The nectar sources and flower preferences of the Poweshiek Skipperling (*Oarisma poweshiek*) in Manitoba.

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Abstract

The Poweshiek Skipperling, *Oarisma poweshiek,* is a threatened butterfly found within the 2300 ha Tall Grass Prairie Preserve in southern Manitoba. Land management practices to maintain prairie habitats in a natural state, primarily burning and grazing, have been linked to a reduction in Poweshiek Skipperling habitat. Management activities in prairie habitat can change flowering plant composition, which may reduce the amount or type of nectar sources available for adult butterflies. In this study nectar plant diversity and adult Poweshiek Skipperling flower utilization between two sites with different burn histories was assessed. Preferred nectar flower sources of the Poweshiek Skipperling included *Rudbeckia hirta* and *Solidago ptarmicoides*, with skipperlings showing a strong preference for a 2002 burn site versus a 2008 burn site. Flowering plant diversity increased in the 2002 burn over the Poweshiek Skipperling flight period in comparison to the 2008 burn. The 2002 burn had shorter, less dense grass cover as well as a greater number of flowering stems of *R. hirta* and *S. ptarmicoides*. The level of competition by arthropods for the nectar or basking area on *R. hirta* was similar between the 2002 and 2008 burn sites. Flower nectar analysis indicated that sugar concentrations in *R. hirta* and *S. ptarmicoides* were relatively low compared to other flowering species during the flight period. The nectar tube length was similar in *R. hirta* and *S. ptarmicoides*. The results of this study support other research showing that time since burn will influence flowering plant composition, and may alter the suitability of prairie habitats for Poweshiek Skipperling. Future burns on the Tall Grass Prairie Preserve should be planned to conserve or enhance Poweshiek Skipperling populations.

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Introduction

The Poweshiek Skipperling, *Oarisma poweshiek* (Parker), is a small species of butterfly in the family Hesperiidae. This species was designated as threatened in 2003 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). The decline in Poweshiek Skipperling populations has been directly attributed to habitat loss caused by human activity, mainly the conversion of natural tall grass prairie to agricultural land (Samson and Knopf 2004) and poorly planned activities such as haying or burning (Swengel 2008). An improved understanding of Poweshiek Skipperling biology and habitat requirements is essential for the recovery and protection of this species.

The Poweshiek Skipperling is found in mostly undisturbed regions of tall grass prairie. The North American range once included an area from Manitoba southward to Iowa, but due to extensive fragmentation and habitat loss it is now restricted to isolated patches of habitat in Michigan, Iowa, Wisconsin, North and South Dakota, Minnesota and Manitoba (Shepherd 2005). In Manitoba, the Poweshiek Skipperling is restricted to the 2300 ha Tall Grass Prairie Preserve located near Tolstoi, Stuartburn, and Gardenton (COSEWIC 2003).

The flight period of the adult Poweshiek Skipperling normally spans two to three weeks from the end of June to early July, with males emerging first (Klassen et al. 1989). Eggs are laid singly on or near the host plant on blades of grass (Shepherd 2005) and take approximately nine days to hatch (Klassen et al. 1989). Larvae feed at the base of grasses from July to October and overwinter in the fifth instar within a silken nest in the litter at the base of the host plant (Scott 1986). Nectar sources for the adults have been reported

for the United States by Swengel and Swengel (1999), however the only flower species reported as a potential nectar source at the Tall Grass Prairie Preserve was *Rudbeckia hirta* L., the Black-eyed Susan (COSEWIC 2003).

The primary threat to Poweshiek Skipperling survival appears to be land management practices. In Manitoba, tall grass prairie has been reduced to less than 1% of the original coverage due to agricultural development (Samson and Knopf 2004). Most remaining tall grass prairie areas are either privately owned or protected (COSEWIC 2003). Management practices to maintain prairie habitat within tall grass prairie remnants include prescribed burns, cattle grazing, or haying. There is evidence to suggest that these practices, when poorly managed, are largely responsible for the decline of Poweshiek Skipperling in remaining prairie habitats (Swengel 2008).

Swengel (1996) showed that habitat specialists such as the Poweshiek Skipperling are often significantly under-represented in recent burns, and that this effect may continue for up to five years. The adult Poweshiek Skipperling flies for short distances which makes it a poor disperser and thus limits the butterfly's ability to recolonize burned habitat (Swengel 1996). Prescribed burns that mimic wildfires where small patches of prairie are burned infrequently were more beneficial to skipperling survival than large rotational burns (Swengel 1996, Swengel 1998). Haying was more promising in managing prairie specialist species when compared to burns in similar age classes (Swengel 1996). Haying needs to be carefully planned as annual haying on a large scale has negative effects on Poweshiek Skipperling populations when compared to haying parts of sites in a given year (Swengel 2008). Cattle grazing caused a reduction in skipperling numbers when the grazing was classified as moderate to heavy (Swengel

2008), likely resulting from the removal of nectar sources and larval host plants by the cattle (COSEWIC 2003).

Understanding the utilization of nectar sources by the adult Poweshiek Skipperling may aid in planning and undertaking land management practices. Nectar is an important source of sugars and amino acids, and many butterflies have specific flower species preferences as a nectar source (Ezzeddine and Matter 2008). Such flower species become indicators of habitat quality for particular butterflies (Ezzeddine and Matter 2008). The presence or absence of this resource may determine the suitability for habitat colonization (Severns and Karacetin 2009). While observations made in Poweshiek Skipperling populations of the United States have shown Ox Eye, (*Heliopsis helianthoides* L.) and Purple Coneflower, (*Echinaceae angustifolia* DC.) to be important nectar sources (Swengel and Swengel 1999), Canadian nectar species for the Poweshiek Skipperling are generally unknown (COSEWIC 2003). The objective of this study was to determine the preferred nectar sources of the Poweshiek Skipperling in Manitoba and to examine the effects of age of burning on the presence of preferred nectar flowers.

Hypotheses

1) There is no difference in the flowering plant composition between two different burn treatments during the flight period of the Poweshiek Skipperling.
2) There is no difference between the number of Poweshiek Skipperling utilizing flowering plants between different burn ages.

Methods

Experimental Areas

Two study sites, one burned in 1998 and 2002 and one burned in 2008, were selected within the Tall Grass Prairie Preserve in south eastern Manitoba (49°05'N, 96°49'W). The Tall Grass Prairie Preserve was established by the provincial Critical Wildlife Habitat Program in 1989. The Nature Conservancy of Canada currently owns the majority of the protected tall grass prairie habitat in the Tall Grass Prairie Preserve (Hamel et al. 2006). Mean temperatures range from 19.8°C in July to -17.1°C in January. Mean annual precipitation is 382.4 mm with 86.7% of the annual precipitation occurring from April through September (Environment Canada 2004). Abundant grass species within the Tall Grass Prairie Preserve include big bluestem (*Andropogon gerardii* Vitman) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) (Henne and Diehl 2002). Deciduous tree species are dispersed within open prairie habitat or in small stands. Tree species include willow (*Salix spp*.), poplar (*Populus spp*.), and oak (*Quercus spp*.) (Henne and Diehl 2002).

The two study sites were burned as part of the land management program in the Tall Grass Prairie Preserve. Each study site covered an area of approximately 2 ha. Site 1 (N49° 05.259', W096° 45.374') had patches burned in 1998 and 2002 and was described as supporting a substantial population of Poweshiek Skipperling (Webster 2003). The plots sampled in Site 1 were within the 2002 burned habitat. The area for Site 2 (N49° 05.231', W096° 43.912') was burned in 2008 and was reported to support a significant population prior to the burn (Webster 2003). The sites were separated by approximately 2 km.

Sample plots (40 x 40 m) within each site were dispersed to give maximum coverage of the open prairie components of the site. The plot borders were established using a compass and measuring tape. Plot corners were marked with flagging tape on pigtail marking pins. Poweshiek Skipperling observations, flowering plant and butterfly census data, and competitor survey data was collected from five plots in Site 1 and four plots in Site 2 (Table 1). For the purposes of this study, use of the term 'flowering plants' refers to non-graminoid flowering plants.

Plant sampling and butterfly observations took place between 17 July and 4 August 2009 (Table 2). The sampling schedule was dependant on weather and adult flight period. Observations of skipperling activity were made mainly on sunny days with low wind speeds. Poweshiek Skipperling nectar preference and non-target butterfly observations took place between 09:00 and 16:00 hrs.

Flower percent cover

Plant composition between study sites was determined using an assessment of percent cover (Schultz and Dlugosch 1999). Quadrats (1 x 1 m) were placed in each plot randomly using a random numbers table and compass. The percent cover of flowering plants was estimated per individual species. Flowering plants that were not in bloom were marked as 'unknown' and a percentage was recorded. 'Unknowns' were identified as the plants bloomed in subsequent sampling days. Grass cover was also estimated in each quadrat. Ten quadrats were sampled per plot in a sample day. A total of 50 quadrats were sampled in Site 1 and 60 quadrats were sampled in Site 2 (Table 1).

Flowering stem counts and non-target butterfly census

Flowering stem counts and non-target butterfly observations were made using 3 x 3 m quadrats. Non-target butterflies included all species except Poweshiek Skipperling. Poweshiek Skipperlings were included in the census if they were observed in the quadrat. Quadrat placement within the plots was determined by random numbers table and a compass. Butterfly observations were made for 15 minutes. Butterflies that flew through or into the quadrat were recorded on a census tally sheet.

Flower stem counts were made after the non-target observations were complete (Schultz and Dlugosch 1999). A count of stems was completed for each flowering species in bloom observed within the quadrat. The height of the inflorescence was measured, and an overall grass height measurement was also taken within the quadrat. Five to twelve quadrats were sampled in a plot per sample day. A total of 39 quadrats were sampled in each site (Table 1).

Poweshiek Skipperling observations

Observations of Poweshiek Skipperling activity were made within the plots in both sites (Table 1). Poweshiek Skipperling activity within the plots included recording skipping flight, direct flight (relatively straight flight path without landing), nectaring, and basking. Flower species utilized were recorded and the activity taking place was timed. Wing quality was estimated as good, intermediate or poor. Flower species that were within the flight path but were avoided were recorded.

The activity of Poweshiek Skipperlings that flew outside the plot boundaries were included in the observations to improve sample size. The observations were accepted if the skipperling was within 10 m of the plot.

Competitor survey

A survey of insects or arachnids that may be competing for resources on flowers with Poweshiek Skipperling was undertaken. Competition was defined as an arthropod taking flower nectar and/or utilizing the landing or basking area of the capitulum. *Rudbeckia hirta* was selected as the flowering species to be surveyed as this species was visited most often by Poweshiek Skipperling. The surveys were completed on days of high Poweshiek Skipperling activity. Subplots (20 x 20 m) were established within the 40 x 40 m study plots at locations of high *R. hirta* density. Each *R. hirta* flower head was counted within the subplot. When an insect or arachnid was encountered on the flower the Family was recorded on a tally sheet. Photographs were taken to aid in identification when the Family could not be identified in the field. Surveys on 27 July took place in five subplots in Site 1 and four subplots in Site 2. Surveys on 29 July took place in six subplots in Site 1 and four sub-plots in Site 2. Data from each site was grouped by sample day for comparison (Table 1).

Flower dissections

Flower heads from plant species in bloom during the Poweshiek Skipperling flight period were collected for dissection in the laboratory. Samples were placed in plastic bags and stored in a refrigerator at 5°C until the dissections took place. During

the dissections, diagrams of the flower anatomy were made. The distance from the opening of a flower or floret to the base or nectary was measured. The presence or absence of visible nectar at the base of the flower was recorded.

Potential nectar value

Sugar concentration measurements were recorded on a relative scale. Flower heads collected in the field were bagged and brought back to the laboratory. The ray and disk florets or flowers with the nectar base were pulled from the stems and crushed using a mortar and pestle. Five milliliters of distilled water was added to the resulting paste to aid in the grinding process. The pulp was pressed, and the liquid runoff was drawn up by syringe. This liquid was then read by a hand-held refractometer and the resulting Brix scale reading was recorded.

Data analysis

Flower percent cover data was grouped for analysis into two sample date ranges. Early flight season sampling (normally dominated by male skipperlings) was from 17 - 21 July 2009, and late flight season sampling (normally dominated by female skipperlings) was from the 23 - 29 July 2009. Each date range included the averaged percent cover for each flower species recorded at 30 quadrats in each site. Flowering stem count data was also grouped into two seasonal date ranges for each site. Early flight season sampling was from 18 – 22 in July 2009. Late flight season sampling was from 23 - 25 in July 2009. Early sampling data represented averaged stem counts per

flowering species for 20 quadrats in each site. Late sampling represented the averaged stem counts for 19 quadrats in each site.

The number of percent flower cover quadrats were uneven between Site 1 and 2 (Table 1). To balance the sample size between Site 1 and 2 for repeated measures analysis the percent cover results for the 50 quadrats in Site 1 were entered into EcoSim (Gotelli and Entsminger 2009). EcoSim can generate a simulation of expected data points based on the distribution of existing data points entered into the software. The simulation was run to produce a distribution of data points for ten quadrats in Site 1 resulting in a balanced design.

Prior to analysis, experimental variables were tested for departure from the normal distribution. Flower percent cover and flowering stem count plant taxon data was entered into a species diversity calculator spreadsheet in Excel based on Magurran (2004).

Simpson's diversity index (1/D), Simpson's evenness, and Berger-Parker dominance (d) were calculated. These indices were selected to explain the distribution of species abundances, and each index expressed species diversity or evenness as a single figure (Magurran 1988). Simpson's diversity combines species richness and evenness into a single value and Berger-Parker dominance calculates the degrees to which a population is dominated by a few species (Magurran 1988).

Simpson's Diversity Index

This index is based on an equation showing the probability that any two individuals, drawn at random from an infinite population, belong to two different species (Magurran 1988).

$$D = \sum p_i^2$$

Here, "p_i = the proportion of individuals to the *i*th species" (Magurran 1988).

The equation is modified to a form that represents a finite community, allowing for the calculation of the index (Magurran 1988).

$$D = \sum \frac{n_i(n_i - 1)}{N(N - 1)}$$

In this form, "n_i = the number of individuals to the *i*th species and N = the total number of individuals" (Magurran 1988).

To allow for the index value to increase as species diversity increases, the result in often expressed as 1/D (Magurran 1988).

Simpson's Evenness

This is a true evenness measure and calculates the abundance of each species within the population (Magurran 1988). High evenness occurs when each species is nearly equal in abundance, and this situation is reminiscent of a highly diverse population (Magurran 1988). Evenness is determined by the equation:

$$E_{1/D} = \frac{(1/D)}{S}$$

where *(1/D)* = Simpson's diversity index and S = the number of species encountered (Magurran 2004).

Simpson's evenness is scored from 0-1, where 1 = a completely even species composition and 0 = a population dominated by one species (Magurran 2004).

Berger-Parker Dominance (d)

This index was intended to show the proportional importance of the most abundant species in the population (Magurran 1988).

$$d = N_{max} / N$$

Here, "N_{max} = the number of individuals in the most abundant species" (Magurran 1988). A decreasing value represents an increase in diversity and a decrease in dominance (Magurran 1988). Dominance is scored from 0-1, where 1 = a population dominated by one species and 0 = a diverse population (Magurran 2004).

For flowering species the mean percent cover and stem counts and mean number of flowering stems were calculated. Repeated measures Analysis of Variance (ANOVA) was used to determine if there was a significant difference in the number of plant species, stem counts, and diversity between the sites over the sampling season. The Wilk's lambda multivariate test was used to compare vegetation composition in the early flight season in Site 1 versus Site 2, as well as vegetation composition in the late flight season in Site 1 versus Site 2, and to compare the changes over the flight season in Site 1 versus Site 2. The Mann-Whitney U test was used to compare *R. hirta* and *Solidago ptarmicoides* (Nees) Boivin flower percent cover and stem counts between sites. Grass cover and height were compared between sites using an independent samples t-test. A Mann-Whitney U test was used to compare the heights of *R. hirta* and *S. ptarmicoides* between sites. Competitor data was recorded as a percentage comparing the density of

competitors occupying *R. hirta* capitula in each site on each sample day. SPSS version 17, 2008 (SPSS Inc. Chicago Illinois) was used for all statistical analysis. Statistical tests were considered significant at $p < 0.05$.

Results

Within the percent cover, flowering plant species were more numerous in the 2002 burn site versus the 2008 burn site (Table 3). Flowering plant diversity tended to be higher and more Poweshiek Skipperlings were present in the 2002 burn site (Table 3). There was no significant difference in the Simpson's diversity index for percent flower cover between sites although the Simpson's index differed significantly by sampling date in both sites (Table 4). There was a strong interaction between site and sampling date for both the number of species and Simpson's diversity where diversity increased (Table 3 and 4). Simpson's evenness increased in the 2002 burn in comparison to the 2008 burn although the increase was not significant (Tables 3 and 4). The Berger-Parker index decreased significantly in the 2002 burn site but remained statistically unchanged in the 2008 burn site (Tables 3 and 4).

A large amount of variability was encountered for the flowering plant stem counts (Table 5). No significant differences were found when flowering plant stem counts were compared between sites (Table 6).

Two flower species were recorded as the main nectar sources for Poweshiek Skipperling during this study. *Rudbeckia hirta* was used during the majority of floral visits by the Poweshiek Skipperling, and *S. ptarmicoides* was also occasionally visited (Table 7). Comparisons of stem counts for *R. hirta* and *S. ptarmicoides* showed that the 2002 burn site had a significantly higher number of stems for both flower species (Table 8). There was no statistical difference in percent cover for *R. hirta* between both study sites. *Solidago ptarmicoides* was poorly represented in the percent cover surveys and as a result the sample size was too small for comparison.

Grass cover was greater in the 2008 burn in comparison to the 2002 burn (Table 9). Grass height was also significantly greater in the 2008 burn (Table 9). There was no significant difference in heights of *R. hirta* between sites (Table 9). *Solidago ptarmicoides* heights were greater in the 2008 burn (Table 9).

No Poweshiek Skipperling were observed in the 2008 burn site during the sampling period, with all Poweshiek Skipperling (n = 11) observations occurring in the 2002 burn (Table 7). Nectaring and/or basking took place on *R. hirta* during the majority of the floral visits, with *S. ptarmicoides* being the secondary choice. *Potentilla fruticosa* L. and *Campanula rotundifolia* L. were most commonly avoided during foraging. Skipperlings were generally in good condition having wings with complete edges and what appeared to be ample wing scale cover.

The most common group of butterflies observed during the Poweshiek Skipperling flight season were in the family Nymphalidae, primarily *Phyciodes spp.* (Table 10). More butterflies were observed in the 2002 burn (n = 86) in comparison to the 2008 burn (n = 21).

There was little difference in arthropod competitors found on *R. hirta* within sites by sample date (Figure 1). The 2002 burn had 0.13 competitors per capitulum on 27 July and 0.12 competitors per capitulum on 29 July. The 2008 burn had 0.10 competitors per capitulum on 27 July and 0.15 competitors per capitulum on 29 July. In the 2002 burn, 12% of *R. hirta* capitula had a competitor on the flower. In the 2008 burn, 13% of the *R. hirta* capitula had competitor arthropods on the flowers. Numbers of ambush bugs (Reduviidae: *Phymata sp.*) that may have been predators of Poweshiek Skipperling were high in the 2002 burn (n = 32) and low in the 2008 burn (n = 1). Crab spiders

(Thomisidae), also likely predators, were not observed in the 2002 burn but were found in the 2008 burn (n = 3).

Rudbeckia hirta and *S. ptarmicoides* had a similar disc floret nectar tube length (Table 11). Nectar was observed within *S. ptarmicoides* but was not observed in *R. hirta*. Relative Brix scale readings of *R. hirta* and *S. ptarmicoides* provided low sugar concentration amounts in nectar when compared to other flowering species in the site area. *Rudbeckia hirta* and *C. rotundifolia* had the lowest recorded sugar concentrations (Table 11). *Castilleja coccinea* (L.) Spreng. had the highest sugar reading but no adult butterflies were observed nectaring on *C. coccinea* during this study.

Table 1. Site layout for the study area, Tall Grass Prairie Preserve, Manitoba, 2009.

Data collection	Sample area	Size	Quantity
–	Site	~ 2 hectares	Site 1 Site 2
All vegetation data collection Poweshiek Skipperling observations Competitor surveys	Plot	40 x 40m	Site 1 - 5 Site 2 - 4
Competitor surveys	Subplot	20 x 20m	Site 1 - 6 Site 2 - 4
Flowering stem counts and heights Non-target butterfly census	Quadrat	3 x 3m	Site 1 - 39 Site 2 - 39
Percent flower cover	Quadrat	1 x 1m	Site 1 - 50 Site 2 - 60

Table 2. Vegetation sampling dates for the 2009 Poweshiek Skipperling flight season.

Descriptor	Site #	Percent flower cover	Flower stem count and butterfly census	Competitor survey	Poweshiek Skipperling observations
2002 burn	1	17-Jul	18-Jul	27-Jul	27-Jul
		18-Jul	22-Jul	29-Jul	28-Jul
		21-Jul	23-Jul		29-Jul
		23-Jul	25-Jul		4-Aug
		24-Jul			
2008 burn	2	17-Jul	18-Jul	27-Jul	27-Jul
		18-Jul	22-Jul	29-Jul	28-Jul
		21-Jul	24-Jul		29-Jul
		24-Jul	25-Jul		4-Aug
		29-Jul			

Table 3. Summary of flowering plants (% cover per 1 m^2) and butterfly census in 2009.

Descriptor	Site #	Sampling date	Number of flowering plant species mean ± SE	Simpson's diversity index 1/D mean ± SE	Simpson's evenness mean ± SE	Berger-Parker dominance d mean ± SE	Number *O. poweshiek* observed during flight period	Total butterflies observed during flight period
2002 burn	1	July17-21	13.0 ± 1.0	8.11 ± 1.01	0.61 ± 0.03	0.27 ± 0.03	2	41
		July 23-29	18.6 ± 0.6	15.91 ± 0.28	0.85 ± 0.01	0.15 ± 0.01	11	34
2008 burn	2	July17-21	16.3 ± 1.2	12.44 ± 0.94	0.75 ± 0.03	0.18 ± 0.02	0	5
		July 23-29	14.3 ± 1.4	12.02 ± 2.27	0.82 ± 0.11	0.18 ± 0.04	0	16

Table 4. Repeated measures ANOVA for flowering plant cover diversity in 2009.

Source	Species	Simpson's diversity index 1/D	Simpson's evenness	Berger-Parker index
Site[1]	$F = 0.170$ $P = 0.701$	$F = 0.020$ $P = 0.895$	$F = 0.812$ $P = 0.418$	$F = 0.564$ $P = 0.494$
Sampling date[2]	$F = 3.270$ $P = 0.145$	$F = 11.800$ $P = 0.026$	$F = 6.036$ $P = 0.070$	$F = 6.516$ $P = 0.063$
Site x Sampling date[2]	$F = 14.297$ $P = 0.019$	$F = 14.612$ $P = 0.019$	$F = 1.750$ $P = 0.256$	$F = 8.090$ $P = 0.047$

[1] Site - ANOVA - test between subject effects.

[2] Sampling date and Site x Sampling date - Wilk's lambda multivariate test.

Table 5. Summary of flowering stem counts (plants per 3 m^2) and butterfly census in 2009.

Descriptor	Site #	Sampling date	Flowering plants stem counts mean ± SE	Number of flowering plant species mean ± SE	Simpson's diversity index 1/D mean ± SE	Simpson's evenness mean ± SE	Berger-Parker dominance d mean ± SE	Number *O. poweshiek* observed during flight period	Total butterflies observed during flight period
2002 burn	1	July18-22	148.5 ± 118.5	8.5 ± 4.5	2.92 ± 0.53	0.42 ± 0.16	0.44 ± 0.02	2	41
		July 23-25	99.0 ± 65.0	11 ± 2.0	4.34 ± 1.62	0.43 ± 0.23	0.45 ± 0.16	11	34
2008 burn	2	July18-22	66.5 ± 25.5	7.5 ± 1.5	3.66 ± 0.36	0.49 ± 0.05	0.38 ± 0.02	0	5
		July 23-25	94.5 ± 48.5	11.5 ± 4.5	3.27 ± 0.68	0.36 ± 0.20	0.48 ± 0.12	0	16

Table 6. Repeated measures ANOVA for flowering plant stem counts in 2009.

Source	Stem counts	Species	Simpson's diversity index 1/D	Simpson's evenness	Berger-Parker index
Site[1]	$F = 0.897$ $P = 0.443$	$F = 0.006$ $P = 0.946$	$F = 0.022$ $P = 0.895$	$F = 0.000$ $P = 1.000$	$F = 0.015$ $P = 0.913$
Sampling date[2]	$F = 0.014$ $P = 0.918$	$F = 0.824$ $P = 0.460$	$F = 0.470$ $P = 0.564$	$F = 0.095$ $P = 0.787$	$F = 0.271$ $P = 0.654$
Site x Sampling date[2]	$F = 0.176$ $P = 0.716$	$F = 0.044$ $P = 0.853$	$F = 1.429$ $P = 0.354$	$F = 0.111$ $P = 0.771$	$F = 0.178$ $P = 0.714$

[1] Site - ANOVA - test between subject effects.

[2] Sampling date and Site x Sampling date - Wilk's lambda multivariate test.

Table 7. Poweshiek Skipperling observations in the 2002 burn during the flight season in 2009.

Date	Time	Poweshiek observation[1]	Activity	Flower species utilized	Time (sec)	Wing quality[2]	Flower species avoided[3]
27-Jul	12:20	1	skipping flight[4]	*R. hirta*	<5	good	*S. ptarmicoides* *P. fruticosa* *Krigia sp.* *C. rotundifolia*
		2	nectaring	*R. hirta*	18	good	n/a
		3	skipping flight	*R. hirta*	<5	intermediate	n/a
			basking on grass		<5		
		4	nectaring	*R. hirta*	18	intermediate	n/a
			nectaring/basking	*R. hirta*	240		
			skipping flight	*R. hirta*	<5		
			nectaring	*S. ptarmicoides*	10		
28-Jul	14:15	5	skipping flight	*R. hirta*	<5	good	n/a
		6	skipping flight	*R. hirta*	<5	good	n/a
29-Jul	13:20	7	skipping flight	*R. hirta*	<5	good	n/a
		8	nectaring/basking	*R. hirta*	45	good	*P. fruticosa*
			skipping flight	*R. hirta*	<5		
		9	direct flight[5]				
		10	basking on grass		30	good	
4-Aug	14:00	11	skipping flight	*R. hirta* *S. ptarmicoides*	<5 <5	intermediate	*P. fruticosa* *C. rotundifolia*
			basking on grass		20		

[1]Activities from first sighting of skipperling until lost from view.
[2]Wing quality: good, intermediate, poor.
[3]Species avoided were within flight path but not utilized.
[4]Short bursts of flight with brief landings on vegetation.
[5]Relatively straight flight path without landing.

Table 8. Comparisons of *Rudbeckia hirta* and *Solidago ptarmicoides* stem counts in 2009.

Descriptor	Site #	Sampling date		*R. hirta* stem counts[1] mean ± SE	*S. ptarmicoides* stem counts mean ± SE
2002 burn	1	July 17-29		0.92 ± 0.21	0.56 ± 0.23
2008 burn	2	July 17-29		0.35 ± 0.12	0.07 ± 0.05
			Z[2]	-2.24	-2.05
			P	0.025	0.04

[1]Plant stems per 3 m^2

[2]Mann-Whitney U value

Table 9. Comparisons of grass cover and height and *R. hirta* and *S. ptarmicoides* heights in 2009.

Descriptor	Site #	Sampling date		Grass cover[1] mean ± SE		Grass height[2] (cm) mean ± SE		*R. hirta* height[3] (cm) mean ± SE	*S. ptarmicoides* height (cm) mean ± SE
2002 burn	1	July 17-29		30.0 ± 2.2		34.7 ± 0.8		34.0 ± 1.5	26.3 ± 0.7
2008 burn	2	July 17-29		66.5 ± 1.7		40.5 ± 0.8		36.5 ± 2.0	39.6 ± 6.3
			t_{108}	-13.259	t_{58}	-4.769	Z^4	-0.359	-2.855
			P	<0.001	P	<0.001	P	0.719	0.004

[1]Percent cover per 1 m^2

[2]Randomly measured in 3 m^2

[3]Plant stems per 3 m^2

[4]Mann-Whitney U value

Table 10. Butterflies observed over the sample period in 2009.

Family	Descriptor	Genus/species	Number of butterflies observed	
			2002 burn (site 1)	2008 burn (site 2)
Hesperiidae	Poweshiek Skipperling	*Oarisma poweshiek*	13	0
Hesperiidae	Skipper	n/a	1	0
Lycaenidae	Acadian Hairstreak	*Satyrium acadica*	1	0
Nymphalidae	Checkerspot	*Chlosyne spp.*	0	1
Nymphalidae	Crescent	*Phyciodes spp.*	52	5
Nymphalidae	Fritillary	*Speyeria spp.*	2	0
Nymphalidae	Viceroy	*Limenitis archippus*	0	1
Pieridae	Sulphur	*Colias spp.*	16	10
Satyridae	Common Wood Nymph	*Cercyonis pegala*	1	4

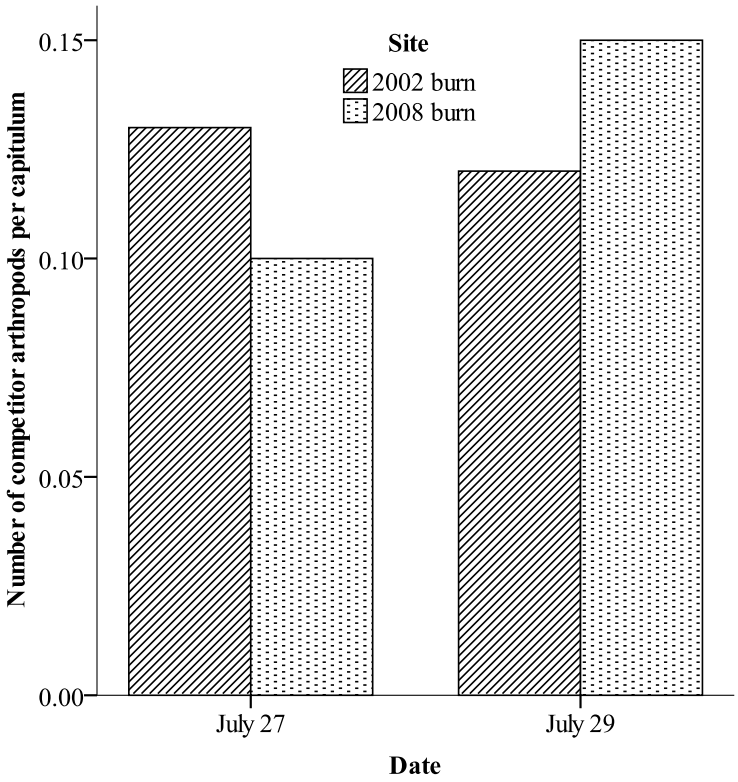

Figure 1. Number of arthropod competitors per *R. hirta* capitulum between sites of differing burn ages in 2009.

Table 11. Flower nectar dissections and Brix (%) readings from both sites pooled in 2009.

Family	Species	Distance to nectar (mm)[1]	Visible nectar	Mean Brix (%) reading[2]
Asteraceae	*A. glauca*	35	no	n/a
Asteraceae	*A. millefolium*	n/a	n/a	2.50
Asteraceae	*Crepis sp.*	15	yes	n/a
Asteraceae	*E. philadelphicus*	3	yes	n/a
Asteraceae	*H. helianthoides*	8	no	n/a
Asteraceae	*Krigia sp.*	14	no	n/a
Asteraceae	*L. ligulistylis*	n/a	n/a	3.75
Asteraceae	*R. hirta**	6	no	1.50
Asteraceae	*S. pauperculus*	9	yes	n/a
Asteraceae	*S. ptarmicoides**	5	yes	2.00
Campanulaceae	*C. rotundifolia*	13	no	1.50
Campanulaceae	*L. kalmii*	n/a	n/a	3.25
Fabaceae	*P. purpureum*	5	no	2.75
Fabaceae	*T. hybridum*	n/a	n/a	2.50
Fabaceae	*T. pratense*	n/a	n/a	3.25
Lamiaceae	*P. vulgaris*	15	yes	0.00
Linaceae	*L. rigidum*	n/a	n/a	1.75
Orobanchaceae	*C. coccinea*	15	yes	5.00
Rosaceae	*P. fruticosa*	0	no	4.25
Rubiaceae	*G. boreale*	2	yes	n/a

[1] Distance from top of floret and/or flower to base.

[2]Readings of crushed flower liquid taken by handheld refractometer.

*Flowering plant species utilized by *O. poweshiek* for nectaring.

Discussion

Vegetation and burn site associations

Flowering plant composition was different between the study sites, and this difference may influence Poweshiek Skipperling habitat preferences. In the 2002 burn there was an increase in the number of flowering plant species over the flight season of the Poweshiek Skipperling. Increased plant diversity in the 2002 burn was reflected by lower plant dominance indices. Simpson's evenness increased as diversity increased. Berger-Parker dominance decreased over the flight season in the 2002 burn and stayed the same in the 2008 burn. In the 2008 burn the number of flowering species and diversity remained relatively unchanged over the flight season.

Adult Poweshiek Skipperlings actively seek out nectar sources during the brief flight period of two to three weeks. During this time period, the development of eggs, as well as mate seeking in males, requires that nutritional sources be available to meet the energy requirements for these behaviours and biological processes (Watt et al. 1974, Boggs 1988, Severns and Karacetin 2009). Habitats with an increasing abundance of nectar sources over the flight period, as in the case of the 2002 burn, offer a more diverse array of nutritional choices to skipperlings. As a result, adult skipperlings may prefer areas with the greatest number of flowering plant species. More flowering species may offer a larger choice of nectar sources, and thus more food, to foraging adults. Females are on the wing for the second half of the flight period and require nutrients for egg maturation and egg laying activity. Sites with increased nectar source availability would be preferred versus those with less appropriate flower species. The 2008 burn did not provide the same levels of nectar source diversity as the 2002 burn. In the 2008 burn the

number and density of flowering plants was unchanged over the flight period. The number of flowering plants available to foraging adults in the 2008 burn may not have been adequate when adult skipperlings were on the wing and energy demands were high, especially for females.

Trends in flowering stem abundance did not mirror flowering plant percent cover measurements. Variability was extremely high for some plant species stem counts between quadrats. The number of plant species in each site based on stem counts was not significantly different, and diversity remained unchanged over the flight period. It is likely that sample size may have had a role in this variability. Percent cover analysis was based on observations from 120 quadrats, while stem count data was based on 78 quadrats. Another factor in the difference between stem counts and percent cover results may have been related to the bloom times of certain species. Flowering stem counts included only stems with open flowers, which was necessary for the bloom height measurements. Flowering species that were present in the quadrat with closed blooms were not measured. Thus percent cover of flowering plants represented all nectar sources available to skipperlings while stem counts only included opened flowers available during the stem counts.

The stem count data was useful, however, when determining the general abundance of preferred nectar sources for Poweshiek Skipperling. Observations of Poweshiek Skipperling behaviour showed that *R. hirta* and *S. ptarmicoides* were the primary flowering species utilized as nectar sources over the flight period. Poweshiek Skipperlings have also been recorded utilizing *R. hirta* as a nectar source in Iowa, Minnesota, and North Dakota (Swengel and Swengel 1999). Stem count comparisons

between sites showed that the 2002 burn contained a greater number of both flowering plant species over the flight period in comparison to the 2008 burn.

The percent cover data for *R. hirta* and *S. ptarmicoides* was less useful for comparison between sites. *Rudbeckia hirta* cover was similar between sites, and *S. ptarmicoides* was not detected in adequate numbers for analysis. While percent cover surveys were sensitive to the change in overall diversity between sites, they were not necessarily sensitive to changes on a species by species basis. This result emphasizes the importance of multiple vegetation surveying techniques to better measure differences in flowering plant composition.

Time between burns probably influenced grass composition between the sites. Grass species were more dense in the 2008 burn with greater mean heights compared to the 2002 burn. Burning has been found to increase the cover of certain grass species, including big bluestem (Gibson 1988, Hulbert 1988, Towne and Kemp 2003), which was abundant in the 2008 burn. Burning removes the dead and decaying litter layer, allowing for the penetration of sunlight to the soil surface (Hulbert 1988). This increase in sunlight may result in higher soil temperatures, a condition related to optimal growth for many species of grass (Rice and Parenti 1978, Hulbert 1988). Sites that have not been burned for a considerable period of time generally seem to suffer a reduction in grass abundance due to increased litter (Howe 2000). This suggests that the 2002 burn site, with a heavier litter layer and lower available sunlight, was not as suitable for supporting dense grass growth.

The degree of grass cover may also affect the abundance of flowering plants within the sites. Dickson and Busby (2009) found that when examining methods for

prairie rehabilitation, sowing grass seeds at high densities suppressed forb cover over a two year study period. Areas of low density grass seeding or no seeding had an increase in forb cover over the study period. In the 2002 burn, grass cover was lower in comparison to the 2008 burn. The 2002 burn also contained a greater number of *R. hirta* and *S. ptarmicoides* stems compared to the 2008 burn, which could mean these species are better able to colonize sites as the litter layer increases and grass cover declines.

The reduction in grass cover in the 2002 burn could also be related to competition with flowering plant roots in the soil. Species such as heath aster (*Aster ericoides* L.) and stiff goldenrod (*Solidago rigida* L.) with dense rhizomes have been shown to significantly decrease big bluestem cover by up to 50% when compared to areas of homogenous big bluestem stands (Dwyer 1958). In Dwyer's study the herbaceous rhizomes were heavily tangled among the roots of the big bluestem. Flowering plants with taproots, however, do not appear to affect grass density (Dwyer 1958). In the 2002 burn, with greater flowering plant diversity, competition between grass and flowering plants may reduce the amount of grass that could potentially occupy the site. The reduction in grass in turn could allow for a more substantial community of flowering plants.

The mean flower height of *R. hirta* was not different between sites. The mean flower height of *S. ptamicoides* was greater in the 2008 burn. Competition with grass for light may be a factor in the increase in height for *S. ptarmicoides* in the 2008 burn. In a study comparing the growth of a grass species versus a sedge species in differing light conditions it was reported that both species had the ability to increase their shoot length in shade conditions (Perry and Galatowitsch 2004). This may have been the case for *S.*

ptarmicoides, while *R. hirta* may not have this flexibility in growth. Light competition may also have influenced the number of nectar species in the 2008 burn, as the biomass of certain species may be reduced with a combination of species competition and shade (Perry and Galatowitsch 2004).

The height of grasses and flowering plants may affect site choice by Poweshiek Skipperling based on foraging behavior. Poweshiek Skipperling fly at grass height when seeking nectar plants (Klassen et al. 1989). Nectar sources that are below grass height may be obscured, and more difficult to detect during foraging. There is also the possibility of a height threshold to which a Poweshiek Skipperling is adapted to flying. Very tall grass that covers nectar sources may be a deterrent to foraging behaviour. While I did not measure maximum grass height in relation to actual Poweshiek Skipperling foraging behaviour, this may be useful to investigate in future research.

Butterfly site preferences

Poweshiek Skipperlings were observed only in the 2002 burn during this study. This butterfly species has been recognized as a habitat specialist in regards to its behaviour and habitat preferences (Swengel 1996, Swengel 1998, Swengel and Swengel 1999).

The abundance of specialist butterflies including the Poweshiek Skipperling are dictated by habitat condition and availability. Most specialist species appear to prefer habitat with infrequent, less intrusive disturbances (Swengel 1998). In particular, management that does not involve burning, such as haying, fosters higher survival of specialist butterflies in prairie ecosystems (Swengel 1996, Swengel 1998). Burns remove

all nectar sources for variable lengths of time, and are followed by a heavy regrowth of vegetation. Lower intensity management such as haying or light grazing leaves behind more nectar sources available to foraging insects (Swengel 1996). Burn timing can have negative effects on Poweshiek Skipperling. For example, burning in the spring or fall can kill larvae within the litter layer (COSEWIC 2003). Also, the interval between burns affects Poweshiek Skipperling abundance. Poweshiek Skipperling populations are often extremely low in the growing season after a burn (Swengel 2008). Specialist butterflies often experience low abundance for three to five years or more in burned sites (Swengel 1996). This five year time frame of population suppression has also been recorded in habitats undergoing restorative grazing. Certain butterfly populations did not recover after five years of restoration and it was found that grazed habitat required more time to mature before being acceptable to specialist butterflies (Pöyry et al. 2005). The 2002 burn site had grown for seven years since the last major disturbance, and likely offered sufficient plant diversity and structure to support the Poweshiek Skipperling. Long stretches of idling or multiple years without intrusive land management treatments support larger Poweshiek Skipperling populations (Swengel 2008).

The dispersal capabilities of Poweshiek Skipperling are limited. The butterfly flies in slow skipping flights over short distances (Swengel 1996). It has been estimated that the Poweshiek Skipperling will only fly about 0.5 to 3 km beyond suitable prairie habitat and nectar sources (COSEWIC 2003). Habitat fragmentation from large scale burns becomes problematic when a butterfly species is unable to colonize suitable habitat that may be too far removed from newly burned areas. Therefore, maintenance of prairie

habitat appropriate for Poweshiek Skipperling requires small patchy burns to create small habitat mosaics rather than large scale repeated fires (Swengel 1996, Swengel 1998).

Natural barriers in the landscape may add to the impediment of Poweshiek Skipperling dispersal. Stasek (et al. 2008) showed that prairie butterflies of different species and body sizes have different dispersal patterns when surrounded by forest edges. Pearl Crescents (*Phycoides tharos* Drury) were more likely to remain isolated in patches of habitat due to small wing size and low flight height when compared to a larger butterfly, the Great Spangled Fritillary (*Speyeria cybele* F.). A low flight height may make it difficult to maneuver through forest vegetation (Stasek et al. 2008). The 2002 burn which supported the most Poweshiek Skipperlings was surrounded by encroaching trees such as poplar and oak. This may limit dispersal to other prairie patches as a result of the Poweshiek Skipperling small size and low height foraging flights.

Burns are a beneficial disturbance within the tall grass prairie as they maintain the prairie landscape and discourage encroaching forest. Fire serves to regenerate the vegetation on older prairie land and maintains the natural habitat by suppressing woody plants (Gibson 1988, Trager et al. 2004). Not all butterfly species are negatively affected by fire, and no land treatment is ideal for each species of butterfly present (Swengel 1998, Swengel 2008). The 2008 burn was dominated by Sulphurs (*Colias spp*), Crescents (*Phycoides spp*.), and the Common Wood Nymph (*Cercyonis pegala* F.). These three species are habitat generalists and were common in burned sites (Swengel 1996). However, the 2002 burn had more butterflies in comparison to the 2008 burn, and this may have been due to a greater diversity of nectar plants, larval host plants, or vegetative structure.

Competing arthropods and site associations

Competition for *R. hirta* between the Poweshiek Skipperling and other resident arthropods was not a likely factor in site preference. While the 2002 burn supported a larger number of *R. hirta*, and a greater number of competitors, there was no difference in the percentage of occupied capitula between sites. Previous observations of arthropods preying on Poweshiek Skipperling included Ambush bugs on *R. hirta* and a crab spider on Ox Eye (*Heliopsis helianthoides*) (Swengel and Swengel 1999). There were two large goldenrod crab spiders (*Misumena spp.*) and one smaller species of crab spider observed in the 2008 burn. It is difficult to conclude whether this density of crab spiders likely influenced skipperling habitat preference. Ambush bugs were observed in relatively higher numbers in the 2002 burn compared to the 2008 burn. A greater abundance of floral visitors in the 2002 burn may favor a larger population of Ambush bugs in this site. Also, more *R. hirta* were available in the 2002 burn as potential hunting platforms for Ambush bugs. The greater abundance of Ambush bugs did not appear to influence habitat preference as all observed Poweshiek Skipperlings were found in the 2002 burn.

Nectar sugar analysis and flower dissections

The difficulties encountered when attempting to measure nectar sugar concentrations stemmed from extremely small volumes of nectar produced by the flowers. Attempts were made to collect nectar on filter paper triangles suspended on insect pins (McKenna and Thomson 1988). The sample is required to be resuspended chemically for analysis by spectrophotometer in the laboratory. In this study nectar collected on the tips of the filter paper triangles was barely detectable, and it was unlikely

that a sufficient volume was collected for appropriate analysis. It was also difficult to determine if the substance collected was nectar or water condensation. The nectar tubes of some disk florets, primarily *R. hirta* and *S. ptarmicoides*, were extremely small and easily damaged. It was difficult to insert a triangle tip for collection without contamination by pollen.

Attempts were made to collect pure nectar by the use of an insulin hypodermic needle. The needle was able to fit into the florets, but generally did not draw up nectar that could be detected in the syringe. The needle often tore through the nectar tube, or punctured the tissues below the flower. When liquid was able to be drawn, the volume was not enough to cover the prism of the refractometer.

Crushing flowers in the laboratory and measuring the liquid runoff by hand-held refractometer provided a relative scale of sugar concentrations. Accuracy was attempted by using only floral parts that were involved in nectar production. The lowest sugar concentration recorded was 1.5%, and the highest recorded was 5%. Using this range, the nectar sugar concentration of *R. hirta* and *S. ptarmicoides* was low compared to other flowers. The method of extraction was imprecise, and presumably the concentration was not representative of nectar sugar concentrations in the field.

Low sugar concentrations (2.5%) have been recorded in resins fed on by female Great Coppers (*Lycaena xanthoides* Boisduval), however it was likely that the chemical deterrents in the resin were a trade off for the reduced nutritional value (Severns and Karacetin 2009). Some nectar that is low in sugar is high in other substances such as monosaccharides and water (Watt et al. 1974). Stress by dehydration may be reduced in an active butterfly species when water is provided at the time of nectaring and extra

foraging for water may be avoided (Watt et al. 1974). It was interesting to note that flowers with the highest nectar sugar concentration values were not utilized by Poweshiek Skipperling. No butterflies were observed utilizing *C. coccinea,* the flower with the highest nectar sugar concentration in this study. *Potentilla fruticosa* L. seemed to be preferred by Coppers (*Lycaena spp.*).

Due to the travel time from site to laboratory, and time required to dissect plant structures under a dissecting microscope, the freshness of the samples could not be maintained. Many samples were stored in a refrigerator for several days. Condensation that gathered in the sample bags could have been mistaken for nectar if the liquid pooled in the base of nectar tubes. Flowers that contained minute volumes of nectar may have been negative for visible nectar due to evaporation during transport or refrigeration. Ideally all samples would have been dissected soon after collection. Growing flowering species of interest within the laboratory may reduce these errors in the future.

For butterflies that depend on nectar sources, the nectar must provide nutritional resources useful for that particular species. Factors in nectar choice can include nutrients and sugar content, and can also be related to viscosity (Watt et al. 1974, Boggs 1988, Ezzeddine and Matter 2008). A more viscous nectar may be difficult for a butterfly to consume due to the narrow diameter of the proboscis (Watt et al. 1974). The chemical composition and viscosity of nectar utilized by Poweshiek Skipperling was not studied in this project. Future work in this area could provide more insight into other nutritional factors that attract Poweshiek Skipperling to *R. hirta* or *S. ptarmicoides.*

Butterfly abundance has been related to nectar abundance in different sites. When peak flowering of a nectar plant species occurs at the same time as the flight period of a

particular butterfly species, those flower species may have a greater chance of being utilized by those butterflies based on floral density (Prior et al. 2009). *Rudbeckia hirta* and *S. ptarmicoides* flowers increased in numbers in both sites over the flight season, but a more comprehensive survey of the flowering times of these species over many years is required to test this assumption. However, the number of flowering plants may not always determine butterfly density, as the abundance of Fender's blue butterflies (*Icaricia icarioides fenderi* Macy) have been correlated with native nectar sugar density, not total flower density (Schultz and Dlugosch 1999). Nectar preference observations of Poweshiek Skipperling populations in the United States have shown substantial variability. This has been attributed to variation between sites, differing plant abundance between years and over flight periods, as well as pressure to find alternate sources during years of greater crowding at a preferred nectar source (Swengel and Swengel 1999).

The flower structure of *R. hirta* and *S. ptarmicoides* was somewhat similar. Flowers of both species are composed of ray florets surrounding densely packed disc florets. Flowers of both species had disc floret nectar tube lengths in the 5 to 6 mm range. False sunflower (*Heliopsis helianthoides*) and Philadelphia fleabane (*Erigeron philadelphicus* L.) had similar morphologies, but their nectar tube lengths were 8 mm and 3 mm, respectively. One interpretation of these observations could be that flower preference is related to proboscis length (Boggs 1988). The proboscis length of the Poweshiek Skipperling has not been reported in the literature and skipperlings were not sacrificed in this study to measure proboscis length. During behavioural observations it appeared that the proboscis of Poweshiek Skipperling was sufficient length to reach to

the bottom of nectar tubes, and that they could have reached nectar in much longer nectar tubes than those of *R. hirta* and *S. ptarmicoides.*

Other aspects of flower morphology or physiology may be a strong attractant to butterflies. Watt et al. (1974) reported that *Colias* butterflies seemed to prefer flowers with a similar UV signature. These 'target' patterns were believed to signal a dilute, monosaccharide rich nectar, with flowers resembling an ultraviolet bulls-eye with the central region of the flowers substantially darker than the outer petals in photographs (Watt et al. 1974). Watt et al. (1974) suggested that the preference for these patterns could be learned over the flight period by trial and error. Younger adult *Colias* butterflies were observed visiting flowers with false nectaries. Identifying a preference for UV patterns by Poweshiek Skipperling would be interesting to pursue. The potential ability of adult butterflies to learn which UV patterns are most beneficial could explain some of the variability in nectar preferences across the range of the Poweshiek Skipperling.

Conclusion

Poweshiek Skipperling were more abundant in a site burned in 2002 compared to a site burned in 2008. The 2002 burn had an increasing diversity of flowering plants over the flight season. Poweshiek Skipperling were only observed in the 2002 burn and absent in the 2008 burn. The preferred nectar sources for Poweshiek Skipperling were *R. hirta* and *S. ptarmicoides.* These two flowering species were more abundant in the 2002 burn, perhaps making this site more preferable to adult skipperlings. More non-target butterflies were observed in the 2002 burn than in the 2008 burn. Grass was more dense and taller in the 2008 burn versus the 2002 burn, potentially suppressing nectar sources. Competition by arthropods for resources offered by *R. hirta* was not different between sites. *Rudbeckia hirta* and *S. ptarmicoides* were found to have similarities in nectar tube length, as well as nectar sugar concentrations.

The number of years between burns created differences in flowering plant composition and may have influenced the habitat choice by Poweshiek Skipperling in the Tall Grass Prairie Preserve. The more mature 2002 burn habitat offered a greater variety and abundance of nectar sources to adult skipperlings. Future burns on the Tall Grass Prairie Preserve should be planned to conserve or enhance Poweshiek Skipperling populations.

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