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FATES OF OVULES IN GROUNDPLUM MILK-VETCH (*ASTRAGALUS CRASSICARPUS* NUTT.) IN SOUTH DAKOTA

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ABSTRACT

Groundplum milk-vetch (*Astragalus crassicarpus* Nutt.) is a native legume found on gravelly to sandy prairie uplands throughout central North America. The large and fleshy fruits, or pods, of this species were consumed by indigenous people and European settlers, and are cached by rodents. Our previous research on native legumes of the northern Great Plains indicated seed predation by insects, notably bruchid beetles belonging to the genus *Acanthoscelides* Schilsky, was a dominant factor determining viable seed production in natural and artificial plant assemblages. However, although we have studied the reproductive biology of several species of *Astragalus* L., none had fleshy pods. Therefore, the objective of this study was to quantify the factors influencing viable seed production in natural populations of GPMV in South Dakota. Mature pods were collected from two natural populations on the South Dakota State University Oak Lake Field Station during July 2000 and from one natural population on the Grand River National Grassland near Lodgepole, SD, in July 2003. Pods were dissected and number of ovules that: 1) produced viable seed, 2) were unfertilized, 3) produced aborted seed, and 4) produced seeds that were predated by larvae of the bruchid beetle *Acanthoscelides fraterculus* (Horn) (Coleoptera: Chrysomelidae: Bruchinae) were recorded for each of the two valves of individual pods. Our results indicated average seed set, i.e., percent of ovules that produced viable seed not predated, was about 55%, averaged across populations. Unfertilized ovules were 42% of the total. Predation by the bruchid beetle occurred in about 2% of the developed seeds, with aborted seed making up the rest of the ovule total. About 35% of pods infested contained larvae, or adults that were immured within the pod while attempting to exit, presumably due to the thick and leathery layers of the ovary wall. Predispersal seed predation was a relatively minor factor in the reduction of viable seed set in GPMV in South Dakota.

Keywords

Bruchid beetle, *Acanthoscelides fraterculus*, seed predation, unfertilized ovules, seed set

INTRODUCTION

Groundplum milk-vetch (*Astragalus crassicarpus* Nutt.) (GPMV) is a native legume commonly found in sandy-gravelly prairies throughout South Dakota (Van Bruggen 1985). Its natural range in the United States is west of a line from Arkansas to Minnesota (Barneby 1964). Pods of this species have long been eaten, fresh or dried, by indigenous Americans and European settlers, and rodents cache the pods for consumption during the winter (Stubbendieck and Conard 1989).

There is increasing interest in North America in the use of native legumes for forage, biofuel, conservation, revegetation, biodiversity enhancement, roadside beautification, and pollinator habitat (Cane et al. 2013; Kaye 1999). However, seed predation by insects may limit the availability of viable seed (Boe et al. 1988). Seed beetles in the genus *Acanthoscelides* Schilsky (Coleoptera: Chrysomelidae: Bruchinae) are primary seed predators of native legumes (Johnson 1970) in the northern Great Plains (Boe and Johnson 2008a,b; Boe and Johnson 2015; Boe and Johnson 2016).

Johnson (1979) reported *Acanthoscelides fraterculus* (Horn) collected from pods of GPMV in Colorado and Manitoba. Kingsolver (2004) also listed GPMV, as well as 12 other species of *Astragalus* Linnaeus as a host for *A. fraterculus*, but did not provide collection site information. Trelease and Trelease (1937) reported *A. fraterculus* predation on seeds of two-grooved poisonvetch (*A. bisulcatus* (A. Gray)) containing 1475 ppm of selenium. We also collected *A. fraterculus* adults that emerged from pods of two-grooved poisonvetch growing in seleniferous soil on rangeland in northeastern Montana (unpublished data).

Although pods of ground-plum milk-vetch are of food value to humans and wildlife, little is known about its reproductive biology, including seed production and seed predators. Pods of GPMV have several unique morphological characteristics (Barneby 1964) relative to the other 25 or so species of *Astragalus* that occur in South Dakota (Van Bruggen 1985). In particular, the tardy indehiscence of the pods of GPMV provide a unique opportunity to conduct a comparative study of the various factors that influence viable seed production in native legumes. Therefore, the objectives of this study were to: 1) determine rates of ovule fertilization and normal seed development, unfertilized ovules, seed abortion, and seed predation in natural populations of GPMV in eastern and western South Dakota, and 2) identify and elucidate aspects of the life histories of seed predators of GPMV.

MATERIALS AND METHODS

Mature pods were collected from plants growing on shoulder/backslope positions of south-facing slopes on natural grasslands in eastern and western South Dakota. The eastern SD sites were located in tallgrass prairie on glacial moraine (Buse-Langhei loam soil) on the South Dakota State University Oak Lake Field Station (OLFS) (44.5115 N, 96.5311 W). Two populations of GPMV, hereafter referred to as OLFS-S (south) and OLFS-N (north), separated by about

2 km, were sampled. The western SD site was located in mixed-grass prairie on the Grand River National Grassland (GRNG) (45.8150 N, 102.5494 W) on a Reeder-Lantry loam soil. Collections were made during July 1999 on the OLFS and during July 2003 on the GRNG.

At each site, pods were collected from racemes lying on the soil surface. For the OLFS-S population, pods from six individual plants were placed in separate paper bags; whereas for the OLFS-N population pods were collected from only one plant. Not all of the plants in a population possessed pods, presumably due to pod predation by rodents. At the GRNG site, pods from two plants were placed in a single bag. Pods were stored in paper bags at room temperature until the following spring, at which time 30 individual pods from each population were split with a scalpel into their two complete valves along the medial suture between the septa (Figure 1). The exposed leathery endocarpal walls that covered the locules were removed with forceps to expose the contents (Figure 2), which were examined for determination of frequencies of ovule-fate categories (i.e., normal seed, predated seed, aborted seed, and unfertilized ovules (Figure 3).

Fifty randomly selected individual pods from each of the two populations at OLFS were weighed on an electronic balance with centigram accuracy to determine variation between and within populations for pod weight.

Chi-squared analysis was used to determine if frequencies of occurrence of the four ovule-fate categories were independent of population. Analyses of variance were conducted to determine the relative importance of among population, among pods within populations, and between valves within pods within population sources of variation for explaining variation for of each of the four ovule-fate categories (i.e., normal seed, aborted seed, predated seed, and unfertilized ovule).

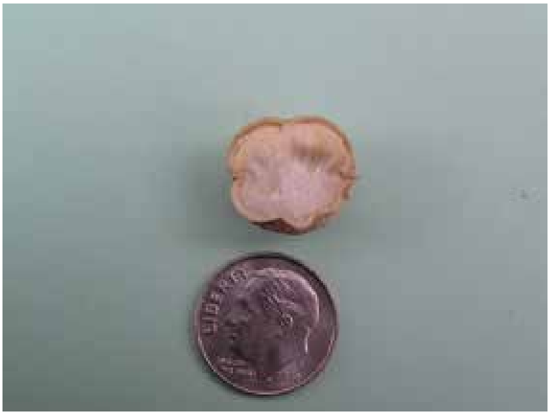

Figure 1. Individual valve of pod from Grand River National Grassland; endocarp (i.e., septum) covering the locule is adnate for about 2/3 of the inner margin.

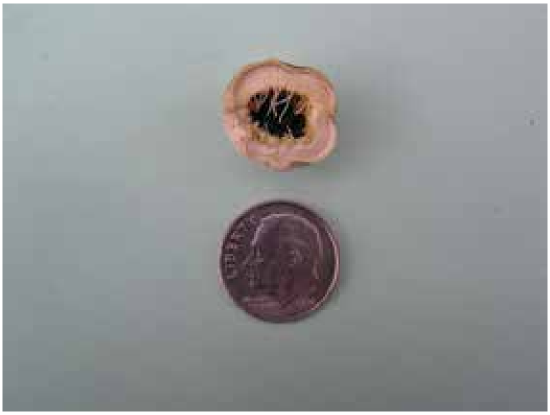

Figure 2. Placentae attached to the ovary wall with ovules extending into the locule of the valve for the pod of groundplum milk-vetch from the Grand River National Grassland.

Figure 3. Normal seed (left), predated seed (center) and unfertilized ovules and aborted seed (right) of groundplum milk-vetch from the Grand River National Grassland.

RESULTS

At the OLFS site, plants from both populations had fully developed fleshy green pods, which were turning red on the exposed surface by 15 June 1999. These pods contained rapidly developing green seeds, as well as unfertilized ovules, in the same valve. On 26 June, seeds appeared fully developed, size wise, but were still green and the contents watery; also, early instar larvae of *Acanthoscelides fraterculus* were feeding on seeds inside the pods. On 3 July, pods had begun to dry and wrinkle, and by 12 July, pods were characteristically shriveled, rugose, and firm. On 26 July when mature pods were collected for ovule-fate data collection and analysis, late instar larvae and pupae of *A. fraterculus* were active within pods. Similar phenological observations for pod, seed, and insect development were not possible for the population at the GRNG site due to a single evaluation/collection activity.

Acanthoscelides fraterculus (Figures 4) was the only seed predator observed feeding on seeds of GPMV during this study. Also, no hymenopterous parasitoids of *A. fraterculus* were encountered. We observed the empty egg chorions of *A. fraterculus*, usually oviposited singly, in depressions in the outer layer of the rugose pod. Entrance holes made by early instar larvae were nearby. If larvae survived to produce adults, they consumed the cotyledons of several, but usually not most, of the seeds in the locule, leaving relatively intact testas in the locule (Figure 3).

Pod weight varied widely within populations. However, no difference occurred between the two populations at OLFS for pod weight. Mean pod weight for a sample of 96 pods (48 from each of the two populations) was 0.36 ± 0.009 g, ranging from 0.15 g to 0.71 g.

Chi-squared analysis indicated that frequency of ovule fate was not independent of population ($\chi^2 = 149.4$, $P < 0.001$). For example, OLFS-N had a four times higher frequency (0.15) for aborted seed and a three times lower frequency (0.03) for predated seeds than the other two populations (0.05). Frequencies for normal seeds and unfertilized ovules were similar for the three populations. Mean number of ovules • valve^{-1} ranged from 16.6 for GRNG to 28.7 for OLFS-S (Table 1).

Intraclass correlations calculated from variance components generated from analysis of variance indicated about 55% of the variation for number of normal seeds • valve^{-1} was due to variation among pods within populations. Whereas, 35 % of the variation was associated with variation between valves within pods, and 10 % was associated with variation among populations. Similar associations were found for variation in number of unfertilized ovules and number of predated seeds valve^{-1}.

About 35% of the pods infested by *A. fraterculus* contained dead early instar larvae in at least one of the valves or dead adults that expired within a tunnel they had chewed in the wall of the pod during attempted exits (Figure 5).

Due to their arrangement, individual placentae were readily noted under low magnification (3X), from their attachment on the wall of the ovary to suspension of their ovules in the locule of the valve. Number of placentae did not always match the number of ovules due to what appeared to be placental and/or ovular atrophy.

Table 1. Means and standard errors (in parenthesis) for fates of all ovules for individual valves for 30 pods from each of three populations of groundplum milk-vetch from South Dakota.

	Ovule Fate (No. • valve^{-1})			
Population	**Normal seed**	**Aborted seed**	**Unfertilized ovules**	**Predated seed**
Grand River	8.6 (0.7)	0.6 (0.1)	6.3 (0.5)	1.1 (0.3)
Oak Lake S.	13.2 (0.9)	1.7 (0.2)	10.9 (2.1)	2.9 (0.8)
Oak Lake N.	10.7 (0.6)	3.5 (0.3)	8.8 (0.4)	0.6 (0.3)

Figure 4. **Acanthoscelides fraterculus*****; top dorsal habitus, lower lateral habitus.***

Figure 5. Adult **Acanthoscelides fraterculus** ***immured in valve of*** **Astragalus crassicarpus*****.***

DISCUSSION

Although Johnson (1979) listed *A. fraterculus* as a seed predator of GPMV, no estimates of this species' impact on seed production of GMPV were available prior to the current study. The levels of seed predation reported here were much lower than we have observed for other bruchid beetle-legume seed associations in the northern Great Plains (e.g., Boe et al. 1988; Boe and Johnson 2015; Boe and Johnson 2016) and were also lower than what others observed for *A. fraterculus* in native species of *Astragalus* in the western USA (Green and Palmblad 1975; Cane et al. 2013). Also, this is only the second bruchid beetle-legume seed interaction (Boe and Johnson 2015) for which we have not encountered chalcidoid (Hymenoptera: Chalcidoidea) parasitoids of the bruchid seed predator. However, the current collections were quite limited in terms of numbers of pods, geographic range, and number of years (Kaye 1999).

The significance of lack of fertilization as a factor reducing seed production was much greater than in a study on *Astragalus australis* (L.) Lam. var. olympicus Isely by Kaye (1999) in the Pacific Northwest. In that study, seed production was reduced by predispersal seed predation by a *Tychius* sp. (*Coleoptera: Curculionidae*), seed abortion, and lack of fertilization, in that order of importance.

The relatively high frequency of the failure of adults of *A. fraterculus* to exit the pods of GPMV suggested that the morphology (i.e., three layers) and thickness of the wall of the valve of GPMV (Barneby 1964) provided formidable impediments to adult emergence. The inner and outer layers of the valve are firm and leathery, whereas the central and much thicker layer is pithy. Several of the expired adults that we observed had traversed the inner and central layers and had begun chewing through the outer layer, but failed to exit. This produced what appears to be a case of immurement involving the inability of *A. fraterculus* adults to leave the confines of their host pod.

The large variation among individual pod morphologies and weights within individual plants within populations was indicative of wide variation for valve/locule size and number of ovules • valve^{-1}. However, even the smallest pods with less than 10 ovules • valve^{-1} still had unfertilized ovules. The analysis of variance and estimates of variance components was also informative regarding variation among pods within populations, since most of the variation in ovule and seed numbers could be explained by this factor.

Similar to several other native legumes (e.g., Boe and Johnson 2016), germination tests of seeds of GPMV from a native prairie in eastern South Dakota indicated high levels of hard seed, but high germination rates after scarification (Sorensen and Holden 1974). Because of its adaptation to erodible land and its palatability to livestock and wildlife, GPMV is an attractive candidate for revegetation, roadside habitat enhancement, and other conservation activities. Results of this study quantified the major factors that would limit seed production for commercial purposes and identified ineffective pollination as having a greater negative impact than seed predators (i.e., *A. fraterculus*) on potential seed yield.

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