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ABSTRACT — Field and laboratory studies of the silky prairie clover (*Petalostemon villosum* Nutt.) in central Minnesota indicated that seeds of this plant require scarification but do not require an overwintering period, to germinate. Germination and primary root growth are rapid; shoot growth appears to be quite slow. Methods of associating shoot growth with age are suggested and several notes on the life history of the plant are presented.

During the spring of 1962 laboratory and field studies of the silky prairie clover (*Petalostemon villosum* Nutt.) were undertaken. Laboratory studies continued in the fall of 1962. The results of these studies are here presented.

The genus *Petalostemon* is peculiar to North America and includes 13 species (Fernald 1950, Rydberg 1954). *P. villosum* is distinguished from the other species by its densely villous leaves and stems and by the presence of 7-19 leaflets on each leaf. It appears that this prairie clover has received little save taxonomic attention. Weaver and Albertson (1956) wrote that *P. villosum* is prominent in sand hills flora and sometimes is a pioneer in blowouts. They also provided information on the nature and extent of the root system of the species. Stevens (1950) and Rydberg (1954) indicated the loose, sandy soils of Texas, eastern New Mexico, eastern Colorado, Kansas, Missouri, Nebraska, Iowa, South and North Dakota, Minnesota, Wisconsin and Saskatchewan as the geographic range of *P. villosum*. One record from Montana (see Appendix A) seems to have gone unnoticed by most workers, and there are several records from Manitoba. There is but one collection of the species from Missouri, and only two records from Iowa, both from Blackhawk Co. (Grant 1953). The distribution of *P. villosum* in North America is indicated by the data in Appendix A. The distribution of the species in Minnesota and adjacent counties of neighboring states is shown in Figure I.

I know of no other published information on this prairie clover. Weaver (1954), Drew (1947) and Gibbens (1954) have presented ecological information on *P. purpureum* and *P. candidum*, but these species are ecologically and morphologically different and easily distinguishable from *P. villosum*. Perhaps the lack of ecological information on *P. villosum* results at least in part from the generally poor agricultural qualities of its habitats.

Materials and Methods

Field study, collection of seed, and collection of plants were conducted in the dune-blowout area of the Nature Conservancy's "Winger Savanna" Tract in Anoka Co., Minnesota. Seed for germination tests was collected several times in April, and on October 14, before the first freeze. The April seed, probably representing the 1961 crop, was largely obtained from accumulations of debris near the bases of the mature plants, but some seed was collected from fruiting spikes. Most of the seed collected in October was taken from fruiting spikes and thus was

FIGURE 1. Distribution of *Petalostemon villosum* in Minnesota and adjacent counties in Wisconsin. All Wisconsin records represented in the University of Wisconsin herbarium. All Minnesota records represented in the University of Minnesota herbarium, except that the Sherburne Co. record is from the St. Cloud State College herbarium.

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The prairie clover work was done under the guidance of Dr. Donald B. Lawrence, while the author was a student in his advanced plant ecology class in 1962, and as a program of independent study in the Fall of that year.

The invaluable assistance and encouragement given by Dr. Donald B. Lawrence in all phases of this study are gratefully acknowledged. The curators of many herbaria in the midwestern, southwestern and Rocky Mountain states, and in several Canadian provinces, kindly provided their locality records of *P. villosum*. Mr. Donald K. Wemble of Iowa State University provided an extensive list of distribution records of specimens temporarily in his care. Dr. Thomas Morley, of the University of Minnesota, identified a number of species of plants from the study area.

1962 crop. A small amount of seed of unknown age (1961?) was collected from accumulations on the ground.

Once collected, seed was treated in one of three ways. Some seeds were scarified by rubbing them gently between two pieces of waterproof garnet paper, grit no. 6/0, until the seed coats appeared to be partially removed. It is likely that some embryos were damaged by this treatment. Some seeds were not scarified, but the flower parts were removed from them by gently rubbing the seed between the thumb and forefinger. For one fall germination test the seeds were used with the flower parts adhering to them.

Field studies of germination were begun in April and conducted through May. Six seeds were planted about one inch deep and one inch apart in the sand within each of three cylindrical ¼-inch mesh hardware cloth exclosures one foot high and one foot in diameter placed in the study area adjacent to successfully established *P. villosum*. Unscarified seeds with the flower parts removed were planted in two of the exclosures; scarified seeds were planted in the third. Three scarified seeds were planted about one inch deep and one inch apart beside each of three wooden stakes set in the same area. All seed plots were examined at least once a week during late April and through May.

On May 3, I planted three scarified and three unscarified seeds behind a piece of plate glass set vertically in the soil. One scarified seed was planted at a depth of three inches, one at two inches, and one at one inch in the soil behind the left half of the observation glass. Three unscarified seeds were similarly planted behind the right side of the glass. In order to insure temporary adherence of the seeds to the glass for planting, the seeds were slightly moistened with saliva. Although saliva, owing to its variable chemical nature, was not a desirable adhesive for this purpose, it apparently did not inhibit germination. Sand replaced on the observation side of the glass was removed periodically in order to observe the seeds.

Laboratory tests of germination were conducted by placing seeds between sheets of wet filter paper set in the bottoms of covered Petri dishes or fingerbowls, and in soil samples as indicated in Table 1. Scarified and unscarified seeds were kept in separate vessels for germination tests using moist filter paper, but in the soils the scarified and unscarified seeds were planted side by side in common vessels. Seeds used in the spring germination tests were moistened with tap water. In the fall tests distilled water was used. For two of the tests in the fall, paper towels were used instead of filter paper. In the fall, seed was tested for ability to germinate in the dark by placing half of a sample of scarified seeds in a germination chamber within a closed warming oven. The other half of the sample served as a control.

During May, many seedlings from germination tests were examined and preserved in 70% ethanol. In the field, seedlings and older plants were dug up with a garden trowel, examined, and similarly preserved. Care was taken to avoid damaging the roots of the plants, but unintentionally some roots were invariably cut or broken from the very extensive root systems of the older plants.

In order to determine whether or not *P. villosum* develops annual growth rings, sections of the root and several stems of a very large plant were cut with a hand saw, and the cut surfaces polished with fine-texture sandpaper. The polished surfaces were then examined, using a spectacle loupe for magnification. Very thin sections of plants believed to be in their second season of growth were also cut and examined microscopically to ascertain the presence of the first annual growth ring.

Results

Results of both field and laboratory germination tests in the spring suggested strongly that most of the seed of *P. villosum* must be scarified before germination can occur. Of the seeds planted one inch deep in the exclosures and beside the stakes in the study area, none produced seedlings that emerged aboveground. Study of the seeds planted behind the observation glass suggested a possible explanation. While none of the three unscarified seeds behind the glass germinated, all three of those scarified did. The scarified seeds produced rapidly elongating hypocotyls and radicles — one grew to a length of 40 mm in nine days — and expanded their cotyledons, but by the end of May none of the cotyledons had appeared aboveground. In early August, a visit to the area revealed that all three of the seedlings had died without having emerged from beneath the soil surface. Since all the other seeds planted in the field had been set about an inch underground, it is possible that at least some had germinated but were undetected because they died before they appeared aboveground. Of the three scarified seeds behind the observation glass, the seed three inches deep germinated first. During the time the seeds were planted, the sand remained damp below about 2½ inches beneath the soil surface. The seeds planted at depths of one and two inches germinated following a rain.

In laboratory germination tests in the spring, all of eight scarified seeds placed on wet filter paper germinated; only one of eight unscarified seeds germinated. The results of germination tests in various soil types are summarized in Table 1.

Fall germination tests, using large numbers of 1962 seeds, provided results similar to those obtained in the spring tests. Of about 200 seeds collected from spikes, scarified, placed in a covered fingerbowl on wet paper toweling, and kept under constant artificial light at 75°F, more than 85% germinated. Of the same number of unscarified seeds, with flower parts adherent, two germinated. Of 100 seeds, unscarified but with flower parts removed, only two germinated. It was noted that, among the scarified seeds, the radicles of some embryos emerged within 24 hours after the seeds were placed on wet paper. Germination was found to occur as rapidly and as successfully in total darkness as in constant light. The work demonstrated that *P. villosum* does not require an overwintering period for germination, and that germination can occur extremely rapidly, in the presence of abun-

TABLE 1. Results of laboratory tests for germination in soil samples.

Soil sample from:	No. of seeds germinated/no. planted	
	Scarified seeds	Unscarified seeds
gopher mound in prairie	4/6	0/0
ant hill in prairie (including vegetative litter)	4/4	1/4*
study area, near mature *P. villosum*	8/8	0/8
bare sand dune in study area	4/4	0/4
gopher mound in study area	5/5	0/5

* It is possible that the germinated seed might have been one that occurred in the litter from the ant hill. The sample was not screened to exclude extraneous seed.

dant water and suitable temperature, without regard for light.

Examination of the seedlings from the laboratory germination experiments showed that the primary root is 1-1½ inches long before the cotyledons emerge from the seed coat. Under field conditions, when the cotyledons emerged aboveground the seedlings could be recognized and collected. Such seedlings collected during May were found to have their seed coats attached about ½-inch below the soil surface. This suggests the optimal planting depth for experimental studies. The seedlings possessed simple, unbranched tap roots 3-4 inches long, usually with 1-3 nodules on their lower parts. Aerial parts consisted of the cotyledons, foliage leaves bearing several leaflets, and, by the end of the first season of growth, an aerial shoot two inches or less in length. Two other size classes of small *P. villosum* were found. Members of one group, assumed to have germinated the previous spring, had a simple unbranched root 7-8 inches long and a stem about 1½-2 inches high, now dead, but from the base of which arose two new living branches, each about one inch long in May. A second group, assumed to be 2-year-old plants, consisted of larger plants with well-developed root systems with 3-5 branch roots and an aerial shoot consisting of two dead branches, each 3-4 inches long, from the base of each of which arose two new branches. In one plant of this class, the total length of the roots was 40 inches. Only two such plants were found.

Examination of a large mature plant for annual xylem growth rings revealed five conspicuous rings in the root crown and two conspicuous rings in the basal portion of the shoot. It is thought that there may be at least one undetected additional ring, representing the first year's growth, in the root crown. The disparity between the numbers of rings in the root and shoot suggests that the oldest portion of the shoot may have been killed, leaving only the 2-year-old portion. Snow cover, as recorded at the Minneapolis City Weather Bureau, was extremely light (15 inches total) during December, January and February of 1960-1961. It seems possible that part of the plant may have been killed by freezing during these cold months.

A number of incidental ecological observations were made and are reported here.

Several plants, both immature (small, non-flowering) and mature (having borne flowers at least once) were found to be connected by single horizontal roots. Also, a 2-inch-long segment of root about ¼-inch in diameter that had been severed from the parent plant and buried during a digging operation was recovered a month later and found to bear several new branches. Whether the branches were roots or shoots was not determined. These observations suggest that propagation by adventitious buds may be an important means of vegetative propagation in this species, and thus a mechanism of increase in stand density.

A vertical observation glass was set in front of four small (one mm diameter) roots whose ends had been cut. The sand piled in front of the glass was removed, the roots examined, and the sand replaced once weekly during late April and May. The initiation of branch roots, which increased in number and length throughout the summer, was first noted when the soil temperatures at three and six inches below the surface were 27° and 23°C respectively. This suggests that the threshold temperature for branch root initiation may be around 20°C.

On August 4, 1962, when the plants were in flower, a visit to the study area revealed that the flowers were visited by several kinds of dipterans and hymenopterans. There appeared to be a standard pattern of flowering on a given inflorescence. A basal band of florets opened first, then withered as those immediately above them opened. Thus, the band of open flowers moved up the spike, and by the time the apical band of florets was open, those below them were withered. I did not investigate the possibility of a similar wave of fruit maturation, but that would be the expected result of the flowering sequence.

Plant associates of *P. villosum* on the study area included *Cladonia* (lichen), *Tradescantia* (*virginiana*?), *Solidago gramnifolia*, *Carex* sp., and *Sporobolus cryptandrus*. *Sporobolus* often grew in very close association with *P. villosum* and the two plants often caused a microdune to form about them.

Under certain conditions, *P. villosum* can become extremely abundant in a small area. One selected square meter quadrat, sloping very slightly toward the west, was found to contain 15 mature *P. villosum* shoots and 15 immature shoots of the species. It is not known how many of these were independent individuals and how many were derived adventitiously from an interconnecting root system.

Discussion and Conclusions

These preliminary studies are surely too fragmentary to make broad generalizations possible. However, one may safely conclude that germination of the seed of *P. villosum* is usually dependent upon adequate scarification and abundant water. The texture of dune sand suggests that aeration may also affect germination, but this was not tested. Seed may be shed anytime from its maturation in early fall until the initiation of new branches by the parent plant in spring. Scarification is perhaps accomplished in nature by a combination of chemical, biological, and (especially) mechanical agents (such as blowing sand). No after-ripening or cold period is needed for maturation of the seed, and no light is required during germination. Germination proceeds very rapidly in the presence of adequate water and air.

A primary root about two inches long is produced before the cotyledons emerge from the seed coat and grow toward the surface. Emergence of the cotyledons above the soil surface occurs when the seed has been buried ½-inch deep but not if the seed is planted one inch deep or more. The rapid elongation of the primary root, a characteristic feature of xerophytes, would seem to insure a continuous supply of moisture and adequate anchorage in the loose, sandy soil.

A single short shoot develops during the first season of growth and dies at the end of the season. By this time the root has elongated to 7-8 inches, but remains largely unbranched. One to three nodules may have developed on the lower part of the root. The relatively small size of the shoot compared to that of the root would appear to be adaptive from the viewpoints of water economy and anchorage.

At the onset of the second season of growth, two new branches develop from the base of the old shoot axis, which probably weathers and later drops off. Root growth is characterized by the production of prominent secondary and tertiary roots that result in a five-fold increase in total root length. At the end of the second season of growth the two shoot branches die. Ideally, each of them would give rise to a new pair of branches the following spring, but such ideal plants are rarely found. After the third season of growth the pattern of branching becomes too complex to follow. The factors responsible for this complexity have not been resolved.

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Appendix A

Records of *Petalostemon villosum* from selected herbaria.

Abbreviations as follows:

Can—National Herbarium of Canada
CC—Colorado College
CSU—Colorado State University
CU—University of Colorado
Duke—Duke University
FSU—Florida State University
Ill—University of Illinois
ISU—Iowa State University
Mich—University of Michigan
Minn—University of Minnesota
NDU—University of North Dakota
NMU—University of New Mexico
NY—New York Botanical Garden
OU—University of Oklahoma
Sask—University of Saskatchewan
SDU—University of South Dakota
SMU—Southern Methodist University
SUI—State University of Iowa
Tex—University of Texas
Wis—University of Wisconsin
Wyo—University of Wyoming
US—U. S. National Museum

CANADA: *Saskatchewan*: 3 mi W Caron (ISU, Sask, SMU, Tex); Mortlach, about 65 mi W Regina (Can). *Manitoba*: Aweme (Central Experimental Farm at Ottawa); Lauder (Can); Grande Clariere (Can).

U. S. (by county): *Montana*: Dawson (US). *North Dakota*: Barnes (NDU, SDU); Benson (Ill, NDU, Wyo); McHenry (NDU, Wyo); Cass (NDU); Ransom (NDU); Richland (NDU); Sargent (NDU). *South Dakota*: Bennett (SDU); Fall River (Wyo); Harding (SDU Wyo); Pennington (SDU); Walworth (SDU); Washabaugh (SDU). *Minnesota*: see Figure 1. *Wisconsin*: Buffalo (Wis); Burnett (Minn, Wis); La Crosse (SUI, Wis); Pepin (Mich, Minn, Wis); Pierce (Wis); Polk (Wis); St. Croix (Wis); Trempealeau (Minn, Wis); Waukesha (Wis). *Nebraska*: Antelope (Tex. US, Wis); Box Butte (ISU, US); Brown (US); Chase (ISU); Cherry (ISU); Franklin (US); Grant (NY, Wyo); Holt (ISU, Tex, SUI); Hooker (US); Lincoln (SUI); Loup (ISU); McPherson (Wis); Merrick (Ill); Sheridan (ISU); Sioux (US); Thomas (ISU, US). *Iowa*: Blackhawk (State College of Iowa, Ill, SUI). *Colorado*: Baca (CU); El Paso (CC); Las Animas (CU, US); Logan (Wyo); Phillips (CSU, CU); Pueblo (CSU); Sedgewick (CU, Wyo); Washington (CSU, US); Weld (CSU, CU, Ill, Tex); Yuma (NY, US, Wyo). *Kansas*: Cloud (Ill, Wis); Dickenson (US); Finney (ISU, SMU, US); Hamilton (ISU, NY); Harper (NY, US, Wyo); Kearney (NY); Miami (NY); Pratt (Duke, FSU, Ill, ISU, OU, SMU, SUI, Tex, US, Wyo); Reno (Ill, ISU, SMU, US, Wis); Rooks (Wyo); Sedgwick (ISU). *Missouri*: Franklin (US). *New Mexico*: Quay (NMU). *Oklahoma*: Beaver (NY, OU, Tex); Blaine (OU); Cleveland (OU); Custer (OU); Garfield (OU); Grady (OU); Greer (OU); Harper (ISU, OU, Tex); Kay (OU); Kingfisher (OU); Major (Ill, OU, SMU, US); Muskogee (OU); Oklahoma (NY, OU); Payne (US); Roger Mills (OU, SMU); Tillman (OU); Washita (OU); Woods (Ill, OU. SMU, US, Wyo); Woodward (OU, Tex). *Texas*: Bailey (FSU, NY, SMU, Tex); Callahan (FSU); Collingsworth (Tex, US); Hall (NY, US); Hemphill (OU, US); Hockley (US); Jones (SMU); Lamb (US); Lipscomb (SMU, US); Mitchell (ISU, SMU); Roberts (OU, SMU); Wheeler (Tex, US).