



Ruby Valley pointvetch (*Oxytropis riparia* Litv.) karyotype, germplasm registration, and seed coat characterization  
by Deborah Jean Solum

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in  
Agronomy  
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**Abstract:**

Ruby Valley pointvetch (*Oxytropis riparia* Litv.) is a perennial forage legume with high salt and drought tolerance. However, pointvetch has a high hardseed content, which makes uniform stand establishment difficult. Studies were conducted to characterize the chromosome complement, register the germplasm, and identify the seed coat components. The chromosome complement of pointvetch is  $2n=2x=16$ , and the chromosomes are generally smaller than other plants and animals.

The pointvetch germplasm was registered in 1990 and distributed upon request to researchers for further studies. The seed coat surface pattern and cell layer components were characterized as a basis for further studies involving hardseed mechanism(s). The seed components were similar to other related small seeded legumes, consisting of cuticle, palisade cells, hourglass cells, parenchyma, endosperm, and cotyledons progressing from exterior to interior. Four pointvetch seed lots were utilized, and all had similar exogenous and endogenous characteristics which indicated species stability in diverse environments. This is useful for taxonomic use in establishing evolutionary relationships.

RUBY VALLEY POINTVETCH (Oxytropis riparia Litv.)  
KARYOTYPE, GERMPLASM REGISTRATION, AND  
SEED COAT CHARACTERIZATION

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A thesis submitted in partial fulfillment  
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APPROVAL  
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## ABSTRACT

Ruby Valley pointvetch (*Oxytropis riparia* Litv.) is a perennial forage legume with high salt and drought tolerance. However, pointvetch has a high hardseed content, which makes uniform stand establishment difficult. Studies were conducted to characterize the chromosome complement, register the germplasm, and identify the seed coat components. The chromosome complement of pointvetch is  $2n=2x=16$ , and the chromosomes are generally smaller than other plants and animals. The pointvetch germplasm was registered in 1990 and distributed upon request to researchers for further studies. The seed coat surface pattern and cell layer components were characterized as a basis for further studies involving hardseed mechanism(s). The seed components were similar to other related small seeded legumes, consisting of cuticle, palisade cells, hourglass cells, parenchyma, endosperm, and cotyledons progressing from exterior to interior. Four pointvetch seed lots were utilized, and all had similar exogenous and endogenous characteristics which indicated species stability in diverse environments. This is useful for taxonomic use in establishing evolutionary relationships.

## CHAPTER 1

## INTRODUCTION

Water stress and increasing soil and water salinity levels are adversely affecting food and feed production worldwide. Sedimentation and salinity are affecting water quality, agricultural production, and general land use in Montana (Water Quality Bureau, 1982). The Soil Conservation Service National Resource Inventory estimated in 1987 that 1.0 million ha of Montana land were eroded by water at a rate greater than normal (R. Nadwornick, personal communication). Additionally, the Water Quality Bureau (1975) reported that 2,944 km of streams in Montana were degraded by sediment, and 1,545 km were affected by salt. The development and utilization of plant species with high drought and salt tolerance as well as erosion control capabilities could provide a partial solution to these problems.

Ruby Valley pointvetch (*Oxytropis riparia* Litv.) is a perennial, small seeded legume which is a palatable livestock forage. Pointvetch also has potential for use in reclamation situations since it is highly drought and salt tolerant and has a prostrate growth habit for erosion control. However, pointvetch has a high hardseed content which makes establishment difficult in field conditions. The hard seed coat

inhibits the uptake of water by the seed, which is necessary for germination, and is often the major factor causing hardseededness in legumes. The hard seed coat may be caused by genotypic and/or environmental factors.

Chromosome number and morphology are important in a genotypic study since they determine the ease of developing a line with uniform germination, increased salt or drought tolerance, and/or greater forage and seed production potential. Chromosomes are often characterized by an ideogram of a karyotype. A karyotype is the particular chromosome complement of an individual or a group of related individuals as defined by chromosome size, morphology, and number (Schulz-Schaeffer, 1980).

After the chromosome complement has been characterized and a seed source collected, the germplasm can be registered with the Crop Science Society of America and stored at a Plant Introduction Station and the National Seed Storage Laboratory. Seed is then distributed, upon request, to researchers for further study.

Seed coat characterization is necessary to properly identify components so further studies may be conducted to identify which components may be responsible for hardseededness by utilizing various environmental conditions and genotypes to obtain a wide range of hardseed percentages. Identified hard and non-hard seeds may also be compared for morphological differences to identify the hardseed mechanism(s). Some small seeded legumes exhibit visible seed coat component differences between hard and non-hard seeds while others do not.

Environmental conditions are an important factor in hardseed development. Hot and dry growing conditions during seed formation increase hardséededness in many crops, especially legumes. Generally, conditions which favor formation of small seeds also favor hardseed development.

The objectives of this study were to 1) verify the chromosome number and characterize the chromosome morphology and size, 2) register the pointvetch germplasm for breeding and experimental purposes, and 3) characterize the seed coat components for both hardseed studies and taxonomic comparisons.

## CHAPTER 2

## LITERATURE REVIEW

Ruby Valley Pointvetch Description

Ruby Valley pointvetch (Oxytropis riparia Litv.) is a member of the Leguminosae family in the Galegeae tribe with other genera such as Wisteria, Indigofera, and Astragalus (Smith, 1977). O. riparia is a deep taprooted, prostrate, perennial legume (Booth and Wright, 1966) with opposite leaves (Komarov, 1972) comprised of subsessile, oblanceolate to acutish leaflets (Barneby, 1952). Flowers are small, purplish, and papilionaceous, with a banner approximately 6 mm long (Barneby, 1952). Seed pods are nodding, stipitate, 10 to 20 mm long, and approximately 5 mm wide (Komarov, 1972).

The chromosome number of pointvetch is  $2n=2x=16$  (Astanova and Abdusaliamova, 1981; Schulz-Schaeffer et al., 1990). Astanova and Abdusaliamova (1981) also reported plants with  $2n=4x=32$ . Ledingham and Rever (1963) reported that all Oxytropis species were either diploid ( $2n=16$ ) or polyploid with multiples of 8 chromosomes.

Ruby Valley pointvetch is native to Russian Turkestan (Barneby, 1952) and was found growing in the Ruby Valley of southwestern Montana in 1930 (Green and Morris, 1935). It was originally named Astragalus rubyi

Green and Morris by Green and Morris (1935) and was reclassified by Barneby (1952) as Oxytropis riparia Litv. Pointvetch has been identified along the Green River northwest of Green River, Wyoming; along the Snake River on the Fort Hall Indian Reservation north of Pocatello, Idaho (Williams and Molyneux, 1988); and near Ice Slough, Wyoming (Lichvar and Dorn, 1982).

Benson (1954) reported that pointvetch grew well on alkaline soil. Eslick and Vogel (1959) determined that pointvetch emergence increased in soil moisture tensions greater than field capacity (-0.03 MPa) down to 7.5% soil moisture (-1.5 MPa). Delaney et al. (1986) reported that pointvetch had 90% germination in -2.0 MPa NaCl solutions while -1.0 MPa NaCl decreased alfalfa (Medicago sativa L.) germination 70%.

Pointvetch has a higher NaCl toxicity tolerance and a greater tolerance to higher osmotic stress than alfalfa (Russo, 1985). Germination of O. riparia has been reported to be temperature insensitive between 5 and 35°C due to rapid germination potential (Townsend and McGinnies, 1972).

Toxicity is often a concern since two other species of Oxytropis contain swainsonine which is responsible for loco syndrome in livestock. O. riparia tested negative for swainsonine toxicity and contained no cyanide, soluble oxalates, or aliphatic nitro compounds (Williams and Molyneux, 1988).

The feeding value of pointvetch was reported as similar to the protein content, nitrogen-free extract, crude fiber, ether extract, and ash of alfalfa. However, pointvetch phosphorus content was higher than alfalfa grown in the same area (Green and Morris, 1935). Feeding

studies involving lambs fed alfalfa hay compared to pointvetch hay showed similar results (Post, 1937).

#### Hardseededness

A high hardseed content is common in O. riparia (Delaney et al., 1986; Hicks et al., 1987, 1990; Post, 1937). Delaney et al. (1986) reported up to 96% hardseed, and Post (1937) found 95% hardseed. Hicks et al. (1990) reported that mechanical scarification for 30 s in a Forsberg scarifier<sup>1</sup> (Forsberg's Inc., Thief River Falls, MN) reduced pointvetch hardseededness better than either sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) or hot water bath treatments.

Hardseededness benefits the preservation of viability over extended periods of time. However, this may be a disadvantage in agricultural situations which require rapid, uniform stand establishment. Hardseededness is common in the Leguminosae family (Quinlivan, 1971) and depends on the genetic nature of the species as well as environmental conditions during seed maturation and storage (Hartmann and Kester, 1983). Grant Lipp and Ballard (1964) studied seed of subterranean clover (Trifolium subterraneum L.) and concluded that large seed and soft seed had lower physiological dormancy than small seed and hard seed. Hardseededness in subterranean clover increased with growing season length and less variable temperatures (Quinlivan, 1965). Kowithayakorn and Hill (1982) reported that alfalfa hardseed increased to 87% when seed ripened during hot, dry weather, and

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<sup>1</sup>Mention of a specific brand, trade, or chemical name does not imply endorsement of that product over others of a similar nature or function.



decreased to 60% if seed ripened during cool, wet conditions. Additionally, crimson clover (Trifolium incarnatum L.) percent hardseed increased when conditions favored the production of small seeds (Crocker and Barton, 1953).

### Seed Coat Morphology

Impermeable seeds or hardseeds are common in many families other than Leguminosae, such as Gramineae, Malvaceae, Chenopodiaceae, and Solanaceae (Barton, 1965; Rolston, 1978; Watson, 1948). Seed coat structure determines the degree of hardseededness in legumes. However, there is disagreement over which part(s) of the seed coat are responsible for impermeability (Watson, 1948). The general structure of a leguminous seed coat from the exterior progressing inward is cuticle, Malpighian cells (palisade cells), osteosclereid cells (hourglass cells), and parenchyma layer (nutrient layer) (Barton, 1965; Rolston, 1978; Watson, 1948). The cuticle and Malpighian cells are most commonly considered impermeable (Rolston, 1978). McKee et al. (1977) punctured crownvetch seeds to a depth of at least 98  $\mu\text{m}$  to induce rapid germination. This depth included all of the seed coat components, aleurone layer, and part of the exterior endosperm. The crownvetch impermeable region included the cuticle and Malpighian cells, while the sub-Malpighian layers may have contained germination inhibitors which must also be disrupted to achieve maximum imbibition potential.

Other seed coat structures such as the hilum and strophiole may also be responsible for hardseededness (Hagon and Ballard, 1970; Hyde, 1954). The hilum is the funicular attachment point to the ovule. The hilar

groove, located in the long axis of the hilum (Corner, 1951), affects imbibition by opening and closing in response to relative humidity (RH) (Hyde, 1954). The groove closes when RH increases due to swelling of the surrounding tissue and opens as RH decreases (Hyde, 1954). Rolston (1978) suggested the hilar groove acts as a hygroscopic valve, allowing moisture loss from the seed when ambient RH is less than the endogenous moisture content. Hyde (1954) reported that the groove began to function when seed moisture decreased below 25%, and desiccation below 14% moisture occurred only through the hilar groove. Up to this point all or part of the seed moisture was lost through the testa.

The strophiole is an external raphe enlargement related to seed dispersal (Rolston, 1978). Gopinathan and Babu (1985) described the strophiole as a seed coat area allowing water imbibition. They found that imbibition was not restricted when Vigna umbellata (Thunb.) Ohwi and Ohashi seeds were coated with araldite except for the strophiole region. However, seeds with only the strophiole zone covered with araldite did not imbibe. Hagon and Ballard (1970) reported similar results with subterranean clover. Miklas et al. (1987) determined that 98% of imbibition after mechanical scarification of cicer milkvetch occurred at the strophiole and seed tip, and 75% of imbibition after H<sub>2</sub>SO<sub>4</sub> scarification was accounted for by the strophiole. Ballard (1976) reported that the strophiole became permeable after other parts of the testa were mechanically stressed, suggesting the strophiole and testa are an integrated water absorption system.

### Electron Microscopy

Seed coat studies often utilized electron microscopy for seed component developmental and morphological research (Adams et al., 1985; Baker et al., 1987; Bragg, 1982, 1983; Bridges and Bragg, 1983; McDaniel, 1987; Trivedi and Gupta, 1987; Wolf and Baker, 1972; Yaklich et al., 1986, 1987). Lersten (1981) compared Leguminosae species in the Papilionoideae subfamily and found significant seed coat patterns between and within specific tribes, but could not conclude evolutionary trends. Trivedi and Bagchi (1982) reported similarities in testa structures within and between three tribes of the Papilionoideae subfamily. All of the Trifolieae tribe had one pattern while members of the Genisteae and Phaseoleae tribes had variable patterns. Even with these variations the authors concluded that the testa pattern was important for identifying species and establishing relationships.

Extensive soybean developmental studies have been conducted. Stages of soybean testa development were studied from early differentiation to seed maturity (Baker et al., 1987). Adams et al. (1985) reported on soybean seed protein body development, and Yaklich et al. (1987) examined pit and antipit structural changes in soybean seeds and seedling development.

## CHAPTER 3

## RUBY VALLEY POINTVETCH KARYOTYPE

Increasing levels of soil salinity and soil erosion have resulted in the need for the development of plant species to aid in control of these problems. Ruby Valley pointvetch is a highly salt tolerant plant species and has a prostrate growth habit which aids in erosion control. The plant carcasses from the previous growing season remain as a dense ground cover on the soil surface providing continual soil protection.

Information is limited on the development of pointvetch for forage and/or reclamation purposes. The genetic potential of pointvetch is not known and is necessary prior to initiation of a breeding program for an improved genotype.

The objective of this study was to identify the chromosome number and characterize the chromosome morphology of this species.

Materials and Methods

O. riparia seeds from accession PI 525497 (MT-101) (Solum et al., 1990) were collected from wild stands at Silver Star, Montana during October 1987 and were used for chromosome number and morphology identification. These seeds were scarified for 50 seconds in a Forsberg

scarifier<sup>1</sup> (Forsberg's Inc., Thief River Falls, MN). Root tips were pretreated in ice water for 20 h, fixed in Carnoy's solution (3:1, 100% ethanol : glacial acetic acid) for 30 m, treated in 70% ethanol for 10 m, and hydrolyzed in 5 N HCl at room temperature for 5 m. Root tips were Feulgen stained and macerated in 5% aceto-carmine counter stain. Cells were observed with phase contrast microscopy. The relative chromosome length was expressed as the percentage of the total length of all chromosomes measured in the same cell to eliminate deviations due to differential contractions in individual metaphase cells. Chromosomes were numbered from the longest to the shortest. A total of thirteen cells was analyzed.

### Results and Discussion

The chromosome number of *O. riparia* was confirmed to be  $2n=2x=16$  (Fig. 1). This is in agreement with Astanova and Abdusaliamova (1981), who also reported plants with  $2n=4x=32$ . According to Ledingham and Rever (1963), *Oxytropis* species always have  $2n=16$  or are polyploids with some multiples of 8 chromosomes.

A karyotype of *O. riparia* is shown in Figure 2. Chromosome measurements indicate an average chromosome length of  $3.2 \mu\text{m}$ . The average size of chromosomes in plants and animals in general is  $6 \mu\text{m}$  with some exceeding  $30 \mu\text{m}$  (Schulz-Schaeffer, 1980). Thus, the chromosomes of *O. riparia* are extremely small when compared to those of other species. Relative total chromosome lengths (RTCL) and short arm/long arm ratios (S/L) are presented in Table 1. One chromosome had near equal arm lengths (metacentric chromosome 7 : S/L

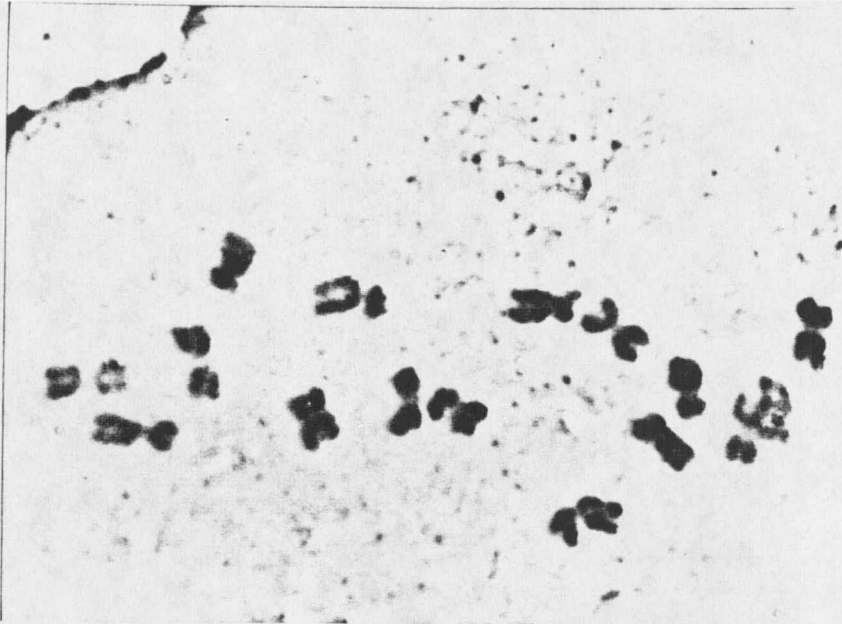


Figure 1: Mitotic metaphase cell of *Oxytropis riparia* ( $2n=16$ ) (x2850).

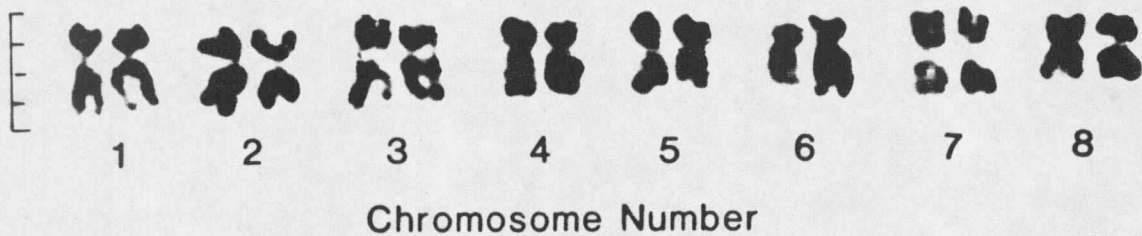


Figure 2: Karyotype of *O. riparia*. Chromosomes are arranged and numbered (1-8) from the longest to the shortest. Photographs of chromosomes were taken from the metaphase cell of Figure 1. Scale units at left represent  $1 \mu\text{m}$ .

Table 1. Means and standard errors of relative total chromosome lengths (RTCL) and short arm/long arm ratios (S/L) of the eight chromosomes (n) of *Oxytropis riparia*. The relative total chromosome length is expressed as the percentage of the total length of all chromosomes in the genome. Chromosomes were numbered from the longest to the shortest.

Measurements	Chromosome number							
	1	2	3	4	5	6	7	8
RTCL	15.0	13.5	12.7	12.3	12.1	12.0	11.9	10.5
SE (+/-)	0.41	0.21	0.24	0.46	0.21	0.27	0.28	0.32
S/L	0.51	0.77	0.55	0.65	0.82	0.52	0.98	0.82
SE (+/-)	0.02	0.02	0.03	0.01	0.02	0.03	0.01	0.02

ratio = 0.98 +/- 0.01). A large non-staining centromere region was observed in this chromosome in every cell that was studied (Fig. 2) and served as a distinctive chromosome marker. In some squash preparations, chromosome 7 separated at the centromere and resembled two separate chromosomes. A chromosome pair with similar centromere characteristics was identified by Ledingham and Rever (1963) in the closely related species *Astragalus mongholicus* Bge. (2n=16). They found one chromosome pair with a large non-staining centromere in each cell but it could not always be recognized in cells where the chromosomes were more contracted. Chromosomes 2, 5, and 8 were nearly metacentric with S/L ratios from 0.77 to 0.82. Additionally, chromosome 8 was the smallest chromosome (10.5  $\mu\text{m}$  +/- 0.32). Chromosome 4 ranked between the near metacentric and submetacentric groups with an S/L ratio of 0.65 +/- 0.01. Chromosomes 1, 3, and 6 were submetacentric and had an S/L ratio close to 0.50, indicating that the short arm was approximately half as long as the long arm. Chromosome 1 was also the largest chromosome

(15.0  $\mu\text{m}$  +/- 0.41). One cell revealed a satellite and short arm configuration in which the satellite was 25 %, the short arm 25 %, and the long arm 50 % of the total length of chromosome 1.

### Conclusions

The O. riparia chromosome number is consistent with findings reported for other Oxytropis species. Chromosomes were also similar to Astragalus mongholicus with a large non-staining centromere region. O. riparia chromosomes were relatively smaller than chromosomes in other plants and animals.

O. riparia is a diploid, which is a desirable trait for breeding purposes.



## CHAPTER 4

## GERMPLASM REGISTRATION

Ruby Valley pointvetch was studied for forage potential in the 1930's and 1940's (Benson, 1954; Green and Morris, 1935; Post, 1937). Pointvetch continued to grow in wild stands near Silver Star, Montana, and research was reinitiated in the early 1980's by Delaney et al. (1986), Hicks et al. (1987, 1990), Lichvar and Dorn (1982), and Russo (1985). Lichvar and Dorn (1982) determined pointvetch grew in both Idaho and Wyoming in addition to southwestern Montana. Hicks et al. (1987, 1990) studied hardseededness and mechanical scarification methods for pointvetch. Delaney et al. (1986) and Russo (1985) researched the ability of pointvetch to germinate under temperature and osmotic stress. Pointvetch is a very versatile plant and germplasm registration would provide for a variety of studies under many environmental conditions. A crop germplasm is registered to provide a known seed source to interested researchers for further study.

Materials and Methods

Pointvetch germplasm seeds were collected in October 1987 from a wild stand in the Ruby Valley of southwestern Montana. A REM flail-type

forage harvester<sup>1</sup> (REM Manufacturing Inc., Swift Current, Saskatchewan, Canada) was used to collect all plant material, which was then dried and threshed in a Hege 125C combine<sup>1</sup> (H & N Equipment Inc, Colwich, KS) and cleaned in a Clipper M-2B seed cleaner<sup>1</sup> (A.T. Ferrell & Co., Saginaw, MI) to obtain clean pointvetch seed.

Solum et al. (1990) named the germplasm Montana-101 when released from the Montana Agricultural Experiment Station. A seed germplasm sample was sent to the Plant Introduction Station in Pullman, Washington and assigned P.I. No. 525 497. Another seed sample was sent to the National Seed Storage Laboratory and was assigned NSSL Serial No. 239 102.01. The germplasm was then registered with Crop Science Society of America and given Registration No. GP-0081 (Solum, et al., 1990).

Preliminary studies, plant observations, and review of the literature resulted in information for the germplasm registration.

### Results and Discussion

Montana-101 is a perennial, indeterminate legume with a deep taproot and prostrate growth habit (Delaney et al., 1986). Plants have acute, sessile, opposite leaves with small, purplish, papilionaceous flowers (Barneby, 1952). The chromosome number of O. riparia has been generally reported as  $2n=2x=16$ . However, Astanova and Abdusaliyeva (1981) found both  $2n=2x=16$  and  $2n=4x=32$ . The MT-101 chromosome number is  $2n=2x=16$ .

O. riparia is native to Russian Turkestan (Barneby, 1952) and was first found in the Ruby Valley area of Montana in 1930 (Green and Morris, 1935). It is capable of growing on highly alkaline soil

(Benson, 1954). Green and Morris (1935) named the plant Ruby Valley milkvetch (Astragalus rubyi Green and Morris), but the taxonomy was changed to Oxytropis riparia Litv. by Barneby (1952).

Hardseed content in pointvetch has been reported as high as 96 % (Delaney et al., 1986). Poor stand establishment due to hardseededness can be eliminated with seed scarification. Hicks et al. (1990) found mechanical scarification with a Forsberg scarifier<sup>1</sup> (Forsberg's Inc., Thief River Falls, MN) for 20 to 30 s to be effective in removing the hard seedcoat. Delaney et al. (1986) found that pointvetch seed germinated under saline conditions, with over 90 % germination in -2.0 MPa NaCl solutions while alfalfa seed germination decreased 70 % in -1.0 MPa NaCl solutions.

Preliminary studies indicate that time of seed harvest is very important since seed shattering is a major problem. Optimum harvest conditions occur as the seed pods begin to turn brown.

Montana-101 planted into fallow soil at Manhattan, Montana, in 1987 and harvested in 1988 produced 4.48 Mg ha<sup>-1</sup> of forage during a severe drought season when crop year precipitation was only 47 mm. Consequently, MT-101 appears to have a highly efficient root system capable of extracting deep soil moisture. Quality of pointvetch forage was similar to that of alfalfa (Medicago sativa L.) (Green and Morris, 1935). Forage of O. riparia tested negative for swainsonine, aliphatic nitro compounds, soluble oxalates, and cyanide (Williams and Molyneux, 1988).

Conclusions

Montana-101 has potential as a forage crop in droughty, alkaline areas. It may be useful in reducing evaporation and soil erosion due to its prostrate growth habit. Additionally, the highly salt tolerant characteristic of MT-101 may be beneficial in reclaiming saline seep areas.

## CHAPTER 5

## RUBY VALLEY POINTVETCH SEED COAT CHARACTERIZATION

Ruby Valley pointvetch (Oxytropis riparia Litv.) is native to Russian Turkestan (Barneby, 1952) and was found growing in the Ruby Valley of southwestern Montana in 1930 (Green and Morris, 1935). Sites in Idaho and Wyoming have recently been identified (Lichvar and Dorn, 1982; Williams and Molyneaux, 1988). O. riparia is a deep taprooted, prostrate, perennial legume (Booth and Wright, 1966) with good salt tolerance (Delaney et al., 1986) and is a member of the Galegeae tribe of the Leguminosae subfamily Papilionoideae.

The prostrate growth habit and salt tolerance of O. riparia make it highly suitable for land reclamation and saline seep control. However, plants with rapid stand establishment and stand renewal either by vegetative or sexual propagation are usually required for land reclamation situations. The high hardseed content of O. riparia (Delaney et al., 1986; Hicks et al., 1987; Post, 1937), which directly affects water imbibition, often results in slow stand establishment due to germination of the seeds over an extended time period. Consequently, O. riparia genotypes with less hardseededness need to be developed or selected. Identification and characterization of O. riparia seed coat

structures could be useful in the development and selection of genotypes with improved stand establishment potential and for taxonomic classification.

Histological research on O. riparia seed coat structures is extremely limited. Lersten (1981) described the seed coat surface pattern of Oxytropis hirta Bunge as multi-reticulate. Endogenous seed coat components have not been reported for Oxytropis species.

Scanning electron microscopy (SEM) and light microscopy have been used in seed coat anatomy studies of many Papilionoideae genera, including Astragalus, Glycine, Melilotus, Lupinus, Trifolium, Medicago, Lotus, Sophora, Sesbania, Strophostyles, Vicia, Lathyrus, Lens, Pisum, and Coronilla (Baker et al., 1986, 1987; Barton, 1965; Bragg, 1983; Bridges and Bragg, 1983; Lersten and Gunn, 1982; McKee et al., 1977; Miklas et al., 1987; Watson, 1948; Yaklich et al., 1986). The general structures of papilionaceous seed coats were found to be similar with the exception of the cell layers interior to the hourglass (osteosclereid) cells. These cell layers have been described as endosperm remnants (Lersten and Gunn, 1982), parenchyma and endothelium (Baker et al., 1986, 1987), mesophyll (Corner, 1951); hypodermis (Bragg, 1982), and nutrient layers or tissue (Barton, 1965; Miklas et al., 1987). Some terms are more specific than others, but a generally accepted description has not been utilized in the literature.

Seed coat structural differences which affect permeability have been found for hardseed and non-hardseed of some legume species. Miklas et al. (1987) did not find any anatomical seed coat differences between hardseed and non-hardseed of Astragalus cicer L. using SEM. However,

Coe and Martin (1920) reported that sweetclover (Melilotus spp.) hardseed and non-hardseed differed structurally.

It is important to evaluate several different seed lots from diverse environments when characterizing seed coat structures and surface patterns. Additionally, comparisons of anatomical structures among closely related genera and species are beneficial for identification and for establishing taxonomic associations.

The objectives of this study were to 1) identify and characterize both exogenous and endogenous seed coat structures of O. riparia as a basis for further hard seed coat development studies and 2) make comparisons with three Papilionoideae members, O. campestris L. (Galegeae tribe), Astragalus cicer (Galegeae tribe), and Medicago sativa L. (Trifolieae tribe). Since research is limited on Oxytropis seed coat structures, this study will provide a basis for characterization of both external and internal seed coat components and information of taxonomic significance for other comparable genera and species.

#### Materials and Methods

Four O. riparia seed lots comprised of 1986 T-5637 seeds from the Bridger Plant Materials Center, Bridger, MT, 1987 and 1989 seeds collected in the Ruby Valley of southwestern Montana, and 1936 seeds collected in Oklahoma were evaluated. M. sativa and A. cicer seeds were from the Montana State University Plant & Soil Science Department collection, and the O. campestris seeds were obtained from The Vavilov Institute of Plant Industry, Leningrad, USSR. Seeds were observed whole or after transverse sectioning through the hilum. Seeds for seed coat

surface pattern determinations were washed in 10% ethanol for 1 m, rinsed in distilled water for 30 s, and dried at 20°C for 2 h prior to mounting for SEM observations. The wash procedure removed external dirt and debris from the seed coat without affecting surface pattern integrity. All seed surfaces were examined near midseed using SEM.

Seeds with complete, undamaged seed coats were soaked in distilled water at 20°C for 24 h. Non-hardseed were differentiated from hardseed by separating seeds that imbibed during the soak period. The hardseed were punctured with a hypodermic needle and soaked in 20°C distilled water for 24 h. Both the imbibed hardseed and non-hardseed were sectioned with an AO model 975C HISTOSTAT cryostat microtome<sup>1</sup>.

Unimbibed seeds were utilized for exogenous and endogenous seed coat characterizations to avoid cell distortion during imbibition. Unimbibed seeds were sectioned with a double edged razor blade, since sectioning with the cryostat microtome compressed the seed coat cell layers and/or shattered the seeds. All seeds were mounted on copper specimen holders with double stick tape and gold sputter coated for 4 m in a Pelco sputter coater<sup>1</sup>. A minimum of 10 seeds per treatment of each seed lot or species was viewed with a JEOL JEM-100CX<sup>1</sup> electron microscope with an ASID-4D scanning attachment<sup>1</sup> at an accelerating voltage of 20 kV.

### Results and Discussion

All O. riparia seed lots and O. campestris seeds were similar in length (2 mm), while M. sativa and A. cicer were larger (3 and 4 mm, respectively) (Table 2). Seed color varied among species. O. riparia



seeds were tan-green to rust-brown, and the brown seeds of O. campestris had a tan hilum-area with approximately 1/3 of the seeds exhibiting black mottling. M. sativa seeds were tan to rust-brown, and A. cicer seed color ranged from yellow-green to tan-brown. O. riparia seeds were semiquadrangular and relatively flat, O. campestris and A. cicer were generally heart-shaped, and M. sativa seeds were kidney-shaped.

Table 2. Exogenous, unimbibed seed characteristics of four Oxytropis riparia seed lots, O. campestris, Medicago sativa, and Astragalus cicer averaged over ten seeds per seed lot or species.

Seed	Seed Size (mm)	Color	Shape	Surface Pattern
1936 <u>Oxytropis riparia</u>	2	tan-green to rust-brown	semiquadrangular relatively flat	rugulate
1986 <u>O. riparia</u>	"	"	"	"
1987 <u>O. riparia</u>	"	"	"	"
1989 <u>O. riparia</u>	"	"	"	"
<u>O. campestris</u>	2	brown with black mottling and tan hilum	heart-shaped	lophate
<u>Medicago sativa</u>	3	tan to rust-brown	kidney-shaped	obscure papillose
<u>Astragalus cicer</u>	4	yellow-green to tan-brown	heart-shaped	levigate

Seed coat surface patterns for O. riparia and O. campestris have not been previously reported. All O. riparia seed lots had rugulate surface patterns (Table 2) (Figs. 3-6). Minor variation was evident in size and/or continuity of ridges or furrows over the surface. The 1987 seed lot (Fig. 5) exhibited narrower ridges and wider, continuous

furrows while the 1989 seed lot (Fig. 6) showed prominent ridges and discontinuous furrows. The 1936 and 1986 seed lots (Figs. 3 and 4, respectively) were similar with weak, discontinuous ridges and furrows. The *O. campestris* surface pattern was lophate (Table 2) (Fig. 7), while *M. sativa* showed an obscure papillose surface pattern with papillae surrounded by an unorganized series of ridges and furrows in the valleys between papillae (Table 2) (Fig. 8). The *A. cicer* seed coat had shallow ridges and furrows but was considered levigate (Table 2) (Fig. 9).

Examination of transverse seed sections showed differences in seed coat cell and cell layer thicknesses among *O. riparia* seed lots and species (Table 3). The average *O. riparia* palisade cell length ranged from 50 to 68  $\mu\text{m}$  among seed lots. *M. sativa* had the shortest palisade cell length at 33  $\mu\text{m}$ , and both *O. campestris* and *A. cicer* were similar to the 1936 and 1987 *O. riparia* seed lots. Hourglass cell lengths of *O. riparia* seed lots varied from 12 to 19  $\mu\text{m}$ . *M. sativa* hourglass cell lengths were markedly shorter than *O. campestris* or *A. cicer*, which were similar to the 1986 and 1989 *O. riparia* seed lots. Parenchyma and endosperm layer thicknesses were more difficult to determine than the other seed coat cell layers. The parenchyma cell layer is designated as the area immediately interior to the hourglass cells, and the endosperm layer includes cells between the parenchyma layer and the cotyledons. *O. riparia* parenchyma layer cell lengths ranged from 20 to 31  $\mu\text{m}$ , and endosperm thicknesses ranged from 11 to 24  $\mu\text{m}$  among seed lots. *M. sativa* and *A. cicer* were similar to *O. riparia* in parenchyma layer thickness, while *O. campestris* was slightly thicker. *M. sativa* endosperm was less than half the thickness of *A. cicer*, but both were

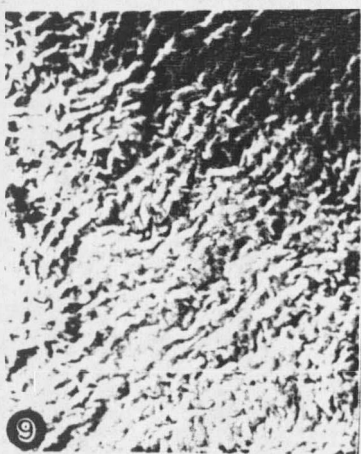
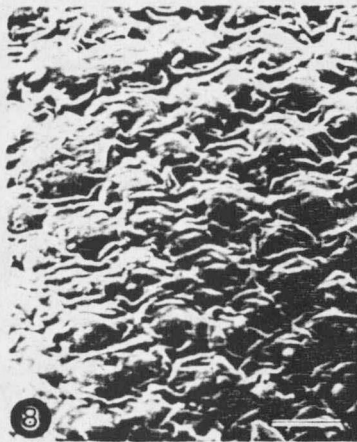
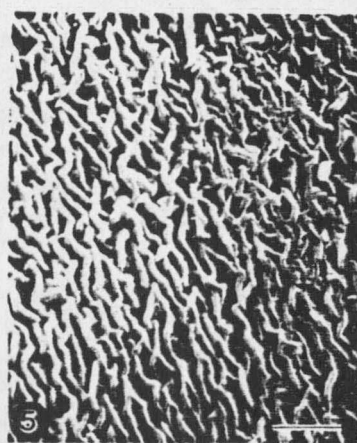
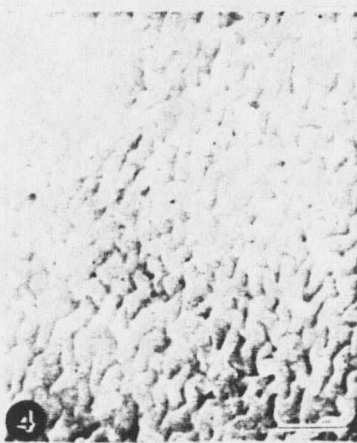
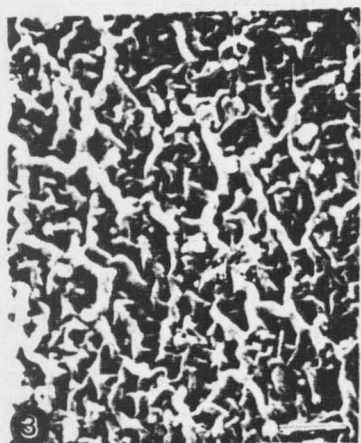


Figure 3. Surface pattern of 1936 Oxytropis riparia - rugulate. Bar = 8  $\mu\text{m}$ .

Figure 4. Surface pattern of 1986 O. riparia - rugulate. Bar = 8  $\mu\text{m}$ .

Figure 5. Surface pattern of 1987 O. riparia - rugulate. Bar = 8  $\mu\text{m}$ .

Figure 6. Surface pattern of 1989 O. riparia - rugulate. Bar = 8  $\mu\text{m}$ .

Figure 7. Surface pattern of O. campestris - lophate. Bar = 8  $\mu\text{m}$ .

Figure 8. Surface pattern of Medicago sativa - obscure papillose.

Bar = 8  $\mu\text{m}$ .

Figure 9. Surface pattern of Astragalus cicer - levigate. Bar = 8  $\mu\text{m}$ .

Table 3. Endogenous, unimbibed seed coat characteristics of four Oxytropis riparia seed lots, O. campestris, Medicago sativa, and Astragalus cicer averaged over ten seeds per seed lot or species.

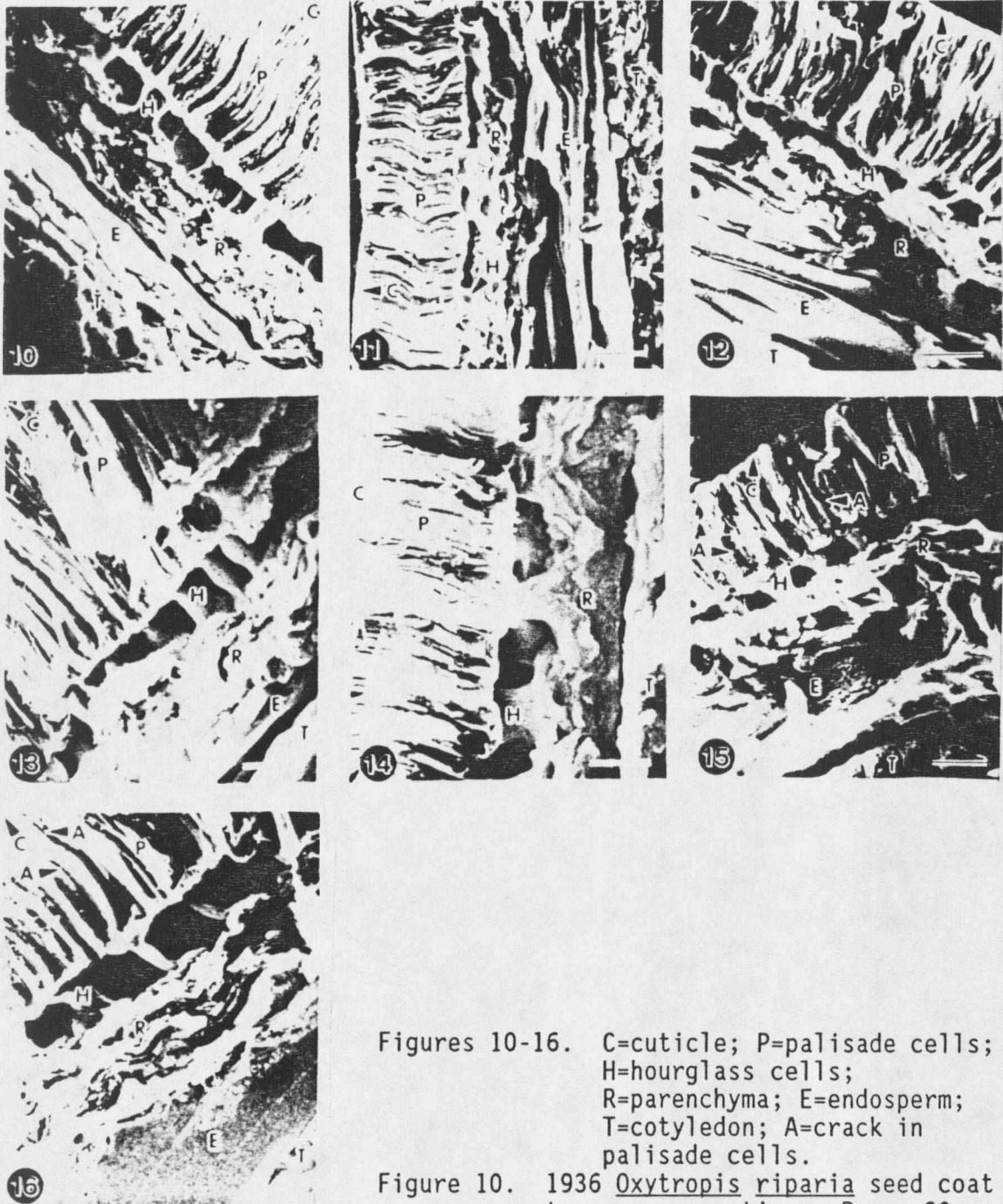
Seed	Palisade cell length ( $\mu\text{m}$ )	Hourglass cell length ( $\mu\text{m}$ )	Parenchyma thickness ( $\mu\text{m}$ )	Endosperm thickness ( $\mu\text{m}$ )
1936 <u>Oxytropis riparia</u>	51	13	23	11
SE (+/-)	3	2	2	6
1986 <u>O. riparia</u>	65	19	29	24
SE (+/-)	4	3	10	3
1987 <u>O. riparia</u>	50	12	20	20
SE (+/-)	7	2	9	3
1989 <u>O. riparia</u>	68	18	31	21
SE (+/-)	7	7	4	4
<u>O. campestris</u>	55	18	35	-
SE (+/-)	4	6	2	-
<u>Medicago sativa</u>	33	12	24	12
SE (+/-)	3	3	6	7
<u>Astragalus cicer</u>	52	21	30	28
SE (+/-)	4	5	6	8

similar to O. riparia. The O. campestris endosperm was not distinguishable from the parenchyma layer.

The palisade cells of O. riparia seed lots (Figs. 10-13) and O. campestris (Fig. 14) were very dense and exhibited no cracks between

cells. M. sativa had small cracks between cells which did not penetrate through the palisade layer, but could connect and form a continuous break in the cell layer (Fig. 15). Wider cracks which did not extend through the cell layer were present between palisade cells in A. cicer (Fig. 16). These cracks in A. cicer could also interconnect and form a continuous break in the palisade layer. The hourglass cells were similar in the four species with cell ends wider than the mid-cell region. A. cicer and O. campestris hourglass cells collapsed more during sectioning than O. riparia or M. sativa. O. riparia hourglass cells were more branched at the cell ends. Overall, the seed layers were quite similar progressing from exterior to interior with cuticle, palisade cells, hourglass cells, parenchyma, endosperm, and cotyledon. Differences between hard and non-hard seed coat structures were not distinguishable.

Seed coat surface patterns varied among the four species, but were similar for the O. riparia seed lots (Figs. 3-6). The rugulate surface pattern has not been previously reported for O. riparia but has been reported in other members of the Galegeae tribe, Caragana arborescens Lam. and Galega officinalis L. (Lersten, 1981). The 'slight' variation in the rugulate pattern among O. riparia seed lots may have been caused by environmental and/or genotypic differences. The lophate surface pattern (Fig. 7) has not been previously reported for O. campestris but has been reported for other Galegeae tribe members, such as Biserrula Pelicinus L. [reclassified by Barneby (1964) as Astragalus Pelicinus (L.) Barneby] and Astragalus asper Jacq. (Lersten, 1981). Lersten (1981) reported that seed coat surface patterns of the Galegeae tribe members were



Figures 10-16. C=cuticle; P=palisade cells;  
H=hourglass cells;  
R=parenchyma; E=endosperm;  
T=cotyledon; A=crack in  
palisade cells.

Figure 10. 1936 *Oxytropis riparia* seed coat  
transverse section. Bar = 20  $\mu\text{m}$ .

Figure 11. 1986 *O. riparia* seed coat  
transverse section. Bar = 20  $\mu\text{m}$ .

Figure 12. 1987 *O. riparia* seed coat transverse section. Bar = 20  $\mu\text{m}$ .

Figure 13. 1989 *O. riparia* seed coat transverse section. Bar = 20  $\mu\text{m}$ .

Figure 14. *O. campestris* seed coat transverse section. Bar = 20  $\mu\text{m}$ .

Figure 15. *Medicago sativa* seed coat transverse section. Bar = 20  $\mu\text{m}$ .

Figure 16. *Astragalus cicer* seed coat transverse section. Bar = 20  $\mu\text{m}$ .

highly variable, including levigate, rugulate, simple-reticulate, multi-reticulate, lophate, and simple-foveolate. M. sativa (Fig. 8) and A. cicer (Fig. 9) surface patterns were similar to those described in the literature. Lersten and Gunn (1982) reported that M. sativa had an obscure papillose surface pattern near the hilum which became nonpapillose at midseed. We observed that the obscure papillose pattern of M. sativa was more distinct near the hilum but also visible at midseed. Miklas et al. (1987) reported no distinct midseed surface pattern for A. cicer as was confirmed by this study.

Interspecific surface pattern variation has been reported in Lupinus (Bragg, 1983), Sophora (Bridges and Bragg, 1983), Vigna (Gopinathan and Babu, 1985), and Prosopis (Bragg, 1982) and is a means of identifying and/or separating seeds within a genus. This variation is also present in the Oxytropis genus with a multi-reticulate surface pattern previously reported for O. hirta (Lersten, 1981), and the lophate and rugulate patterns of O. campestris and O. riparia, respectively, observed in this study.

The internal seed coat structures of the O. riparia seed lots, O. campestris, M. sativa, and A. cicer (Figs. 10-16, respectively) were similar to Mucuna pruriens (L.) DC. var. utilis (Papilionoideae), Viciaeae tribe, and Trifolium spp. seed coat components reported by Corner (1951), Lersten and Gunn (1982), and Rolston (1978), respectively. Lersten and Gunn (1982) described the parenchyma layer as an area of crushed cells possibly consisting of endosperm remnants. Corner (1951) described the area only as mesophyll, while Rolston (1978) reported the parenchyma layer to be separate from the endosperm.



Distinguishable parenchyma and endosperm cell layers were present in O. riparia, M. sativa, and A. cicer seeds, confirming Rolston's (1978) findings. However, no distinguishable endosperm layer was observed in O. campestris seeds.

Seed coat cell lengths and cell layer thicknesses varied among seed lots and species. Differences among the O. riparia seed lots may have been caused by environmental, genotypic, and/or seed maturity differences. The other species were represented by one seed lot per species, thus interspecific comparisons were not valid. An association was evident between seed size and cell length and cell layer thickness in A. cicer. A. cicer seeds were the largest and had the longest hourglass cells and thickest endosperm layer. Other species showed no such relationship (Tables 2 and 3). O. campestris was dramatically different than the other species with no detectable endosperm layer. However, since O. campestris had the thickest parenchyma layer the endosperm layer may have been present and unidentifiable.

Structural differences between hardseed and non-hardseed were not distinguished using SEM. Seed coat structures of the imbibed seeds were difficult to differentiate due to cell distortion.

### Conclusions

Brisson and Peterson (1976) cautioned against classifying an entire species with seeds from one geographic location. The four O. riparia seed lots utilized in this study were from three diverse locations. Each of the O. riparia seed lots had similar exogenous and endogenous characteristics which indicated stability in diverse



environments within the species. No structural differences were distinguishable between hard and non-hard seeds of O. riparia with SEM. Further studies of other Oxytropis species are necessary for establishing relationships within the tribe and subfamily.

## CHAPTER 6

## SUMMARY

The O. riparia chromosome number was verified and is consistent with other Oxytropis species. Chromosomes were similar to a closely related species, Astragalus mongholicus Bge., and were relatively smaller than chromosomes of other plants and animals.

O. riparia germplasm was registered with Crop Science Society of America as Montana-101 Ruby Valley pointvetch after the chromosomes were characterized. Pointvetch is a diploid with a low chromosome number, both desirable breeding traits. Dry matter production (4.48 Mg ha<sup>-1</sup>) and salt and drought tolerance suggest this germplasm may have potential for forage and reclamation of saline and/or erodible areas.

The O. riparia seed coat surface pattern was identified and the seed coat components were characterized, providing a basis for further hardseededness studies and for taxonomic use in establishing evolutionary relationships. The seed coat surface patterns were previously unreported for O. riparia and O. campestris, and intraspecific stability was evident in the four seedlots of O. riparia which all exhibited the same pattern. M. sativa and A. cicer patterns were similar to those reported in the literature. The seed coat cell

layers were the same as those reported for other small-seeded legumes and were similar for the species in this study, with the exception of O. campestris in which there was no identifiable endosperm layer.

No morphological differences were observed between seed coats of hard and non-hard O. riparia seeds.

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