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A 20 y Analysis of Weather and Management Effects on a Small White Lady's-slipper (Cypripedium candidum) Population in Manitoba

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Abstract.—The small white lady's-slipper, *Cypripedium candidum*, is a rare perennial orchid with a limited distribution in Canada, occurring as isolated populations in remnant tallgrass prairie in southern Manitoba and Ontario. The species is listed as endangered in both provinces and as threatened federally. Despite its status, information on how environmental conditions and land management affect population size and persistence of this species is limited. We used 20 y of monitoring data collected for a subset of the largest population in Canada to evaluate the response of small white lady's-slipper to land management and weather. Long-term monitoring suggests the population is in decline and may not persist under the current climate and management regime. Temperature appears to regulate vegetative growth and flowering proximately. Warm temperatures early in the spring, when shoots are emerging, appear favored, but high temperatures during anthesis appear detrimental, reducing both vegetative growth and flowering. In contrast, precipitation appears to have a lag effect on growth and flowering. However, snow depth was identified as a positive influence on vegetative growth, suggesting precipitation in early spring, when shoots are emerging, is also important for above-ground growth. Some grazing appears to benefit the species presumably by reducing competition and shading, but frequent grazing may increase the risk of direct damage to individuals from cattle consumption and trampling and does not provide sufficient time for individuals to recover following grazing events. Our findings add to the knowledge of orchid conservation and management, highlighting the importance of long-term monitoring in detecting population trends in species with erratic life cycles and fluctuating populations, such as the small white lady's-slipper.

Introduction

The small white lady's-slipper *Cypripedium candidum* Muhl. ex Willd. is a rare perennial orchid with a limited distribution in Canada, occurring as isolated populations in southern Manitoba and Ontario, where it is found in remnant tallgrass prairie, savannah, and calcareous fens (COSEWIC, 2014). It grows in clumps (where the term "clump" refers to a genetic individual or genet) of one to many stems (or ramets) and propagates primarily by creeping rootstocks or rhizomes (Curtis, 1943; Bowles, 1983). The species is capable of prolonged dormancy, surviving underground for as long as 6 y, though 2 to 4 y may be more common, and as little as one year has been observed (Falb and Leopold, 1993; Shefferson, 2006). Prolonged dormancy is likely a response to environmental stressors (Shefferson *et al.*, 2005). Sexual reproduction also occurs in this species through a system of deceptive

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pollination whereby flowers do not provide any reward (edible pollen, nectar), but falsely advertise those rewards to pollinators in order to attract them (Van der Pijl and Dodson, 1966; Dafni, 1984; Ackerman, 1986). Small white lady's-slipper flowers also act as one-way traps, guiding pollinators through the flower in a singular direction towards the stigma and anthers (Stoutamire, 1967; Catling and Knerer, 1980; Catling and Catling, 1991; Ames *et al.*, 2005; Li *et al.*, 2006). Pollen occurs as a sticky mass that smears onto the backs of the pollinators as they move past the reproductive organs. Due to the restrictive architecture of the floral features, only insects with the appropriate morphology will contact the reproductive organs and effect pollen transfer; therefore, many insects can be floral visitors, but not effective pollinators (Stoutamire, 1967; Pearn, 2012; Grantham *et al.*, 2019). Andrenid and halictine bees were identified to be the most important pollinators of a study population in an Ontario prairie (Catling and Knerer, 1980). Potential pollinators in Manitoba include the European honey bee *Apis mellifera*, the sweat bee *Lasioglossum zonulum*, mining bees in the *Andrena* genus, and the fly *Odontomyia pubescens* (Grantham *et al.*, 2019).

The small white lady's-slipper is listed as endangered in both provinces where it occurs and federally as threatened under Canada's *Species at Risk Act*. Small white lady's-slipper is also imperiled in most of the 17 U.S. states in which it is believed extant and is listed as threatened or endangered in eight of those states [NatureServe, 2019; United State Department of Agriculture (USDA), 2019]. It was added to the International Union for Conservation of Nature (IUCN) Red List as vulnerable in 2014 (Rankou, 2014). Historically, much of the population decline and range contraction of this species was attributed to habitat loss and degradation; early European settlers cultivated most of the highly fertile tallgrass prairie, suppressed fire, and implemented domestic livestock grazing on land unsuitable for plowing (Steinauer and Collins, 1996; Allen and Palmer, 2011; Henderson and Koper, 2014). Fire suppression and unmanaged grazing have resulted in extensive woody encroachment and poor diversity in many remnant tallgrass prairie patches (Howe, 1994; Briggs *et al.*, 2002; Lett and Knapp, 2005). Encroachment of woody vegetation, competitive exclusion from other vegetation and particularly invasive species, and thatch accumulation in the absence of land management are currently identified as major threats to the species' persistence (Environment Canada, 2014). However, information on how environmental conditions and land management affect population size and persistence is limited, as evidenced by the paucity of published literature on these aspects of the species' ecology. These knowledge gaps are particularly concerning given the conservation status of the species.

Climate (most notably temperate and precipitation) has been observed to be particularly important in regulating population abundance and dynamics in terrestrial orchids (Djordjević and Tsiftsis, 2020). Studies on other species of lady's-slipper (*e.g.*, *C. calceolus*, *C. reginae*) and on the sympatric prairie fringed-orchids, *Platanthera leucophaea* and *P. praeclara*, demonstrate the importance of temperature and precipitation during various phenological stages in regulating populations of these species (Bowles *et al.*, 1992; Kéry and Gregg, 2004; Willson *et al.*, 2006; Blinova, 2008; Bleho *et al.*, 2015; Morrison *et al.*, 2015; Hurskainen *et al.*, 2017). It is therefore reasonable to assume these climatic variables also play an important role in population regulation of small white lady's-slipper. Similarly, given land management (grazing, burning) has been shown to regulate populations of various grassland orchid species globally (Lunt, 1994; Pleasants, 2005; Coates *et al.*, 2006; Alexander *et al.*, 2010; Catorci *et al.*, 2013; Sonne and Hauser, 2014; Bleho *et al.*, 2015), it is reasonable to assume land management also plays an important role in population regulation of small white lady's-slipper.

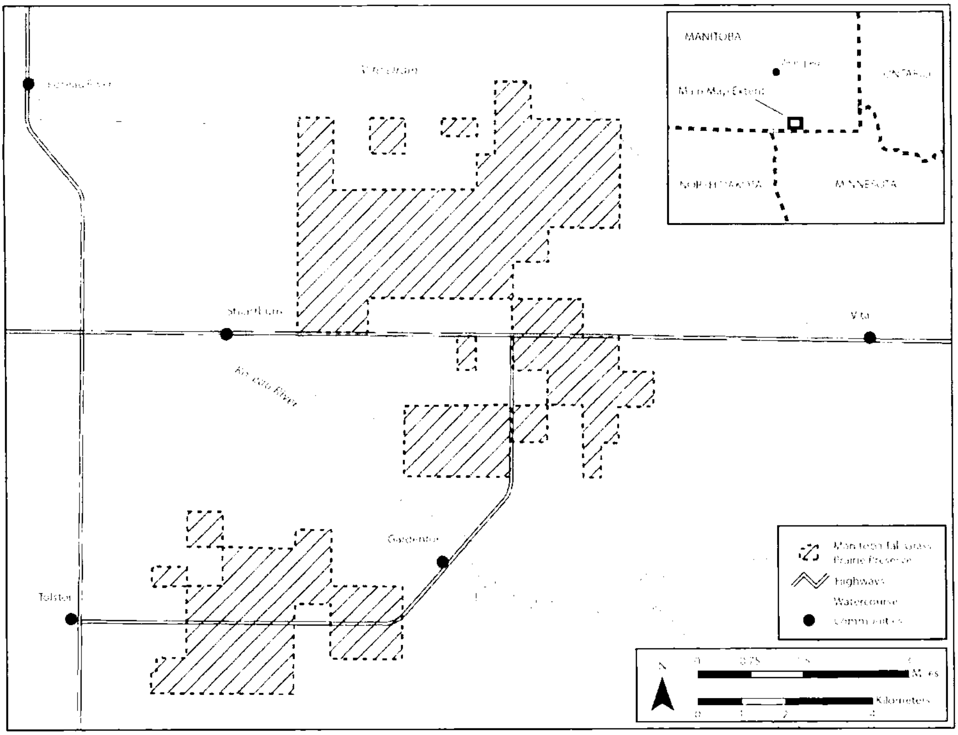

FIG. 1.—Location of the Manitoba Tall Grass Prairie Preserve

The Manitoba Tall Grass Prairie Preserve (MTGPP) in southeastern Manitoba protects most of the known population of small white lady's-slipper in Manitoba and possibly Canada (Environment Canada, 2014). Monitoring of a subset of the small white lady's-slipper population at the MTGPP has been ongoing since 1993, and information on land management at the MTGPP has also been gathered over that period. This presents an opportunity to address some of the knowledge gaps identified above. Our study objectives specifically were to evaluate the response of small white lady's-slipper to land management and weather, with the following questions: (1) Do population fluctuations correspond with weather patterns within defined phenological stages? and (2) Which land management practices best support the population? Understanding the effects of weather on small white lady's-slipper populations can improve management by enabling managers to predict population fluctuations and adjust management activities accordingly.

METHODS

STUDY AREA

The 12,728 acre (5170 ha) MTGPP is located near Tolstoi, Stuartburn, and Gardenton, Manitoba (49°04′45″N, 96°43′53″W; Fig. 1) and protects much of the remaining relatively intact tallgrass prairie in Canada. Most of the MTGPP lands (78%) are owned by the Nature Conservancy of Canada (NCC), with the balance being owned by Nature Manitoba, Province of Manitoba, and Manitoba Habitat Heritage Corporation. The lands are jointly managed through a Management Committee that includes NCC, Manitoba Sustainable Development, Environment and Climate Change Canada, Nature Manitoba, and the Manitoba Habitat Heritage Corporation.

The MTGPP occurs within a landscape characterized by a complex of shallow wetlands and mesic prairie. Regional hydrology is thought to be complex, with surface water flow supplemented by near-surface calcium-rich groundwater at many sites (P. Gerla, pers. comm.). Spared complete conversion to cropland due to stony soils and the high water table, the MTGPP supports dozens of tallgrass prairie species that are dependent on this endangered landscape.

Management activities at the MTGPP include prescribed burning and grazing by cattle (*Bos taurus*). Long-term fire management plans call for each MTGPP property to be burned through prescribed fire once every five years, typically in spring or fall. Properties are not grazed in the year prior to prescribed fire. Actual fire frequency often departs from prescription due to occasional wildfires and seasonal weather conditions not conducive to the use of prescribed fire (*e.g.*, too wet, too dry). A twice-over rotational grazing system has been used with regularity (*i.e.*, at least one property grazed each year, more typically six to eight properties) for management at the MTGPP since 1995. Prior to 1995, grazing was irregular with some properties grazed using a once-over rotational grazing system, and at one property season-long grazing was used. Grazing, fire and other management techniques (*e.g.*, haying) are applied at various MTGPP properties for varying numbers of years as part of a broad approach to sustain a diversity of habitat and structural types; long rest periods between treatments are typical for any given property.

DATA COLLECTION

Four permanent 5 m x 15 m plots were established at the MTGPP in the late 1990s to monitor annual production of small white lady's-slipper vegetative and flowering stems: three plots in 1997 and a fourth in 1999. Plots were in separate MTGPP properties. General plot locations were selected based on the occurrence of small white lady's-slipper on a property and summer staff selecting a general area that best represented the habitat at the time for most of the occurrence. Once the general area was chosen, staff tossed a pin flag over their shoulder to randomly select the first corner of the plot. The plots were censused annually between 1997 and 2016 during the flowering period (late May to mid-June; Table 1). Eight transects spaced approximately 1-2 m apart were walked widthwise through each plot and the number of flowering and vegetative stems was recorded for all clumps of orchids observed within the plot. As it is often difficult to determine to which clump a lone stem may belong, the number of orchid clumps was not recorded.

Weather data for the years 1996 to 2016 were obtained from the National Climate Data and Information Archive from the Emerson station (approximately 35 km west from the study area), with data from the Gretna station (approximately 60 km west from the study area) supplementing missing data for two months in 2009 and one month in 2011, and data from the Winnipeg station (approximately 100 km northwest from the study area) supplementing missing data for one month in each of 1996, 1997, and 2009 (ECCC, 2017). Emerson station was the nearest station to the study area that had weather records for all 21 y included in our study. Weather data obtained were mean monthly temperature, total monthly precipitation, and depth of snow cover at the end of each month. Correlations between weather data from Emerson station and from each of the Gretna and Winnipeg stations were high (precipitation: $r = 0.806$ and $r = 0.759$, respectively; temperature: $r = 0.998$ and $r = 0.997$, respectively), justifying substitution where data from Emerson station were

TABLE 1.—Phenological stages of the small white lady's-slipper (*Cypripedium candidum*) in Manitoba

Stage	Description	Date[1]
Emergence	Emergence of stems, vegetative growth, flower bud development	Late April to mid-May
Anthesis	Flowering, continued vegetative growth	Late May to mid-June
Propagation	Seed production and dispersal	Late June to late August
Senescence	Termination of above-ground growth, below-ground bud development	Late August to September
Dormancy	Dormant below-ground rhizome with perennating buds and flower primordia	October to late April

[1] Based on phenological information observed from Manitoba populations (M. Grantham, pers. obs.; C. Borkowsky, pers. obs.; Punter 1999) and supplemented with literature sources (Curtis 1946, 1954; Bowles 1983), with consideration of the Manitoba climate

unavailable. Land management information was compiled from pasture assessments, fire prescriptions, annual monitoring reports, and annual staff report records maintained by NCC and MTGPP staff. Land management information consisted of the occurrence of grazing or burning each year by property and season of burn (spring or fall). Cattle stocking densities were not available. Land management across the years at each property is detailed in Supplemental Table 1.

STATISTICAL ANALYSIS

We conducted all statistical analyses using SAS v. 9.3 (SAS Institute, 2010). Significance was determined for all analyses at alpha of 0.05. To develop an understanding of how the population was trending generally, we used linear regression using the REG procedure to analyze the effect of year on total stems within each plot. We used generalized linear mixed models (GLMMs) using the GLIMMIX procedure to analyze the effects of temperature, precipitation, and snow depth on vegetative, flowering and total stem count. GLMMs are appropriate for long-term monitoring studies such as ours, because they accommodate clustering of data in space and time. For example, there may be more similarity between data collected from the same site across years than between data collected from different sites. These models also accommodate non-normal distributions typical of count data. Temperature, precipitation, and snow depth were the independent or predictor variables in the models, with plot treated as a random, nesting variable. Stem counts (vegetative, flowering, total) were the dependent or response variables in the models.

For each of temperature and precipitation, we constructed a global model containing mean values within each of six time periods based on phenological stage (Table 1) judged to be potentially associated with stem counts: previous-year emergence, previous-year anthesis, previous-year propagation and senescence, winter dormancy, current-year emergence, and current-year anthesis. We then compared the global model to other candidate models containing various subsets of the six variables. We used Akaike's Information Criterion corrected for small sample size (AIC_c) to select best fitting models, where the best model had the smallest AIC_c score, but models with $\Delta AIC_c \leq 2$ were also included as top models (*i.e.*, also considered to have substantial support; Burnham and Anderson, 2002). Parameter estimates were determined from the highest-scoring model in which that variable appeared.

Snow depth data were limited. We excluded October, April, and years 1998–2009 and 2012–2013, because data were unavailable or otherwise snow depth was zero, which precluded comparisons within these periods. Due to high correlations in snow depth among months, we used the month with the greatest end-of-month snow depth to represent maximum snow depth in each dormancy period (November-March).

We were also interested to know if climate had changed over the 20 y monitoring period as this may inform our interpretation of results. We used linear regression using the REG procedure to analyze the effects of year on temperature and precipitation means within the phenological stages of the small white lady's-slipper.

Analysis of the grazing management data was limited by the fact that grazing history at the MTGPP prior to 1993 is unknown, but sufficient data were available to permit some analyses. We used GLMMs to analyze the effects of time (years) since last grazed and frequency of grazing (number of years) within the last 10 y on number of stems. We removed two plots that were ungrazed throughout the study period due to problems with models converging when these plots were included. Fire data were insufficient to conduct statistical analyses. Burn history at the MTGPP prior to 1992 is unknown. Further, the extent of burn was not recorded for burns that occurred before 2006 and it is unknown if the study subpopulations were burned.

RESULTS

GENERAL POPULATION TRENDS

Total number of stems within plots varied at times widely among years, but declined over time (Fig. 2; Table 2; Supplemental Table 2). Correlation in total stem count across years was moderate to high between plots (mean $r = 0.623$, range 0.350-0.704). Percentage of stems that were flowering in a given year ranged between approximately 30% and 60% and also varied widely among years (Supplemental Table 2). Correlation in percent flowering stems across years was low to moderate and in two instances negative between plots (mean $r = 0.125$, range -0.385-0.549).

WEATHER

Mean temperature and precipitation values within phenological stages did not differ significantly among years ($P > 0.5$). Temperature during emergence and anthesis of the current growing season best explained total number of stems and number of vegetative stems (Table 3). Temperature during emergence was strongly positively correlated with both total number of stems and number of vegetative stems, whereas temperature during anthesis was strongly negatively correlated with both dependent variables (Table 4). Temperature during dormancy was also in top models for both total number of stems and number of vegetative stems (Table 3), and was positively correlated with total number of stems (Table 4). Temperature during dormancy best explained number of flowering stems, with more flowering stems observed following warmer dormant periods (Tables 3 and 4). Temperature during anthesis was also in the best fitting model for number of flowering stems and approached significance, suggesting increased temperatures during anthesis may discourage flowering (Tables 3 and 4).

Few patterns were observed for precipitation. More variables were present in top models for all stem count variables (Table 3), but most relationships were not significant (Table 4). Only precipitation during previous-year emergence was significant for flowering stems, with more flowering stems observed in the following growing season when higher precipitation

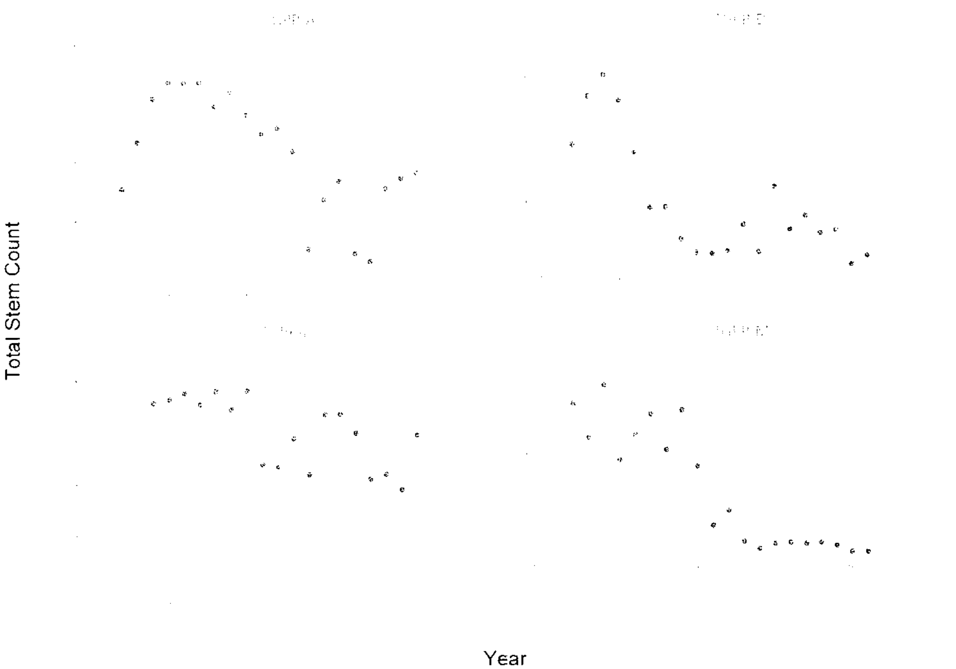

FIG. 2.—Population trends for each small white lady's-slipper (*Cypripedium candidum*) study plot showing total stem count by year for the duration of the study (1997-2016) and with linear regression trend line

was reported during the previous emergence period, but the relationship was weak (Table 4). No significant relationships were detected between precipitation and number of vegetative or total stems, although precipitation during previous-year propagation/senescence approached significance for total number of stems, suggesting a positive relationship between precipitation during previous-year propagation/senescence and overall growth (number of stems) the following growing season (Table 4).

TABLE 2.—Parameter estimates and calculated probability for effect of year on small white lady's-slipper (*Cypripedium candidum*) total stem count in study plots (n = 4) at the Manitoba Tall Grass Prairie Preserve, 1997–2016

	Total number of stems			
Model variable	β	SE	CI	P
TGPP-A				
Year	-10.635	3.058	-17.058, -4.211	0.003
TGPP-D				
Year	-11.750	2.524	-17.053, -6.446	<0.001
TGPP-L				
Year	-8.578	2.279	-13.409, -3.746	0.002
TGPP-M				
Year	-9.250	1.083	-11.525, -6.974	<0.001

TABLE 3.—Best model (lowest AIC_c) and top models ($\Delta AIC_c \leq 2$) by weather variable (temperature, precipitation) for each of total, vegetative, and flowering stem count of small white lady's-slipper (*Cypripedium candidum*) in study plots (n = 4) at the Manitoba Tall Grass Prairie Preserve, 1997-2016. Log-likelihood value [log(L)], number of parameters (K), minimum corrected Akaike's Information Criterion (AIC_c) value, delta AIC_c and Akaike's weight (w_i) are provided

Model	log(L)	K	Minimum AIC_c	ΔAIC_c	w_i
Temperature[1]					
Total stems (n = 78)					
EM + AN	456.23	5	923.29	0	0.40
DOR + AN	456.87	5		1.27	0.21
DOR + EM + AN	455.69	6		1.27	0.21
Vegetative stems (n = 78)					
EM + AN	417.84	5	846.51	0	0.34
DOR + EM + AN	417.39	6		1.45	0.17
AN	419.99	4		2.02	0.12
Flowering stems (n = 78)					
DOR + AN	416.68	5	844.19	0	0.43
DOR	418.57	4		1.49	0.20
Precipitation[1]					
Total stems (n = 78)[2]					
pPRSE + EM	99.94	5	210.71	0	0.18
pAN + pPRSE	100.23	5		0.57	0.14
EM	101.81	4		1.46	0.09
pEM + pAN + pPRSE	99.56	6		1.6	0.08
AN	99.56	4		1.76	0.08
pEM + EM	101.96	5		1.84	0.07
DOR	100.86	4		1.88	0.07
pEM + pAN	102.02	5		1.96	0.07
Vegetative stems (n = 78)					
AN	420.28	4	849.11	0	0.17
EM	420.29	4		0.02	0.17
pPRSE + EM	419.33	5		0.39	0.14
pEM + EM	419.74	5		1.2	0.09
EM + AN	420.04	5		1.79	0.07
DOR + AN	420.07	5		1.85	0.07
Flowering stems (n = 78)					
pEM + pAN	420.79	5	852.41	0	0.17
pEM + pAN + pPRSE	419.88	6		0.52	0.13
pPRSE + EM	421.30	5		1.02	0.10
pAN + pPRSE	421.33	5		1.08	0.10
AN	422.53	4		1.2	0.09
DOR	422.79	4		1.72	0.07
EM	422.80	4		1.73	0.07

[1] pEM = previous-year emergence; pAN = previous-year anthesis; pPRSE = previous-year propagation/senescence; DOR = dormancy; EM = emergence; AN = anthesis

[2] Count data transformed (divided by 100) for all total stem count models for precipitation

Greater snow depths corresponded with increases in the number of vegetative stems (0.907 ± 0.371 SE; CL = 0.128, 1.686; P = 0.025) and total stems (1.462 ± 0.530; CL = 0.349, 2.575; P = 0.013). However, snow depth was not significant for flowering stem count (0.531 + 0.323 SE; CL = -0.147, 1.210; P = 0.117).

TABLE 4.—Parameter estimates and calculated probability for temperature and precipitation variables included in top models of weather effects on small white lady's-slipper (*Cypripedium candidum*) stem count in study plots (n = 4) at the Manitoba Tall Grass Prairie Preserve, 1997–2016. Parameter estimate for each weather variable is from the highest-scoring model in which that variable appears

	Number of flowering stems				Number of vegetative stems				Total number of stems			
Weather variable	β	SE	CL	P	β	SE	CL	P	β	SE	CL	P
Temperature[1]												
Previous-year emergence	–	–	–	–	–	–	–	–	–	–	–	–
Previous-year anthesis	–	–	–	–	–	–	–	–	–	–	–	–
Previous-year propagation/ senescence	–	–	–	–	–	–	–	–	–	–	–	–
Dormancy	**8.062**	**2.427**	**3.224, 12.900**	**0.001**	-2.771	2.910	-8.572, 3.031	0.344	**8.984**	**3.995**	**1.020, 16.948**	**0.028**
Current-year emergence	–	–	–	–	**5.427**	**2.579**	**0.286, 10.569**	**0.039**	**10.650**	**4.193**	**2.292, 19.008**	**0.013**
Current-year anthesis	-9.686	4.926	-19.506, 0.134	0.053	**-11.883**	**5.087**	**-22.023, -1.743**	**0.022**	**-22.215**	**8.269**	**-38.699, -5.731**	**0.009**
Precipitation[1,2]												
Previous-year emergence	**0.620**	**0.304**	**0.013, 1.226**	**0.045**	0.249	0.237	-0.222, 0.721	0.295	0.576	0.499	-0.418, 1.570	0.252
Previous-year anthesis	-0.384	0.237	-0.857, 0.089	0.110	–	–	–	–	0.037	0.297	-0.554, 0.628	0.901
Previous-year propagation/ senescence	0.451	0.333	-0.213, 1.115	0.180	0.446	0.324	-0.200, 1.091	0.173	1.031	0.526	-0.017, 2.079	0.054
Dormancy	0.249	0.589	-0.925, 1.423	0.673	0.451	0.636	-0.818, 1.720	0.481	0.232	0.944	-1.649, 2.114	0.806
Current-year emergence	-0.095	0.240	-0.573, 0.383	0.694	0.373	0.233	-0.090, 0.837	0.113	0.291	0.381	-0.469, 1.050	0.448
Current-year anthesis	-0.160	0.189	-0.536, 0.216	0.400	0.292	0.180	0.066, 0.651	0.109	0.129	0.303	-0.475, 0.734	0.671

[1] Phenological stages calculated from monthly averages as follows: emergence (April, May), anthesis (May, June), propagation/senescence (July, August, September), dormancy (October to March)

[2] Count data transformed (divided by 100) for all total stem count models. Model output values multiplied by 100 to permit direct comparisons of results

– Variable did not occur in top models

Values in bold are significant

GRAZING

Time since last grazed had a strong negative influence on vegetative and total stem count, but was not significant for flowering stem count (Table 5). All three stem count variables were strongly negatively correlated with frequency of grazing in a 10 y period (Table 5).

DISCUSSION

GENERAL POPULATION TRENDS

Because of the potential for prolonged dormancy and difficulty in distinguishing genetic individuals, there is uncertainty associated with our population estimates throughout the monitoring period. Nevertheless, our data suggest the small white lady's-slipper population at the MTGPP is in decline and may not persist under the current climate and management regime. Moderate to high correlation observed between plots in total stem counts across years suggests broader scale environmental factors (*e.g.*, weather) are driving population trends more so than local scale (*e.g.*, land management) or microhabitat factors.

Percent flowering rates are within the range of percentages observed in other studies for these and other Manitoba populations, as well as elsewhere, and are similarly highly variable among years (Curtis, 1954; Bowles, 1983; Falb and Leopold, 1993; Pearn, 2012). However, whereas Curtis (1954) observed uniformity in the proportion of flowering stems across study locations within a given year, we found much variation in percent flowering stems across our study plots within years, suggesting local scale (*e.g.*, land management) or microhabitat factors are driving flowering trends more so than broader scale environmental factors (*e.g.*, weather).

WEATHER

Warm temperatures during emergence appeared to encourage vegetative growth, but significant reductions in vegetative and total stem counts were observed at higher temperatures during anthesis. Warmer spring temperatures extend the growing season by advancing phenological events such as shoot emergence (Peñuelas and Filella, 2001) and increase rates of photosynthesis and respiration (Went, 1953; Liang *et al.*, 2013), providing opportunity for greater vegetative growth. The negative relationship observed between total and vegetative stem counts and temperature during anthesis is difficult to interpret. Mean temperatures during anthesis ranged annually from 11.7 C to 16.2 C (average 14.6 C), which are well below temperatures expected to induce symptoms of heat stress such as reduced growth (Wahid *et al.*, 2007). However, maximum temperatures would better indicate potential heat stress. It is possible that the observed negative relationship could be partly driven by short periods of high daily maximum temperatures resulting in a negative growth response during anthesis. Mixed responses to current-year growing season temperatures were found in a long-term study of northern Eurasian orchids, although most populations responded positively to warmer temperatures by producing more stems (Blinova, 2008). However, the growing season spans multiple phenological stages, limiting the ability to make comparisons between those findings and our results.

Flowering increased following warm winter dormancy periods. Flower primordia (*i.e.*, the first histologically differentiated stage of development) develop late in the previous growing season (Bowles, 1983; Table 1) and are at risk of damage caused by low soil temperatures during dormancy. Frost late in the previous growing season (*i.e.*, during senescence) was a strong deterrent of flowering by lady's-slipper orchid, *C. calceolus,* in northern Russia

TABLE 5.—Parameter estimates and calculated probability for grazing variables on small white lady's-slipper (*Cypripedium candidum*) stem count in study plots (n = 4) at the Manitoba Tall Grass Prairie Preserve, 1997–2016

	Number of flowering stems				Number of vegetative stems				Total number of stems			
Grazing variable	β	SE	CL	P	β	SE	CL	P	β	SE	CL	P
Last graze	0.661	2.335	-4.139, 5.461	0.779	**-10.945**	**2.294**	**-15.660, -6.231**	**<0.001**	**-10.310**	**3.823**	**-18.169, -2.451**	**0.012**
Graze frequency in 10-year period	**-17.174**	**4.886**	**-27.218, -7.129**	**0.002**	**-11.556**	**4.743**	**-21.306, -1.806**	**0.022**	**-29.307**	**7.909**	**-45.565, -13.049**	**0.001**

Values in bold are significant

(Blinova, 2002), alluding to the importance of temperature during this period of development. We did not detect effects of temperature during this stage on flowering the following year; however, we used mean not minimum temperatures.

Warm temperatures during the anthesis stage may reduce flowering, although this relationship was not significant. Temperature during the floral induction period when the plant transitions from vegetative to reproductive growth has been found to have a strong effect on flowering in commercial orchid species, with warmer temperatures promoting vegetative growth over flowering and eventual inhibition of flowering at high temperatures (Lopez and Runkle, 2005; Newton and Runkle, 2009). Flower bud blasting (abortion) and inflorescence abortion have also been observed at high temperatures in various orchid species (Lopez and Runkle, 2005; Djordjević and Tsiftsis, 2020). These observations suggest that while the ability of the orchid individual to flower may be predetermined in the previous growing season, whether the plant ultimately flowers may depend on temperatures after flower primordia have developed. Although studies on the effects of weather on primordial development in lady's-slippers or other geophytic orchids are lacking, research on orchid floral morphogenesis is increasingly demonstrating the fluidity of floral development, the importance of different environmental triggers over the course of the floral induction period, and the ability of orchids to revert to vegetative growth (*i.e.*, floral reversion) in periods of stress (Lopez and Runkle, 2005; Teixeira da Silva *et al.*, 2014; Wang *et al.*, 2019).

Precipitation appeared to have little influence on small white lady's-slipper growth, consistent with the findings of Falb and Leopold (1993), who did not detect any correlations between previous growing season precipitation and flowering of this species. However, we did detect a weak positive association between precipitation during the previous-year emergence period and flowering, and precipitation during the previous-year propagation/ senescence period may promote overall growth, though the latter relationship was not significant. Precipitation during the previous growing season may better predict flowering and overall growth than precipitation during the current growing season since the initiation of flower primordia and acquisition and storage of nutrient reserves occur during the previous growing season (Bowles, 1983). Precipitation during the previous growing season has been shown to be a strong predictor of flowering in the sympatric western prairie fringed-orchid, *P. praeclara* (Willson *et al.*, 2006; Bleho *et al.*, 2015; Morrison *et al.*, 2015), which has a comparable life cycle and below-ground morphology (Bowles, 1983), and precipitation the previous spring had a positive effect on survival the following year for the related showy lady's-slipper (*C. reginae*; Kéry and Gregg, 2004). No effects of precipitation in the current growing season were apparent. In contrast, spring precipitation during the current growing season had a positive effect on overall growth of lady's-slipper orchid, *C. calceolus* (Hurskainen *et al.*, 2017), and flowering of western prairie fringed-orchid, *P. praeclara* (Bleho *et al.*, 2015; Morrison *et al.*, 2015).

Increased snow depth during the dormant period appeared to benefit small white lady's-slipper vegetative growth. Because snow typically remains on the ground throughout the winter season in Manitoba because of consistent freezing temperatures, snow accumulates over time and only dissipates into the soil in spring. Therefore, maximum snow depth within the dormant period can be a good measure of the amount of moisture from winter precipitation that is available for the forthcoming growing season. Moisture availability from snowmelt is not well reflected in precipitation measures due to the time lag between snowfall and snowmelt, which may explain why neither precipitation during the dormant period nor precipitation during emergence influenced growth. Alternatively, the near-surface water table may act as a relatively stable source of water and thus obscure the effects of seasonal precipitation fluctuations. Depth to water table has been identified as an important determinant of habitat suitability for small white lady's-slipper (Phillips-Mao *et al.*, 2016). Blinova (2008) also observed a positive response (number of stems) to snow depth for some terrestrial orchids in northern Eurasia, but most species including lady's-slipper orchid, *C. calceolus*, responded negatively, which the author hypothesized could be due to plant rot following excessive spring melt. Conversely, Hurskainen *et al.* (2017) found spring snow depth to have a positive effect on overall growth of that species.

Snow cover also insulates the soil and thereby protects plant roots from ambient temperatures. For example, paired data loggers recently placed in one of the properties included in this study recorded mean January 2018 temperatures at ground level (below snow) between -7.3 C and -7.4 C (range -24.0 C to 0.1 C), compared to mean above snow temperatures between -14.4 C and -16.5 C (range -39.9 C to 5.8 C; Nature Conservancy of Canada, unpublished data). Lower winter soil temperatures can result in root damage (Schaberg *et al.*, 2008), which in turn reduces plant capacity to absorb nutrients from the soil and overall growth rate the following growing season (Weih and Karlsson, 2002). This may be especially important for small white lady's-slipper, which depends on vegetative reproduction via rhizomes. Further, snow traps atmospheric nutrients that are subsequently deposited into the soil during spring melt and made available for uptake by plant roots during emergence (Clement *et al.*, 2011). Insufficient snow cover depth has been linked to reduced abundance and mortality in other plant species, presumably due to the effect of snow cover on soil temperature (Simons *et al.*, 2010; Molano-Flores and Bell, 2012).

Mean temperature and precipitation values within phenological stages did not differ significantly among years, suggesting the climate has remained relatively stable during the 20 y monitoring period. However, climate projections for this region forecast gradual increases in annual temperature and precipitation, and a decrease in summer precipitation, with overall climate more reminiscent of regions currently farther south upward to several hundred kilometers (Galatowitsch *et al.*, 2009). Such climatic changes may alter timing of phenological stages in the small white lady's-slipper, and possibly desynchronize plant-pollinator interactions (Hegland *et al.*, 2009). A disruption of plant-pollinator interactions may be especially impactful on this species and its genetic diversity; due to their system of pollination and restrictive floral architecture, the number of insects that can effectively pollinate small white lady's-slipper flowers is quite limited compared to the number of floral visitors (Pearn, 2012; Grantham *et al.*, 2019). If climatic changes were to result in fewer or no insects capable of effecting pollen transfer, genetic exchange would be impacted, and could result in a complete reliance on asexual (vegetative) reproduction, thus reducing the genetic diversity of a given population. Changes to climate may also impact regional hydrology, resulting in drier prairies unsuitable for wet prairie obligates like the small white lady's-slipper (Galatowitsch *et al.*, 2009).

GRAZING

Small white lady's-slipper appears to benefit from some grazing, with fewer stems observed as time increased since the last grazing event, but frequent grazing over a 10 y period may be detrimental. Within the two grazed plots, time since last grazed ranged from zero to over 10 y, and frequency of grazing within the preceding 10 y period ranged from zero to six times. Cattle grazing removes grass cover and prevents heavy litter accumulation (Towne *et al.*, 2005), which may otherwise limit the growth of small white lady's-slipper through competitive exclusion and shading (Falb and Leopold, 1993; Punter, 1999; Wake, 2007).

However, heavy grazing resulting from high stocking rates or frequent grazing becomes unselective as cattle run out of preferred graminoids to eat and increases the risk of consumption and trampling of orchids and other less desirable forage species (Howe, 1994; Alexander *et al.*, 2010). Removal of above-ground parts could render small white lady's-slipper individuals unable to store enough below-ground reserves to flower, grow vegetatively, or survive the following growing season (Bowles, 1983). Excessive defoliation could also force individuals to go into prolonged dormancy as a survival mechanism (Shefferson *et al.*, 2005). In the shorter term, consumption or trampling of flowers or fruits could reduce reproductive success within the growing season, and trampling of shallow rhizomes could potentially kill the plant.

SUMMARY

The small white lady's-slipper population at the MTGPP appears to be in decline and may not persist under the current climate and management regime. This stresses the need to identify factors that affect plant survival and growth and ultimately population size of this endangered orchid, and use this information to implement effective conservation measures through strategic management. Temperature appears to proximately regulate vegetative growth and flowering. Warm temperatures early in the spring when shoots are emerging appear favored, but high temperatures during anthesis appear detrimental, reducing both vegetative growth and flowering. In contrast, precipitation appears to have a lag effect on growth and flowering. Given much of the below-ground development of the small white lady's-slipper (*e.g.*, flower primordia) occurs in the previous growing season, it is not altogether surprising that precipitation may be most influential during that period since water is the avenue by which plants acquire nutrients used for development. However, snow depth was identified as having a positive influence on vegetative growth, suggesting moisture availability in early spring when shoots are emerging is also important for above-ground growth.

Some grazing appears to benefit the species presumably by reducing competition and shading, but frequent grazing may increase the risk of direct damage to individuals from cattle consumption and trampling and provide insufficient time for individuals to recover following grazing events. Only two of the four subpopulations were subjected to grazing during the study period or within 10 y prior, and details of grazing such as extent, timing, and intensity within the season were not recorded, limiting our ability to analyze the data and interpret our results, and fire data were inadequate for analysis. In recent years and going forward, more detailed data are being collected on the extent (*e.g.*, fine scale mapping), timing, frequency, and intensity (*e.g.*, stocking rate, substrate burn severity) of grazing and fire management at the MTGPP. Evaluation of fire management to curb woody encroachment, a major threat to the species (Environment Canada, 2014), is required. We were unable to analyze the fire data, which may have confounded our grazing analysis. Interactive effects of fire and grazing and potential for integrated management (*i.e.*, patch-burn grazing) should also be explored. We note that on three occasions properties in our study were subjected to both grazing and burning within a single growing season (Supplemental Table 1), although it is unknown if the plots themselves were burned.

Long-term monitoring is necessary to detect general population trends in the small white lady's-slipper due to its erratic life cycle and fluctuating populations, which complicate studies of this species. Prolonged dormancy is a potential confounding factor that is being addressed in more recent investigations at the MTGPP by marking individuals, which will also permit demographic investigations. Long-term monitoring is also essential to identify lag effects that environmental variables may have on small white lady's-slipper populations, and future research on this species should continue to explore this important concept.

Although our study had some limitations (*i.e.*, small sample size and data gaps), our long-term monitoring study can inform other orchid researchers and conservation practitioners of trends observed to date in the MTGPP population and to guide future management and research at the MTGPP and elsewhere where this species persists. Furthermore, our findings add to the knowledge of orchid conservation and management.

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