprifoliacieae	Lonicera caerulea	0.0003±0.0003	Scrophulariaceae	Orthocarpus luteus	0.0012±0.0011
Compositae	Senecio pauperculus	0.0003±0.0003	Compositae	Erigeron annuus	0.0010±0.0005

**Appendix Va.** Early July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Asclepiadaceae	Asclepias ovalifolia	0.0002±0.0002	Compositae	Chrysopsis villosa	0.0009±0.0009
Leguminosae	Astragalus striatus	0.0002±0.0002	Liliaceae	Lilium philadelphicum	0.0009±0.0004
Compositae	Cirsium arvense	0.0002±0.0001	Rosaceae	Potentilla anserina	0.0008±0.0007
Compositae	Solidago rigida	0.0002±0.0001	Compositae	Liatris ligulistylis	0.0006±0.0006
Ranunculaceae	Thalictrum venulosum	0.0002±0.0001	Compositae	Cirsium arvense	0.0006±0.0005
Asclepiadaceae	Asclepias speciosa	0.0001±0.0001	Labiatae	Stachys palustris	0.0004±0.0004
Compositae	Eupatorium maculatum	0.0001±0.0000	Compositae	Cirsium flodmanii	0.0004±0.0001
Compositae	A. ericodes or A. falcatus	0.0000±0.0000	Compositae	Erigeron philadelphicus	0.0002±0.0001
Liliaceae	Allium stellatum	0.0000±0.0000	Compositae	Tragopogon dubius	0.0002±0.0001
Compositae	Aster ciliolatus	0.0000±0.0000	Iridaceae	Sisyrinchium montanum	0.0001±0.0001
Leguminosae	Astragalus canadensis	0.0000±0.0000	Compositae	Aster ericoides	0.0001±0.0001
Compositae	Chrysopsis villosa	0.0000±0.0000	Umbelliferae	Cicuta maculata	0.0000±0.0000
Compositae	Cirsium spp.	0.0000±0.0000	Scrophulariaceae	Castilleja coccinea	$0.0000 \pm 0.0000$
Compositae	Erigeron asper	0.0000±0.0000	Scrophulariaceae	Pedicularis canadensis	0.0000±0.0000
Compositae	Erigeron glabellus	0.0000±0.0000	Scrophulariaceae	Penstemon gracilis	0.0000±0.0000
Compositae	Erigeron lonchophyllus	0.0000±0.0000	Saxifragaceae	Parnassia glauca	0.0000±0.0000
Compositae	<i>Erigeron</i> spp.	0.0000±0.0000	Saxifragaceae	Parnassia palustris	0.0000±0.0000
Crucifera	Erysimum inconspicuum	0.0000±0.0000	Rosaceae	Potentilla fruticosa	0.0000±0.0000
Compositae	Gaillardia aristata	0.0000±0.0000	Rosaceae	Potentilla pensylvanica	0.0000±0.0000
Gentianaceae	Gentiana affinis	0.0000±0.0000	Ranunculaceae	Thalictrum venulosum	0.0000±0.0000
Gentianaceae	Gentiana crinita	0.0000±0.0000	Onagraceae	Oenothera biennis	0.0000±0.0000
Compositae	Grindelia squarrosa	0.0000±0.0000	Nyctaginaceae	Mirabilis hirsuta	0.0000±0.0000
Compositae	Helianthus laetiflorus	0.0000±0.0000	Lobeliaceae	Lobelia kalmii	$0.0000 \pm 0.0000$
Compositae	Helianthus maximilianii	0.0000±0.0000	Liliaceae	Allium stellatum	0.0000±0.0000
Compositae	Helianthus nuttallii	0.0000±0.0000	Leguminosae	Astragalus striatus	0.0000±0.0000
Compositae	Hieracium umbellatum	0.0000±0.0000	Leguminosae	Psoralea argophylla	0.0000±0.0000
Amaryllidaceae	Hypoxis hirsuta	0.0000±0.0000	Leguminosae	Trifolium pratense	0.0000±0.0000
Compositae	Lactuca pulchella	0.0000±0.0000	Leguminosae	Vicia americana	0.0000±0.0000
Compositae	Liatris ligulistylis	0.0000±0.0000	Labiatae	Lycopus asper	$0.0000 \pm 0.0000$
Liliaceae	Lilium philadelphicum	0.0000±0.0000	Labiatae	Monarda fistulosa	0.0000±0.0000
Linaceae	Linum lewisii	0.0000±0.0000	Gentianaceae	Gentiana affinis	0.0000±0.0000
Caprifoliacieae	<i>Lonicera</i> spp.	0.0000±0.0000	Gentianaceae	Gentiana crinita	$0.0000 \pm 0.0000$

**Appendix Va.** Early July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Labiatae	Lycopus asper	0.0000±0.0000	Crucifera	Erysimum inconspicuum	0.0000±0.0000
Leguminosae	Medicago lupulina	0.0000±0.0000	Compositae	A. ericodes or A. falcatus	0.0000±0.0000
Nyctaginaceae	Mirabilis hirsuta	0.0000±0.0000	Compositae	Antennaria spp.	$0.0000 \pm 0.0000$
Labiatae	Monarda fistulosa	0.0000±0.0000	Compositae	Aster ciliolatus	$0.0000 \pm 0.0000$
Onagraceae	Oenothera biennis	0.0000±0.0000	Compositae	Cirsium spp.	$0.0000 \pm 0.0000$
Scrophulariaceae	Orthocarpus luteus	0.0000±0.0000	Compositae	Erigeron asper	$0.0000 \pm 0.0000$
Saxifragaceae	Parnassia palustris	$0.0000 \pm 0.0000$	Compositae	Erigeron glabellus	$0.0000 \pm 0.0000$
Scrophulariaceae	Pedicularis canadensis	0.0000±0.0000	Compositae	Erigeron lonchophyllus	0.0000±0.0000
Scrophulariaceae	Penstemon gracilis	0.0000±0.0000	Compositae	Eupatorium maculatum	0.0000±0.0000
Polygalaceae	Polygala senega	0.0000±0.0000	Compositae	Grindelia squarrosa	0.0000±0.0000
Rosaceae	Potentilla anserina	0.0000±0.0000	Compositae	Helianthus laetiflorus	0.0000±0.0000
Rosaceae	Potentilla arguta	0.0000±0.0000	Compositae	Helianthus maximilianii	0.0000±0.0000
Rosaceae	Potentilla pensylvanica	0.0000±0.0000	Compositae	Helianthus nuttallii	0.0000±0.0000
Compositae	Prenanthes racemosa	0.0000±0.0000	Compositae	Hieracium umbellatum	0.0000±0.0000
Leguminosae	Psoralea argophylla	0.0000±0.0000	Compositae	Prenanthes racemosa	0.0000±0.0000
Compositae	Ratibida columnifera	0.0000±0.0000	Compositae	Senecio pauperculus	0.0000±0.0000
Compositae	Solidago canadensis	0.0000±0.0000	Compositae	Solidago canadensis	0.0000±0.0000
Compositae	Solidago gigantea	0.0000±0.0000	Compositae	Solidago gigantea	0.0000±0.0000
Compositae	Solidago missouriensis	0.0000±0.0000	Compositae	Solidago mollis	0.0000±0.0000
Compositae	Solidago mollis	0.0000±0.0000	Compositae	Solidago rigida	0.0000±0.0000
Compositae	Solidago spp.	0.0000±0.0000	Caprifoliacieae	Lonicera caerulea	0.0000±0.0000
Labiatae	Stachys palustris	0.0000±0.0000	Caprifoliacieae	Lonicera spp.	0.0000±0.0000
Caprifoliaceae	Symphoricarpos occidentalis	0.0000±0.0000	Caprifoliaceae	Symphoricarpos occidentalis	0.0000±0.0000
Compositae	Tragopogon dubius	$0.0000 \pm 0.0000$	Asclepiadaceae	Asclepias ovalifolia	$0.0000 \pm 0.0000$
Leguminosae	Vicia americana	$0.0000 \pm 0.0000$	Amaryllidaceae	Hypoxis hirsuta	$0.0000 \pm 0.0000$

**Appendix Va.** Early July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Compositae	Rudbeckia hirta	0.3243±0.1462	Lobeliaceae	Lobelia spicata	0.2048±0.0690
Saxifragaceae	Parnassia glauca	0.0936±0.0439	Leguminosae	Melilotus alba	0.1979±0.0818
Lobeliaceae	Lobelia kalmii	0.0754±0.0641	Leguminosae	Petalostemon candidum	0.1593±0.0550
Lobeliaceae	Lobelia spicata	0.0505±0.0231	Compositae	Crepis runcinata	0.1584±0.0526
Compositae	Solidago ptarmicoides	0.0439±0.0108	Campanulaceae	Campanula rotundifolia	0.1368±0.0337
Campanulaceae	Campanula rotundifolia	0.0373±0.0115	Compositae	Solidago ptarmicoides	0.1282±0.0402
Compositae	Solidago spathulata	0.0365±0.0118	Compositae	Rudbeckia hirta	0.0987±0.0372
Compositae	A. ericodes or A. falcatus	0.0358±0.0233	Leguminosae	Petalostemon purpureus	0.0686±0.0281
Leguminosae	Petalostemon purpureus	0.0253±0.0113	Leguminosae	Medicago sativa	0.0606±0.0552
Leguminosae	Petalostemon candidum	0.0209±0.0182	Compositae	Lactuca pulchella	0.0368±0.0352
Liliaceae	Allium stellatum	0.0173±0.0115	Leguminosae	Melilotus officinalis	0.0346±0.0218
Compositae	Erigeron lonchophyllus	0.0130±0.0072	Compositae	Achillea millefolium	0.0300±0.0186
Compositae	Crepis runcinata	0.0114±0.0107	Polygalaceae	Polygala senega	0.0239±0.0212
Compositae	Cirsium flodmanii	0.0106±0.0059	Compositae	Sonchus arvensis	0.0199±0.0110
Compositae	Sonchus arvensis	0.0104±0.0041	Rosaceae	Rosa arkansana	0.0131±0.0106
Leguminosae	Melilotus alba	0.0084±0.0042	Scrophulariaceae	Orthocarpus luteus	0.0105±0.0073
Leguminosae	Medicago sativa	0.0083±0.0048	Compositae	Solidago spathulata	0.0103±0.0048
Compositae	Solidago rigida	0.0062±0.0026	Leguminosae	Glycyrrhiza lepidota	0.0091±0.0041
Rosaceae	Potentilla fruticosa	0.0039±0.0015	Compositae	Solidago missouriensis	0.0082±0.0061
Compositae	Erigeron annuus	0.0038±0.0019	Compositae	Erigeron annuus	0.0074±0.0050
Scrophulariaceae	Castilleja coccinea	0.0028±0.0013	Compositae	Chrysopsis villosa	0.0066±0.0066
Compositae	Helianthus maximilianii	0.0019±0.0011	Compositae	Liatris ligulistylis	0.0060±0.0042
Compositae	Liatris ligulistylis	0.0019±0.0009	Leguminosae	Psoralea argophylla	0.0057±0.0057
Compositae	Achillea millefolium	0.0018±0.0005	Compositae	Ratibida columnifera	0.0055±0.0036
Leguminosae	Trifolium pratense	0.0014±0.0014	Liliaceae	Allium stellatum	0.0040±0.0023
Compositae	Erigeron philadelphicus	0.0012±0.0010	Compositae	Cirsium flodmanii	0.0038±0.0011
Compositae	Agoseris glauca	0.0012±0.0005	Liliaceae	Zygadenus elegans	0.0026±0.0016
Scrophulariaceae	Pedicularis canadensis	0.0011±0.0011	Caprifoliaceae	Symphoricarpos occidentalis	0.0021±0.0021
Compositae	Helianthus nuttallii	0.0010±0.0009	Compositae	Agoseris glauca	0.0020±0.0008
Compositae	Cirsium arvense	0.0008±0.0008	Asclepiadaceae	Asclepias speciosa	0.0018±0.0010
Caprifoliacieae	Lonicera caerulea	0.0006±0.0006	Rosaceae	Potentilla arguta	0.0017±0.0016
Compositae	Aster ciliolatus	0.0006±0.0003	Rosaceae	Potentilla pensylvanica	0.0017±0.0016

**Appendix Vb.** Mid-July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Compositae	Hieracium umbellatum	0.0006±0.0002	Labiatae	Stachys palustris	0.0013±0.0009
Umbelliferae	Cicuta maculata	0.0005±0.0004	Linaceae	Linum lewisii	0.0012±0.0011
Compositae	Prenanthes racemosa	0.0005±0.0003	Compositae	Erigeron glabellus	0.0012±0.0008
Gentianaceae	Gentiana crinita	0.0004±0.0004	Compositae	Helianthus maximilianii	0.0012±0.0005
Leguminosae	Melilotus officinalis	0.0003±0.0003	Scrophulariaceae	Pedicularis canadensis	0.0011±0.0011
Compositae	Grindelia squarrosa	0.0002±0.0002	Compositae	Helianthus laetiflorus	0.0010±0.0007
Scrophulariaceae	Orthocarpus luteus	0.0002±0.0001	Leguminosae	Astragalus canadensis	0.0009±0.0009
Labiatae	Monarda fistulosa	0.0001±0.0001	Compositae	Cirsium arvense	0.0008±0.0008
Rosaceae	Rosa arkansana	0.0001±0.0001	Nyctaginaceae	Mirabilis hirsuta	0.0005±0.0005
Compositae	Solidago gigantea	0.0001±0.0001	Labiatae	Lycopus asper	0.0004±0.0004
Compositae	Solidago mollis	0.0001±0.0001	Compositae	Solidago canadensis	0.0004±0.0003
Compositae	Antennaria spp.	$0.0000 \pm 0.0000$	Leguminosae	Medicago lupulina	0.0003±0.0003
Asclepiadaceae	Asclepias ovalifolia	$0.0000 \pm 0.0000$	Compositae	A. ericodes or A. falcatus	0.0002±0.0002
Asclepiadaceae	Asclepias speciosa	0.0000±0.0000	Compositae	Solidago rigida	0.0002±0.0002
Compositae	Aster ericoides	0.0000±0.0000	Compositae	<i>Erigeron</i> spp.	0.0002±0.0001
Leguminosae	Astragalus canadensis	0.0000±0.0000	Onagraceae	Oenothera biennis	0.0002±0.0001
Leguminosae	Astragalus striatus	0.0000±0.0000	Liliaceae	Zygadenus gramineus	0.0002±0.0001
Compositae	Chrysopsis villosa	0.0000±0.0000	Compositae	Gaillardia aristata	0.0001±0.0001
Compositae	Cirsium spp.	$0.0000 \pm 0.0000$	Compositae	Antennaria spp.	0.0000±0.0000
Compositae	Erigeron asper	$0.0000 \pm 0.0000$	Asclepiadaceae	Asclepias ovalifolia	0.0000±0.0000
Compositae	Erigeron glabellus	$0.0000 \pm 0.0000$	Compositae	Aster ciliolatus	0.0000±0.0000
Compositae	<i>Erigeron</i> spp.	$0.0000 \pm 0.0000$	Compositae	Aster ericoides	0.0000±0.0000
Crucifera	Erysimum inconspicuum	$0.0000 \pm 0.0000$	Leguminosae	Astragalus striatus	0.0000±0.0000
Compositae	Eupatorium maculatum	$0.0000 \pm 0.0000$	Scrophulariaceae	Castilleja coccinea	0.0000±0.0000
Compositae	Gaillardia aristata	0.0000±0.0000	Umbelliferae	Cicuta maculata	0.0000±0.0000
Gentianaceae	Gentiana affinis	0.0000±0.0000	Compositae	Cirsium spp.	0.0000±0.0000
Leguminosae	Glycyrrhiza lepidota	0.0000±0.0000	Compositae	Erigeron asper	0.0000±0.0000
Compositae	Helianthus laetiflorus	0.0000±0.0000	Compositae	Erigeron lonchophyllus	0.0000±0.0000
Amaryllidaceae	Hypoxis hirsuta	$0.0000 \pm 0.0000$	Compositae	Erigeron philadelphicus	0.0000±0.0000
Compositae	Lactuca pulchella	$0.0000 \pm 0.0000$	Crucifera	Erysimum inconspicuum	0.0000±0.0000
Liliaceae	Lilium philadelphicum	$0.0000 \pm 0.0000$	Compositae	Eupatorium maculatum	0.0000±0.0000
Linaceae	Linum lewisii	$0.0000 \pm 0.0000$	Gentianaceae	Gentiana affinis	0.0000±0.0000

**Appendix Vb.** Mid-July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

Family	Scientific Name	Interlake (# stems)	Family	Scientific Name	SW Manitoba (# stems)
Caprifoliacieae	Lonicera spp.	0.0000±0.0000	Gentianaceae	Gentiana crinita	0.0000±0.0000
Labiatae	Lycopus asper	0.0000±0.0000	Compositae	Grindelia squarrosa	0.0000±0.0000
Leguminosae	Medicago lupulina	0.0000±0.0000	Compositae	Helianthus nuttallii	0.0000±0.0000
Nyctaginaceae	Mirabilis hirsuta	0.0000±0.0000	Compositae	Hieracium umbellatum	0.0000±0.0000
Onagraceae	Oenothera biennis	0.0000±0.0000	Amaryllidaceae	Hypoxis hirsuta	0.0000±0.0000
Saxifragaceae	Parnassia palustris	0.0000±0.0000	Liliaceae	Lilium philadelphicum	0.0000±0.0000
Scrophulariaceae	Penstemon gracilis	0.0000±0.0000	Lobeliaceae	Lobelia kalmii	0.0000±0.0000
Polygalaceae	Polygala senega	0.0000±0.0000	Caprifoliacieae	Lonicera caerulea	0.0000±0.0000
Rosaceae	Potentilla anserina	0.0000±0.0000	Caprifoliacieae	Lonicera spp.	0.0000±0.0000
Rosaceae	Potentilla arguta	0.0000±0.0000	Labiatae	Monarda fistulosa	0.0000±0.0000
Rosaceae	Potentilla pensylvanica	0.0000±0.0000	Saxifragaceae	Parnassia glauca	0.0000±0.0000
Leguminosae	Psoralea argophylla	0.0000±0.0000	Saxifragaceae	Parnassia palustris	0.0000±0.0000
Compositae	Ratibida columnifera	0.0000±0.0000	Scrophulariaceae	Penstemon gracilis	0.0000±0.0000
Compositae	Senecio pauperculus	0.0000±0.0000	Rosaceae	Potentilla anserina	0.0000±0.0000
Iridaceae	Sisyrinchium montanum	0.0000±0.0000	Rosaceae	Potentilla fruticosa	0.0000±0.0000
Compositae	Solidago canadensis	0.0000±0.0000	Compositae	Prenanthes racemosa	0.0000±0.0000
Compositae	Solidago missouriensis	0.0000±0.0000	Compositae	Senecio pauperculus	0.0000±0.0000
Compositae	Solidago spp.	0.0000±0.0000	Iridaceae	Sisyrinchium montanum	0.0000±0.0000
Labiatae	Stachys palustris	0.0000±0.0000	Compositae	Solidago gigantea	0.0000±0.0000
Caprifoliaceae	Symphoricarpos occidentalis	0.0000±0.0000	Compositae	Solidago mollis	0.0000±0.0000
Ranunculaceae	Thalictrum venulosum	0.0000±0.0000	Compositae	Solidago spp.	0.0000±0.0000
Compositae	Tragopogon dubius	0.0000±0.0000	Ranunculaceae	Thalictrum venulosum	0.0000±0.0000
Leguminosae	Vicia americana	0.0000±0.0000	Compositae	Tragopogon dubius	0.0000±0.0000
Liliaceae	Zygadenus elegans	0.0000±0.0000	Leguminosae	Trifolium pratense	0.0000±0.0000
Liliaceae	Zygadenus gramineus	0.0000±0.0000	Leguminosae	Vicia americana	0.0000±0.0000

**Appendix Vb.** Mid-July 2010 flower survey species ranked (number of stems per m<sup>2</sup>, mean±SE)

State	County	Approx. Location	Site #	Site Code	Site Name	Approx. Distance to SW MB Sites	Approx. Distance to IL Sites
Minnesota	Clay	West-central MN	1	FP	Felton Prairie	425km	425km
Minnesota	Lincoln	Southwestern MN	2	HM	Hole-in-the-Mountain	650km	675km
Minnesota	Pipestone	Southwestern MN	3	PC	Prairie Coteau	675km	725km
North Dakota	McHenry	North-central ND	4	MCC	Mount Carmel Camp	150km	300km
North Dakota	McHenry	North-central ND	5	SLS	Smokey Lake School Sect.	150km	300km
North Dakota	McHenry	North-central ND	6	SSS	Swearson School Sect.	150km	300km
South Dakota	Roberts	Northeastern SD	7	SFP	Scarlet Fawn Prairie	525km	550km
South Dakota	Roberts	Northeastern SD	8	KNP	Knapp Pasture	525km	550km
South Dakota	Hamlin	Northeastern SD	9	CXL	Cox Lake WMA	600km	650km

Appendix VI. Summary of Royer et al.'s (2008) study sites and distance between study regions

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $											
Site ID       Field Code       Site S       Site S       Field S       Site S       Site S       Site S<					Temp	perature at the	soil level in 20	10 (°C; mean	ESE)		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			21	Week 26 (June 20 - 26)	eek 27 27 - July		4 E	Week 30 (July 18 - 24)	Week 31 (July 25 - 31)	Week 32 (Aug 1 - 7)	Week 33 (Aug 8 - 14)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	А	IL19	18.10±0.47	19.97±0.41	19.61±0.39	20.65±0.38	19.46±0.34	19.80±0.36	21.34±0.38	20.27±0.45	21.00±0.42
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	В	IL24	18.78±0.58	20.56±0.48	20.07±0.47	21.89±0.48	19.85±0.39	21.34±0.48	23.26±0.49	21.63±0.48	21.56±0.39
MeanInterlake18.68±0.2720.30±0.2220.04±0.2221.54±0.2319.94±0.2220.60±0.2422.43±0.2521.04±0.2621.47±0.23EOK118.04±0.5421.61±0.4223.03±0.4921.30±0.4520.96±0.4620.16±0.4722.47±0.4521.63±0.4022.25±0.41FOK618.48±0.7020.62±0.5222.67±0.5919.8±0.5120.08±0.5419.25±0.5522.28±0.5421.14±0.4621.70±0.455GOK718.08±0.5321.18±0.4522.41±0.4921.05±0.4920.32±0.4420.30±0.4722.42±0.4521.63±0.4221.11±0.45HOK1418.49±0.6121.62±0.5323.36±0.6021.06±0.5621.16±0.5620.66±0.5622.92±0.5522.58±0.4821.70±0.44SWManitoba18.27±0.3021.26±0.2422.87±0.2720.81±0.2520.63±0.2520.09±0.2622.52±0.2521.75±0.2221.69±0.22t*1.01-2.92-8.132.12-2.071.44-0.25-2.10-0.68P0.3120.004<0.001	С	IL39	19.75±0.53	21.36±0.44	20.91±0.42	22.74±0.45	21.23±0.48	21.64±0.48	23.69±0.55	22.22±0.58	22.46±0.55
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	D	IL50	18.09±0.56	19.31±0.45	19.58±0.45	20.87±0.53	19.23±0.49	19.61±0.54	21.44±0.55	20.02±0.52	20.87±0.47
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Mean	Interlake	18.68±0.27	20.30±0.22	20.04±0.22	21.54±0.23	19.94±0.22	20.60±0.24	22.43±0.25	21.04±0.26	21.47±0.23
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Е	OK1	18.04±0.54	21.61±0.42	23.03±0.49	21.30±0.45	20.96±0.46	20.16±0.47	22.47±0.45	21.63±0.40	22.25±0.41
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		OK6	18.48±0.70	20.62±0.52	22.67±0.59	19.8±0.51	20.08±0.54	19.25±0.55	22.28±0.54	21.14±0.46	21.70±0.45
SW Mean         18.27±0.30         21.26±0.24         22.87±0.27         20.81±0.25         20.63±0.25         20.09±0.26         22.52±0.25         21.75±0.22         21.69±0.22           t*         1.01         -2.92         -8.13         2.12         -2.07         1.44         -0.25         -2.10         -0.68           P         0.312         0.004         <0.001	G	OK7	18.08±0.53	21.18±0.45	22.41±0.49	21.05±0.49	20.32±0.44	20.30±0.47	22.42±0.45	21.63±0.42	21.11±0.45
Mean         Manitoba         18.27±0.30         21.26±0.24         22.87±0.27         20.81±0.25         20.63±0.25         20.09±0.26         22.52±0.25         21.75±0.22         21.69±0.22           t*         1.01         -2.92         -8.13         2.12         -2.07         1.44         -0.25         -2.10         -0.68           P         0.312         0.004         <0.001	Н	OK14	18.49±0.61	21.62±0.53	23.36±0.60	21.06±0.56	21.16±0.56	20.66±0.56	22.92±0.55	22.58±0.48	21.70±0.44
t*         1.01         -2.92         -8.13         2.12         -2.07         1.44         -0.25         -2.10         -0.68           P         0.312         0.004         <0.001											
P         0.312         0.004         <0.001         0.034         0.039         0.149         0.800         0.036         0.499	Mean	Manitoba	18.27±0.30	21.26±0.24	22.87±0.27	20.81±0.25	20.63±0.25	20.09±0.26	22.52±0.25	21.75±0.22	21.69±0.22
		t*	1.01	-2.92	-8.13	2.12	-2.07	1.44	-0.25	-2.10	-0.68
			0.312	0.004	<0.001	0.034	0.039	0.149	0.800	0.036	0.499

				Tem	perature at the	soil level in 2	010 (°C; mear	ı±SE)		
Site ID	Field Code	Week 34 (Aug 15 - 21)	Week 35 (Aug 22 - 28)	Week 36 (Aug 29 - Sep 4)	Week 37 (Sep 5 - 11)	Week 38 (Sept 12 - 18)	Week 39 (Sep 19 - 25)	Week 40 (Sep 26 - Oct 2)	Week 41 (Oct 3 - 9)	Week 42 (Oct 10 - 16)
		n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
Α	IL19	15.12±0.45	18.59±0.47	14.66±0.27	12.86±0.34	10.33±0.27	9.54±0.27	12.04±0.39	12.36±0.40	9.21±0.39
В	IL24	16.20±0.58	18.96±0.56	15.35±0.39	13.21±0.44	10.98±0.44	9.89±0.37	11.96±0.51	11.66±0.52	8.70±0.48
С	IL39	16.75±0.56	19.70±0.55	15.47±0.37	13.90±0.43	11.70±0.39	10.40±0.34	12.83±0.44	13.00±0.45	10.12±0.44
D	IL50	16.34±0.53	20.07±0.48	16.32±0.34	13.98±0.36	12.13±0.37	10.60±0.31	13.34±0.43	12.77±0.40	10.10±0.40
Mean	Interlake	16.10±0.27	19.33±0.26	15.45±0.17	13.49±0.20	11.28±0.19	10.11±0.16	12.54±0.22	12.44±0.22	9.53±0.21
Е	OK1	18.51±0.56	19.83±0.55	15.57±0.40	13.88±0.36	11.47±0.41	10.06±0.31	13.33±0.55	12.80±0.57	9.99±0.50
F	OK6	17.72±0.58	19.10±0.57	15.01±0.43	13.62±0.37	10.82±0.42	9.92±0.27	12.74±0.52	12.22±0.56	9.45±0.51
G	OK7	16.66±0.56	17.95±0.52	14.33±0.39	12.93±0.34	10.38±0.40	10.18±0.37	14.92±0.67	14.23±0.68	11.72±0.64
Н	OK14	16.19±0.38	17.02±0.37	13.95±0.26	12.97±0.25	10.61±0.29	9.09±0.22	10.27±0.41	9.60±0.51	7.55±0.42
	SW									
Mean	Manitoba	17.27±0.26	18.48±0.26	14.72±0.19	13.35±0.17	10.82±0.19	9.81±0.15	12.82±0.28	12.21±0.30	9.68±0.27
	ť P	-3.11	2.33	2.86	0.53	1.73	1.33	-0.77	0.62	-0.42
	P (0.05)	0.002	0.020	0.004	0.597	0.084	0.183	0.442	0.533	0.677

		Temp	erature at the	e soil level in	2010 (°C; me	ean±SE)
Site ID	Field Code	Week 43 (Oct 17 - 23)	Week 44 (Oct 24 - 30)	Week 45 (Oct 31 - Nov 6)	Week 46 (Nov 7 - 10)	2010 Mean
		n = 1344	n = 1344	n = 1344	n = 768	n = 28992
А	IL19	4.57±0.44	3.27±0.33	3.95±0.35	4.22±0.45	14.33±0.13
В	IL24	3.93±0.38	3.51±0.30	2.70±0.39	3.30±0.47	14.74±0.15
С	IL39	4.44±0.45	2.98±0.35	3.11±0.33	3.70±0.41	15.41±0.15
D	IL50	4.88±0.44	3.96±0.33	3.10±0.35	4.43±0.41	14.79±0.14
Mean	Interlake	4.46±0.21	3.43±0.16	3.21±0.18	3.91±0.22	14.82±0.07
Е	OK1	5.05±0.45	2.25±0.31	3.67±0.36	3.98±0.37	15.30±0.15
F	OK6	4.65±0.50	2.10±0.32	3.31±0.33	3.84±0.35	14.78±0.15
G	OK7	6.59±0.64	2.51±0.31	4.74±0.46	4.58±0.44	15.22±0.15
Н	OK14	2.50±0.39	1.72±0.30	1.08±0.26	2.64±0.28	14.26±0.15
	SW					
Mean	Manitoba	4.70±0.26	2.15±0.16	3.20±0.19	3.76±0.19	14.89±0.07
	ť*	-0.72	5.67	0.05	0.52	-0.72
	Р	0.471	<0.001	0.964	0.601	0.475
*t tost (	P<0.05)					

				Temp	erature at the	soil level in 20'	11 (°C; mean±	SE)		
Site ID	Field Code	Week 19 (May 5 - 7)	Week 20 (May 8 - 14)	Week 21 (May 15 - 21)	Week 22 (May 22 - 28)	Week 23 \(May 29 - Jun 4)	Week 24 (June 5 - 11)	Week 25 \(June 12 - 18)	Week 26 (June 19 - 25)	Week 27 (June 26 - July 2)
		n = 768	n = 1345	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	11.32±0.50	9.56±0.37	16.05±0.62	12.35±0.50	13.77±0.40	16.33±0.44	19.19±0.36	19.85±0.35	21.51±0.51
В	IL24	12.68±0.83	10.01±0.48	16.25±0.82	12.26±0.65	13.71±0.51	15.29±0.72	19.35±0.54	19.53±0.55	21.20±0.62
С	IL39	14.04±0.80	9.97±0.40	17.56±0.73	13.40±0.54	14.18±0.48	16.95±0.64	21.18±0.51	20.84±0.48	22.94±0.55
D	IL50	11.96±0.60	9.90±0.34	15.83±0.57	12.80±0.43	14.33±0.41	16.02±0.48	18.94±0.35	19.29±0.35	20.87±0.40
Mean	Interlake	12.50±0.35	9.86±0.20	16.42±0.35	12.70±0.27	14.00±0.23	16.15±0.29	19.66±0.23	19.88±0.22	21.63±0.26
Е	OK1	12.72±0.78	10.09±0.41	15.25±0.74	14.64±0.62	13.22±0.48	16.68±0.68	17.65±0.42	19.37±0.50	21.44±0.54
F	OK6	13.78±0.84	11.02±0.41	16.23±0.70	15.32±0.57	13.80±0.48	17.26±0.60	18.18±0.39	19.44±0.39	21.91±0.48
G	OK7	12.16±0.61	9.45±0.31	15.23±0.56	13.40±0.53	12.79±0.41	16.14±0.57	17.56±0.38	18.75±0.40	20.91±0.45
Н	OK14	12.51±0.71	10.20±0.40	15.58±0.66	14.63±0.60	13.36±0.47	16.92±0.67	17.86±0.39	19.12±0.44	21.24±0.50
Mean	SW Manitoba	12.79±0.37	10.19±0.19	15.57±0.33	14.50±0.29	13.29±0.23	16.75±0.32	17.82±0.20	19.17±0.22	21.38±0.25
	ť*	-0.57	-1.19	1.76	-4.53	2.19	-1.41	6.15	2.27	0.7
	Р	0.566	0.233	0.078	<0.001	0.029	0.16	<0.001	0.023	0.487

				Temp	erature at the	soil level in 20'	11 (°C; mean±	SE)		
Site ID	Field Code	Week 28 (July 3 - 9)	Week 29 (July 10 - 16)	Week 30 (July 17 - 23)	Week 31 (July 24 - 30)	Week 32 (July 31 - Aug 6)	Week 33 (Aug 7 - 13)	Week 34 (Aug 14 - 20)	Week 35 (Aug 21 - 27)	Week 36 (Aug 28 - Sep 3)
		n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	22.56±0.54	22.13±0.55	23.51±0.55	22.24±0.59	22.49±0.66	20.47±0.70	20.11±0.68	20.11±0.66	17.98±0.41
В	IL24	21.99±0.69	21.52±0.69	22.71±0.65	21.56±0.70	21.65±0.79	20.10±0.80	19.54±0.82	19.96±0.82	17.97±0.54
С	IL39	24.05±0.57	24.49±0.70	25.17±0.66	23.86±0.65	24.00±0.72	21.16±0.67	21.09±0.70	21.93±0.79	19.69±0.56
D	IL50	22.08±0.47	21.49±0.55	22.89±0.57	21.75±0.57	21.67±0.61	18.74±0.50	18.49±0.56	18.76±0.60	17.46±0.40
Mean	Interlake	22.67±0.29	22.41±0.32	23.57±0.31	22.35±0.32	22.45±0.35	20.11±0.34	19.81±0.35	20.19±0.36	18.28±0.24
Е	OK1	22.45±0.49	21.84±0.46	22.20±0.45	20.24±0.40	22.41±0.41	20.53±0.38	20.97±0.46	21.05±0.48	19.64±0.39
F	OK6	23.67±0.50	22.69±0.41	23.47±0.40	21.86±0.39	21.96±0.33	19.96±0.32	19.95±0.36	19.68±0.37	18.79±0.30
G	OK7	22.21±0.44	21.52±0.47	22.10±0.49	20.25±0.40	20.80±0.42	18.48±0.40	18.47±0.47	18.60±0.51	17.21±0.41
Н	OK14	22.12±0.45	21.35±0.41	21.88±0.41	20.19±0.34	20.81±0.34	18.59±0.31	18.92±0.38	19.19±0.41	18.02±0.32
Mean	SW Manitoba	22.61±0.24	21.85±0.22	22.41±0.22	20.64±0.19	21.49±0.19	19.39±0.18	19.58±0.21	19.63±0.22	18.42±0.18
	ť*	0.15	1.46	3.05	4.63	2.41	1.89	0.56	1.31	-0.46
	Р	0.881	0.145	0.002	<0.001	0.016	0.059	0.574	0.189	0.646

U Week 45 (Oct 30 - Nov 5)
2.27±0.37
2.68±0.39
3.37±0.38
2.32±0.38
2.66±0.19
NA
3.01±0.22
2.19±0.34
2.87±0.33
2.69±0.17
-0.11
0.913

		Temperature a	Temperature at the soil level in 2011 (°C; mean±SE								
Site ID	Field Code	Week 46 (Nov 6 - 12)	Week 47 (Nov 13 - 19)	Week 48 (Nov 20 - 22)	2011 Mean						
		n = 1176	n = 1175	n = 504	n = 37478						
А	IL19	0.25±0.24	-3.10±0.29	-7.13±0.38	14.09±0.15						
В	IL24	0.97±0.22	-1.17±0.16	-2.23±0.12	14.16±0.15						
С	IL39	0.73±0.22	-1.82±0.19	-2.45±0.13	15.42±0.16						
D	IL50	0.26±0.22	-2.04±0.20	-5.18±0.32	13.70±0.14						
Mean	Interlake	0.55±0.11	-2.03±0.11	-4.25±0.18	14.34±0.07						
Е	OK1	NA	NA	NA	18.15±0.13						
F	OK6	-0.19±0.14	-2.10±0.16	-2.46±0.19	14.44±0.13						
G	OK7	-1.65±0.22	-4.37±0.26	-4.54±0.30	13.16±0.14						
Н	OK14	-0.42±0.19	-2.31±0.23	-1.64±0.09	13.91±0.13						
Mean	SW Manitoba	-0.75±0.11	-2.92±0.14	-2.88±0.15	14.65±0.07						
	ť	8.01	5.14	-5.68	-3.02						
	Р	<0.001	<0.001	<0.001	0.003						

				Relativ	e humidity at t	he soil level in	2010 (%; mea	an±SE)		
Site ID	Field Code	Week 25 (June 13 - 19)	Week 26 (June 20 - 26)	Week 27 (June 27 - July 3)	Week 28 (July 4 – 10)	Week 29 (July 11 - 17)	Week 30 (July 18 - 24)	Week 31 (July 25 - 31)	Week 32 (Aug 1 - 7)	Week 33 (Aug 8 - 14)
		n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	86.71±0.80	88.79±0.61	92.40±0.37	90.03±0.46	90.55±0.50	92.12±0.34	91.34±0.32	88.21±0.80	89.62±0.76
В	IL24	80.99±1.45	83.41±1.11	84.14±1.01	78.95±1.12	81.41±1.10	80.78±1.12	76.86±1.08	80.14±1.02	88.40±0.83
С	IL39	82.44±1.18	85.47±0.88	88.01±0.66	82.91±0.92	84.94±0.97	84.66±0.92	80.05±1.08	78.76±1.29	86.25±1.24
D	IL50	79.71±1.49	88.87±0.84	93.84±0.33	86.76±0.77	86.33±0.87	86.49±0.80	84.88±0.82	87.20±0.74	90.72±0.68
Mean	Interlake	82.46±0.64	86.63±0.45	89.60±0.36	84.67±0.45	85.80±0.46	86.01±0.45	83.28±0.49	83.58±0.52	88.75±0.46
Е	OK1	85.90±1.22	89.42±0.94	87.38±0.91	88.78±0.77	85.58±0.80	89.26±0.80	89.95±0.70	89.95±0.66	92.55±0.65
F	OK6	81.96±1.56	87.54±1.17	85.45±1.16	85.34±1.06	80.59±1.22	86.53±1.10	84.60±1.16	87.53±0.96	90.69±0.84
G	OK7	89.37±0.98	91.23±0.85	87.44±0.91	88.81±0.87	85.34±0.92	89.02±0.97	88.15±0.98	88.36±0.87	94.58±0.67
Н	OK14	81.58±1.34	83.79±1.22	81.41±1.21	81.25±1.13	81.39±1.23	85.93±1.21	86.74±1.11	86.17±1.05	93.28±0.84
Mean	SW Manitoba	84.70±0.66	87.99±0.54	85.42±0.54	86.04±0.50	83.23±0.54	87.68±0.52	87.36±0.51	88.00±0.45	92.78±0.38
	ť*	-2.45	-1.95	6.49	-2.05	3.65	-2.44	-5.80	-6.44	-6.79
	Р	0.015	0.052	<0.001	0.041	<0.001	0.015	<0.001	<0.001	<0.001

		Relative humidity at the soil level in 2010 (%; mean±SE)								
Site ID	Field Code	Week 34 (Aug 15 - 21)	Week 35 (Aug 22 - 28)	Week 36 (Aug 29 - Sep 4)	Week 37 (Sep 5 - 11)	Week 38 (Sept 12 - 18)	Week 39 (Sep 19 - 25)	Week 40 (Sep 26 - Oct 2)	Week 41 (Oct 3 - 9)	Week 42 (Oct 10 - 16)
		n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	87.23±0.83	87.76±0.78	94.80±0.39	89.42±0.57	89.76±0.47	92.30±0.40	87.54±0.61	87.29±0.57	84.15±0.60
В	IL24	82.03±1.07	84.18±0.98	89.24±0.78	81.86±1.03	83.31±0.77	84.24±0.79	81.52±0.96	79.46±0.93	78.99±0.83
С	IL39	83.10±1.14	82.67±1.21	91.21±0.72	85.31±0.92	84.54±0.83	89.60±0.64	86.54±0.75	85.46±0.73	83.23±0.73
D	IL50	85.86±0.76	85.14±0.74	89.97±0.51	86.24±0.63	86.00±0.47	88.47±0.50	86.16±0.58	83.37±0.58	83.79±0.54
Mean	Interlake	84.56±0.49	84.94±0.48	91.31±0.32	85.71±0.42	85.90±0.34	88.65±0.32	85.44±0.38	83.89±0.38	82.54±0.35
Е	OK1	85.79±1.05	87.64±1.05	91.46±0.84	92.61±0.79	91.95±0.80	92.03±0.78	80.85±1.30	81.83±1.28	80.90±1.29
F	OK6	82.23±1.26	84.67±1.21	89.78±0.96	90.19±0.90	89.25±0.98	90.68±0.76	79.13±1.21	79.35±1.26	74.86±1.44
G	OK7	88.23±0.87	92.62±0.70	94.80±0.58	96.18±0.40	94.97±0.49	91.31±0.94	78.98±1.48	78.75±1.49	74.56±1.60
Н	OK14	93.37±0.40	96.81±0.25	97.33±0.26	98.24±0.31	95.54±0.40	98.23±0.25	90.89±0.82	90.46±0.72	86.59±0.90
Mean	SW Manitoba	87.41±0.50	90.43±0.48	93.34±0.37	94.30±0.35	92.93±0.37	93.06±0.38	82.47±0.64	82.60±0.64	79.23±0.69
	ť*	-4.09	-8.15	-4.14	-15.85	-14.07	-8.87	3.99	1.75	4.27
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.080	<0.001

		Relativ	e humidity at t	he soil level in	1 2010 (%; me	an±SE)
Site ID	Field Code	Week 43 (Oct 17 - 23)	Week 44 (Oct 24 - 30)	Week 45 (Oct 31 - Nov 6)	Week 46 (Nov 7 - 10)	2010 Mean
		n = 1344	n = 1344	n = 1344	n = 768	n = 28992
А	IL19	75.57±1.14	90.19±0.64	83.58±0.95	88.88±0.83	88.55±0.15
В	IL24	75.37±0.84	87.70±0.71	82.64±1.07	87.45±0.98	82.31±0.22
С	IL39	77.18±1.20	89.73±0.70	86.30±0.82	91.69±0.63	84.87±0.21
D	IL50	82.11±0.75	92.40±0.47	89.90±0.53	91.14±0.56	86.98±0.16
Mean	Interlake	77.56±0.51	90.01±0.32	85.61±0.44	89.79±0.39	85.68±0.10
Е	OK1	80.70±1.12	93.23±0.59	87.25±0.84	93.95±0.79	88.02±0.21
F	OK6	73.53±1.35	90.45±0.58	85.68±0.83	93.30±0.58	84.99±0.25
G	OK7	71.71±1.54	91.63±0.70	83.67±1.05	91.95±1.04	87.72±0.24
Н	OK14	85.74±0.75	95.32±0.42	93.12±0.46	99.05±0.14	89.92±0.21
	SW					
Mean	Manitoba	77.92±0.65	92.66±0.30	87.43±0.43	94.56±0.38	87.66±0.12
	ť	-0.44	-6.03	-2.94	-8.18	-13.22
	Р	0.660	<0.001	0.003	<0.001	<0.001

				Relativ	e humidity at t	he soil level in	2011 (%; me	an±SE)		
Site ID	Field Code	Week 19 \(May 5 - 7)	Week 20 (May 8 - 14)	Week 21 \(May 15 - 21)	Week 22 (May 22 - 28)	Week 23 (May 29 - Jun 4)	Week 24 \(June 5 - 11)	Week 25 (June 12 - 18)	Week 26 (June 19 - 25)	Week 27 (June 26 - July 2)
		n = 768	n = 1345	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	85.35±1.01	86.54±1.06	68.56±1.68	79.69±1.36	89.42±0.68	92.12±0.48	92.94±0.63	92.87±0.54	81.94±1.06
В	IL24	66.90±1.95	82.40±1.34	65.97±1.96	75.46±1.69	82.47±1.32	77.35±1.41	84.99±1.17	81.94±1.12	78.27±1.34
С	IL39	74.05±1.41	80.34±1.37	69.00±1.61	74.24±1.44	85.96±0.98	79.43±1.25	87.24±0.85	86.47±0.85	84.15±0.98
D	IL50	73.57±1.60	90.73±0.61	74.00±1.54	82.54±1.13	89.02±0.67	87.27±0.71	91.37±0.56	91.16±0.47	90.27±0.61
Mean	Interlake	74.97±0.83	85.01±0.59	69.38±0.86	77.98±0.72	86.72±0.49	84.04±0.57	89.14±0.44	88.11±0.43	83.66±0.54
Е	OK1	70.01±2.44	88.48±1.34	69.85±2.09	76.97±1.64	87.52±1.36	78.03±1.49	94.10±0.78	88.16±1.09	83.86±1.21
F	OK6	67.22±2.53	84.54±1.54	66.04±2.14	73.00±1.69	85.49±1.35	77.92±1.40	92.78±0.76	90.32±0.86	88.07±0.91
G	OK7	69.00±2.19	85.76±1.35	66.01±2.02	73.15±1.62	83.82±1.31	78.16±1.46	92.49±0.82	89.73±0.90	84.57±1.07
Н	OK14	77.06±1.84	88.83±1.18	70.76±1.89	76.41±1.54	89.47±1.21	80.69±1.41	95.93±0.65	90.16±0.85	88.54±0.94
Mean	SW Manitoba	70.82±1.14	86.90±0.68	68.17±1.02	74.88±0.81	86.58±0.66	78.70±0.72	93.83±0.38	89.59±0.47	86.26±0.52
	ť*	2.93	-2.11	0.91	2.86	0.17	5.84	-8.11	-2.35	-3.45
	Р	0.004	0.035	0.36	0.004	0.865	<0.001	<0.001	0.019	0.001

				Relativ	e humidity at t	he soil level in	2011 (%; mea	an±SE)		
Site ID	Field Code	Week 28 (July 3 - 9)	Week 29 (July 10 - 16)	Week 30 (July 17 - 23)	Week 31 (July 24 - 30)	Week 32 (July 31 - Aug 6)	Week 33 (Aug 7 - 13)	Week 34 (Aug 14 - 20)	Week 35 (Aug 21 - 27)	Week 36 (Aug 28 - Sep 3)
		n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344	n = 1344
А	IL19	80.26±1.08	77.18±1.13	81.77±1.12	80.16±1.24	75.55±1.40	74.28±1.54	75.59±1.64	72.67±1.55	83.88±1.02
В	IL24	78.18±1.27	74.69±1.32	78.83±1.31	77.55±1.35	74.12±1.48	72.89±1.71	73.56±1.82	69.58±1.71	81.17±1.30
С	IL39	81.76±1.02	75.93±1.30	77.93±1.37	77.61±1.48	73.58±1.56	73.17±1.58	69.27±1.82	64.62±1.80	76.50±1.54
D	IL50	88.63±0.59	84.81±0.89	85.57±0.95	85.18±0.91	81.34±1.10	83.77±0.89	79.34±1.15	77.31±1.12	87.45±0.79
Mean	Interlake	82.21±0.53	78.15±0.60	81.02±0.61	80.12±0.64	76.15±0.71	76.03±0.75	74.44±0.82	71.05±0.80	82.25±0.62
Е	OK1	85.70±1.16	85.24±1.03	87.42±0.99	87.16±1.04	87.31±0.84	89.34±0.89	83.79±1.04	80.99±1.17	89.34±0.86
F	OK6	87.58±0.94	89.00±0.71	91.66±0.60	91.12±0.68	90.85±0.59	91.89±0.57	89.41±0.58	88.69±0.61	93.86±0.45
G	OK7	86.02±1.05	84.85±1.06	87.59±0.95	88.42±0.95	89.07±0.74	91.45±0.72	86.93±1.01	84.52±0.99	93.20±0.56
Н	OK14	88.63±0.84	87.88±0.82	89.42±0.74	89.49±0.75	90.15±0.65	92.89±0.61	87.62±0.79	84.29±0.90	92.14±0.64
Mean	SW Manitoba	86.98±0.50	86.74±0.46	89.02±0.42	89.05±0.44	89.34±0.36	91.39±0.36	86.94±0.44	84.62±0.48	92.13±0.33
	ť	-6.53	-11.29	-10.78	-11.51	-16.68	-18.47	-13.34	-14.52	-14.17
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

				Relative hum	idity at the soil	level in 2011 (%	; mean±SE)		
Site ID	Field Code	Week 37 (Sep 4 - 10)	Week 38 (Sept 11 - 17)	Week 39 (Sep 18 - 24)	Week 40 (Sep 25 - Oct 1)	Week 41 (Oct 2 - 8)	Week 42 (Oct 9 - 15)	Week 43 (Oct 16 - 22)	Week 44 (Oct 23 - 29)
		n = 1344	n = 1344	n = 1262	n = 1176	n = 1176	n = 1176	n = 1176	n = 1176
А	IL19	75.89±1.49	70.25±1.34	73.13±1.42	63.76±1.57	66.64±1.73	84.38±0.96	77.28±1.02	82.96±0.76
В	IL24	70.88±1.73	68.27±1.53	70.92±1.68	64.25±1.60	67.97±1.55	82.49±1.04	73.44±1.05	80.16±0.83
С	IL39	69.66±1.82	65.63±1.58	69.59±1.75	64.62±1.79	64.28±1.73	81.84±1.13	74.18±1.17	82.08±0.90
D	IL50	82.85±0.93	75.20±1.15	77.86±1.20	70.33±1.35	69.70±1.54	83.50±0.99	75.08±0.98	83.72±0.61
Mean	Interlake	74.82±0.79	69.84±0.72	72.88±0.77	65.74±0.80	67.15±0.82	83.05±0.52	74.99±0.53	82.23±0.39
Е	OK1	84.29±0.99	84.04±0.88	95.52±0.57	NA	NA	NA	NA	NA
F	OK6	89.68±0.47	88.80±0.56	93.23±0.51	86.96±0.65	85.84±0.99	93.99±0.43	86.87±0.62	93.47±0.40
G	OK7	90.54±0.68	84.92±0.98	88.59±0.96	77.20±1.28	77.16±1.39	92.05±0.67	78.42±1.28	87.09±0.89
Н	OK14	84.53±0.95	79.15±1.06	86.36±1.04	76.63±1.22	74.89±1.30	90.69±0.66	78.30±0.99	84.40±0.72
Mean	SW Manitoba	87.26±0.41	84.23±0.46	90.29±0.46	80.26±0.66	79.30±0.74	92.24±0.35	81.20±0.60	88.32±0.44
	ť*	-13.95	-16.86	-18.75	-13.41	-10.61	-13.72	-7.69	-10.28
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

		Relati	ve humidity at t	he soil level in	2011 (%; mean	t±SE)
Site ID	Field Code	Week 45 (Oct 30 - Nov 5)	Week 46 (Nov 6 - 12)	Week 47 (Nov 13 - 19)	Week 48 (Nov 20 - 22)	2011 Mean
		n = 1176	n = 1176	n = 1175	n = 504	n = 37478
А	IL19	79.18±1.16	90.91±0.37	90.05±0.31	86.45±0.51	80.19±0.24
В	IL24	77.00±1.10	89.66±0.48	89.15±0.33	86.41±0.49	76.51±0.27
С	IL39	78.72±1.16	90.71±0.40	88.89±0.41	89.62±0.42	76.83±0.27
D	IL50	79.63±0.92	93.46±0.23	90.50±0.28	86.75±0.63	83.13±0.20
Mean	Interlake	78.63±0.54	91.19±0.20	89.65±0.17	87.31±0.27	79.17±0.13
Е	OK1	NA	NA	NA	NA	84.67±0.29
F	OK6	90.05±0.61	95.22±0.32	89.41±0.59	89.64±0.82	87.68±0.20
G	OK7	79.65±1.23	93.56±0.35	90.88±0.40	92.36±0.52	84.99±0.22
Н	OK14	81.40±1.06	92.70±0.50	91.19±0.57	95.13±0.21	85.81±0.21
Mean	SW Manitoba	83.70±0.61	93.83±0.23	90.49±0.30	92.38±0.36	85.88±0.11
	ť	-6.17	-8.65	-2.57	-11.45	-39.48
	Р	<0.001	<0.001	0.010	<0.001	<0.001

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 11, 2010	16:00	F	<b>1</b> ♀	OK6 Voucher #1 (2010)		Caught while flying.
Hesperia dacotae	July 12, 2010	10:55	Н	1♂	No	Nectaring on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	
Hesperia dacotae	July 12, 2010	11:50	Η	<b>1</b> ♀	OK14 Genetic Sample #1	Female observed nectaring on shorter plants of white sweet-clover ( <i>Medicago</i> <i>alba</i> ), purple prairie-clover ( <i>Petalostemon purpureus</i> ) and black- eyed Susan ( <i>Rudbeckia hirta</i> ). Seemed to select only shorter plants only even when taller plants present.	Female seemed to select only shorter plants only even when taller plants present. Female also willing to perch on a finger when gently handled. Caught while perched. No ovipositing observed.
Hesperia dacotae	July 12, 2010	12:00	Н	<b>1</b> ♀	No	Nectaring on black-eyed Susan (Rudbeckia hirta).	
Hesperia dacotae	July 12, 2010	13:11	Η	<b>1</b> ♂	OK14 Voucher #3 (2010)	Nectaring on a short (>30cm) white sweet-clover ( <i>Medicago alba</i> ).	
Hesperia dacotae	July 12, 2010	13:20	Н	2♂ & 2♀	No	Pair landed on white prairie-clover ( <i>Petalostemon candidum</i> ), smooth camas ( <i>Zygadenus elegans</i> ) and an unknown species of <i>Carex</i> .	Observed two pairs of DS closely following each other. Pairs not attached to each other but one male was very close to a female (~1 to 40cm) and the pairs were close to each other (~10 to 50cm). No copulation observed but pair was within reach of each other at times.
Hesperia dacotae	July 12, 2010	~13:30	Н	1♂ & 1♀	No	One male and one female nectaring on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	
Hesperia dacotae	July 12, 2010	~13:30	Η	<b>1</b> ♀	No	Female DS observed flying around and down into grasses. No egg laying observed even though I looked for it. Species of grass unknown.	

Appendix IX. Dakota Skipper adult behavioural observations and plant interactions

				Sex			
Species	Date	Time	Site	& No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 12, 2010	all day	Η	NA	NA	DS really seem to select shorter white sweet-clover ( <i>Medicago alba</i> ) plants (>40cm) even when taller plants present. Also seemed to select shorter flowers of other species, even when taller ones present.	
Hesperia dacotae	July 18, 2010	14:31	Н	<b>1</b> ♀	OK14 Voucher #4 (2010)	Perched on a young trembling aspen ( <i>Populus tremuloides</i> ).	Caught while perched.
Hesperia dacotae	July 20, 2010	14:11	Η	<b>1</b> ♀	OK14 Voucher #5 (2010)	Perched on the leaves of a sweet- clover ( <i>Melilotus alba</i> or <i>M. officinalis</i> ) that was not in flower	Caught while perched. Afternoon was very hot (~32°C), humid (muggy) and calm (Beaufort 0 to 1), which seemed ideal for the butterflies.
Hesperia dacotae	July 20, 2010	15:30	Η	<b>1</b> ở	OK14 Genetic Sample #2 & Voucher #6 (2010)	Nectaring on upland white goldenrod ( <i>Solidago ptarmicoides</i> ).	Caught while nectaring. Male was very worn and old but still had 5 legs. Collected a mid-leg as a genetic sample. Afterwards released male was very lethargic and unwilling to fly. Believed male may die soon, so then took male as a voucher specimen. Afternoon was very hot (~32°C), humi (muggy) and calm (Beaufort 0 to 1), which seemed ideal for the butterflies.
Hesperia dacotae	July 20, 2010	15:45	Η	1♀	OK14 Genetic Sample #3	Caught while walking down in grasses.	Caught by hand (covered). No ovipositioning observed. Afternoon was very hot (~32°C), humid (muggy) and calm (Beaufort 0 to 1), which seemed ideal for the butterflies.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 20, 2010	17:00	Η	<b>1</b> ♀	OK14 Genetic Sample #4	Caught while perched on a blade of grass. When released placed on a meadow blazingstar ( <i>Liatris ligulistylis</i> ) but realized that she probably does not nectar on this species, so moved her to a black-eyed Susan ( <i>Rudbeckia hirta</i> ). Female still did not nectar so then gently moved her to a flower head of yarrow ( <i>Achillea millefolium</i> ), followed by a white prairie-clover ( <i>Petalostemon candidum</i> ), purple prairie-clover ( <i>Petalostemon purpureus</i> ), upland white goldenrod ( <i>Solidago ptarmicoides</i> ) and white sweet-clover ( <i>Melilotus alba</i> ). She actively nectared upon the white prairie-clover (video taken) but none of the other flower species.	Observed for a bit but female was not very active and seemed to be cooling down for the day. She was a bit worn but otherwise in good shape and mobile when desired. Nectared on white prairie-clover. No ovipositing observed. Afternoon was very hot (~32°C), humid (muggy) and calm (Beaufort 0 to 1), which seemed ideal for the butterflies.
Hesperia dacotae	July 21, 2010	16:15	Η	<b>1</b> ♀	OK14 Genetic Sample #7	Observed perching on upland white goldenrod ( <i>Solidago ptarmicoides</i> ) and a yellow goldenrod species (likely moutain goldenrod, <i>Solidago spathulata</i> ).	
Hesperia dacotae	July 7, 2011	14:00	D	1♂	None	Perched on grasses.	Male perched on grasses and appeared newly emerged.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 7, 2011	14:10- 14:40	Η	~8∱ &6♀	(Yes, per July 7, 2011 below)	Saw 5 <i>Hesperia dacotae</i> nectaring on black-eyed Susan (R <i>udbeckia hirta</i> ).	First day of flight period observations in SW in 2011. Lots of <i>Hesperia</i> <i>dacotae</i> out, including females. Lots of <i>Polites mystics</i> out too (same amount as DS), plus one <i>Anatrytone logan</i> also seen. Often saw a male pursue a female. Then a 2nd or even 3rd male would join in and the cluster would then fly higher into the air, going up to >5m from the ground until the additional males broke off the pursuit leaving only one male to continue pursuing the female.
Hesperia dacotae	July 7, 2011	14:10	Н	<b>1</b> ♀	OK14 Voucher #10 (2011)		
Hesperia dacotae	July 7, 2011	15:00	Η	<b>1</b> ♂	OK14 Voucher #7 (2011)		Male caught with female (voucher #8) it was pursuing and attempting to entice to mate.
Hesperia dacotae	July 7, 2011	15:00	Н	<b>1</b> ♀	OK14 Voucher #8 (2011)		Female caught with male (voucher #7) it was pursuing and attempting to entice to mate.
Hesperia dacotae	July 9, 2011	13:00	В	<b>2</b> ð	None	Nectaring on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	2 males newly emerged.
Hesperia dacotae	July 9, 2011	14:30- 16:00	F	<b>1</b> ♂	None		Saw a male but could not catch.
None	July 10, 2011	11:00- 12:30	F	0	None		Several Polites mystics and Polites themistocles seen.
Hesperia dacotae	July 10, 2011	14:15	G	1♀ & 1 sex?	OK7 Voucher #1 (2011)	Porcupine grass ( <i>Stipa spartea</i> ) evident but seeds not mature and sharp yet. White sweet-clover ( <i>Melilotus alba</i> ) in flower.	Saw one other DS, plus the voucher.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 12, 2011	13:30- 15:30	H	>40♂ & >20♀	None	Hesperia dacotae perching (P) and nectaring (N) observations: 15N & 19P on black-eyed Susan; 4N & 3P on white sweet-clover; 1N & 1P on spiked lobelia; 6P on horsetail/carex/grass; 1P on upland white goldenrod; 2P on death camas; 4P on wild licorice; 1P on harebell; 1P on prairie rose. Proportion of flowers in bloom (% total area) in habitat is approx: 30% black-eyed Susan, 30% white sweet-clover, 25% spiked lobelia, 5% white prairie-clover, 5% upland white goldenrod, 5% harebell and 1% yarrow.	Males in good condition and females not yet gravid (abdomen distended with eggs). Saw 41 instances of DS pair interactions ( $\mathcal{Q} \otimes \mathcal{J}; \mathcal{J} \otimes \mathcal{J}$ ) including 2 instances of potential courtship with $\mathcal{Q} \otimes \mathcal{J}$ perched in close contact and vibrating wings rapidly. Often followed a $\mathcal{Q}$ who was approached by multiple $\mathcal{J}$ as she flew around. Saw one pair fly a distance 100m together and a max. height of 8m. Observed one male <i>Hesperia dacotae</i> "attacking"/persuing a pair of European skippers, and then interacting (courtship attempt?) with a female <i>Polites mystic</i> . Lots of <i>Polites mystics</i> and a few <i>Polites peckus</i> flying.
Hesperia dacotae	July 13, 2011	10:45	F	<b>1</b> ♀	None	Very fresh female perched on a small, immature Asteraceae species (perhaps a <i>Solidago</i> spp.) that was without flowers. Then flew to several white prairie-clovers ( <i>Petalostemon</i> <i>candidum</i> ) and nectared upon them (photos taken).	Very fresh female actively nectaring.
Hesperia dacotae	July 13, 2011	12:50	Н	1♂	OK14 Genetic Sample #8		
Hesperia dacotae	July 13, 2011	13:15	Н	<b>1</b> ð	OK14 Genetic Sample #9	Caught on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	
Hesperia dacotae	July 13, 2011	13:35	Η	1∂	OK14 Genetic Sample #10		

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 13, 2011	14:37	Η	<b>1</b> ð	OK14 Genetic Sample #11		Male was following a female <i>Polites mystic.</i>
Hesperia dacotae	July 15, 2011	15:00	D	<b>1</b> ♀	None	Perched on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	One very worn female.
Hesperia dacotae	July 16, 2011	12:00- 18:00	Η	>30♀ & 5♂	(per below)	Saw >50 instances of DS nectaring on black-eyed Susan, plus saw 2 females nectaring on white prairie-clover ( <i>Petalostemon candidum</i> ), 1 female nectaring on Floodman's thistle ( <i>Cirsium floodmani</i> ) (took photos), and 1 female possibly nectaring on spiked lobelia ( <i>Lobelia spicata</i> ) (took video; skipper species unclear). Often females seemed to fly from the areas with large flower patches out into the areas with carexes (Creeping spike- rush, <i>Eleocharis palustris</i> ) and grasses. Sometimes they also seemed to prefer those flowers at the edge of the flower patches beside the Carex/grass areas. Photographed these areas.	Did behavioural observations in the "good spot", west of T1. Often saw a female hop from one black-eyed Susan to another while nectaring. Other times females just perched on grass or other vegetation. Watched many females for 5 to 20 minutes, but never saw any ovipositing-like actions. Some female DS were slow flying and worn, while others were in very good shape and stronger flyers. Also saw several males and took a voucher (per below). Saw some DS interact (approach) with other skipper species. Saw many <i>Polites mystics</i> , a few <i>Polites peckus</i> and a <i>Polites</i> <i>themistocles</i> .
Hesperia dacotae	July 16, 2011	13:03	Η	<b>1</b> ♀	OK14 Genetic Sample #12		Moderately worn, very full of eggs.
Hesperia dacotae	July 16, 2011	13:10	Н	<b>1</b> ♀	OK14 Genetic Sample #13	Perched on white sweet-clover ( <i>Melilotus alba</i> ).	
Hesperia dacotae	July 16, 2011	14:25	Η	<b>1</b> ð	OK14 Voucher #11 (2011)	Nectaring on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	Took a voucher to confirm it was a male Dakota Skipper as it was very late in the flight period and very few males seen at this point.
Hesperia dacotae	July 17, 2011	11:45	Н	<b>1</b> ð	None	Nectaring on black-eyed Susan ( <i>Rudbeckia hirta</i> ).	

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 17, 2011	11:45- 12:05	Η	1	None	Observed a very gravid female DS flying around, down in the grasses but she never did any egg laying action.	Observed a very gravid female DS flying around, down in the grasses but she never did any egg laying action. For several minutes she was repeatedly attacked by a robberfly whose intentions were unclear but appeared to directly target this female. The fly knocked the female DS out of the air and down into the grasses at least 3 times. Took photos of female and may have attack on video. Collected a voucher of the attacker at a later date.
<i>Machimus paropus</i> [Walker] (Asilidae)	July 18, 2011	11:45	Н	1ð	OK14 Evil Bastard Sample #1		Specimen is visually identical to a robberfly species attacked a female Dakota Skipper on July 17, 2011. Caught in same location and circumstance as seen the day prior.
Hesperia dacotae	July 17, 2011	12:35	Н	<b>1</b> ð	None	Nectaring on scapose hawk's-beard ( <i>Crepis runcinata</i> ) and white prairie-clover ( <i>Petalostemon candidum</i> ).	
Hesperia dacotae	July 17, 2011	13:21	Н	<b>1</b> ♀	OK14 Genetic Sample #14	· · · · · · · · · · · · · · · · · · ·	Abdomen distended with eggs.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 17, 2011	10:30- 15:30	H	~5♂ & >20♀	(Yes, per voucher below)		Saw 3 female DS at the good patch between Transect 1 and Transect 2. Also saw one other female DS moving her abdomen around like she was going to oviposit but she never laid any eggs. Followed >10 females for a few minutes while they were flying low over grasses and flower but them lost them when they then flew away rapidly over a greater distance (>5m). Saw several males again some were very worn and tattered while others were only moderately worn.
Hesperia dacotae	July 18, 2011	12:45	Η	<b>1</b> ♀	OK14 Voucher #12 (2011)	Plants within ~1ft radius of location where female did the "ovipositing" actions were collected and inventoried. Plant species present included: western wheatgrass ( <i>Agropyron smithii</i> ), switch grass ( <i>Panicum virgatum</i> ), Canada bluegrass ( <i>Poa compressa</i> ), Parry's sedge ( <i>Carex parryana</i> ), creeping spike-rush ( <i>Eleocharis palustris</i> ) and Baltic rush ( <i>Juncus balticus</i> ). The female did the ovipositing action on Canada bluegrass, and western wheatgrass was immediately beside it (so these two species were what was present in the clump the female did her action in).	Female distinctly did ovipositing action with her abdomen upon Canada bluegrass ( <i>Poa compressa</i> ) but did not lay any eggs. Collected and inventoried plants within ~1ft radius of females "ovipositing" action spot.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 18, 2011	~12:00 - 15:00	H	~5♂ & >15♀	None	Saw a male perched on a goldenrod species (took pictures). Females often seem to fly out into the slightly lower/wetter (darker) areas of carex (often dominated by Creeping spike- rush, <i>Eleocharis palustris</i> ) and grasses adjacent to the more upland/dryer areas of flowers. I wonder if the females are looking at the lower areas for ovipositing. Also seems like females do a lot of "resting" where they just perch on a plant and appear to do nothing for a while except occasionally open their wings in the sunshine.	Saw approx. 5 males (still!) and >15 females. Males are very worn, while most females are in moderate condition. Females often seem to fly out into the slightly lower/wetter (darker) areas of carex (often dominated by Creeping spike-rush, <i>Eleocharis palustris</i> ) and grasses adjacent to the more upland/dryer areas of flowers. I wonder if the females are looking at the lower areas for ovipositing. Also seems like females do a lot of "resting" where they just perch on a plant and appear to do nothing for a while except occasionally open their wings in the sunshine.
Hesperia dacotae	July 18, 2011	~12:30	Н	<b>1</b> ♀	None	Saw a female nectaring on spiked lobelia (Lobelia spicata).	· · ·
Hesperia dacotae	July 18, 2011	~12:30	Н	<b>1</b> ♀	None	Saw a female nectaring on upland white goldenrod (Solidago ptarmicoides).	
Hesperia dacotae	July 18, 2011	~12:50	Н	<b>1</b> ♀	None	Saw a female nectaring on spiked lobelia (Lobelia spicata).	
Hesperia dacotae	July 18, 2011	~12:50 - 13:15	Η	<b>1</b> ♀	None	Saw a female nectaring on yellow evening primrose ( <i>Oenothera biennis</i> ), white prairie-clover ( <i>Petalostemon</i> <i>candidium</i> ) and white sweet-clover ( <i>Melilotus alba</i> ).	Observed female for a bit while she nectared on several plant species and did the "ovipositing" action with her abdomen but did not lay any eggs.
Hesperia dacotae	July 21, 2011	~12:30	Н	<b>1</b> ♀	None	````	Saw 1 very worn female Dakota Skipper but could not follow it as it was very windy and she disappeared. It was very windy that day and very hard to see anything flying.

				Sex			
Species	Date	Time	Site	& No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	June 26, 2012	15:15	Η	<b>1</b> 3	None	DS seem ahead of many of the plants flowering this year. Unusually smooth & death camas ( <i>Zygadenus elegans</i> & <i>Z. gramineus</i> ), black-eyed Susan ( <i>Rudbeckia hirta</i> ) not quite flowering but there were also not many black-eyed Susan this year. Wood lilies ( <i>Lilium</i> <i>philadelphicum</i> ) just starting to flower. Harebell ( <i>Campanula rotundifolia</i> ), blanket flower ( <i>Gaillardia aristata</i> ), 3- flowered avens ( <i>Geum triflorum</i> ) and heart-leaved Alexander ( <i>Zizia aptera</i> ) in full bloom. Prairie dandelion ( <i>Agoseris</i> <i>glauca</i> ) almost done blooming. Porcupine grass ( <i>Stipa spartea</i> ) seeds not quite mature yet (not pokey yet).	Saw one very fresh male that appears to be no more than 1 day old. Saw many <i>Polites mystics</i> and only one <i>Polites themistocles</i> .
Hesperia dacotae	June 27, 2012	15:00	Н	1♂	None		Saw one fresh male.
Hesperia dacotae	July 4, 2012	13:14	В	<b>1</b> ð	None		Saw one fresh male in great condition & caught. ~2 to 3 days old.
Hesperia dacotae	July 4, 2012	16:30	D	<b>1</b> ♀	Not for this study		Nick DeSilva caught one fresh female in great condition with only very slight wear. Nick joined us in the field and was conducting surveys to update the Status Report for Dakota Skipper.
Hesperia dacotae	July 4, 2012	16:30	D	1∂	Not for this study		Nick DeSilva caught one fresh male in great condition with only very slight wear. Nick joined us in the field and was conducting surveys to update the Status Report for Dakota Skipper.
Hesperia dacotae	July 4, 2012	16:30	D	1∂	Not for this study		Nick DeSilva caught one fresh male in great condition with only very slight wear. Nick joined us in the field and was conducting surveys to update the Status Report for Dakota Skipper.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 4, 2012	16:30	D	~5∱ & 1♀	None		Observed 5 males and 1 female in total at this location at this approximate time. Males & females were in great condition with only slight wear. Nick DeSilva joined us in the field and was conducting surveys to update the Status Report for Dakota Skipper.
Hesperia dacotae	July 5, 2012	15:30	F	<b>1</b> ♀	None		Saw one very worn female with abdomen distended with eggs. Female seems ~ 5 days old.
Hesperia dacotae	July 5, 2012	16:30- 17:00	Η	saw 2♂ & 7⊊; ~15 total	None		Caught 7 females and 2 male DS at this portion of the property, and estimate there were at least 15 DS present here. Kept all 9 DS until done survey to prevent recount and estimate numbers present. Females in good condition, males moderately worn.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 5, 2012	17:15- 18:00	Н	saw 13♂ & 8⊊; ~30 total	None	Generally appears that some of the flower species can't be rushed to flower this year. It was an "early" season this year but some flowers seems out of sync with each other. For instance the camases ( <i>Zygadenus elegans</i> & Z. <i>gramineus</i> ), wood lily ( <i>Lilium</i> <i>philadelphicum</i> ) and prairie clovers ( <i>Petalostemon candidum</i> & <i>P.</i> <i>purpureus</i> ) did not seem to keep up, while the black-eyed Susan ( <i>Rudbeckia</i> <i>hirta</i> ) was out with the skippers though the numbers of flower heads this year were VERY low compared to prior years. In past years the camases and wood lilies are nearly done flowering by the start of the flight period, but this year they had only started. Plus in past years the prairie clover plants are big and just starting to flower but this year the plants were very small, not bushy and were not yet flowering. Porcupine grass ( <i>Stipa spartea</i> ) seeds were also not yet mature (and poky) this year at start of flight period. These observations were particularly evident in the southwest region. Low densities of black-eyed Susans are a significant concern.	Caught 8 females and 13 male DS at this portion of the property, and estimate there were at least 30 DS present here. Again kept all DS until survey done. Most females in good condition & few in excellent condition (1 to 4 days old), males moderately to very worn (3 to 7 days old). Also saw ~15 Polites mystic, ~10 Polites themistocles, ~10 Polites peckius and a few Thymelicus lineola.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 6, 2012	11:00- 13:45 incl. obs.	Η	Saw 50; ~150 total	None	Overall DS observed nectaring upon: lots of black-eyed Susan ( <i>R. hirta;</i> ~75% of time), spiked lobelia ( <i>Lobelia</i> <i>spicata;</i> 1 $^{\circ}$ ), Flodman's thistle ( <i>Cirsium</i> <i>flodmanii;</i> 1 $^{\circ}$ & 1 $^{\circ}$ ), blanket flower ( <i>Gaillardia aristata;</i> 2 $^{\circ}$ & 4 $^{\circ}$ ), white sweet clover ( <i>P. candidum;</i> 1 $^{\circ}$ ), smooth camas ( <i>Z. elegans;</i> 1 $^{\circ}$ ) and prairie dandelion ( <i>A. glauca;</i> 1 $^{\circ}$ ). Some of these observations are detailed in cells below.	Walked property, counted number of DS and estimated number of adults present during entire time at site. Also estimated ratio of DS to other skippers to be approx. 20 DS: 3 <i>Polites mystic</i> : 1.5 <i>Polites themistocles</i> : 1.5 <i>Polites peckius</i> : 0.5 <i>Thymelicus lineola</i> . Seems like there are less of the other (more generalist) skipper species present at immediate spots where DS are present.
Hesperia dacotae	July 6, 2012	11:15	Н	<b>1</b> ♀	None	1 female observed nectaring upon smooth camas ( <i>Z. elegans</i> ). Female was moderately worn.	
Hesperia dacotae	July 6, 2012	11:30	Η	1♂ & 1♀	None		Saw one $3$ and one $9$ attempting to mate! $3$ pursued $9$ then both perched down on a blade of grass, & $9$ vibrated wings rapidly. Then $3$ perched below 9 & sideways extended genitalia up to 9 genitals & briefly copulated for ~ 2 seconds. Saw $3$ as an open hole. Then $3$ flew off.
Hesperia dacotae	July 6, 2012	11:35	Н	<b>1</b> ♀	None	Saw one female nectaring on blanket flower ( <i>G. aristata</i> ).	
Hesperia dacotae & Misumenops asperratus [Hentz] (Areaneae: Thomisidae)	July 6, 2012	11:42	Η	1♂ DS & 1♀ spider	OK14 Voucher #1 (2012) & Spider		Found a male DS in the clutches of a female northern crab spider ( <i>Misumenops asperatus</i> [Hentz], Araneae: Thomisidae) on a blanket flower ( <i>G. aristata</i> ). Evidence of DS predation! Took spider & DS as a voucher. DS moderately worn. Spider determined by D. Wade, Insect Control Branch, City of Winnipeg.

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 6, 2012	12:20	Н	1♂ & 1♀	None		Saw male & female DS interacting as if attempting to mate but no mating observed. Suspect female rejected male (already mated?). Female in great condition, male moderately worn.
Hesperia dacotae	July 6, 2012	12:35	Η	<b>1</b> ♀	Yes - only eggs & grass (Not female)	Eggs laid on early bluegrass ( <i>Poa cusickii</i> ). Within approx. 30 cm radius of eggs there were: lots of porcupine grass ( <i>S. spartea</i> ), some other bluegrasses ( <i>Poa</i> spp.), some <i>Agropyron</i> spp. and some little bluestem ( <i>Andropogon scoparius</i> ).	Observed a female DS ovipositing 2 eggs on to an early bluegrass ( <i>Poa</i> <i>cusickii</i> ) blade. Eggs were creamy white colour at the time. Eggs were taken back, photographed and reared. Two larvae emerged (on July 14 or 15, 2012) making incubation egg stage 9 to 10 days. Photographed 1st instar larvae (July 15, 2012) as well as egg remains. Newly emerged larvae have big, brown heads and creamy white bodies, and larvae were approx. 3.5 mm long (see photos). Larvae constructed a shelter of grass pieces and their own silk. Attempted to rear larvae but could not relocate them in rearing chamber after one week following hatching and presumed dead.
Hesperia dacotae	July 6, 2012	12.39	Н	<b>1</b> ♀	None	Saw one female nectaring on black- eyed Susan ( <i>R. hirta</i> ) and prairie dandelion ( <i>A. glauca</i> ).	
Hesperia dacotae	July 6, 2012	12:46	Η	<b>1</b> ♀	None	Saw one female nectaring on blanket flower ( <i>G. aristata</i> ).	
Hesperia dacotae	July 6, 2012	13:00	Н	<b>1</b> ♀	None	Eggs laid on bluegrass (Poa spp.). Adjacent grasses could not be identified at the time.	Saw a female DS ovipositing 1 egg on to a bluegrass ( <i>Poa</i> spp.) blade.
Hesperia dacotae	July 6, 2012	13:25	Н	<b>1</b> ∂ੈ	None	Saw one male nectaring on spiked lobelia ( <i>L. spicata</i> ).	

Species	Date	Time	Site	Sex & No.	Sample No.	Plant interaction	Behavioural observations
Hesperia dacotae	July 6, 2012	13:43	Η	<b>1</b> ♀	None	Saw one female nectaring on two different stems of white prairie clover ( <i>P. candidum</i> ) and a stem of black-eyed Susan ( <i>R. hirta</i> ).	
Hesperia dacotae	July 9, 2012		All	None	None	Susan ( <i>R. hirta</i> ). Overall there seem to be less black- eyed Susan ( <i>Rudbeckia hirta</i> ) flower heads this year in both the Interlake and southwest sites. The flowers also seem smaller, shorter and have fewer heads per plant. Cause is unclear but prolonged flooding and wetness last year and this spring may have killed many plants, plus early summer this year was very hot and sites dried up very quickly. Increased temperatures early this season advanced some plant phenology but also may have stunted/killed plants. Plus generally saw less nectar plants at all the sites. There seemed to also be less spiked lobelia ( <i>Lobelia spicata</i> ) and sweet clovers ( <i>Melilotus alba</i> and <i>M.</i> <i>officinalis</i> ) especially, and saw almost no prairie clovers ( <i>Petalostemon</i> <i>candidum</i> and <i>P. purpureus</i> ) anywhere. Most of the porcupine grass ( <i>Stipa</i>	No DS seen at this point in Interlake. Generally seems like all the skipper species we see during DS flight period are getting more worn. Also now seeing Duns skipper ( <i>Euphyes vestris</i> Based on 3 years of observations skipper emergence order seems to be approximately: European skipper, long dash skipper, tawny-edged skipper, Peck's skipper, Dakota Skipper, silver spotted skipper, Dun skipper. Peck's skipper emerges just a few days before or at same time as Dakota Skipper.
		er Hesperia (				<i>spartea</i> ) also seems to have disappeared from the Interlake and southwest Manitoba sites, except at Site H (OK14) where it is still abundant.	

DS = Dakota skipper, Hesperia dacotae

#### Appendix X. Photo Appendix



Male Dakota Skipper (*Hesperia dacotae*) showing upper wing surface markings.



Worn male Dakota Skipper (*Hesperia dacotae*) showing lower wing surface with faint markings.



Female Dakota Skipper (*Hesperia dacotae*) showing upper wing surface markings.



Female Dakota Skipper (*Hesperia dacotae*) showing lower wing surface markings.



Dakota Skipper habitat among the aspen parkland at Site D, during the flight period.



Field of black-eyed Susan (Rudbeckia hirta) at Site C.



Dakota Skipper habitat with a few patches of aspen at Site G in southwestern Manitoba during the flight period.



Mature porcupine grass (*Stipa spartea*) fruit during the Dakota Skipper flight period.



Mature porcupine grass (*Stipa spartea*) inflorescence.



Creeping spike-rush (*Eleocharis palustris*).



Purple prairie-clover (*Petalostemon purpureus*).



Upland white goldenrod (Solidago ptarmicoides).



Spiked lobelia (Lobelia spicata).



Meadow blazingstar (*Liatris ligulistylis*).



Male Dakota Skipper dead in the clutches of a female Northern Crab Spider (*Misumenops asperatus*) on a blanket flower (*Gaillardia aristata*).



Female Northern Crab Spider that was feeding upon a male Dakota Skipper.



A robberfly (*Machimus paropus*); a likely predator of Dakota Skipper.



Male (bottom) and female (top) Dakota Skippers during "courtship" and just before attempted copulation.



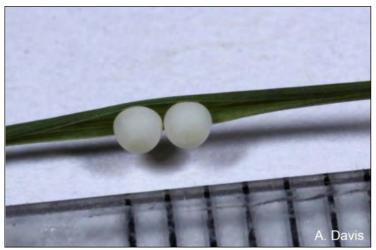
A Dakota Skipper female perched down in the grasses and making ovipositing motions with her abdomen, but no eggs were deposited.



Two fresh Dakota Skipper eggs deposited on a blade of early bluegrass (*Poa cusickii*).



Another Dakota Skipper female perched down in the sedges and grasses and making ovipositing motions with her abdomen, but no eggs were deposited.



Two Dakota Skipper eggs measuring approximately 1 mm in diameter.



First instar Dakota Skipper larvae two or three days after emergence and measuring 3.5 to 4.0 mm in length.



First instar Dakota Skipper larvae two or three days after emergence, showing prothoracic shield.



First instar Dakota Skipper larvae two or three days after emergence.



Female Dakota Skipper feeding on black-eyed Susan (*Rudbeckia hirta*).



Female Dakota Skipper feeding on white prairie-clover (*Petalostemon candidum*).



Male Dakota Skipper feeding on scapose hawk's-beard (*Crepis runcinata*).



Female Dakota Skipper feeding on white sweet-clover (*Melilotus alba*).



Female Dakota Skipper perched on a short, white sweetclover (*Melilotus alba*).



Female Dakota Skipper being released immediately after capture.



Male Dakota Skipper showing identifying markings.



Female Dakota Skipper heavy with eggs in her abdomen, and showing typical wing position when perched and markings.



Site F during the 2011 flight period, lush with vegetation.



Site F in 2010, showing large clumps of the nectar flower, white prairie-clover (*Petalostemon candidum*).



Site F in 2010, showing large clumps of the nectar flower, purple prairie-clover (*Petalostemon purpureus*).



Site F during the 2010 flight period, lush with vegetation and nectar flowers.



Site F during the 2010 flight period, lush with vegetation and nectar flowers.



Site F following the 2011 flight period, lush with vegetation and flowers.



Site F in 2012 during the flight period, after cattle were pastured on the site in early summer. Note the complete lack of nectar sources.



Tuberous-rooted sunflowers (*Helianthus nuttallii*) in 2010 at Site F.



Site F in 2012 during the flight period, after cattle were pastured on the site in early summer. Note the complete lack of nectar sources.