SEED SET AND SEED-INSECT INTERACTIONS IN NATURAL AND CULTIVATED POPULATIONS OF PURPLE PRAIRIE CLOVER

Arvid Boe[1*], Paul J. Johnson[1,2], and Abigail P. Martens[1,2]
Agronomy, Horticulture, and Plant Science Department[1]
Insect Biodiversity Laboratory[2]
South Dakota State University
Brookings, SD 57000
*Corresponding author e-mail: arvid.boe@sdstate.edu

ABSTRACT

Purple prairie clover (*Dalea purpurea* Vent.) is a perennial legume native to prairies throughout the central USA and Canada. Seed set in natural populations can be very low, and the reasons are not well known. Our objectives were: 1) describe the reproductive morphology in natural populations of purple prairie clover, 2) identify seed predators and parasitoids associated with natural populations, and 3) determine seed set in natural and cultivated populations of purple prairie clover in eastern SD. Inflorescences were collected from natural and cultivated populations on the South Dakota State University Oak Lake Field Station (OLFS), a cultivated population near Aurora, SD, and from a cultivated population in McCrory Gardens at Brookings, SD. In natural populations at OLFS, normal seed set was ca. 10 %, frequency of unfertilized ovules was ca. 20 %, and seed predation was ca. 15 %. The most easily verifiable seed predator was *Acanthoscelides seminulum* (Horn). However, the most common insects on the spikes were adults of *Kissingeria capitone* (Kissinger) and larvae of an unidentified gall midge of genus *Contarinia* (Cecidomyiidae). The overwhelmingly most common seed predators in the cultivated populations were *K. capitone* and *Contarinia* sp. The most common parasitoid reared from beetle larvae was *Eurydinoteloides incerta* (Ashmead). The chalcidoid wasps *Baryscapus* sp. and *Aprostocetus marylandensis* (Girault) parasitized gall midge larvae, with *Baryscapus* sp. the most abundant by far. In addition, a few individuals of *Bracon* sp. and *Inostemma* sp., hosts unknown, were collected from immature inflorescences. Normal seed set in the McCrory Gardens population was 55% compared with <15% in the other cultivated populations. This study identified a previously unknown guild of insects associated with inflorescences in purple prairie clover and revealed probable reasons for a paucity of viable seed production in natural and cultivated populations.

Keywords

Bruchid beetle, *Acanthoscelides seminulum*, seed weevil, *Kissingeria capitone*, seed predation, parasitoid, *Aprostocetus marylandensis*, *Eurydinoteloides incerta*, *Inostemma*, *Bracon*, unfertilized ovules

INTRODUCTION

Purple prairie clover (*Dalea purpurea*) is a perennial legume native to droughty, coarse-textured soils throughout the eastern two-thirds of the US and Canada, with the exception of the Eastern Seaboard (Stubbendieck and Conard 1989). It is palatable and nutritious forage for domestic livestock and wildlife (Larson and Johnson 1999; Wynia 2008) and increases forage yield, protein concentration, and biodiversity, while reducing nitrogen inputs in forage (Posler et al. 1993) and biofuel plantings (Zilverberg et al. 2016). Several selected populations have been developed for forage (Wynia 2008), roadside plantings, revegetation, beautification (Lindgren 1992), and pollinator habitat (Cane 2006), and seed of this drought tolerant legume is usually commercially available.

At least two seed predators, *Kissingeria capitone* Kissinger (Coleoptera: Apionidae: Apioninae) (e.g., Kissinger 1968) and *Acanthoscelides seminulum* (Horn) (Coleoptera: Chrysomelidae: Bruchinae) (e.g., Kingsolver 2004) have been reported from the genus *Dalea*. *Kissingeria capitone* occurs in multiple ecological regions in North Dakota (Balsbaugh and Aarhus 1990) and Iowa (Hendrix 1992). Although levels of seed predation have been found to be lower than in several other native prairie legumes, seed production in natural stands of purple prairie clover can be reduced by insects (Hendrix 1992). The objectives of this study were: 1) describe the reproductive morphology of natural populations of purple prairie clover, 2) identify species of insects associated with flowers and seeds of natural and cultivated populations purple prairie clover, and 3) determine percentage seed set and the relative importance of several factors that determine fates of ovules and thereby ultimately determine normal seed set in natural and cultivated populations of purple prairie clover in eastern South Dakota.

MATERIALS AND METHODS

Plant Reproductive Morphology, Fates of Ovules, and Seed Set of Natural Populations at the Oak Lake Field Station—Ten randomly selected plants of purple prairie clover having shoots with intact mature inflorescences were harvested at ground level from each of three natural populations at the South Dakota State University Oak Lake Field Station (OLFS) on 26 August 1999. The populations ranged in size from about 50 to 75 plants growing on shoulder positions of glacial moraines. Distance between populations was about 5 km. The soil type at each location was a Singsaas silty-clay loam (fine-loamy, mixed, superactive, frigid, Haplic Vermudolls) with Buse loam (fine-loamy, mixed, superactive, frigid

Typic Calciudolls) complex. For two of the populations, number of shoots and number of inflorescences per shoot were determined for each plant. Weights of individual inflorescences for all shoots of individual plants were summed to derive inflorescence weight per plant.

For the third population, each inflorescence from the 10-plant sample was hand threshed separately using a rubboard equipped with rubber stair tread matting and an associated hand tool equipped with the same matting to extract seeds from the seed units (i.e., mature flowers). This method simulates, on a greatly reduced scale, machine harvesting and threshing of the inflorescences. Seeds were separated from the threshed floral material (i.e., chaff and fractured abnormal seeds) by using a South Dakota Seed Blower®. Normal seeds were counted for each inflorescence.

For two of the populations, two inflorescences were randomly selected from each of three shoots for three plants from each population for determination of fate of ovules (Boe and Johnson 2017). Two sub-samples of 10 flowers (Figure 1) randomly selected from each inflorescence were dissected at 7X–40X under a binocular microscope to ascertain the fates of their ovules. Visually identifiable fates were: A) fertilization and mature intact seed, B) fertilization but shriveled or otherwise abnormal seed, C) unfertilized ovules, D) seed predation by either a curculionid beetle (*Kissingeria capitone*) or bruchid beetle (*Acanthoscelides seminulum*), E) parasitism of beetle larva by a chalcidoid wasp (*Eurydinoteloides incerta*) (Hymenoptera: Pteromalidae), and F) no intact mature seed production; cause unknown. Fate D was reliably identified by beetle larva feeding damage and fecal material. *Kissingeria capitone* larvae feed on green seeds, pupation takes place in the flower, and adults emerge in early August; whereas larvae of *Acanthoscelides seminulum* enter a physiologically mature seed in early August and overwinter as advanced instars, with adult emergence taking place during the following spring. Fate E was reliably identified by presence of golden-colored wasp exuviae and remnants of the carcass of the beetle larva.

During mid-flower to physiological maturity stages (i.e., early August to mid-September) in 1999, 10 inflorescences were collected at four dates (5 August, 14 August, 11 September, and 14 September) from each of 10 plants for each population and placed in plastic Whirl-Pak® bags for recovery of insects associated with inflorescences during flowering, seed development and maturation. Adult and immature insects were collected from bags daily until activity ceased. Collected insects were placed in 80% ethanol for subsequent identification and counting.

Analyses of variance were conducted to determine if differences occurred among populations for shoots per plant, inflorescences per shoot, total plant inflorescence weight, and individual inflorescence weight. Chi-squared analysis was used to determine if frequencies of ovule fates varied among populations.

Fate of Ovules and Seed Set of Cultivated Populations—In June 2009 a small plot (15 m x 25 m) of a mixture of purple prairie clover, Canada tickclover (*Desmodium canadense* (L.) DC.), big bluestem (*Andropogon gerardii* Vitman), and switchgrass (*Panicum virgatum* L.) was seeded at a combined rate of 2.5 g of seed m^{-2}, with 0.25 g being purple prairie clover, at the South Dakota State University Crop Improvement Association Farm near Aurora, SD (44.3042 N,


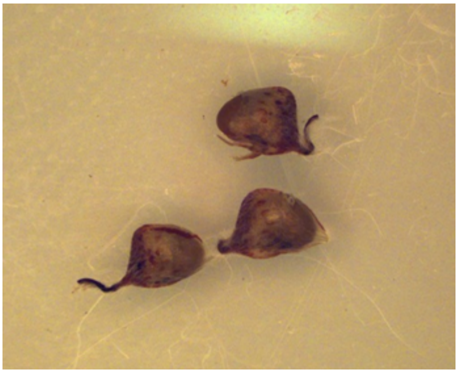

Figure 1. Left: Mature seed units of purple prairie clover from a cultivated population in Brookings, SD. The seed unit is composed of a durable villous persistent calyx that encloses a 1-seeded legume. Right: Mature legumes of purple prairie clover. Note that the style is persistent.

96.6696 W). This population is hereafter referred to as the Aurora cultivated population. Soil type was a Brandt silty clay loam (fine-silty, mixed, superactive, frigid Calcic Hapludolls). The plot was harvested after a killing frost for biomass determinations during each of 2010 through 2014. From 2015 through 2018, standing biomass was burned during April. The purple prairie clover was a relatively small component of the total biomass of the grass-legume mixture from the onset, with only scattered plants available for sampling for seed insect biodiversity and activity during 2018.

At about 10-day intervals beginning 20 July and ending 20 August 2018, samples of 20 of the most mature (indicated by length of the spike displaying flowers) inflorescences were randomly selected, clipped, and placed in gallon-sized plastic storage bags. Bags were subsequently stored in a laboratory at room temperature and checked daily for insects, which were collected and immediately placed in 80% ethanol. Individual insects were identified to species, when possible, and counted.

In June 2012, 1.5 hectares of previously long-term-tilled cropland on the OLFS (44.5150 N, 96.5310 W) were planted to a 16-species mixture of native grasses and legumes that included purple prairie clover using conventional farm equipment at a rate of 18 kg of pure live seeds per hectare; hereafter referred to as the OLFS cultivated population. The soil type was a Singsaas-Waubay silty clay loam (fine-loamy, mixed, frigid Hapludic Vermiborolls-fine-silty, mixed, frigid Pachic Udic Haploborolls). Purple prairie clover was well established and a major component of the forb community in the grass-forb mixture by 2015.

As was the procedure for the Aurora population, at 10-day intervals beginning 20 July and ending 20 August 2018, samples of 20 of the most mature (indicated by length of the spike displaying flowers) inflorescences from the OLFS cultivated population were randomly selected, clipped, and placed in gallon-sized plastic storage bags. Bags were subsequently stored in a laboratory at room temperature and checked daily for insects which were collected and immediately placed in 80% ethanol. Individual insects were identified to species, when possible, and counted.

During spring 2015, seed of Bismarck Germplasm purple prairie clover (Wynia 2008) was planted in a mixture of native grasses and legumes on introduced topsoil at McCrory Gardens (44.3142 N, 96.7689 W) on the campus of South Dakota State University during spring 2015; hereafter referred to as the McCrory Gardens cultivated population. The area covered was about 30 m^2. It was weeded, watered, and fertilized as needed to facilitate establishment and promote vigorous growth of reproductive shoots. At the end of the 2017 growing season, purple prairie clover was the dominant species for cover and biomass. On 19 January 2018, we hand-collected inflorescences from about 50 random shoots for ovule fate analysis. Similar to the sampling methods described above at OLFS and Aurora, inflorescence collections were also made at McCrory Gardens during July and August 2018.

During late August 2018, three random inflorescences from the last collection date for each of the three cultivated populations (i.e., Aurora, OLFS, and McCrory Gardens) were sampled for fate of ovules analyses. For each inflorescence, 10 random flowers from each of proximal, middle, and distal segments were dissected under 10 X to 30 X magnification for determination of ovule fate, as previously described here for the natural populations. Identification of the results of flower-occupation activities of particular insect species, whether seed predator or parasitoids of seed predators, was based on techniques similar to those described by Finlayson (1962). In addition to observations made at OLFS during 1999, dates of phenological events, including plant and insect activities, were recorded for the OLFS, Aurora, and McCrory Gardens cultivated populations during June through August 2018.

Insects were identified with keys and descriptions in Johnson (1970), Kingsolver (2004) and Kissinger (1968) for Coleoptera, Burks (1943) and Gibson et al. (2006) for Hymenoptera, and Gagné (1989) for Cecidomyiid larvae. Comparisons were also made to previously determined specimens in the Severin-McDaniel Insect Research Collection, South Dakota State University, and the National Museum of Natural History, Washington, D.C.

RESULTS

Plant Reproductive Morphology and Fate of Ovules of Natural Populations at the Oak Lake Field Station—Plants of purple prairie clover at OLFS began flowering during the first week of July in 1999. Flowering was acropetal, with about 25% of the spikes displaying petals, anthers, and stigmas on the proximal 50% of their axes on terminal inflorescences by 15 July. Flowering was completed on most inflorescences and physiological maturity (dry calyxes and pods with mature seeds) was reached by 10 September.

Significant differences (P = 0.03, $F_{2, 27}$ = 3.98) were found among populations at OLFS for total plant inflorescence weight (range was 5.4 g to 10.2 g), but not for shoots per plant (grand mean = 9.0) or inflorescences per plant (grand mean = 22.4).

Figure 2. Upper: adult female of Kissingeria capitone (actual length 2 mm). Middle: adult female of Aprostocetus marylandensis (actual length 1.5 mm). Lower: adult male (lt.) and female of Eurydinoteloides incerta (rt.) (actual length 2 mm). All were collected from inflorescences of purple prairie clover at the Oak Lake Field Station in eastern South Dakota.

Analysis of individual inflorescence weight indicated significant population ($P < 0.001$, $F_{2, 634}$) and plants-within-populations ($P < 0.001$, $F_{27. 634}$) effects. Population means ranged from 102 to 163 mg per inflorescence.

A total of 519 individual insects were collected from inflorescences of purple prairie clover sampled during peak flowering through seed maturity from three natural populations at OLFS in 1999. Greater than 85% of the total number were collected from flowering inflorescences during early to mid-August. Greater than 90% of the individuals reared from mature inflorescences were adults of *Eurydinoteloides incerta* (Figure 2), a parasite of *Kissingeria capitone* (Figure 2) and *Acanthoscelides seminulum* (Table 1). This is the first report of these host-parasite associations. In contrast, most of the adults of *Aprostocetus marylandensis* (Figure 2) and *Baryscapus* sp. were obtained from immature inflorescences collected during the first half of August (Table 1). Collectively, *E. incerta* and *Baryscapus sp*. composed nearly 90% of the total number of parasitoids collected (Table 1). Parasitoids collected, in addition to the chalcids (Hymenoptera: Chalcidoidea), were small numbers of unidentified *Bracon* sp. (Hymenoptera: Braconidae) and an *Inostemma* sp. (Hymenoptera: Platygastridae) (Table 1).

Table 1. Numbers of insects collected from fresh inflorescences placed in Whirl-Pak plastic bags immediately after removal from shoots in the field at two developmental stages at OLFS in 1999 and stored at room temperature until emergence of insects ceased.

	Plant Developmental Stage		
Insect	**Flowering[1]**	**Mature Seed[2]**	**Total**
Floral/Seed Predators			
Kissingeria capitone	166	2	168
Acanthoscelides seminulum	1	0	1
Contarinia sp.	74	0	74
Parasitoids			
Eurydinoteloides incerta	63	61	124
Aprostocetus marylandensis	17	0	17
Baryscapus sp.	116	3	119
Bracon sp.	12	0	12
Inostemma sp.	4	0	4
Total	**453**	**66**	**519**

[1] Collections made 5 and 14 August; emergence ceased 28 August.

[2] Collections made 11, 14, and 19 September; emergence ceased 25 January 2000.

Frequency of normal seed set was about 10% for the two natural populations at OLFS during 1999. The two largest ovule fate categories were abnormal seed and unfertilized ovules, collectively accounting for about 60% of the flowers examined (Table 2). The abnormal seeds had shrunken cotyledons and embryo axes resulting in a space between them and the surrounding testa. These seeds fractured from light pressure applied from a forceps. Out of the 229 individual inflorescences collected from OLFS 3 that were hand-threshed, 155 contained no normal seeds. Five was the highest number of normal seeds obtained from any individual hand-threshed inflorescence.

Table 2. Frequencies of fates of ovules for two natural stands from the Oak Lake Field Station (OLFS) in eastern South Dakota in 1999.

	Population	
Fate of Ovule	**OLFS 1¶**	**OLFS 2¶**
Seed	7	13
Abnormal seed	40	36
Unfertilized	26	17
K. capitone	2	0
A. seminulum	13	14
E. incerta (parasitoid)	13	1
Other	9	29

¶ Sample size was 110 mature seed units (seed unit is a mature pod surrounded by dry persistent calyx) selected at random.

The 'Other' category of ovule fate (Table 2) represents what appeared to be predominantly destructive insect-related activity (Finlayson 1962) that was unattributable to any particular species. Unfertilized ovules were absent and fecal pellets or other evidence of damage due to insect feeding was generally present.

Fate of Ovules in Cultivated Populations**—**The only insects reared from the 18 January 2018 collection of inflorescences that were produced during 2017 and retained mature seed units during the 2017-2018 winter up to 18 January in the McCrory Garden population were adults of *Acanthoscelides seminulum*, which emerged in the laboratory at room temperature during February 2018. No adults of *Kissingeria capitone* or any of the parasitoids were reared from this collection.

All three cultivated populations of purple prairie clover were in early bloom by 25 June 2018. By mid-August, seeds were mature along the entire rachis in inflorescences of the Aurora and OLFS populations and in all but the most distal segments of the inflorescences of the McCrory Gardens population. In 2018, unopen flowers in the terminal inflorescences of plants in the cultivated population at McCrory Gardens had fully developed floral structures by 20 June. The petals were bright purple, the anthers were deep orange, the stigmas were plumose, and the ovules were plump and translucent. The first open flowers were observed on 26 June. About 50% of terminal inflorescences were ≥50% flowered and axillary inflorescences were beginning to flower by 10 July. Flowering was complete for both terminal and axillary inflorescences by August 15. Adults of both *Kissingeria capitone* (Figure 2) and *Acanthoscelides seminulum* were present and active during late June. As was the case for the natural populations at OLFS in 1999, adult individuals of *K. capitone* greatly outnumbered those of *A. seminulum*.

Based on comparative numbers of insects reared from August and September inflorescence collections during 1999 (Table 1), it was clear that to accurately estimate insect biodiversity (Table 3) and relative importance of various seed predators and parasitoids in determining fates of ovules, we needed to examine inflorescences in full to late bloom during mid-July to mid-August. Therefore, we collected inflorescences from the three cultivated populations during early August 2018. That sampling time enabled us to observe the behavior of the major seed predators, i.e., *Kissingeria capitone* and *Contarinia* sp. During early August, larvae of *K. capitone* were in the final instar, pupa, or early adult stage. Adults were emerging from the flowers during that time. The physical ramification of the full development of the larva in a flower was evident so, for future study, a flower that provided a successful progression from early instar to emergent adult could be identified.

Table 3. Numbers of individuals reared from inflorescences of flowering purple prairie clover collected from cultivated populations at Aurora, the Oak Lake Field Station (OLFS) and McCrory Gardens in eastern South Dakota during July and August 2018.

	Population		
Species	**Aurora**	**OLFS**	**McCrory Gardens**
Seed predators			
K. capitone	75	162	185
A. seminulum		7	
Contarinia sp.	187	604	
Parasitoids			
Baryscapus sp.	113	100	8
Aprostocetus marylandensis	15	44	
Eurydinoteloides incerta	18	7	2
Total	**408**	**924**	**195**

Association of midge larvae with flowers was also possible by slitting the persistent calyx and examining the basal area around the ovary. Midge larvae positioned themselves adnately to the base of the ovary and presumably fed on juices from the ovary wall and perhaps the developing seed, as well. No seeds occurred where a midge larva had developed to the final instar, ultimately indicated by a somewhat shrunken seedless pod.

The list of insect species reared from three cultivated populations in 2018 was similar to the list reared from natural populations in 1999 (Tables 1 and 3). Of the two beetle seed predators, *Kissingeria capitone* occurred in much greater numbers than *Acanthoscelides seminulum* in both natural and cultivated populations. Similarly, for the two midge parasitoids, *Baryscapu*s sp. was reared in much greater numbers than *Aprostocetus marylandensis* from both natural and cultivated populations in 1999 and 2018, respectively.

Significant ($P < 0.01$) differences were found among cultivated populations for frequencies of normal seed (χ = 95.9, df =3), unfertilized ovules (χ = 35.8, df =3) and seed predation by *Kissingeria capitone* (χ =18.8, df = 2) (Table 4). The McCrory Gardens population had a five-fold higher seed set than either of the Aurora or OLFS populations. It also had a lower unfertilized component than the Aurora or OLFS population and no prevention of seed development by *Contarinia* sp.

Table 4. Frequencies (%) of fates of ovules for purple prairie clover from three cultivated populations at seed maturity in late August in eastern South Dakota in 2018.

	Population			
Fate of Ovules	**Aurora**	**OLFS**	**McCrory Gardens 2017¶**	**McCrory Gardens 2018**
Seed	6	13	56	54
Unfertilized	44	26	8	21
Contarinia sp..	18	44		0
Contarinia sp. parasitoid	0	4	0	0
K. capitone	21	12	0	20
A. seminulum	0	0	30	2
Other	9	1	3	3

¶ Inflorescences produced during the 2017 growing season and collected 19 January 2018.

DISCUSSION

Molano-Flores (2004) counted number of stems and determined number of inflorescences per stem and individual inflorescence weights for individual plants of leafy prairie clover (*Dalea foliosa* (A. Gray) Barneby) in Illinois. Interestingly, those data were similar to the same traits for purple prairie clover in eastern South Dakota. Leafy prairie clover plants produced 9 stems per plant compared to 9 stems per plant for purple prairie clover. Leafy prairie clover plants produced 26 inflorescences per plant compared with 22 for purple prairie clover. However, inflorescence biomass was 3.5 times greater for leafy prairie clover (500 mg) compared with purple prairie clover (140 mg). Also, a strong negative correlation was found between percent unfertilized flowers and inflorescence biomass (r = -0.48, P = 0.04). Lindgren (1992) counted shoot number in three natural populations of purple prairie clover in a cultivated nursery in Nebraska and found populations means of 4.2, 8.7, and 20.2, with a grand mean of 11 shoots per plant.

Molano-Flores (2004) also recorded reproductive output, seed set, and seed predation in a natural population of federally endangered leafy prairie clover over a 3-year period in Illinois. In that study annual seed set, which ranged from 70 to 88%, was very high compared with the natural and cultivated populations of purple prairie clover in the present study in South Dakota. Percent unfertilized ovules for leafy prairie clover was less than 20%, and seed predation was less than 1%.

Hand threshing of the 1999 material at OLFS on a rubber tread stair matting rubboard fractured most of the seeds that had abnormal cotyledons and/or thin testas. In that situation, fragments of fragile, under-filled seeds would end up in the chaff fraction after airflow separation with the South Dakota Seed Blower. Although testas were often intact, the cotyledons and embryo axis of the abnormal seeds were lacking or drastically reduced. Such seeds are not viable. Cause(s) of the relatively high frequency of abnormal seed in the natural populations of purple prairie clover at OLFS in 1999 is unknown. However, similar symptoms in alfalfa seeds are signs of lygus bug (*Lygus hesperis* Knight) (Hemiptera: Lygaéidae) feeding (App and Manglitz 1972). Hemipterans were also found feeding on the internal contents of seeds of *Astragalus cibarius* Sheld. in Utah (Green and Palmbald 1975).

The overwintering behavior of *Acanthoscelides seminulum* was similar to that of *A. aureolus* (Horn) in American licorice (*Glycyrrhiza lepidota* Pursh), in that both species survive as full-grown larvae within individual seeds within indehiscent pods (Boe and Johnson 2016). This contrasts to *A. perforatus* (Horn) that overwinters as a mature larva in a cocoon within partially dehiscent pods of Canada milk-vetch (*Astragalus canadensis* L.) (Boe and Johnson 2008a), where it has consumed all or parts of several small seeds. Several species of *Apion* (Coleoptera: Apionidae) also overwinter as larvae in seeds of other legumes (Tuttle 1954).

Eurydinoteloides incerta has been associated with more than 30 hosts, many of which are larvae of Curculionidae or Bruchidae (Peck 1963), including species of *Apion* to which *K. capitone* was formerly associated. Boe and Johnson (2008b) found this chalcidoid wasp (Hymenoptera: Pteromlidae) to be the major parasitoid of *Acanthoscelides submuticus* (Sharp) larvae in pods of false indigo (*Amorpha fruticosa* L.) on shorelines of lakes in eastern South Dakota. Burks (1943) described the range of *Aprostocetus marylandensis* as the eastern one-half of the US, and reported it reared from several unidentified Cecidomyiidae (Diptera).

Low frequency of normal seed production in natural populations in 1999 and cultivated populations in 2018 were results of several factors, including lack of fertilization, abnormal seed development post fertilization, seed predation by

Kissingeria capitone, and prevention of seed formation by *Contarinia* sp. Collectively, those four categories of ovule fate accounted for about 80% of the ovules sampled from the natural populations. On the other hand, abnormal seed development post fertilization were relatively minor fates for ovules in the cultivated population.

Accurate estimates of the impacts of the unidentified midge (Cecidomyiidae) may not be possible from the types of evidence gathered during this study. That is, estimates of prevention of normal seed production by *Contarinia* sp. were based on the presence of one or more midge larvae feeding at the base of an ovary. In general, the calyx and ovary wall were discolored when a midge larva was present. However, during the sampling if a flower had those symptoms, but did not contain a larva, it was included in the 'other' category. Similarly, the frequency of flowers with a parasitized larva of *Contarinia* sp was based on the presence generally of a pupa of either of the two chalcid (Hymenoptera: Chalcidoidea: Eulophidae) species.

The low frequency of normal seed set for the three natural populations in this study may not be reflective of natural populations from the northern Great Plains, in general. For example, a natural population of purple prairie clover near Redstone, Montana (Sheridan County), sampled on 21 November 1999 had 52% normal seed set and only 8% abnormal seed (Boe, unpublished data). However, it did have 26% unfertilized ovules, similar to the OLFS populations.

No midges (Cedidomyiidae: Dipera) and only a few eulophids (Chalcidoidea: Eulophidae) were reared from the McCrory Gardens population. The high seed set and lack of seed predation by midges in the McCrory Gardens population may reflect its young age (4 years old) relative to the Aurora (9 years old) and OLFS (7 years old) populations, or possibly its arboretum-type setting within an urban environment.

ACKNOWLEDGMENTS

This research was funded by a grant from the Oak Lake Research Incentive Program. We are grateful to Dr. Nels Troelstrup, Director of the Oak Lake Field Station (OLFS), for his support of the project and access to field and laboratory facilities at OLFS. We also acknowledge the support of USDA-NIFA through the South Dakota Agricultural Experiment Station.

LITERATURE CITED

App, B.A., and G.R. Manglitz. 1972. Insects and related pests. Pages 527-554 *in* C.H. Hanson, editor. Alfalfa science and technology. Agronomy No. 15. American Society of Agronomy, Madison, WI.

Balsbaugh, E.U., and D.G. Aarhus. 1990. Checklist and new state records of Curculionidae (Broad sense) (Coleoptera) for North Dakota. Journal of the Kansas Entomological Society 63:227-236.

Boe, A., and P.J. Johnson. 2008a. Seed predators of Canada milk-vetch and their parasitioids. Proceedings of the South Dakota Academy of Science 87:229-236.

Boe, A., and P.J. Johnson. 2008b. Seed predation by *Acanthoscelides submuticus* and *A. pallidipennis* (Coleoptera: Bruchidae) in false indigo in the northern Great Plains. Proceedings of the South Dakota Academy of Science 87:223-228.

Boe, A., and P.J. Johnson. 2016. Reproductive biology of American licorice (*Glycyrrhiza lepidota* Pursh) in western South Dakota with new seed insect associations. Proceedings of the South Dakota Academy of Science 95:91-103.

Boe, A., and P.J. Johnson. 2017. Fates of ovules in groundplum milk-vetch (*Astragalus crassicarpus* Nutt.) in South Dakota. Proceedings of the South Dakota Academy of Science 96:129-137.

Burks, B.D. 1943. The North American parasitic wasps of the genus *Tetrastichus*-—A contribution to biological control of insect pests. Proceedings of the United States National Museum 93:505-608.

Cane, J.H. 2006. An evaluation of pollination mechanisms for purple prairieclover, *Dalea purpurea* (Fabaceae: Amorpheae). The American Midland Naturalist 156: 193-197.

Finlayson, T. 1962. Taxonomy of cacoons and puparia, and their contents, of Canadian parasites of *Diprion similis* (Htg.) (Hymenoptera: Diprionidae). The Canadian Entomologist 94:271-283.

Gagné, R.J. 1989. The plant-feeding gall midges of North America. Cornell University Press. Ithaca, NY.

Gibson, G.A.P, M.W. Gates, and G.D. Buntin. 2006. Parasitoids (Hymenoptera: Chalcidoidea) of the cabbage seedpod weevil (Coleopera: Curculionidae) in Georgia, USA. Journal of Hymenoptera Research 15:187-207.

Green, T.W., and I.G. Palmbald. 1975. Effects of insect seed predators on *Astragalus cibarius* and *Astragalus utahensis*. Ecology 56:1435-1440.

Hendrix, S.D. 1992. Effects of population size on fertilization, seed production, and seed predation in two prairie legumes. Pages 115-121 *in* R.G. Wickett et al., editors. Proceedings of the Thirteenth North American Prairie Conference. 6-9 August 1992, Windsor, Ontario, Canada.

Johnson, C.D. 1970. Biosystematics of the Arizona, California, and Oregon species of the seed beetle genus *Acanthoscelides* Schilsky (Coleoptera: Bruchidae). University of California Publications in Entomology 59:1-116.

Kingsolver, J.M. 2004. Handbook of the Bruchidae of the United States and Canada (Insecta: Coleoptera). US Department of Agriculture Technical Bulletin 1912.

Kissinger, D.G. 1968. Curculionidae Subfamily Apioninae of North and Central America, with reviews of the World genera of Apioninae and World subgenera of *Apion* Herbst (Coleoptera. Taxonomic Publications, South Lancaster, Massachusetts.

Larson, G.E., and J.R. Johnson. 1999. Plants of the Black Hills and Bear Lodge Mountains. B732. South Dakota State University, Brookings, SD.

Lindgren, D.T. 1992. Variation in ornamental traits of *Dalea purpurea* Vent. (Purple Prairie Clover). HortScience 27:927.

Molano-Flores, B. 2004. Reproductive success of the federally endangered leafy prairie clover (*Dalea foliosa* (A. Gray) Barneby (Fabaceae) in Illinois: a third year of data. Castanea 69:9-14.

Peck, O. 1963. A catalogue of the Nearctic Chalcidoidea (Insecta: Hymenoptera). The Canadian Entomologist Supplement 30.

Posler, G.L., A.W. Lenssen, and G.L. Fine. 1993. Forage yield, quality, compatibility, and persistence of warm-season grass-legume mixtures. Agronomy Journal 85:554-560.

Stubbendieck, J., and E.C. Conard. 1989. Common legumes of the Great Plains. University of Nebraska Press, Lincoln, NE.

Tuttle, D.M. 1954. Notes on the bionomics of six species of *Apion*. Annals Entomological Society of America 47:301-307.

Wynia, R. 2008. Plant guide for purple prairieclover (*Dalea purpurea* Vent.). USDA-NRCS, Manhattan Plant Materials Center, Manhattan, KS.

Zilverberg, C.J., K. Teoh, A. Boe, W.C. Johnson, and V. Owens. 2016. Strategic use of native species on environmental gradients increases diversity and biomass relative to switchgrass monocultures. Agriculture, Ecosystems and Environment 215:110-121.