

POTENTIAL OF BASALT MILKVETCH (*ASTRAGALUS FILIPES* TORR. EX A.
GRAY) POPULATIONS AND RHIZOBIAL STRAINS FOR REVEGETATION
AND RESTORATION OF INTERMOUNTAIN WEST RANGELANDS

by

Kishor Bhattarai

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

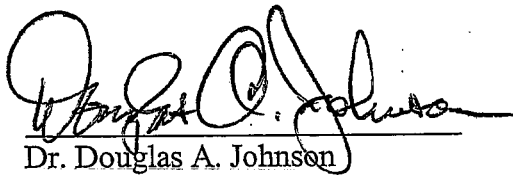
in

Ecology

Approved:



Dr. Ronald J. Ryel
Academic Advisor



Dr. Douglas A. Johnson
Research Advisor



Dr. H. Charles Romesburg
Committee Member



Dr. Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2007

ABSTRACT

Potential of Basalt Milkvetch (*Astragalus filipes* Torr. Ex A. Gray) Populations and
Rhizobial Strains for Revegetation and Restoration of Intermountain West
Rangelands

by

Kishor Bhattarai, Master of Science

Utah State University, 2007

Academic Advisor: Dr. Ronald J. Ryel
Research Advisor: Dr. Douglas A. Johnson
Department: Wildland Resources

Astragalus filipes Torr. Ex A. Gray (basalt milkvetch) is a leguminous plant species that has a wide distribution in western North America. A series of greenhouse and field experiments were conducted to identify rhizobial strains and populations (accessions) of basalt milkvetch that have potential for use in revegetation and restoration programs in the western U.S.A. In the first greenhouse experiment, six rhizobial strains were evaluated with several basalt milkvetch accessions for their N-fixation characteristics. Rhizobial treatments and basalt milkvetch accessions exhibited no significant interactions for N-fixation characteristics, suggesting that the best rhizobial strain was the best across all basalt milkvetch accessions. Plants inoculated with different rhizobial strains differed in nodule weight, and number of nodules, but did not differ in shoot biomass and root biomass. Significant correlations were detected between nodule

weight and total N pool ($r = 0.481$, $P < 0.0001$), and number of nodules and total N pool ($r = 0.456$, $P < 0.0001$). Total N pool was highly correlated with plant biomass ($r = 0.934$, $P < 0.001$), and biomass per unit N was negatively correlated to total biomass indicating that plants were more efficient in using N as grew bigger. Plants inoculated with rhizobial strain I-49 had the greatest nodule weight and number of nodules compared to plants inoculated with other rhizobial inocula. In the second greenhouse experiment, plants inoculated with I-49 also had the highest shoot N pool, total N pool, and number of nodules compared to all other rhizobial inocula, except I-48. As a result, rhizobial strain I-49 was the best rhizobial strain in both greenhouse experiments.

Common garden field experiments were conducted at two sites in northern Utah (Evans Farm and Millville) in 2005 and 2006. The results from Evans Farm showed that accessions from north central Oregon exhibited high biomass yield in summer and fall in both years. Accessions from Nevada, Washington, and Utah, and some accessions from Oregon and Idaho showed poor biomass production in both summer and fall. Forage quality was measured by crude protein concentration, neutral detergent fiber (NDF), and acid detergent fiber (ADF). ADF and NDF were positively correlated with biomass yield. Accessions with high and low biomass differed little for crude protein concentration, ADF, and NDF. Accessions with high biomass yields (Af-5, Af-29, Af-33) did not differ from accessions with low biomass yields (Af-20, Af-54, Af-69) for ADF and NDF. Also, accessions with high biomass yield had a high crude protein pool. At Millville, accessions from north central Oregon also showed high biomass and seed yield. Seed weight per 100 seeds varied among basalt milkvetch accessions, and positive correlations of seed weight and biomass indicated that accessions with high biomass yields had a

tendency to produce heavier seeds. No significant interaction between insecticide treatment and basalt milkvetch accessions for seed yield indicated that there was no preferential seed predation for one accession over another. Significant seed predation was observed in 2006, but not in 2005, which could be due to the high seed yields and greater numbers of seed predators. High correlations between number of stems and biomass and between number of stems and seed yield indicated that number of stems is a good predictor of high biomass and seed production in basalt milkvetch. Results indicated that I-49 rhizobial strain and accessions from north central Oregon have the most promise for plant improvement efforts with basalt milkvetch.

(128 pages)

ACKNOWLEDGMENTS

I would like to express my gratitude to the many people who have contributed to make this thesis possible.

First and foremost, I would like to acknowledge my research advisor, Dr. Douglas Johnson, for his incessant guidance, help, suggestions, and discussions during my research and making funding available to me from the 'Native Plant Selection and Increase Project' for this research. It was a wonderful experience to share his work experience, considerate attitude, and patience during the study, which was unforgettable. Also, I am equally grateful to my academic advisor, Dr. Ronald Ryel, and my committee member, Dr. Charles Romesburg, for their invaluable suggestions and guidance during my study and research.

It is my pleasure to express my gratitude to Kevin Connors for his help, support, and friendship. Tom Jones and Tom Monaco deserve special praise for helping me to make my research run smoothly. The assistance of the lab and field assistants and other staff of the Forage and Range Research Lab is greatly appreciated. I would like to acknowledge professors, staff, and colleagues of the Wildland Resources Department for providing me excellent friendship and a solid intellectual platform.

I would like to thank Ramesh Khanal, Keshab Parajuli, Eamonn Leonard, Brian Bell, Jayanti Mukharjee, Koketso Tshireletso, and Tanushree Biswas for their friendship and making my life easier in Logan. I owe my special admiration to Katie Swoboda for her encouragement, help, support, and friendship especially during thesis preparation.

Finally, I wish to thank my parents, Ganga Dhar Bhattarai and Bidhya Kumari Bhattarai, and my sisters, Milan and Anju, for everything they have given me. To them, my thesis is dedicated.

Kishor Bhattarai

CONTENTS

vii

	Page
ABSTRACT.....	ii
ACKNOWLEDGMENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	xii
CHAPTER	
1. BACKGROUND	1
LITERATURE CITED	6
2. RHIZOBIAL STRAIN EVALUATION FOR <i>ASTRAGALUS FILIPES</i> : A NATIVE LEGUME FOR RANGELANDS OF WESTERN NORTH AMERICA.....	13
INTRODUCTION	13
MATERIALS AND METHODS.....	17
Rhizobial Isolations	17
Greenhouse Experiment A.....	20
Greenhouse Power Test Experiment.....	22
Greenhouse Experiment B	23
Statistical Analysis.....	24
RESULTS	25
Greenhouse Experiment A.....	25
Greenhouse Experiment B	27
DISCUSSION.....	32
MANAGEMENT IMPLICATIONS	36
LITERATURE CITED	37
3. BIOMASS YIELD, SEED PRODUCTION, AND FORAGE QUALITY OF <i>ASTRAGALUS FILIPES</i> : A NATIVE LEGUME FOR RANGELANDS OF WESTERN NORTH AMERICA	45

INTRODUCTION	45
MATERIALS AND METHODS.....	49
Statistical Analysis.....	55
RESULTS	56
Forage Production.....	56
Forage Quality	61
Seed Yield and Seed Weight.....	63
Plant Mortality	65
Morphological Characteristics	67
Cluster Analysis	69
DISCUSSION	70
MANAGEMENT IMPLICATIONS	78
LITERATURE CITED	79
4. SUMMARY.....	88
LITERATURE CITED	91
APPENDIXES	92
Appendix A: Supplemental Tables	93
Appendix B: Permission-to-Use Letter.....	115

LIST OF TABLES

Table	Page
2-1 Climatic and geographic information of basalt milkvetch accessions and rhizobial inocula with state, elevation, latitude, longitude, mean annual maximum (max) and minimum (min) temperatures, and mean annual precipitation (precip).....	19
2-2 Analysis of variance results for Greenhouse Experiment A.....	26
2-3 Mean comparisons for 6 rhizobial inoculants (I-16, I-30.1, I-41, I-48, I-49, I-68) and 2 control (with N = C/N, no N = C) treatments for number of nodules, shoot weight, root weight, nodule weight, and nodule rating for Greenhouse Experiment A.....	27
2-4 Mean comparisons for basalt milkvetch accessions (Af-16, Af-30.1, Af-41, Af-53, Af-68, Af-89) for shoot weight, root weight, nodule weight, number of nodules, and nodule rating for Greenhouse Experiment A	28
2-5 ANOVA results for Greenhouse Experiment B	28
2-6 Pearson correlation coefficients (r) for number of nodules (Nn), nodule rating (Nr), nodule weight (Nw), shoot weight (Sw), root weight (Rw), total biomass (Tb), shoot N pool (Sn), root N pool (Rn), and total N (Tn)	32
3-1 Climatic and geographic information for basalt milkvetch accessions with state, elevation, latitude, longitude, mean annual maximum (max) and minimum (min) temperatures, and mean annual precipitation (precip).....	51
3-2 Mean summer (May-August) maximum and minimum temperature and precipitation in 2004, 2005, and 2006 at Evans Farm and Millville	52
3-3 Values of the root transformation used in transferring data to meet normality and homoscedasticity criteria for field data from Evans Farm and Millville in 2005 and 2006.....	55
3-4 Analyses of variance of basalt milkvetch accessions for summer biomass (biomass), fall regrowth (regrowth), plant height (height), number of stems (stem), number of inflorescences (inflores.), plant vigor (vigor), seed weight (seed wt.), crude protein concentration (CPC), crude protein pool (CPP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) in 2005,	

	2006, and combined across 2005 and 2006 (combined) at Evans Farm and Millville.....	57
3-5	Mean, standard deviation (SD), and range of basalt milkvetch accessions for summer biomass (biomass), fall regrowth (regrowth), plant height (height), number of stems (stem), number of inflorescences (inflores.), plant vigor (vigor), seed yield, seed weight (seed wt.), crude protein concentration (CPC), crude protein pool (CPP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) in 2005, 2006, and combined across 2005 and 2006 (combined) at Evans Farm and Millville	58
3-6	Mean comparisons of acid detergent fiber (ADF) and neutral detergent fiber (NDF) for the June biomass harvest of basalt milkvetch accessions in 2006 at Evans Farm	63
3-7	Analyses of variance of basalt milkvetch accessions for seed yield in 2005, 2006, and combined across 2005 and 2006 (combined) for accession (A), insecticide treatment (T), and interaction between accession and insecticide treatment (A X T) at Millville.....	64
3-8	Pearson correlation coefficients (r) and their associated significance levels for Millville in 2005 and 2006. Variables in the matrix include seed weight (Sw), seed yield (Sy), and August biomass (Ab).....	66
3-9	Pearson correlation coefficients (r) and their associated significance levels for Evans Farm in 2005 and 2006. Variables in the matrix include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), June biomass (Jb), and October regrowth (Or)	68
3-10	Pearson correlation coefficients (r) and their associated significant levels for Millville in 2005 and 2006. Variables in the matrix include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), August biomass (Ab), October regrowth (Or), and seed yield (Sy)	69
3-11	Site characteristics for 4 groups of basalt milkvetch accessions separated based on cluster analysis of measured variables from Evans Farm and Millville in 2005 and 2006.....	72
A-1	Mean comparisons of June biomass and October regrowth of basalt milkvetch accessions from Evans Farm in 2005 and 2006.....	94
A-2	Mean comparisons of August biomass and October regrowth of basalt milkvetch accessions from Millville in 2005 and 2006.....	96

A-3	Mean comparisons of crude protein concentration (CPC) and crude protein pool (CPP) of basalt milkvetch accessions from Evans Farm in 2005 and 2006.....	98
A-4	Mean comparisons of seed weight (per 100 seeds) and seed yield of basalt milkvetch accessions from Millville in 2005 and 2006	100
A-5	Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Evans Farm in 2005	102
A-6	Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Evans Farm in 2006	104
A-7	Mean comparisons of plant height, number of stems per plant (stems), number of inflorescence per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Millville in 2005	106
A-8	Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Millville in 2006	108
A-9	Evans Farm data used to construct cluster dendrogram.....	110
A-10	Millville data used to construct cluster dendrogram.....	113

LIST OF FIGURES

Figure		Page
2-1	The relationship of power to total sample size for the Greenhouse Power Test Experiment.....	23
2-2	Results of Greenhouse Experiment B for shoot weight (A), root weight (B), shoot N pool (C), and total N pool (D). Treatments include 6 rhizobial strains (I-30.1, I-16, I-41, I-49, I-68, I-48), Control with no N (C), and Control with N (C/N).	29
2-3	Results of Greenhouse Experiment B for number of nodules (A), nodule weight (B), and nodule rating (C). Treatments included 6 rhizobial strains (I-30.1, I-16, I-41, I-49, I-68, I-48), Control with no N (C), and Control with N (C/N).	31
2-4	Linear regression of total plant biomass against total N pool for Greenhouse Experiment B.	31
3-1	Plant mortality percentage of basalt milkvetch accessions in various plant mortality classes (% mortality of individual accession) at Evans Farm (A) and Millville (B)	66
3-2	Cluster dendrogram based on all measurements obtained at Evans Farm and Millville during 2005 and 2006 for 67 basalt milkvetch accessions.....	71

CHAPTER 1

BACKGROUND

The objective of my studies was to evaluate various collections of basalt milkvetch for their possible differences in biomass production, forage quality, and seed production. In addition, I was interested in determining the best rhizobial strains for maximizing biological N fixation in basalt milkvetch. Also, I was interested to know if there was an effect of seed predation on seed yield, preferential seed predation of seed predators across basalt milkvetch accessions, and different performance of rhizobial strains for N fixation across basalt milkvetch accessions. Studies were conducted at two field sites and in a greenhouse in northern Utah in 2005 and 2006. Data collected in the studies will form the basis for identifying the most promising collections of basalt milkvetch and their associated rhizobial strains for eventual use in revegetation and restoration efforts on rangelands of the western U.S.A.

Rangelands cover about half of the total land area of the world (Horn 1995). About 400 million ha (42 %) of total land area in the U.S.A. is used for grazing (Bovey 1987), out of which 177 million ha are public rangelands in the western U.S.A. (Baca 1995). Areas classified as rangeland are often too dry, rocky, steep, or cold to farm or practice forestry (World Resources Institute 1986). However, rangelands provide 40 % of the cattle feed in the U.S.A. (Horn 1995) and are important for wildlife, recreation, minerals, and watersheds. The Great Basin lies in the Intermountain Region of the western U.S.A. and is located between the Rocky Mountains on the east and Sierra-Nevada and Cascade Mountains on the west. Sagebrush steppe and more arid semi-desert

ecosystems (West 1983) cover an estimated total land area of 40 million ha (MacMahon 1979).

Precipitation, fire, and herbivory are the external factors that drive communities of annual grasses, perennial grasses, and woody shrubs in rangeland ecosystems (Noy-Meir 1973). Overgrazing, invasion by exotic weedy species, redistribution of water resources, fragmentation of habitat, and alteration of fire regimes since European settlement have changed the botanical composition of the Great Basin from diverse native plant communities to expansive areas dominated by invasive weeds (Miller et al. 1994). In some cases, fire suppression has resulted in juniper (*Juniperus* spp.) encroachment in higher elevations of the Intermountain Region, which has reduced forage availability and increased soil erosion (Miller et al. 1994). In other cases, cheatgrass (*Bromus tectorum* L.) invasion has resulted in increased fire frequency (Whisenant 1990), which has decreased native perennial plant species. These burned areas are highly susceptible to erosion, which has a negative impact on watershed values. Although the importance of native plant species for wildlife habitat and forage was realized as early as the 1930s (Richards et al. 1998), most rehabilitation projects prior to 1980 used introduced species to protect soils and watersheds and increase forage availability (Johnson et al. 1989; Pyke et al. 2003). Native plant species were typically difficult to establish, less commercially available, and had a high cost (Rumbaugh 1983; Pellant and Monsen 1993).

Despite high amounts of N in the atmosphere (about 79 %), plant growth is often limited by a lack of N because atmospheric N is unusable to most living organisms (Major 1973). Low vegetation cover coupled with wind erosion make soils in arid and semiarid areas low in organic matter (Diaz et al. 1994). As a result, N is particularly

limiting on arid and semiarid rangelands (Sprent 1985; Zahran 1999; Trubet et al. 2006).

Site productivity can often be improved by adding N to the site if other ecological factors are not limiting. Addition of N can be done by either adding N fertilizer to the soil or through increased biological N fixation (BNF). BNF is the process that changes inert N_2 to biologically usable NH_3 (Major 1973) and contributes about 60 % of total fixed N per year (Zahran 1999). In terrestrial systems, legume-rhizobial symbiosis is prominent and alone accounts for at least 70 million tons of fixed N per year (Brockwell et al. 1995). The application of fertilizer to rangelands is not economically feasible and ecologically sustainable. Fertilization can acidify soils and leach nitrates, which can cause eutrophication of water bodies (Bantilan and Johansen 1995). Also, soil acidification can change species competition in plant communities, which can change local biodiversity.

The incorporation of legumes into pasture and rangeland ecosystems is a sustainable approach that can help increase soil N and improve forage N content (Haynes 1980; Legard and Steele 1992; Posler et al. 1993; Aydin and Uzun 2005), improve wildlife forage and habitat (Madison and Robel 2001), and increase biodiversity, which in turn increases ecosystem stability (Tilman 2000). Høgh-Jensen and Schjoerring (2000) showed that legumes can increase productivity of non-leguminous neighbors by releasing symbiotically fixed N through mycorrhizal hyphae. Similarly, van der Heijden et al. (2006) documented that *Lotus corniculatus* L., *Ononis repens* L., and *Trifolium repens* L. increased productivity of associated grasses and forbs by reducing the competition for limited soil N. Also, legumes contained more protein and less fiber than grasses at similar stages of growth (Cherney and Allen 1995).

Failure to form root nodules with effective rhizobia on host legume plants impedes legume establishment in rangelands and makes them less desirable in revegetation programs (Gonzalez-Andres and Ortiz 1996). Infective and effective rhizobia are essential for BNF. For example, N concentration in several legumes (*Lotus corniculatus* L., *Ononis repens* L., and *Trifolium repens* L.) was almost twofold greater when inoculated with rhizobia compared to no rhizobial inoculation (van der Heijden et al. 2006). Indigenous rhizobial populations are typically more competitive compared to introduced rhizobial strains because they are well adapted to the local environment. For example, plants of *Astragalus sinicus* L. that were inoculated with an indigenous rhizobial strain 1 week after inoculation with an introduced strain did better than plants inoculated with the introduced strain (Malek et al. 1998). Although the introduced strain formed active nodules when inoculated alone, it was less competitive than the indigenous strain. This has been documented in several other studies (Icgen et al. 2002; Musiyiwa et al. 2005). Another study that examined the survival of rhizobial strains revealed that a rhizobial strain of mesquite (*Prosopis* spp.) isolated from desert soil survived for 1 month in desert soil, whereas a commercial, introduced strain was unable to survive under these conditions (Shoushtari and Pepper 1985).

With increased emphases on restoration of ecosystem integrity in western rangelands of the U.S.A. (Roundy et al. 1995), there has been increased demand for native plant species for revegetation and restoration projects (Richards et al. 1998). Restoration of native vegetation in degraded landscapes can allow regeneration of ecosystem function (Hobbs and Harris 2001), which is a primary goal of conservation ecology (Young 2000; Hobbs and Harris 2001). Soil rhizobial populations form a

symbiotic relationship with leguminous plants that converts atmospheric N into a biologically usable N form (Major 1973), which promotes plant growth in N-limited environments. Restoring symbiotic elements into degraded rangelands is an important step in re-establishing native vegetation (Clay and Holah 1999; Requena et al. 2001; Bruno et al. 2003; van der Heijden et al. 2006) because rhizobia are considered ecosystem engineers (Crooks 2002). Rhizobial strains that are capable of infecting host plants (infectiveness) and provide for high levels of N fixation (effectiveness) increase the chance of successful nodulation with the host plant and their long-term persistence in the soil (Shoushtari and Pepper 1985). Restoring native plants in rangeland ecosystems makes ecosystem services sustainable. Although some native grasses and shrubs are available for revegetating rangelands in the Intermountain West, native forbs (particularly legumes) are not widely used because of low seed availability and difficulty of establishment (Rumbaugh 1983). Pyke et al. (2003) compared the establishment success of native plant species to introduced species in revegetation programs in the Great Basin and found that a number of native species established as well as introduced ones. Legumes in particular have the ability to grow in N-deficient soils, which are common in semiarid regions (Zahran 1999).

Astragalus filipes Torr. ex A. Gray, commonly called threadstalk milkvetch or basalt milkvetch, is a leguminous species that has a wide distribution in the western U.S.A. and is found in northern Mexico to southern British Columbia, Canada. Basalt milkvetch grows in sites that range from 200 to 2,500 m elevation and with annual precipitation ranging from 127 to 741 mm. It is possibly one of the most abundant and widely distributed *Astragalus* species in the western U.S.A. (Isely 1998). Most

Astragalus species are toxic to livestock (Rumbaugh 1983; DiTomaso 2000) and may have precluded basalt milkvetch from being considered as a candidate for rangeland revegetation programs. Recent biochemical analyses, however, showed that levels of nitrotoxins, selenium, and swainsonine were non-detectable or extremely low in field samples of basalt milkvetch (D. A. Johnson, unpublished data). Also, field observations in Nevada and Oregon indicated that basalt milkvetch does well after fire and competes well with cheatgrass (D.A. Johnson, unpublished data).

Because basalt milkvetch is a native legume with a wide geographic distribution in western North America, it may have potential for use in revegetation and restoration programs in the Intermountain West, where fire frequency has increased (Whisenant 1990). Heterogeneity in basalt milkvetch populations is likely, given its wide-scale geographic distribution and adaptation to diverse climates. Evaluation of various populations of basalt milkvetch and the subsequent selection of plant populations with desirable characteristics is an important first step in developing breeding populations of this species. Also, evaluation of associated rhizobial strains is critical to identify rhizobial strains that maximize BNF in N-limited rangeland environments.

LITERATURE CITED

- Aydin, I., and F. Uzun. 2005. Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. *European Journal of Agronomy* 23(1):8-14.

- Baca, S. V. 1995. Managing rangelands for an improved environment and for production of food, fiber, recreation, and aesthetic values. *In*: N. E. West [ed.]. Proceedings- rangelands in a sustainable biosphere. Denver, CO: Society for Range Management. p 3-4.
- Bantilan, M. C. S., and C. Johansen. 1995. Research evolution and impact analysis of biological nitrogen fixation. *Euphytica* 36:903-911.
- Bovey, R.W. 1987. Weed control problems, approaches, and opportunities in rangeland. *Review of Weed Science* 3:57-91.
- Brockwell, J., R. R. Gault, D. F. Herridge, L. J. Morthorpe, and R. J. Roughly. 1995. Studies on alternative means of legume inoculation: Microbiological and agronomic appraisals of commercial procedures for inoculating soybeans with *Bradyrhizobium japonicum*. *Australian Journal of Agricultural Research* 39:965-972.
- Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119-125.
- Cherney, J. H., and V.G. Allen. 1995. Forages in a livestock system. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: The science of grassland agriculture. Volume I. Ames, IA: Iowa State University Press. p 175-188.
- Clay, K., and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742-1744.
- Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* 97:153-166.

- Diaz, E., A. Roldan, A. Lax, and J. Albaladejo. 1994. Formation of stable aggregates in degraded soils by amendment with urban refuse and peat. *Geoderma* 63:277-288.
- DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. *Weed Science* 48(2):255-256.
- Gonzalez-Andres, F., and J. M. Ortiz. 1996. Specificity of rhizobia nodulating *Genista monspessulana* and *Genista linifolia* in vitro and in field situations. *Arid Soil Research and Rehabilitation* 13:223-237.
- Haynes, R. J. 1980. Competitive aspects of the grass-legume association. *Advances in Agronomy* 33:227-261.
- Hobbs, R. J., and J. A. Harris. 2001. Restoration ecology: Repairing the earth's ecosystem in the new millennium. *Restoration Ecology* 9(2):239-246.
- Hogh-Jensen, H., and J. K. Schjoerring. 2000. Below-ground nitrogen transfer between different grassland species: Direct quantification by N-15 leaf feeding compared with indirect dilution of soil N-15. *Plant and Soil* 227(1-2):171-183.
- Horn, F. D. 1995. The importance of rangelands in sustaining the economics of rural communities. In: N. E. West [ed.]. Proceedings- rangelands in a sustainable biosphere. Denver, CO: Society for Range Management. p 1-2.
- Icgen, B., G. Ozcengiz, and N. G. Alaeddinoglu. 2002. Evaluation of symbiotic effectiveness of various *Rhizobium cicer* strains. *Research in Microbiology* 153:369-372.
- Isely, D. 1998. Native and naturalized Leguminosae (Fabaceae) of the United States. Provo, UT: Brigham Young University. 1007 p.

Johnson, D. A., T. M. Ford, M. D. Rumbaugh, and B. L. Richardson. 1989.

Morphological and physiological variation among ecotypes of sweetvetch

(*Hedysarum boreale* Nutt.). *Journal of Range Management* 42(6):496-501.

Legard, S. F., and K. W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* 14:137-153.

MacMahon, J. A. 1979. North American deserts: Their floral and faunal components. *In*:

D. W. Goodall and R. A. Perry [eds.]. *Arid-land ecosystems: Structure, functioning, and management*. Cambridge, United Kingdom: Cambridge University Press. p

1:21-82.

Madison, L. A., and R. J. Robel. 2001. Energy characteristics and consumption of several

seeds recommended for northern bobwhite food plantings. *Wildlife Society*

Bulletin 29:1219-1227.

Major, J. 1973. Review: Nitrogen regime of alpine vegetation in Germany. *Ecology*

54(4):960-961.

Malek, W., M. Inaba, H. Ono, Y. Kaneko, and Y. Murooka. 1998. Competition for

Astragalus sinicus root nodule infection between its native microsymbiont

Rhizobium huakuii bv. rene B3 and *Rhizobium* sp. ACMP18 strain, specific for

Astragalus cicer. *Applied Microbiology and Biotechnology* 50:261-265.

Miller, R. F., T. J. Svejcar, and N. E. West. 1994. Implications of livestock grazing in the

Intermountain sagebrush region: Plant composition. *In*: M. Vavra, W. A. Laycock

and R. D. Pieper [eds.]. *Ecological implications of livestock herbivory in the*

west. Denver, CO: Society for Range Management. p 101-146.

- Musiyiwa, K., S. Mpeperekwi, and K. E. Giller. 2005. Symbiotic effectiveness and host ranges of indigenous rhizobia nodulating promiscuous soybean varieties in Zimbabwean soils. *Soil Biology and Biochemistry* 37(6):1169-1176.
- Noy-Meir, I. 1973. Desert ecosystems: Environment and producers. *Annual Review of Ecology and Systematics* 4:25-51.
- Pellant, M., and S. Monsen. 1993. Rehabilitation on public rangelands in Idaho, USA: A change in emphasis from grass monocultures. *In*: D. Eldridge and D. Freudenberger [eds.]. People and rangelands building the future. Queensland, Australia: Proceedings of the XVII International Rangeland Congress. p 778-779.
- 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.
- Pyke, D. A., T. O. McArthur, K. S. Harrison, and M. Pellant. 2003. Coordinated Intermountain restoration project-fire, decomposition and restoration. *In*: N. Allsopp, A. R. Palmer, S. J. Milton, K. P. Kirkman, G. I. H. Kerley, C. R. Hurt, C. J. Brown [eds.]. Durban, South Africa: Proceedings of the VIIIth International Rangeland Congress. p 1116-1124.
- Requena, N., E. Perez-Solis, C. Azcon-Aguilar, P. Jeffries, and J. M. Baries. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology* 67:495-498.
- Richards, R. T., J. C. Chambers, and C. Ross. 1998. Use of native plants on Federal lands: Policy and practice. *Journal of Range Management* 51:625-632.

- Roundy, B. A., E. D. McArthur, J. S. Haley, and D. K. Mann. [eds.]. 1995. Proceedings of the wildland shrub and arid land restoration symposium. Ogden, UT: USDA Forest Service, Gen. Rep. INT-GTR-315. 384 p.
- Rumbaugh, M. D. 1983. Legumes- their use in wildland plantings. *In*: S. B. Monsen and N. Shaw (compilers). Managing Intermountain rangelands-Improvement of range and wildlife habitats. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-157. p 115-122.
- Shoushtari, N. H., and I. L. Pepper. 1985. Mesquite rhizobia isolated from the Sonoran desert: Competitiveness and survival in soil. *Soil Biology and Biochemistry* 17:803-806.
- Sprent, J. I. 1985. Nitrogen fixation in arid environments. *In*: G. E. Wickens, J. R. Goodin, and D. V. Field [eds.]. Plants for arid lands. London, United Kingdom: Kluwer Academic Publishers. p 215-229.
- Tilman, D. 2000. Causes, consequences and ethics of biodiversity. *Nature* 405:208-211.
- Trubat, R., C. Jordi, and A. Vilagrosa. 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.). *Trees – Structure and Function* 20(3):334-339.
- van der Heijden, M. G. A., R. Bakker, J. Verwaal, T. R. Scheublin, M. Rutten, R. van Logtestijn, and C. Staehelin. 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiology Ecology* 56:178-187.

- West, N. E. 1983. Western Intermountain sagebrush steppe. *In*: N. E. West [ed.].
Ecosystem of the World 5: Temperate deserts and semi-deserts. New York, NY:
Elsevier Scientific Publication Co. p 351-397.
- Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River Plains:
Ecological and management implications. *In*: E. D. McArthur, E. M. Romney, S.
D. Smith, and P. T. Tueller [eds]. Proceedings- Symposium on cheatgrass
invasion, shrub die-off, and other aspects of shrub biology and management.
Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-276. p 4-7.
- World Resources Institute. 1986. World resources, 1986. New York, NY: Basic Books.
353 p.
- Young, T. P. 2000. Restoration ecology and conservation biology. *Biological
Conservation* 92:73-83.
- Zahran, H. H. 1999. *Rhizobium*-legume symbiosis and N₂ fixation under severe
conditions and in an arid climate. *Microbiology and Molecular Biology Reviews*
63(4):968-989.

CHAPTER 2

RHIZOBIAL STRAIN EVALUATION FOR *ASTRAGALUS FILIPES*: A NATIVE LEGUME FOR RANGELANDS OF WESTERN NORTH AMERICA¹

INTRODUCTION

Despite high amounts of N in the atmosphere (about 79 %), growth of plants is often limited by a lack of N because atmospheric N is unusable to most living organisms (Major 1973). Soil N is especially limiting in semiarid and arid areas (Sprent 1985; Zahran 1999), which typically have shallow soils and low precipitation. Low vegetation cover coupled with wind erosion and harsh climate typically results in soils with low organic matter (Diaz et al. 1994). As a result, N deficiency often limits plant productivity on arid and semiarid rangelands (Sprent 1985; Trubat et al. 2006).

Overgrazing, changing the elements of the natural food web of local systems, introducing exotic species, redistributing water resources, fragmenting habitat, and controlling fire regimes have changed the natural biological composition of the Intermountain Region of the western U.S.A. (Miller et al. 1994). Consequences of such activities include invasive species problems, loss of ecosystem function, and desertification of natural habitats (Pellant et al. 2004). Regeneration of ecosystem function is thus a primary goal of conservation ecology (Young 2000; Hobbs and Harris 2001), which can be achieved by restoring native vegetation in a degraded landscape

¹ Coauthored by Kishor Bhattarai and Douglas A. Johnson

(Hobbs and Harris 2001). Restoring plant-rhizobial elements into such degraded landscapes could be a very important step to reestablish native vegetation (Clay and Holah 1999; Requena et al. 2001; Bruno et al. 2003; van der Heijden et al. 2006) because rhizobia are considered ecosystem engineers (Crooks 2002).

Productivity of a site can often be improved by adding N to the site if other ecological factors are not limiting. Addition of N can be done by either adding N fertilizer to the soil or through biological N fixation (BNF). BNF is the process that changes atmospheric N_2 to biologically usable NH_3 (Major 1973). BNF in the world is estimated to be twofold greater than N fixation by non-biological processes (Lodwig et al. 2003). As a result, BNF in natural systems is important and significant. The application of fertilizer on rangelands is not economically feasible or ecologically sustainable. Also, fertilizer application can acidify soils and leach nitrates, which can cause eutrophication of water bodies (Bantilan and Johansen 1995). In addition, soil acidification can alter species competitiveness in a community, which can change local biodiversity. The addition of legumes and their associated rhizobial strains into pasture and rangeland ecosystems represents a sustainable approach that can help increase soil N and improve forage N content (Legard and Steele 1992).

Failure of legumes to develop nodules with effective rhizobial strains makes legumes less desirable in revegetation and restoration programs (Gonzalez-Andres and Ortiz 1996). Infective and effective rhizobia significantly increase N content in legumes and thus promote their growth compared to non-effective rhizobial strains. For example, N concentration in legumes was twofold greater when inoculated with rhizobia compared

to no rhizobial inoculation in microcosms simulated to represent species-rich dune grasslands in Holland (van der Heijden et al. 2006).

Expression of host and microsymbiont genes is critical in nodule initiation and development. Variation in both host and bacterial genomes may affect the sequence of nodule development and the expression of the genes involved in nitrogenase activity and regulation (Starker et al. 2006). Development of lateral roots is particularly important for nodulation. In some cases, root hairs and nodulation occur only after lateral roots emerge (Dongre et al. 1985). Before infection of the root hair, *Rhizobium* binds to the host root and elicits root hair curling. The host cell wall is degraded in a pocket formed by the curled root hair, and the bacteria invade the cell through the degraded portion of the wall (Callaham and Torray 1981). Plant cell wall material deposited around the bacteria forms the infection thread. Meanwhile, meristematic activity in the root cortex is induced and gives rise to cells that form the nodule. The infection thread grows into many of these new cells, and the rhizobia are then released into the cytoplasm. Bacteria change into a bacteroid state by losing their outer membrane, which is evident by a change from a spherical to rod shape (Hirsch 1992). At the same time, plant cells start differentiating, and nitrogenase activity begins (Cleyet-Marel et al. 1990). Rhizobia obtain carbon in the form of dicarboxylic acid from the host plant (Udvardi et al. 1988; Lodwig et al. 2003). If bacterioids cannot obtain dicarboxylic acid from the host plant, they cannot fix N inside the nodules (Udvardi et al. 1988). Change in root morphology in response to rhizobial inoculation has been observed as early as 6 h after inoculation (Ehrhardt et al. 1996), and nitrogenase activity inside nodules is initiated about 1 week after inoculation (Starker et al. 2006).

Environmental variables and naturalized rhizobial populations may affect the competitive ability of rhizobial strains. Ineffective root nodules that do not fix N also may be the result of mutations in the host or bacterial genomes (Starker et al. 2006). Number of nodules, nodule weight, and interior nodule color can indicate the status of N fixation in a legume plant. Effective rhizobial strains tend to produce large numbers of big nodules (Wadisirisuk and Weaver 1985; Laberge et al. 2005; Simms et al. 2006). However, some studies found that number of nodules and nodule weight are not always good indicators for assessing efficiency of symbiotic N fixation in legumes (Gallardo et al. 1996; Lodwig et al. 2003; Laberge et al. 2005). Therefore, estimating total N content of the host plant is a more reliable method of evaluating the effectiveness of particular legume-rhizobial strain combinations (Hardarson and Atkins 2003). A functional nodule has a pink to dark-red coloration, which is an indication of the presence of leghaemoglobin, whereas a greenish color typically indicates ineffective nodulation (Gwata et al. 2003; Starker et al. 2006).

Indigenous rhizobial populations are typically more competitive than introduced rhizobial strains because they are adapted to the particular environment. For example, *Astragalus sinicus* L. inoculated with an indigenous rhizobial strain 1 week after inoculation with an introduced rhizobial strain was able to outcompete the introduced strain (Malek et al. 1998). Although the introduced strain formed active nodules when inoculated alone, the introduced strain was less competitive than the native strain. Another study that examined the survival of rhizobial strains revealed that a rhizobial strain of mesquite (*Prosopis* spp.) isolated from desert soil survived for 1 month in desert soil, whereas a commercial strain was unable to survive under desert conditions

(Shoushtari and Pepper 1985). This same pattern has been observed in other studies (Icgen et al. 2002; Musiyiwa et al. 2005).

Rhizobial strains that are capable of infecting host plants (infectiveness) and provide for high levels of N fixation (effectiveness) increase the establishment success of legumes in natural environments (van der Heijden et al. 2006). Because indigenous rhizobia are well adapted to their natural environment, selection of effective rhizobial strains from indigenous populations increases the chance of successful nodulation with the target host and their long-term persistence in the soil (Shoushtari and Pepper 1985). The present study was conducted to identify the most infective and effective rhizobial strains of basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray), a native legume that occurs across a six-state area in the western U.S.A. We were interested in testing the hypothesis that indigenous rhizobia from across the range of distribution of basalt milkvetch differed in their ability to infect and fix N. Also, we were interested in knowing if rhizobial isolates differed in N fixation across basalt milkvetch accessions. We used host biomass, N pool, number of nodules, and nodule weight to assess infectiveness and effectiveness of N fixation.

MATERIALS AND METHODS

Rhizobial Isolations

Soil samples were collected beneath plants of basalt milkvetch from 79 field sites across 6 states of the western U.S.A. including Utah, Idaho, Nevada, Washington,

Oregon, and California. After removing the top 1 to 3 cm of surface material to eliminate litter, soil was excavated from a soil volume about 25 cm in diameter and 25 cm deep. Soil samples were brought back from the field and air-dried in a greenhouse. Soils from all sampling sites were mixed together and sieved through a series of screens in a sieve shaker (Model Aggra-Shaker, Soil Test Inc., Evanston, IL) to homogenize the soil samples and remove roots, rocks, litter, and seeds. We used this sieved soil as a rhizobial inoculant source.

Seeds of 6 basalt milkvetch accessions (Table 2-1) were placed on moistened blotter paper, germinated, and transplanted to cone-shaped plastic conetainers (3.8-cm diameter, 21-cm length) filled with a sand:peat moss (3:1) mixture. The seedlings were grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory at Logan, UT (41° 45' N, 110° 48' W, 1,350 m above sea level), under a day/night temperature regime of about 30/15 °C and were watered to maintain growth. Seedlings were inoculated with homogenized, sieved soil (from above). After about 5 weeks of growth, plants of each accession were harvested by carefully washing the roots free of soil. Washed plants of each accession with attached roots and nodules were placed between a moistened paper towel, sealed in a Ziploc plastic bag, and sent by overnight mail to the Nitragin Company (Milwaukee, WI). One large, pinkish nodule from each of 2 plants for each of 6 basalt milkvetch accessions was selected and surface sterilized by placing in 70 % ethanol for 4 min. The nodule was then removed from the 70 % ethanol and rinsed thoroughly in sterile, de-ionized water. Four sterile Petri dishes (each containing a large drop of sterile de-ionized water) were arranged in sequence, and the

Table 2-1. Climatic and geographic information of basalt milkvetch accessions and rhizobial inocula with state, elevation, latitude, longitude, mean annual maximum (max) and minimum (min) temperatures, and mean annual precipitation (precip). Means are averages across 30 years.

Inoculum	Accession	State	Elevation	Latitude (N)	Longitude (W)	Temperature		Precip
						Max	Min	
			m			-----°C-----		mm
I-16	Af-16	CA	1,530	41° 53'	120° 18'	15	0	399
I-30.1	Af-30.1	OR	625	44° 55'	120° 31'	14	3	363
I-41	Af-41	NV	1,774	41° 48'	115° 56'	10	-2	741
I-48	-	NV	2,347	39° 16'	115° 25'	17	0	275
I-49	-	NV	2,545	39° 32'	114° 38'	16	0	296
-	Af-53*	NV	2,258	39° 19'	117° 07'	17	1	213
I-68	Af-68	ID	2,201	44° 20'	113° 31'	11	-5	342
-	Af-75*	OR	265	45° 37'	119° 43'	16	3	258
-	Af-89*	BC**	915	50° 11'	120° 07'	13	1	489

* Not used to isolate rhizobial strains

**British Columbia, Canada

surface-sterilized nodule was placed in the drop of water in the first dish and crushed using a sterilized probe. An inoculating loop was used to mix the crushed nodule suspension, and a loopful of this suspension was then transferred to the drop of water in the next dish of the sequence. This procedure was repeated until a portion of the nodule suspension had been serially transferred to all 4 Petri dishes. Yeast Extract Mannitol (YEM) media was prepared by mixing 0.5 g/L K_2HPO_4 , 0.2 g/L $MgSO_4$, 0.1 g/L NaCl, 1.0 g/L yeast extract, and 10.0 g/L mannitol. Molten YEM agar at about 50 °C was poured into each of the 4 Petri dishes. The Petri dishes were swirled; allowed to solidify in a laminar flow hood, and then incubated until colonies appeared. From each series of Petri dishes, a typical, single rhizobial colony was selected and streaked on a YEM agar plate. One isolate representing each of the nodules was thus selected. Plates were evaluated for uniform and typical rhizobial growth characteristics, and any questionable

isolates were discarded. One isolate was then chosen for each of the 6 plant accessions. Each of the 6 rhizobial isolates was then grown in YEM broth, mixed in a milled sedge peat carrier, placed in separate plastic bags, and shipped to Logan for testing. Each rhizobial isolate was isolated by a basalt milkvetch accession and was named after the basalt milkvetch accession. Site number given to each inoculum, therefore, did not necessarily indicate that the isolated rhizobial strain was from that particular site.

Greenhouse Experiment A

Greenhouse Experiment A was conducted in 2005 with 6 basalt milkvetch accessions (Af-16, Af-30.1, Af-41, Af-53, Af-68, Af-89) and 8 treatments: 6 rhizobial strains from the Nitragin Co. (I-16, I-30.1, I-41, I-48, I-49, I-68) and 2 control treatments (Control with N and Control without N). The 6 basalt milkvetch accessions were selected based on their seed availability and representativeness across its range of distribution. This experiment was a randomized complete block design of 6 X 8 factors with 5 replications.

Black plastic pots (25-cm deep, 6-cm wide, D40 Deepot, Stuewe and Sons, Inc. Corvallis, OR) were filled with commercial washed sand with the bottom of the pots covered from inside the pot with landscape cloth to eliminate sand loss from the pot. Seeds were rinsed with 95 % ethanol for 10 sec. and washed with de-ionized water for 6 successive times to minimize rhizobial contamination on the seed coat. Five seeds were sown in each pot. A 1-cm layer of Perlite (Miracle-Gro Lawn Products, Inc., Marysville, OH) was added to the top of each pot to minimize contamination by airborne rhizobia.

Plants were inoculated with respective rhizobial strains using a slurry mix because slurry inoculation typically produces earlier nodulation (Brockwell et al. 1988), and liquid inoculation methods generally produce more successful nodulation than seed inoculation or application of alginate bead inoculant (Odee et al. 2002). A 5-mL slurry with 1 g rhizobial peat mix per 250 mL of de-ionized water was used for inoculation. The rhizobial slurries were applied to each pot by syringe injection into the sand, which was done during 3 consecutive weeks after seed sowing or seed transplanting. A total of 50 mL of water was injected twice a week.

A stock macronutrient solution was prepared by mixing 200 g of KCl, 50 g of $\text{CaSO}_4 \times 2\text{H}_2\text{O}$, 50 g of $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, and 50 g of $\text{Ca}_5\text{HO}_{13}\text{P}_3$ in a mortar with a pestle. A ferric citrate solution was made by boiling 6.7 g of ferric citrate solution in 1 L of de-ionized water. A micronutrient stock solution was made by mixing 0.57 g of H_3Bo_3 , 0.31 g of $\text{MnSO}_4 \times \text{H}_2\text{O}$, 0.09 g of $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, 0.08 g of $\text{CuSO}_4 \times 5\text{H}_2\text{O}$, 0.016 g of molybdic acid (85 % MoO_3), and 0.0008 g of $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ in 1 L of de-ionized water. A solution containing 1.31 g of macronutrient solution, 0.5 mL of ferric citrate solution, and 5 mL of micronutrient solution was mixed to make 1 L of nutrient solution. An appropriate amount of HCl was added to the final nutrient solution to keep the pH of the solution between 6.5 to 7.0. A total of 76.5 mL of nutrient solution was applied to each container to meet the recommended amount of 50 kg/ha of K_2O for legume and grass seedling establishment in nutrient-poor soils. For the N solution, 0.06 g of NH_4NO_3 per L of solution was used to achieve the recommended concentration of N (50 kg/ha) for nutrient-deficient soil (Finck 2005) in Greenhouse Experiment A. Because nodulation was observed in the Control with N treatment in Greenhouse Experiment A, N

concentration was doubled (0.12 g per L) in Greenhouse Experiment B after no leaf burning was observed from this fertilizer rate in a preliminary greenhouse experiment. Nutrient solution was injected into the containers every 2 weeks.

The number of root nodules was determined at 10 weeks after seed sowing. Roots and shoots were separated at the cotyledonary node, and roots were gently washed over a fine screen to remove attached sand. Nodules were severed at their point of root attachment and counted. Roots, shoots, and nodules were oven dried at 60 °C for 48 h, and dry weight was determined.

Greenhouse Power Test Experiment

A greenhouse experiment was conducted in 2005 to provide data to determine the number of replications needed with a power of 80 % to show statistical significance for nodule weight. Nodule weight was used because nodule weight is a better indicator of N fixation than number of nodules (Gallardo et al. 1996; Er et al. 2004). This experiment involved the same 6 rhizobial treatments and 2 control treatments as above; however, only 1 basalt milkvetch accession was used (Af-30.1 from Clarno, OR). The experimental design of this experiment was a completely randomized block with 8 replications. The same procedures were used as above. Based on this experiment, the required sample size for determining differences in nodule weight was calculated to be 13 replications (80 % power at $\alpha = 5\%$) (Fig. 2-1). This result was obtained by using GLMPOWER of SAS V 9.0 (SAS Institute Inc., Cary, NC) with the effect weight of 0.36 g after data were log transformed to meet normality and homoscedasticity assumptions. The effect weight was

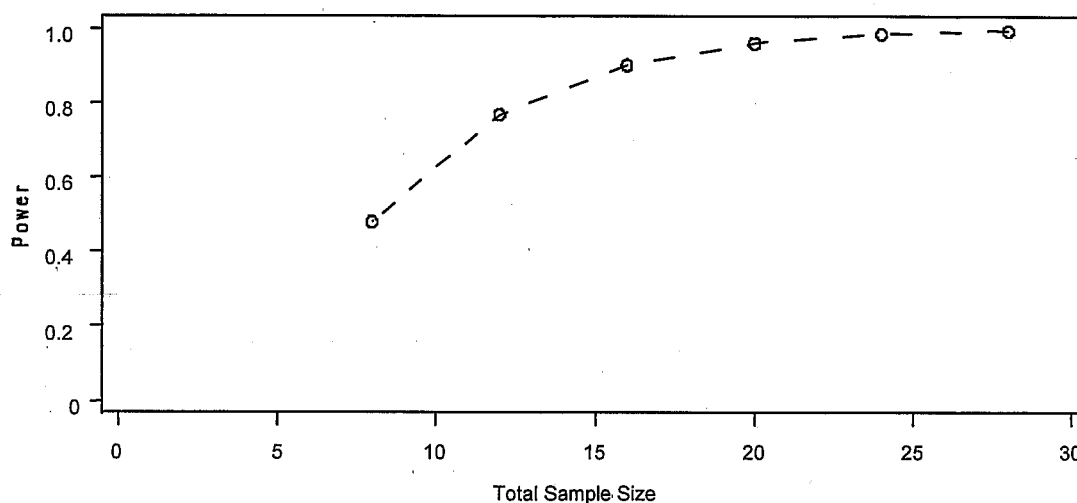


Figure 2-1. The relationship of power to total sample size for the Greenhouse Power Test Experiment.

the difference between treatment I-49 (most effective rhizobia) and Control with N treatment.

Greenhouse Experiment B

For Greenhouse Experiment B, which was conducted in 2006, only 1 basalt milkvetch accession (Af-75 from Boardman, OR) was used. Only one accession was used in this experiment because no significant interaction was observed between basalt milkvetch accessions and rhizobial strains in Greenhouse Experiment A. Af-75 was chosen because it had a high germination percentage and we had a large number of seeds of this accession. This experiment was designed as a randomized complete block with 13 replications, and the same 8 treatments were used as above. The same pots and sand were also used. For this experiment, seeds were placed on moistened blotter paper, germinated,

and 3 seedlings were transplanted to minimize differences due to germination time. A 1-cm layer of Perlite was added to the top of each pot to minimize contamination by airborne rhizobia.

Plants were inoculated with respective rhizobial strains using a 10-mL slurry (1 g rhizobial peat mix per 200 mL of de-ionized water). The rhizobial slurries were applied to each pot by syringe injection as above. A total of 50 mL of water was injected 3 times a week using individual syringes for each treatment as above. Nutrient solution (as above) also was injected into the pots every 2 weeks. About 8 weeks after seedling transplanting, plants were harvested and shoot weight, root weight, number of nodules, and nodule weight were measured in Greenhouse Experiment B. In addition, dried shoots and roots were ground with a small electric coffee grinder (KRUPS, Model F 203O-70) with a modified top that minimized sample loss while grinding. Ground samples were analyzed for total N by Utah State University Analytical Laboratories using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI).

Statistical Analysis

Analysis of variance was conducted on the measured variables to determine statistical differences among accessions and treatments using GLM procedure of SAS V 9.0 (SAS Institute Inc., Cary, NC). Data were transformed, to meet normality and homoscedasticity assumptions prior to significance testing. In Greenhouse Experiment A, squared root transformation was done for shoot weight, and quarter root transformation was done for root weight, number of nodules, and nodule weight. In Greenhouse

Experiment A, basalt milkvetch accessions and rhizobial inocula were considered fixed factors and blocks were considered random factors. In Greenhouse Experiment B, quarter root transformation was done for shoot weight, number of nodules, and nodule weight, whereas quarter root transformation was used for shoot N and total N pool. In Greenhouse Experiment B, rhizobial inocula were fixed factors and blocks were random factors. Type III sums of squares were used for the analysis. To control the familywise error rate due to multiple comparisons at $\alpha = 5\%$, the Ryan-Einot-Gabriel-Welch (REGWQ) Method was used as a post hoc mean comparison method. A linear regression analysis was done to analyze the relationship between total N content and total biomass using SigmaPlot V 10.0 (Systat Software Inc., Richmond, CA). Correlation analysis was conducted among measured parameters within rhizobial inocula using SAS. All differences discussed in the Results and Discussion sections are significant at $P < 0.05$ unless otherwise noted.

RESULTS

Greenhouse Experiment A

Treatments differed significantly for nodule weight and number of nodules (Table 2-2). Significant differences were observed among accessions for shoot weight, root weight, nodule weight, and number of nodules. No significant treatment differences were observed for shoot weight or root weight. In Greenhouse Experiment A, the treatment by accession (T X A) interaction was not significant for any of the measured variables.

Table 2-2. Analysis of variance results for Greenhouse Experiment A.

Source of variation	d. f.	F value	P
Shoot weight			
Treatment (T)	7	1.17	ns
Accession (A)	5	27.42	**
T X A	35	0.88	ns
Root weight			
Treatment (T)	7	1.47	ns
Accession (A)	5	6.2	**
T X A	35	1.02	ns
Nodule weight			
Treatment (T)	7	14.92	**
Accession (A)	5	4.19	*
T X A	35	1.38	ns
Number of nodules			
Treatment (T)	7	14.36	**
Accession (A)	5	4.87	**
T X A	35	1.2	ns

ns = not significant, * = $P < 0.05$, ** = $P < 0.01$

Mean comparisons for number of nodules and nodule weight indicated that I-49 rhizobial inoculum had the highest nodule weight (1.55 mg/plant) and highest number of nodules (6 per plant compared to other inoculation treatments (Table 2-3). As expected, the 2 control treatments differed significantly from the inoculation treatments and had the lowest nodule weight and lowest number of nodules. I-30.1, I-68, I-41, I-16, and I-48 were significantly different from the other treatments in nodule weight and number of nodules, but did not differ among themselves. These rhizobial inocula were intermediate between I-49 and the 2 control treatments, which had number of nodules ranging from 1.2 to 1.8 and nodule weights from 2.0 to 2.5.

Mean comparisons indicated that Af-30.1 and Af-53 had almost double the shoot weight (20 and 19 mg) of Af-16, Af-41, Af-68, and Af-89 (range: 9 to 11 mg) (Table

Table 2-3. Mean comparisons for 6 rhizobial inoculants (I-16, I-30.1, I-41, I-48, I-49, I-68) and two control (with N = C/N, no N = C) treatments for number of nodules, shoot weight, root weight, and nodule weight for Greenhouse Experiment A.

Treatment	Shoot weight	Root weight	Nodule weight	Number of nodules
	-----mg-----			
I-30.1	14.8 a ¹	93.8 a	0.64 b	2.1 b
I-68	13.3 a	66.6 a	0.72 b	2.5 b
I-41	11.3 a	53.2 a	0.36 b	2.0 b
I-49	15.9 a	86.6 a	1.55 a	6.0 a
I-16	13.8 a	75.0 a	0.51 b	2.1 b
I-48	13.2 a	77.7 a	0.53 b	2.0 b
C	14.2 a	79.1 a	0.08 c	0.6 c
C/N	14.6 a	82.3 a	0.04 c	0.2 c

¹Numbers followed by the same letter within a column are not statistically significant at $P = 0.05$.

2-4). Af-30.1 had greater root weight (109 mg) than Af-16, Af-41, Af-68, and Af-89 (range: 55 to 67 mg), except Af-53 (91 mg). Also, Af-53 had greater root weight than Af-16 and Af-68. Af-53 produced greater nodule weight (0.9 mg) and number of nodules (4.1) than Af-16, Af-68, and Af-89 (with a range of 0.3 to 0.5 mg for nodule weight and 1.1 to 1.7 for number of nodules). However, Af-30.1 and Af-41 were not significantly different from other accessions for nodule weight and number of nodules.

Greenhouse Experiment B

No significant differences were detected among treatments for root weight (Table 2-5, Fig 2-2B). However, differences were observed among treatments for shoot weight, shoot and total N pool, number of nodules, and nodule weight. As expected, the Control with N treatment had the greatest shoot weight (17 mg) and differed from I-30.1 (9 mg),

Table 2-4. Mean comparisons for basalt milkvetch accessions (Af-16, Af-30.1, Af-41, Af-53, Af-68, Af-89) for shoot weight, root weight, nodule weight, and number of nodules for Greenhouse Experiment A.

Accession	Shoot weight	Root weight	Nodule weight	Number of nodules
	-----mg-----			
Af-16	9.9 b ¹	60.1 c	0.49 b	1.7 b
Af-30.1	20.3 a	109.4 a	0.84 ab	2.2 ab
Af-41	11.3 b	63.8 bc	0.34 ab	2.2 ab
Af-53	18.7 a	90.5 ab	0.88 a	4.1 a
Af-68	9.3 b	55.3 c	0.37 b	1.1 b
Af-89	10.6 b	67.2 bc	0.29 b	1.2 b

¹Numbers followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table 2-5. ANOVA results for Greenhouse Experiment B.

Source of variation	d. f.	<i>F</i> value	<i>P</i>
Shoot weight	7	1.35	*
Root weight	7	1.89	ns
Shoot N pool	7	8.99	**
Total N pool	7	8.49	**
Numbers of nodules	7	7.57	**
Nodule weight	7	8.14	**

ns = not significant, * = $P < 0.05$, ** = $P < 0.01$

I-41 (9 mg), and I-68 (9 mg) (Fig. 2-2A). I-49 produced a shoot weight of 15 mg weight, which was not different from the control with N treatment. The Control with N (0.41 mg) and I-49 (0.33 mg) treatments exhibited greater shoot N pool than I-30.1 (0.15 mg), I-16 (0.15 mg), I-41 (0.08 mg), I-68 (0.14 mg), and Control (no N) (0.14 mg) treatments (Fig. 2-2C); however, these treatments did not differ from I-48 (0.21 mg). I-41 had a lower

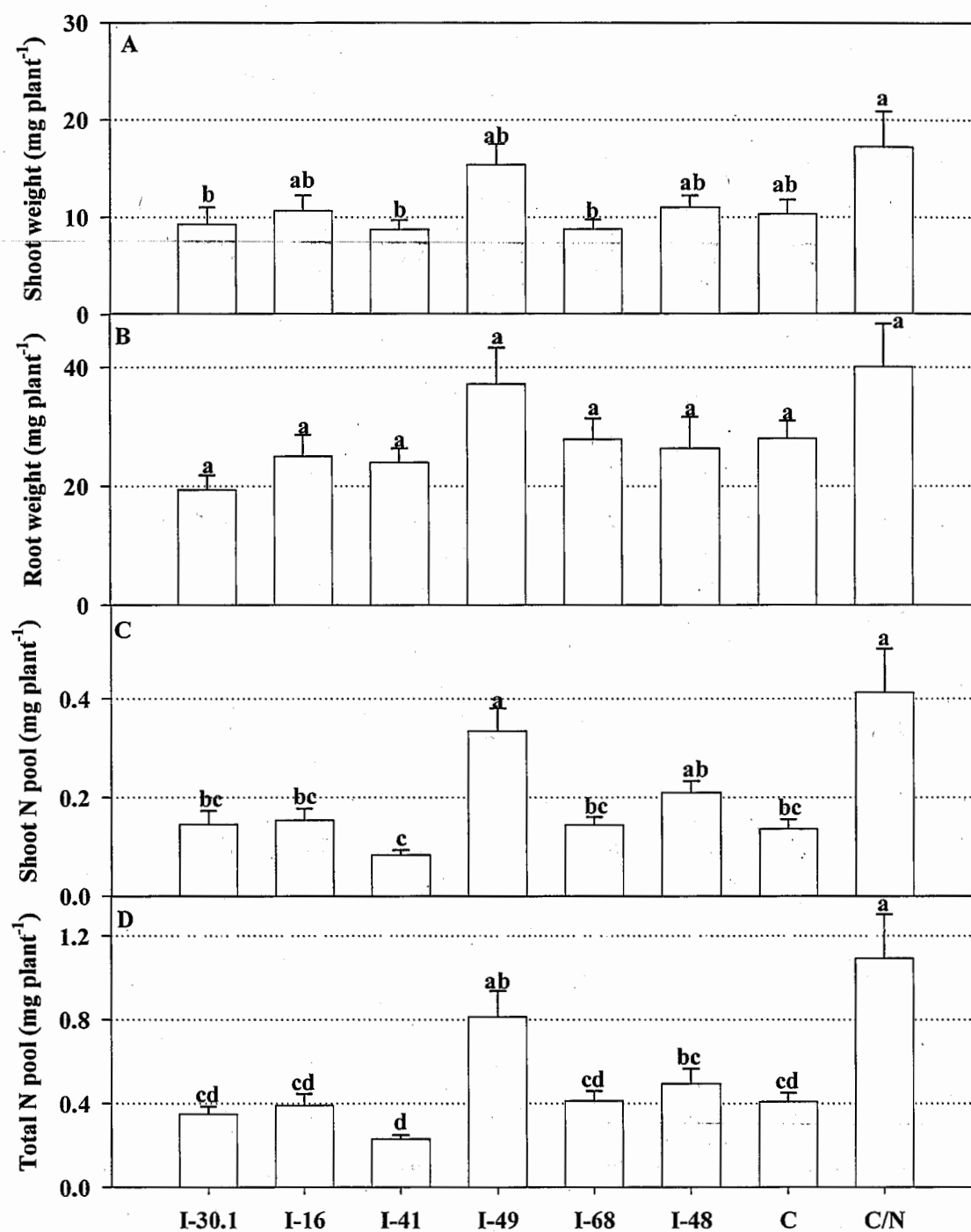


Figure 2-2. Results of Greenhouse Experiment B for shoot weight (A), root weight (B), shoot N pool (C), and total N pool (D). Treatments include 6 rhizobial strains (I-30.1, I-16, I-41, I-49, I-68, I-48), Control with no N (C), and Control with N (C/N). Bars represent means $\pm$ one standard error. Bars with the same letters on top are not statistically different at $P = 0.05$.

shoot N pool than I-49, I-48, and Control with N treatments (Fig. 2-2C). The Control with N treatment had higher total N pool (1.1 mg) than I-30.1 (0.35 mg), I-16 (0.39 mg), I-41 (0.23 mg), I-68 (0.41 mg), I-48 (0.50 mg), and Control (no N) (0.41 mg) treatments. I-49 had the second highest total N pool (0.81) and was different from I-30.1, I-16, I-41, I-68, and Control (no N) treatments (Fig. 2-2D). I-48 had higher total N pool than I-41. Inoculation with I-49 resulted in a greater number of nodules (3.4) than I-30.1 (1.8), I-16 (1.5), I-41 (0.2), I-68 (1.7), and Control (no N) (0.7). As expected, Control with N treatments produced no nodules (Fig. 2-3A). I-49 did not produce more nodules than I-48 (2.6). I-48 exhibited a greater number of nodules than I-41. I-30.1, I-16, I-41, I-68, and Control (no N) did not differ in number of nodules. Inoculation with I-49 (1.3 mg) and I-48 (0.8 mg) resulted in greater nodule weight than I-41 (0.1 mg), and Control (no N) (0.2 mg) (Fig. 2-3B). Nodule weights were not different among the Control (no N) and I-30.1, I-16, I-41, and I-68.

I-49 consistently exhibited the best performance for all the measured variables. However, I-49 did not differ significantly from I-48. I-49 exhibited a 117 % higher total N pool than the third best inoculum, I-16 (Fig. 2-2D), and a 95 % greater number of nodules than I-30 (Fig. 2-3A). Linear regression analysis revealed that total biomass was associated with total N pool ($r = 0.93$, $P < 0.0001$) (Fig. 2-4). In addition, a significant negative correlation was observed between biomass per unit N and total biomass ($r = -0.25$, $P < 0.05$).

Pearson correlation coefficients were significant for regressions of nodule weight and number of nodules against shoot weight, root weight, total biomass, shoot N pool,

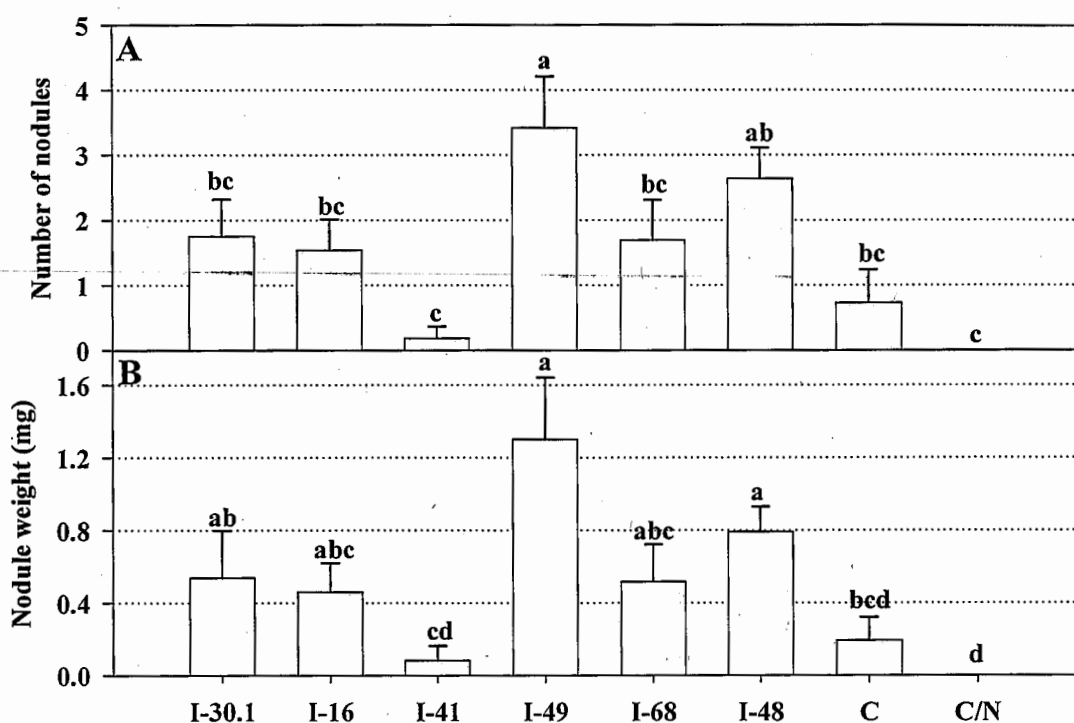


Figure 2-3. Results of Greenhouse Experiment B for number of nodules (A) and nodule weight (B). Treatments included 6 rhizobial strains (I-30.1, I-16, I-41, I-49, I-68, I-48), Control with no N (C), and Control with N (C/N). Bars represent means $\pm$ one standard error. Bars with the same letters on top are not statistically different at $P = 0.05$.

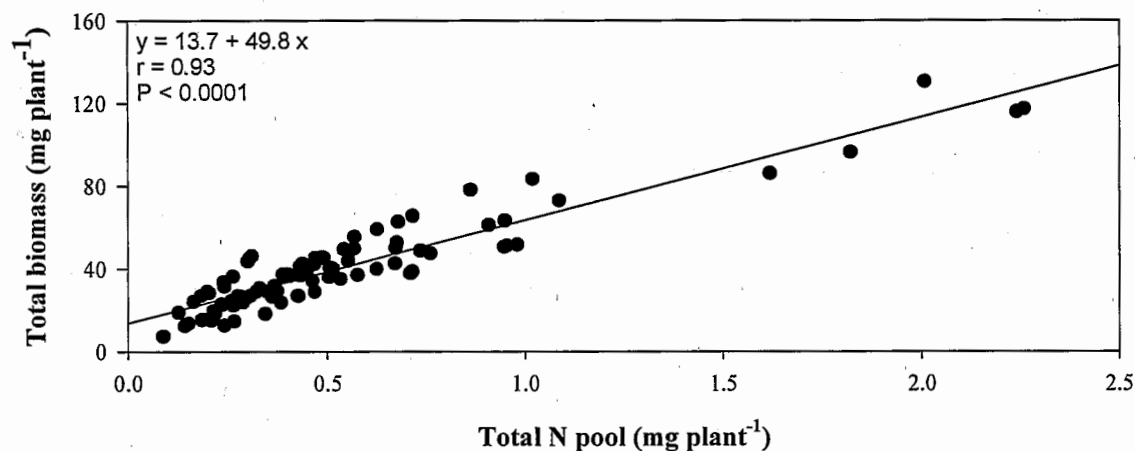


Figure 2-4. Linear regression of total plant biomass against total N pool for Greenhouse Experiment B.

root N pool, and total N pool (Table 2-6). However, nodule weight exhibited overall higher correlations with shoot weight, root weight, total biomass, shoot N pool, root N pool, and total N pool than number of nodules (Table 2-6).

DISCUSSION

Significant differences among rhizobial inoculum treatments for number of nodules, nodule weight, shoot N pool, and total N pool suggested that rhizobial strains varied in their infectiveness and effectiveness for N fixation with basalt milkvetch accessions. In Greenhouse Experiment A, no significant interactions were observed between host plant accession and inoculation treatment for shoot weight, root weight, number of nodules, and nodule weight. Similar nonsignificant results were reported for interactions between alfalfa cultivars and *Sinorhizobium meliloti* strains for nodule dry

Table 2-6. Pearson correlation coefficients (r) for number of nodules (Nn), nodule weight (Nw), shoot weight (Sw), root weight (Rw), total biomass (Tb), shoot N pool (Sn), root N pool (Rn), and total N (Tn).

	Nn	Nw	Sw	Rw	Tb	Sn	Rn
Nn							
Nw	0.431**						
Sw	0.235*	0.346**					
Rw	0.247*	0.374**	0.650**				
Tb	0.263*	0.395**	0.804**	0.975**			
Sn	0.358**	0.440**	0.934**	0.660**	0.791**		
Rn	0.355**	0.467**	0.691**	0.948**	0.946**	0.787**	
Tn	0.376**	0.481**	0.829**	0.881**	0.934**	0.918**	0.967**

* = $P < 0.05$, ** = $P < 0.01$

weight and biomass yield (Bradic et al. 2003). Although many studies have reported significant legume host by rhizobial strain interactions for biological N fixation characteristics (Fernandez and Miller 1987; Awonaike et al. 1992; McKenzie et al. 2001; Mhadhbi et al. 2005), many other studies have shown non-significant interactions between host genotypes and their rhizobial inocula for symbiotic N fixation (Buttery et al. 1997; Bradic et al. 2003; Ballard et al. 2003).

The nonsignificant accession by inoculation treatment interactions in our study indicated that symbiotic effectiveness of rhizobial strains did not vary significantly across basalt milkvetch accessions. This suggests that the best inoculum selected for N fixation should be consistent across basalt milkvetch accessions. However, basalt milkvetch accessions varied considerably for measured variables such as shoot weight, root weight, nodule weight, and number of nodules. Our results agree with the significant cultivar effects of alfalfa (*Medicago sativa* L.) found for nodule dry weight and plant biomass in inoculation studies with strains of *Sinorhizobium meliloti* (Bradic et al. 2003). Our results also showed that basalt milkvetch accessions with a high number of nodules and nodule weight consistently had higher shoot and root biomass. This may be because larger plants have more root area available for rhizobia to initiate nodulation and more carbon reserves for rhizobial growth and nodule development. In return, more nodules provide more fixed N to the host plant (Wadisirisuk and Weaver 1985), which facilitates growth of the host plant.

As expected, a strong positive correlation between total N pool and total biomass in our study (Fig. 2-4) suggested that increasing N availability would improve host growth. Also, a significant negative correlation between biomass per unit N and total

biomass indicated that as plants grew bigger, they were more efficient in using available N. Although there was no significant difference among rhizobial treatments for shoot weight in Greenhouse Experiment A, a significant difference was observed in Greenhouse Experiment B among rhizobial treatments for shoot weight, which could be because of a larger sample size and higher concentration of N treatment in Greenhouse Experiment B than those in Greenhouse Experiment A. In Greenhouse Experiment B, our results showed that significant differences were present among rhizobial inocula for shoot N pool, total N pool, number of nodules, and nodule weight. I-49 had a significantly higher number of nodules, shoot N, and total N pool than other rhizobial inocula, except I-48. In addition, I-49 showed a trend towards greater shoot weight, root weight, and total biomass than the other rhizobial inocula (Fig. 2-2, 2-3). I-49 had comparable shoot weight to the Control with N treatment where the latter treatment had significantly higher shoot weight than other treatments including I-30.1, I-41, and I-68 (Fig. 2-2A). In Greenhouse Experiment A, I-49 produced a greater number of nodules, and nodule weight than other rhizobial inocula (Table 2-3). Analyzing N content of the host plant is a direct way of assessing effectiveness of rhizobial strains (Hardarson and Atkins 2003). Total N pool was greatest with I-49, indicating that I-49 was significantly more infective and effective for N fixation than other rhizobial strains, except I-48. Although an effective rhizobial strain may not always be competitive, competitiveness of a particular rhizobial strain can be enhanced by increasing rhizobial numbers in inocula (Amarger and Lobreau 1982; Hungria et al. 2003; Okazaki et al. 2003).

If plant yield is limited by soil N, number of nodules and nodule weight of legumes may provide a rough guide to level of N fixation (Hardarson and Atkins 2003).

Our results confirmed that number of nodules and nodule weight are positively correlated with symbiotic N fixation (Wadisirisuk and Weaver 1985; Hafeez et al. 2000; Zhang et al. 2002; Mayz et al. 2003). However, having a greater number of nodules and nodule weight does not always confirm rhizobial effectiveness for N fixation. Ludwig et al. (2003) found a strain of *Rhizobium leguminosarum* bv. *viceae* that produced greater numbers of nodules and nodule weight in peas (*Pisum sativum* c.v. Avola and Winner), but actually had low N fixation. Also, Gallardo et al. (1996) found that some *Bradyrhizobium* strains with relatively low nodule dry weight in *Crotalaria juncea* L. and *Cajanus cajan* (L.) Millsp. had high N fixation. Some studies (Gallardo et al. 1996; Er et al. 2004; Simms et al. 2006) have suggested that nodule weight is a more reliable measure of BNF than number of nodules, which was true in our study where we found that nodule weight was more closely correlated with total N pool than number of nodules (Table 2-5). This may be because the increase in N demand of the host plant may be achieved by increasing nodule mass without increasing number of nodules (Herdina and Silsbury 1990).

In our study, plants inoculated with rhizobial strains had a lower N pool than control plants supplied with N. Several studies showed that plants inoculated with the best inoculum could achieve an N pool as high as plants in the control treatment (Gallardo et al. 1996; Mostasso et al. 2002). Our results could be due to the small size of plants in our study. At harvest in our study, total plant biomass was 0.03 to 0.09 g compared to that of Gallardo et al. (1996) where total biomass ranged from 1.19 to 4.31 g in *Crotalaria juncea* L. and 1.6 to 3.2 g in *Cajanaus cajan* (L.) Millsp. Similarly, in the study of Mostasso et al. (2002), shoot biomass ranged from 0.15 to 3.58 g in *Phaseolous*

vulgaris cv. Carioca and 0.12 to 2.71 g in *Phaseleous vulgaris* cv. Negro Argel, respectively. Because energy must be expended by the host plant for nodulation and N fixation, plants provided N in nutrient solution can allocate carbon and nutrients to plant growth rather than nodulation and N fixation.

A review by Triplett and Sadowsky (1992) indicated that plants inoculated with the best rhizobial strains typically exhibited a 5 to 15 % greater biomass or grain yield than plants inoculated with less effective rhizobial strains. In our study, we observed a trend that I-49 produced relatively greater biomass than other rhizobial inocula (Table 2-3, Fig. 2-3A, 2-3B). Moreover, I-49 had a significantly greater (117 %) total N pool than I-16, which was third in ranking among rhizobial strains for total N pool (Fig. 2-3C) in Greenhouse Experiment B. In the same experiment, I-49 had a 95 % greater number of nodules (Fig. 2-2A) than I-30.1. I-49 was the best rhizobial inoculum for number of nodules, and nodule weight in Greenhouse Experiment A (Table 2-3). Although I-49 did not differ statistically from I-48 in Greenhouse Experiment B, there was a consistent tendency for plants inoculated with I-49 to perform better than I-48 (Fig. 2-2, 2-3). As a result, I-49 was the best rhizobial inoculum for N fixation in basalt milkvetch in our study.

MANAGEMENT IMPLICATIONS

Restoration of ecosystem processes in the Great Basin Region of the western U.S.A. is needed because of increasing anthropogenic-induced disturbances (Pellant et al.

2004). Establishment of legumes increases plant diversity, which in turn enhances ecosystem stability (Tilman 2000), promotes soil N, increases soil organic matter, and hydrostable soil aggregates (Requena et al. 2001). In addition, legumes facilitate the growth of non-leguminous plants, thereby increasing forage production and forage quality (Posler et al. 1993). Rhizobial strains play a critical role in legume N fixation, which is important in N-limiting ecosystems of arid and semiarid regions (Gonzalez-Andres and Ortiz 1996). Based on the results of our studies, I-49 would be an effective rhizobial strain for N fixation in basalt milkvetch. Rhizobial strain I-48 was the second best inoculum for increasing N fixation with basalt milkvetch, which can be used in case I-49 is not available, but rhizobial inoculum I-41 was the worst for N fixation with basalt milkvetch accessions.

LITERATURE CITED

- Amarger, N., and J. P. Lobreau. 1982. Quantitative study of nodulation competitiveness in *Rhizobium* strains. *Applied and Environmental Microbiology* 44(3):583-588.
- Awonaike, K. O., G. Hardarson, and K. S. Kumarasinghe. 1992. Biological nitrogen-fixation of *Gliricidia sepium*-*Rhizobium* symbiosis as influenced by plant genotype, bacterial strain and their interactions. *Tropical Agriculture* 69(4):381-385.

- Ballard, R. A., B. R. Shepherd, and N. Charman. 2003. Nodulation and growth of pasture legumes with naturalised soil rhizobia. 3. Lucerne (*Medicago sativa* L.) *Australian Journal of Experimental Agriculture* 43(2):135-140.
- Bantilan, M. C. S., and C. Johansen. 1995. Research evolution and impact analysis of biological nitrogen fixation. *Euphytica* 36:903-911.
- Bradic, M., S. Sikora, S. Redzepovic, and Z. Stafa. 2003. Genetic identification and symbiotic efficiency of an indigenous *Sinorhizobium meliloti* field population. *Food Technology and Biotechnology* 41(1):69-75.
- Brockwell, J., R. R. Gault, D. F. Herridge, L. J. Morthorpe, and R. J. Roughly. 1998. Studies on alternative means of legume inoculation: Microbiological and agronomic appraisals of commercial procedures for inoculating soybeans with *Bradyrhizobium japonicum*. *Australian Journal of Agricultural Research* 39:965-972.
- Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119-125.
- Buttery, B. R., S. J. Park, and P. van Berkum. 1997. Effects of common bean (*Phaseolus vulgaris* L.) cultivar and *Rhizobium* strain on plant growth, seed yield and nitrogen content. *Canadian Journal of Plant Science* 77(3):347-351.
- Callaham, D. A., and J. G. Torray. 1981. The structural basis for infection of root hairs of *Trifolium repens* by *Rhizobium*. *Canadian Journal of Botany* 59:1647-1664.
- Clay, K., and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742-1744.

- Cleyet-Marel, J. C., D. R. Bonito, and D. P. Beck. 1990. Chickpea and its root-nodule bacteria: Implication of their relationships for legume inoculation and biological nitrogen fixation. *Options Mediterraneennes, Serie-seminaires* 9:101-106.
- Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* 97:153-166.
- Diaz, E., A. Roldan, A. Lax, and J. Albaladejo. 1994. Formation of stable aggregates in degraded soils by amendment with urban refuse and peat. *Geoderma* 63:277-288.
- Dongre, A. B., M. Lodha, N. Prakash, and S. L. Mehta. 1985. Ultrastructural studies of infection mechanism of *Rhizobium* strain 6050 in groundnut mutants. *Indian Journal of Experimental Biology* 23:387-392.
- Ehrhardt, D. W., R. Wais, and S. R. Long. 1996. Calcium spiking in plant root hairs responding to *Rhizobium* nodulation signals. *Cell* 85:673-681.
- Er, F., M. Kilic, A. R. Brohi, and M. Ogut. 2004. Nodulation and growth of bean plants inoculated with genetically modified *Rhizobium etli* strains and *Azospirillum brasilense*. *Agrochimica* 48(3-4):124-131.
- Fernandez, G. C. I., and J. C. Miller. 1987. Analysis of host genotype X rhizobial strain interaction in N₂ fixation. *Euphytica* 36:903-911.
- Finck, A. [ed.]. 2005. World fertilizer use manual. Paris, France: International Fertilizer Industry Association. 600 p.
- Gallardo, F. O., E. S. Lopes, and M. C. Diez. 1996. Evaluation of different *Bradyrhizobium* sp. strains on nitrogen fixation capacity in two forage legumes. *Arquivos De Biologia Tecnollogia* 39(4):887-892.

- Gonzalez-Andres, F., and J. M. Ortiz. 1996. Specificity of rhizobia nodulating *Genista monspessulana* and *Genista linifolia* in vitro and in field situations. *Arid Soil Research and Rehabilitation* 13:223-237.
- Gwata, E. T., D. S. Wofford, K. J. Boote, and H. Mushoriwa. 2003. Determination of effective nodulation in early juvenile soybean plants for genetic and biotechnology studies. *African Journal of Biotechnology* 2(11):417-420.
- Hafeez, F. Y., N. H. Shah, and K. A. Malik. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. viciae strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biology and Fertility of Soils* 31(1):65-69.
- Hardarson, G., and C. Atkins. 2003. Optimising biological N₂ fixation by legumes in farming systems. *Plant and Soil* 252(1):41-54.
- Herdina, J., and J. H. Silsbury. 1990. The effect of reduction in the number of nodules on nodule activity of faba bean (*Vicia faba* cv. Fiord). *Annals of Botany* 65:473-481.
- Hirsch, A. M. 1992. Tansley review no. 40: Developmental biology of legume nodulation. *New Phytologist* 122:211-237.
- Hobbs, R. J., and J. A. Harris. 2001. Restoration ecology: Repairing the earth's ecosystem in the new millennium. *Restoration Ecology* 9(2):239-246.
- Hungria, M., R. J. Campo, and I. C. Mendes. 2003. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobium tropici* strains. *Biology and Fertility of Soils* 39:88-93.
- İçgen, B., G. Özcengiz, and N. G. Alaeddinoglu. 2002. Evaluation of symbiotic effectiveness of various *Rhizobium cicer* strains. *Research in Microbiology* 153:369-372.

- Laberge, G., F. Mabood, and P. Segun. 2005. Kura clover early growth is comparable to that of white clover when not nitrogen limited. *Journal of Plant Nutrition* 28:447-457.
- Legard, S. F., and K. W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* 14:137-153.
- Lodwig, E. M., A. H. F. Hosie, A. Bourdes, K. Findlay, D. Allaway, R. Karunakaran, J. A. Downie, and P. S. Poole. 2003. Amino-acid cycling drives nitrogen fixation in the legume-*Rhizobium* symbiosis. *Nature* 422:722-726.
- Major, J. 1973. Review: Nitrogen regime of alpine vegetation in Germany. *Ecology* 54(4):960-961.
- Malek, W., M. Inaba, H. Ono, Y. Kaneko, and Y. Murooka. 1998. Competition for *Astragalus sinicus* root nodule infection between its native microsymbiont *Rhizobium huakuii* bv. rene B3 and *Rhizobium* sp. ACMP18 strain, specific for *Astragalus cicer*. *Applied Microbiology and Biotechnology* 50:261-265.
- Mayz, J., F. Campos, and N. Valladares. 2003. Evaluation of rhizobial (*Rhizobium* sp.) isolates for their effects on cowpea [*Vigna unguiculata* (L.) Walp.] growth and yield. *Tropical Agriculture* 80(1):36-40.
- McKenzie, R. H., A. B. Middleton, E. D. Solberg, J. DeMulder, N. Flore, G. W. Clayton, and E. Bremer. 2001. Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Canadian Journal of Plant Science* 81(4):637-643.
- Mhadhbi, H., M. Jebara, F. Limam, T. Huguet, and M. E. Aouani. 2005. Interaction between *Medicago truncatula* lines and *Sinorhizobium meliloti* strains for

- symbiotic efficiency and nodule antioxidant activities. *Physiologia Plantarum* 124(1):4-11.
- Miller, R. F., T. J. Svejcar, and N. E. West. 1994. Implications of livestock grazing in the Intermountain sagebrush region: Plant composition. *In*: M. Vavra, W. A. Laycock and R. D. Pieper [eds.]. Ecological implications of livestock herbivory in the West. Denver, CO: Society for Range Management. p 101-146.
- Mostasso, L., F. L. Mostasso, B. G. Dias, M. A. T. Vargas, and M. Hungria. 2002. Selection of bean (*Phaseolus vulgaris* L.) rhizobial strains for the Brazilian Cerrados. *Field Crops Research* 73:121-132.
- Musiyiwa, K., S. Mpeperekwi, and K. E. Giller. 2005. Symbiotic effectiveness and host ranges of indigenous rhizobia nodulating promiscuous soybean varieties in Zimbabwean soils. *Soil Biology and Biochemistry* 37(6):1169-1176.
- Odee, D., S. Indieka, and D. Laseur. 2002. Evaluation of inoculation procedures for *Calliandra calothyrsus* Meisn. grown in tree nurseries. *Biology and Fertility of Soils* 36(2):124-128.
- Okazaki, S., K. I. Yuhashi, and K. Minamisawa. 2003. Quantitative and time-course evaluation of nodulation competitiveness of rhizobitoxine-producing *Bradyrhizobium elkanii*. *FEMS Microbiology Ecology* 45(2):155-160.
- Pellant, M., B. Abbey, and S. Karl. 2004. Restoring the Great Basin desert, U.S.A.: integrating science, management, and people. *Environmental Monitoring and Assessment* 99(1-3):169-179.

- 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.
- Requena, N., E. Perez-Solis, C. Azcon-Aguilar, P. Jeffries, and J. M. Baries. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology* 67:495-498.
- Shoushtari, N. H., and I. L. Pepper. 1985. Mesquite rhizobia isolated from the Sonoran desert: Competitiveness and survival in soil. *Soil Biology and Biochemistry* 17:803-806.
- Simms, E. L., D. L. Taylor, J. Povich, R. P. Shefferson, J. L. Sachs, M. Urbina, and Y. Tausczik. 2006. An empirical test of partner choice mechanisms in a wild legume-*Rhizobium* interaction. *Proceedings of the Royal Society B* 273:77-81.
- Sprent, J. I. 1985. Nitrogen fixation in arid environments. In: G. E. Wickens, J. R. Goodin, and D. V. Field [eds.]. *Plants for arid lands*. London, United Kingdom: Kluwer Academic Publishers. p 215-229.
- Starker, C. G., A. L. Parra-Colmenares, L. Smith, R. M. Mitra, and R. Long. 2006. Nitrogen fixation mutant of *Medicago truncatula* fail to support plant and bacterial symbiotic gene expression. *Plant Physiology* 140:671-680.
- Tilman, D. 2000. Causes, consequences and ethics of biodiversity. *Nature* 405:208-211.
- Triplett, E. W., and M. J. Sadowsky. 1992. Genetics of competition for nodulation of legumes. *Annual Reviews of Microbiology* 46:399-428.

- Trubat, R., C. Jordi, and A. Vilagrosa. 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.). *Trees – Structure and Function* 20(3):334-339.
- Udvardi, M., G. D. Price, P. M. Gresshoff, and D. A. Day. 1988. A dicarboxylate transporter on the peribacteroid membrane of soyabean nodules. *FEBS Letters* 231:36-40.
- van der Heijden, M. G. A., R. Bakker, J. Verwaal, T. R. Scheublin, M. Rutten, R. van Logtestijn, and C. Staehelin. 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiology Ecology* 56:178-187.
- Wadisirisuk, P., and R. W. Weaver. 1985. Importance of bacteroid number in nodules and effective nodule mass to dinitrogen fixation by cowpeas. *Plant and Soil* 87:223-231.
- Young, T. P. 2000. Restoration ecology and conservation biology. *Biological Conservation* 92:73-83.
- Zahran, H. H. 1999. *Rhizobium*-legume symbiosis and N₂ fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews* 63(4):968-989.
- Zhang, H., F. Daoust, T. C. Charles, B. T. Driscoll, B. Prithiviraj, and D. L. Smith. 2002. *Bradyrhizobium japonicum* mutants allowing improved nodulation and nitrogen fixation of field-grown soybean in a short season area. *Journal of Agricultural Science* 138:293-300.

CHAPTER 3

BIOMASS YIELD, SEED PRODUCTION, AND FORAGE QUALITY OF *ASTRAGALUS FILIPES*: A NATIVE LEGUME FOR RANGELANDS OF WESTERN NORTH AMERICA¹

INTRODUCTION

Many weedy species have invaded western rangelands and caused a loss of genetic diversity and functional heterogeneity (DiTomaso 2000), which has impaired ecosystem functionality and resilience (Tilman 2000). The shift in species composition from native-dominated rangeland ecosystems to ones dominated by invasive weedy species has resulted in low nutrient availability, reduced productivity, changes in fire regimes, increased soil erosion risk, and loss of biodiversity. Although the importance of using native plant species to improve degraded rangelands was recognized as early as the 1930s, introduced species have been widely used because of their low seed cost, commercial availability, and ease of establishment (Rumbaugh 1983; Shaw and Monsen 1983; Pellant and Monsen 1993; Richards et al. 1998). Shifts in social values and changes in management policies towards restoration of ecosystem functionality of degraded landscapes, however, have increased the demand for native plant species in revegetation and reclamation programs, especially on public rangelands (Roundy et al. 1995).

¹ Coauthored by Kishor Bhattarai, Douglas A. Johnson, and Thomas A. Jones

Use of legumes on rangelands and pastures enhances plant diversity, increases the quantity and quality of forage, and improves wildlife benefits (Haynes 1980; Madison and Robel 2001). Legumes fix atmospheric N in association with *Rhizobium* bacteria and provide N to other plants in the ecosystem (Becker and Crockett 1976). Presence of legumes may directly or indirectly increase the productivity of associated species in plant communities by release of symbiotically fixed N through the mycorrhizal hyphae networks, root exudates, or decaying plant materials (Patra et al. 1986; Haystead et al. 1988; Høgh-Jensen and Schjoerring 2000; van der Heijden et al. 2006). Posler et al. (1993) compared the forage yield and quality of 5 native legumes grown with 3 different grasses to that of grasses grown alone and found that legumes increased yield and protein content compared to grasses grown alone. Leguminous plants generally contain more protein and less fiber than grasses at similar stages of growth and can be an excellent food source for both wildlife and domestic livestock (Cherney and Allen 1995).

Astragalus filipes Torr. ex A. Gray (common name basalt milkvetch or threadstalk milkvetch) is a North American legume that is native to 6 western states including California, Idaho, Nevada, Oregon, Utah, and Washington as well as to northern Mexico and British Columbia in Canada (Isely 1998). Its potential genetic diversity, erect growth form, good seed production, and high density in recently burned areas make it a promising species for revegetating western rangelands (D. A. Johnson, unpublished data). The prominence of basalt milkvetch after fire may be especially important considering the increasing frequency of fire on western rangelands (Whisenant 1990) and the importance of fire as an ecosystem management tool in restoring rangeland functionality. Although most *Astragalus* species are toxic to livestock (Rumbaugh 1983;

DiTomaso 2000), no toxicity problems have been reported for basalt milkvetch. Recent chemical analyses of basalt milkvetch plant samples across its range of distribution also revealed no toxicity concerns (D. A. Johnson, unpublished data). Therefore, basalt milkvetch has considerable potential for use in rangeland revegetation and restoration programs in the western U.S.A.

Biomass yield, seed yield, forage quality, and defoliation tolerance are important characteristics for evaluating possible forage species such as basalt milkvetch (McGraw et al. 2004). Reproductive capacity is critical for maintaining plant stands (Harper 1979) and is an important attribute in conservation programs. As a result, any problems related to flowering, seed production, and seedling establishment may contribute to low species abundance and impede its potential use in revegetation and restoration. Basalt milkvetch reproduces primarily by seeds, which is typical of most *Astragalus* species (Barneby 1964). Consequently, the number of seeds a plant produces and individual seed weight are important characteristics. Seeds that have low weight typically have low predation rates, imbibe water easily, and are more readily dispersed. Also, plants with low seed weights generally produce a large number of seeds for a given amount of carbon and nutrients than plants with high seed weights (Turnbull et al. 1999; Jakobsson and Eriksson 2000). However, plants with high seed weights can exhibit high seedling vigor (Westoby et al. 2002) and emerge from a deep soil depth (Johnson 1985; Wuff 1986), which can lead to improved establishment success under severe environmental conditions (Asay and Johnson 1983; Milberg et al. 1998).

Forage quality is a function of nutrient concentration of the forage, its intake or rate of consumption, digestibility of the forage consumed, and proportion of metabolized

product that remains in the animal (Buxton and Mertens 1995). Forage quality is often estimated by in vitro or chemical methods because such techniques are less expensive and easier to conduct than actual animal evaluations (Andres et al. 2005). Many factors influence forage quality such as: forage species, stage of maturity at harvest, soil fertility, and storage method (Buxton and Mertens 1995). Other factors include: temperature during growth, soil water availability, and forage variety. As plants grow, they become more fibrous, which decreases the relative proportion of nutrients and minerals, reduces forage digestibility, and decreases forage intake in ruminants (Buxton and Mertens 1995). Important forage quality characteristics include: crude protein, acid detergent fiber (ADF), and neutral detergent fiber (NDF) (Sollenberger and Cherney 1995). Crude protein is a measure of the amount of N in forage and is calculated by multiplying N content by 6.25. NDF is a measure of total structural carbohydrate in the plant (Robinson 1999) and is partially digestible and usually negatively correlated with dry matter intake (Van Soest 1994). ADF is a measure of the least digestible plant component, especially cellulose and lignin (Jung 1997). ADF is negatively correlated with digestibility (Van Soest et al. 1991) and is often used to estimate energy content of forages.

Differences in defoliation tolerance among plant ecotypes may be due to genotypic differences (Linhart and Grant 1996). Forage ecotypes that have a high allocation to underground biomass (Volenec et al. 1996), prostrate growth form (Westoby 1989; Lavorel et al. 1999; Hazard et al. 2001), and late maturity (Smith et al. 2000) are generally considered more defoliation tolerant. Morphological variation in response to grazing was documented in various grass ecotypes in several studies (Westoby 1989; Painter et al. 1993; Hazard et al. 2001; Lauault et al. 2005). Grass ecotypes exposed to

high grazing pressure tended to be shorter with higher numbers of tillers than those exposed to low grazing pressure. This relationship was not clear in legumes, although grazing seems to favor shorter plants with leafy stems and decumbent growth habit (Lavorel et al. 1999).

The question that I asked in this study was if there were differences among 67 germplasm collections (accessions) of basalt milkvetch from California, Nevada, Utah, Idaho, Oregon, and Washington for forage and seed production, forage quality, seed weight, and morphological characteristics. I was interested in knowing the scope of variation for forage production and quality characteristics in these diverse accessions of basalt milkvetch to determine opportunities for selection in semiarid environments similar to northern Utah. In addition, I wanted to know if seed predators reduced seed yield of basalt milkvetch and if they preferred one basalt milkvetch accession over other accessions. Also, I was interested in determining which accessions had the greatest potential for inclusion in plant improvement programs.

MATERIALS AND METHODS

Based on herbarium specimen data, a total of 67 seed collections (accessions) of basalt milkvetch were made across 6 western states of the U.S.A. (California, Idaho, Nevada, Oregon, Utah, and Washington). Ripe lomentos from individual sites were placed in paper bags. A 5 to 15-g section of insecticide strip (active ingredient Dichlorvos [2, 2-dichlorovinyl dimethyl phosphate] 18.6 % and related compounds 1.4 %, Hotshot No-

Pest Strip, United Industries Corp., St. Louis, MO) was placed in each bag (based on seed volume) to minimize seed damage from associated insects. Collected lomentos were dried in a greenhouse for 2 months. Seeds were threshed using a Wintersteiger seed thresher (Model LD180, Des Moines, IA), passed through screens (Size 9K and A, Seedburo Equipment Co., Chicago, IL), and cleaned with a seed blower. Cleaned seeds were stored in a dark room maintained at 3 °C temperature and 20-25 % relative humidity until use.

The 67 seed accessions of basalt milkvetch (Table 3-1) were germinated on moistened blotter paper and transplanted to cone-shaped plastic containers (4-cm diameter, 22-cm length) filled with a sand:peat moss (3:1) mixture. The seedlings were grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory at Logan, UT (41° 45' N, 110° 48' W, 1,350 m above sea level) under a day/night temperature regime of about 30/15 °C and were watered and fertilized to maintain growth. After 1 week of growth, about 5 mm of homogenized sieved soil from all 67 collection sites was added to each container for rhizobial inoculation. In addition, a slurry of common rhizobial inoculum (*Astragalus* Special No. 1, Nitragin Co., Milwaukee, WI) was added to each container after about 3 weeks of growth to insure root nodulation. After about 90 days of greenhouse growth, seedlings were then transplanted to field plots at Evans Farm (41° 41' N and 111° 49' W, 1,350 m above sea level) and Millville (41° 39' N and 111° 48' W, 1,370 m above sea level) located about 2 km and 9 km south of Logan, UT, respectively. Climatic data indicated that 2006 was the driest and hottest year during the study period (Table 3-2). 2005 had the highest amount of precipitation during summer.

Table 3-1. Climatic and geographic information for basalt milkvetch accessions with state, elevation, latitude, longitude, mean annual maximum (max) and minimum (min) temperatures, and mean annual precipitation (precip). Means are averages across 30 years.

Accession	State	Elevation m	Latitude (N)	Longitude (W)	Temperature		Precip mm
					Max	Min	
					-----°C-----		
Af-3	NV	1,476	41° 52'	118° 35'	14	0	225
Af-4	WA	519	47° 25'	118° 41'	16	3	228
Af-5	OR	202	45° 35'	119° 56'	16	3	258
Af-6	WA	653	46° 56'	120° 15'	18	4	196
Af-7	OR	1,081	44° 22'	117° 45'	14	0	303
Af-8	OR	1,502	42° 56'	118° 36'	15	0	348
Af-9	OR	1,396	43° 07'	118° 16'	16	1	295
Af-10	NV	1,888	38° 36'	117° 50'	19	1	205
Af-11	CA	1,372	41° 28'	120° 56'	14	1	575
Af-13	OR	1,315	43° 04'	118° 46'	15	0	348
Af-14	OR	1,449	42° 48'	118° 49'	15	0	348
Af-15	CA	1,410	41° 36'	120° 25'	15	0	399
Af-16	CA	1,530	41° 53'	120° 18'	15	0	399
Af-18	OR	1,463	42° 28'	119° 47'	15	0	399
Af-19	OR	1,331	43° 26'	119° 00'	15	0	348
Af-20	OR	1,617	42° 56'	119° 54'	16	0	201
Af-21	OR	1,435	43° 10'	120° 40'	13	-1	517
Af-22	NV	2,088	41° 04'	114° 32'	15	-1	294
Af-23	OR	1,138	44° 10'	120° 43'	16	0	278
Af-24	OR	1,019	44° 09'	120° 28'	14	0	363
Af-25	OR	1,177	44° 03'	120° 45'	16	0	278
Af-26	OR	699	44° 37'	120° 20'	13	1	463
Af-28	OR	611	44° 32'	121° 16'	14	3	363
Af-29	OR	687	44° 51'	120° 49'	14	3	363
Af-30	OR	443	44° 54'	120° 27'	13	1	463
Af-30.1	OR	625	44° 55'	120° 31'	14	3	363
Af-31	OR	424	44° 53'	120° 26'	13	1	463
Af-32	OR	873	44° 55'	120° 15'	13	1	463
Af-33	OR	920	44° 54'	119° 42'	13	1	463
Af-34	OR	1,162	44° 40'	117° 37'	14	0	380
Af-36	OR	952	44° 26'	119° 03'	13	-2	398
Af-37	OR	1,152	44° 44'	119° 06'	15	1	456
Af-38	ID	1,653	44° 14'	112° 12'	13	-1	335
Af-39	ID	1,628	43° 36'	113° 20'	7	-4	612
Af-41	NV	1,774	41° 48'	115° 56'	10	-2	741
Af-42	OR	1250	43° 15'	117° 12'	16	0	367
Af-43	ID	1,717	42° 17'	115° 56'	10	-2	741
Af-44	OR	1,446	43° 08'	117° 28'	14	0	444
Af-45	ID	1,250	43° 26'	116° 58'	14	0	444
Af-45.1	ID	1,234	43° 24'	116° 59'	14	0	444

Table 3-1 (continued)

Accession	State	Elevation	Latitude (N)	Longitude (W)	Temperature		Precip
					Max	Min	
		m			-----°C-----		mm
Af-46	ID	836	44° 04'	116° 38'	18	3	253
Af-47	ID	1,700	43° 08'	116° 43'	14	0	444
Af-48	NV	2,347	39° 16'	115° 25'	17	0	275
Af-49	NV	2,545	39° 32'	114° 38'	16	0	296
Af-50	NV	2,197	39° 35'	117° 51'	13	0	388
Af-51	NV	2,376	39° 36'	117° 53'	13	0	388
Af-52	NV	2,421	39° 19'	116° 31'	17	1	213
Af-53	NV	2,258	39° 19'	117° 07'	17	1	213
Af-54	NV	2,262	39° 15'	117° 09'	17	1	213
Af-55	WA	692	47° 45'	119° 21'	15	3	290
Af-56	WA	552	47° 21'	118° 28'	16	2	367
Af-58	OR	1,055	44° 24'	120° 58'	16	0	278
Af-59	OR	1,317	42° 26'	121° 11'	15	0	429
Af-60	CA	1,711	40° 42'	120° 53'	14	1	575
Af-61	OR	1,354	43° 39'	118° 36'	13	-2	398
Af-62	OR	1,172	44° 17'	118° 57'	13	-2	398
Af-63	OR	1,501	42° 18'	120° 20'	15	0	399
Af-64	OR	1,302	42° 43'	120° 43'	13	-1	517
Af-65	NV	2,088	41° 04'	114° 32'	15	-1	294
Af-66	NV	2,499	41° 41'	117° 34'	15	1	354
Af-67	ID	2,080	43° 24'	113° 02'	14	0	265
Af-68	ID	2,201	44° 20'	113° 31'	11	-5	342
Af-69	UT	1,874	41° 54'	113° 58'	15	0	309
Af-70	OR	408	44° 54'	120° 26'	13	1	463
Af-75	OR	265	45° 37'	119° 43'	16	3	258
Af-76	OR	479	44° 54'	120° 24'	13	1	463
Af-77	OR	1,595	43° 26'	117° 55'	16	1	295

Table 3-2. Mean summer (May-August) maximum and minimum temperature and precipitation in 2004, 2005, and 2006 at Evans Farm and Millville.

Year	Temperature		Precip
	Max	Min	
	-----°C-----		mm
2004	24	12	115
2005	26	12	174
2006	28	13	74

Soil at Evans Farm is a Nibley silty clay loam series (fine, mixed, mesic Aquic Argiustoll). Soil at Millville is a coarse-loamy over sandy or sand-skeletal, mixed, superactive mesic, Calcic Haploxerolls (Ricks Series). The experimental design at Evans Farm was a randomized complete block design with 6 replications. At Millville, an insecticide treatment was also applied on 3 of the 6 replications in a split-plot design with insecticide as the whole plot factor and accessions as a subplot factor. Each plot consisted of 5 plants from each accession with a 0.5-m spacing within and between rows. The 67 accessions were randomly assigned within each replication. Plots were routinely weeded throughout the study. Plots were irrigated after transplanting only during the establishment year. Morphological measurements were obtained for each plant from both sites in both years during late May to early June.

Measurements included plant height, number of stems, number of inflorescences, and plant vigor score (a relative score with 0 for a dead plant and 9 for the largest plant). Winter mortality also was determined for each plot. Dry matter yield and forage quality were determined for plants from Evans Farm. Plants were harvested to a 5-cm stubble height at about 50 % bloom (Miller 1984) during 24-27 June 2005 and on 7 June 2006. Regrowth biomass was also harvested at Evans Farm 17 October 2005 and 18 October in 2006. At Millville, biomass was harvested on 2 August 2005 and 8 August 2006, and regrowth biomass was harvested on 26 October 2005. Negligible regrowth occurred in 2006 so regrowth biomass was not harvested in 2006. Plant samples were oven dried (60 °C for 72 h) and dry weights of each harvest were recorded. Plant samples from the June harvest at Evans Farm were ground (Clycotec 1093 Sample Mill, Sweden) to pass through a 1-mm diameter screen. Ground samples were analyzed for crude protein by

Utah State University Analytical Laboratories using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI). Acid detergent fiber (ADF) was analyzed at the Utah State Analytical Laboratories following AOAC (1990), and neutral detergent fiber (NDF) was analyzed following Goering and Van Soest (1970) as modified by Mertens (1992) from the 2006 ground sample from Evans Farm. All basalt milkvetch accessions of cluster group 3 were analyzed for ADF and NDF. For the other cluster groups, at least one basalt milkvetch accession was analyzed for ADF and NDF to minimize cost for analysis.

Seed production was evaluated at Millville. Admire 2 (a flowable insecticide with 21.4 % of active ingredient Imidacloprid, 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) was applied on 2 June 2005 (Bayer CropScience, Kansas City, MO), and Provado 1.6 (a flowable insecticide with 17.4 % active ingredient as above) was applied on 6 June 2006 for 3 of the 6 replications at Millville to compare the effect of insect predation on seed yield. An amount equivalent to 15.7 mL of Admire and 19.6 mL of Provado was applied to each replication, which allowed a constant application amount of active ingredient in each year. Irrigation was applied after insecticide application to facilitate plant absorption. Seeds were sequentially harvested from July to early August in both years after pods matured. Seeds were threshed, cleaned, and stored as mentioned earlier. Seed production was determined by weighing number of seeds produced for each plot. Seed weight of 100 seeds was also determined.

Statistical Analysis

For Evans Farm, accessions were considered a fixed factor and blocks a random factor. For Millville, insecticide and accessions were considered fixed factors and blocks a random factor. Analysis of variance (ANOVA) was conducted using Mixed Procedure of SAS V 9.0 (SAS Institute Inc., Cary, NC) for both experiments. Data were transformed (when required, table 3-3) to meet normality and homoscedasticity assumptions. Significance tests were done at $\alpha = 5\%$, and post hoc mean separation was conducted using the Tukey Test. Because insecticide was not applied by the time morphological measurements were obtained at Millville, the insecticide effect was not included in the model to analyze plant morphological data. No insecticide effect was observed for biomass yield, therefore, this effect was not included in analysis of biomass

Table 3-3. Values of the root transformation used in transferring data to meet normality and homoscedasticity criteria for field data from Evans Farm and Millville in 2005 and 2006 ('-' means no transformation, 'n/a' means no measurement).

Variable	Evans Farm		Millville	
	2005	2006	2005	2006
Plant height	0.50	-	-	0.75
Number of stems	0.25	0.50	0.50	0.50
Number of inflorescences	0.50	0.50	0.50	0.50
Plant vigor	-	0.75	1.50	0.75
June biomass	0.25	0.33	n/a	n/a
August biomass	n/a	n/a	0.50	0.50
Oct. biomass	0.25	0.33	0.75	n/a
Crude protein concentration	0.25	-0.25	n/a	n/a
Crude protein pool	0.25	0.25	n/a	n/a
Acid detergent fiber	n/a	-	n/a	n/a
Neutral detergent fiber	n/a	-	n/a	n/a
Seed yield	n/a	n/a	0.25	0.33
Seed weight	n/a	n/a	-	-

yield. For seed weight, not all plants produced seed and other plants produced very little seed. Consequently, there were not sufficient degrees of freedom to analyze weight of 100 seeds for effect of insecticide. Therefore, the insecticide effect was not included in the analysis of seed weight. Seed yield from Millville was analyzed with a split-plot analysis. Correlations were conducted using SAS, and cluster analysis was done using R (Ihaka and Gentleman 1996) from the measured variables at Evans Farm and Millville. Ward method was used to construct clusters (Romesburg 2004).

RESULTS

Forage Production

Basalt milkvetch accessions varied significantly for June biomass at Evans Farm in 2005 (Table 3-4), which ranged from 2 to 44 g plot⁻¹ (Table 3-5). Mean comparisons indicated that there was a group of accessions that had significantly greater June biomass yield than the other accessions. Although Af-5 had the highest June biomass yield (44 g plot⁻¹), it did not differ significantly from 17 other accessions Af-31 (44 g plot⁻¹), Af-76 (35 g plot⁻¹), Af-30 (31 g plot⁻¹), Af-29 (29 g plot⁻¹), Af-70 (26 g plot⁻¹), Af-18 (25 g plot⁻¹), Af-30.1 (24 g plot⁻¹), Af-75 (22 g plot⁻¹), Af-36 (20 g plot⁻¹), Af-7 (20 g plot⁻¹), Af-44 (20 g plot⁻¹), Af-26 (19 g plot⁻¹), Af-33 (19 g plot⁻¹), Af-45.1 (18 g plot⁻¹), Af-32 (18 g plot⁻¹), Af-8 (17 g plot⁻¹), Af-67 (16 g plot⁻¹), and Af-42 (16 g plot⁻¹). Most of these accessions were from Oregon, except Af-45.1 and Af-67 were from Idaho. Af-48 had the lowest June biomass yield (2 g plot⁻¹) and was collected from Nevada. There were 39

Table 3-4. Analyses of variance of basalt milkvetch accessions for summer biomass (biomass), fall regrowth (regrowth), plant height (height), number of stems (stem), number of inflorescences (inflores.), plant vigor (vigor), seed weight (seed wt.), crude protein concentration (CPC), crude protein pool (CPP) in 2005 and 2006, and acid detergent fiber (ADF), and neutral detergent fiber (NDF) in 2006; and contrast between years 2005 and 2006 (contrast) for respective variables at Evans Farm and Millville.

Factor	Evans Farm						Millville					
	2005		2006		Contrast		2005		2006		Contrast	
	d. f.	F	d. f.	F	d. f.	F	d. f.	F	d. f.	F	d. f.	F
Biomass, g plot ⁻¹	66, 328	7.62**	66, 326	5.04**	1, 4.9	190.84**	66, 316	11.89**	66, 316	10.77**	1, 5	243.63**
Regrowth, g plot ⁻¹	66, 328	7.68**	66, 332	6.5**	1, 5	122.89**	66, 322	12.9**	-	-	-	-
Height, cm	66, 328	5.33**	66, 328	2.58**	1, 5	0.46 ^{ns}	66, 322	4.12**	66, 313	3.92**	1, 5.1	0.04 ^{ns}
Stem	66, 325	8.35**	66, 327	8.32**	1, 5	336.61**	66, 323	7.65**	66, 314	9.47**	1, 5	1189.4**
Inflores.	66, 328	3.94**	66, 329	3.98**	1, 5	60.7**	66, 325	3.55**	66, 316	4.95**	1, 5.1	517.5**
Vigor	66, 327	5.59**	66, 327	3.02**	1, 5	54.76**	66, 324	3.87**	66, 312	5.41**	1, 4.9	594.2**
Seed wt., g 100 seeds ⁻¹	-	-	-	-	-	-	66, 194	4.33**	66, 251	6.97**	1, 5.1	44.24**
CPC, g kg ⁻¹ DM	66, 328	3.69	66, 323	4.42	1, 5	0.68 ^{ns}	-	-	-	-	-	-
CPP, g plant ⁻¹	66, 326	5.59	66, 323	4.19	1, 5	7.3*	-	-	-	-	-	-
ADF, g kg ⁻¹ DM	-	-	16, 48	4.47	-	-	-	-	-	-	-	-
NDF, g kg ⁻¹ DM	-	-	16, 46	4.22	-	-	-	-	-	-	-	-

ns = not significant, * = $P < 0.05$, ** = $P < 0.01$

Table 3-5. Mean, standard deviation (SD), and range of basalt milkvetch accessions for summer biomass (biomass), fall regrowth (regrowth), plant height (height), number of stems (stem), number of inflorescences (inflores.), plant vigor (vigor), seed yield, seed weight (seed wt.), crude protein concentration (CPC), crude protein pool (CPP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) in 2005, 2006, and combined across 2005 and 2006 (combined) at Evans Farm and Millville.

Measurement	Evans Farm						Millville					
	2005			2006			2005			2006		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Biomass, g plot ⁻¹	13	9	2 - 44	38	24	2 - 114	32	22	4 - 98	58	34	5 - 147
Regrowth, g plot ⁻¹	7	9	1 - 69	14	11	1 - 59	3	4	0 - 17	-	-	-
Height, cm	26	5	16 - 37	26	4	16 - 34	26	4	19 - 37	29	5	18 - 36
Stem	4	2	2 - 10	14	7	4 - 32	5	2	2 - 13	21	10	6 - 37
Inflores.	4	3	0 - 17	19	15	1 - 82	4	4	0 - 30	41	23	2 - 79
Vigor	5	1	3 - 7	4	1	2 - 5	5	1	3 - 7	4	1	2 - 7
Seed yield, g plot ⁻¹	-	-	-	-	-	-	1	2	0 - 10	3	2	0 - 5
Seed wt, g 100 seeds ⁻¹	-	-	-	-	-	-	0.4	0.1	0.2 - 0.8	0.5	0.1	0.2 - 0.7
CPC, g kg ⁻¹ DM	140	17	95 - 179	147	15	118 - 202	-	-	-	-	-	-
CPP, g plant ⁻¹	0.4	0.2	0.1 - 1.0	1.4	0.7	0.3 - 3.5	-	-	-	-	-	-
NDF, g kg ⁻¹ DM	-	-	-	420	31	361 - 461	-	-	-	-	-	-
ADF, g kg ⁻¹ DM	-	-	-	348	24	308 - 380	-	-	-	-	-	-

accessions that did not differ from Af-48 for June biomass.

October regrowth at Evans Farm differed significantly among accessions in 2005 (Table 3-4) and ranged from 1 to 69 g plot⁻¹. Generally, accessions that exhibited high June biomass also had high October regrowth. Af-75 (69 g plot⁻¹) had the highest October regrowth, but was not statistically different from Af-29 (23 g plot⁻¹), Af-31 (19 g plot⁻¹), and Af-76 (18 g plot⁻¹). Af-5, which had the highest June biomass, had a significantly lower October biomass (13 g plot⁻¹) than Af-75. Also, Af-67 was one of the accessions that had high June biomass, but had significantly lower October regrowth (4 g plot⁻¹) than Af-75 and Af-29 (Table A-1). Af-48 exhibited the lowest October regrowth producing less than 1 g plot⁻¹, but did not differ significantly from 40 other basalt milkvetch accessions.

Although accessions varied significantly in June biomass at Evans Farm in 2006 (Table 3-4), more than two thirds of the accessions did not differ statistically from each other (Table A-1). June biomass varied from 2 to 114 g plot⁻¹. Af-75 had the highest June biomass (114 g plot⁻¹). Af-31 (90 g plot⁻¹), Af-30 (89 g plot⁻¹), Af-76 (88 g plot⁻¹), Af-29 (85 g plot⁻¹), Af-5 (83 g plot⁻¹), Af-7 (76 g plot⁻¹), Af-33 (73 g plot⁻¹), and Af-18 (71 g plot⁻¹) were the other accessions with high June biomass in 2006 and did not differ significantly from each other. Af-48 was again the lowest for June biomass in 2006 (2 g plot⁻¹) and did not differ from 38 other accessions.

Although significant differences were observed among basalt milkvetch accessions for October regrowth at Evans Farm in 2006 (Table 3-4), October regrowth did not differ significantly among 23 accessions (Table A-1). October regrowth varied from 1 to 59 g plot⁻¹ with Af-29 having the greatest October regrowth (59 g plot⁻¹). Other

accessions that exhibited large regrowth biomass in 2006 were Af-31 (47 g plot⁻¹), Af-30 (44 g plot⁻¹), Af-76 (37 g plot⁻¹), Af-75 (36 g plot⁻¹), and Af-30.1 (33 g plot⁻¹). Af-5 had 20 g plot⁻¹ for October regrowth, which was not significantly different from the previously mentioned accessions. Af-48 had the lowest October regrowth (1 g plot⁻¹). In 2006, 18 other accessions did not differ from Af-48 for October regrowth. For Evans Farm, the top 10 accessions for June biomass were also the accessions with the highest October regrowth (Table A-1). Similarly, accessions with low June biomass were the lowest for October regrowth. Significant differences were observed between 2005 and 2006 for both June and October regrowth (Table 3-4). Overall, basalt milkvetch accessions produced greater biomass in 2006 than 2005 (Table 3-5).

At Millville in 2005, basalt milkvetch accessions differed significantly for August biomass (Table 3-4). August biomass varied considerably among accessions (4 to 98 g plot⁻¹). Twelve accessions had high August biomass, which did not differ statistically from each other. Af-30 had the highest August biomass (98 g plot⁻¹), whereas Af-31 (93 g plot⁻¹), Af-29 (85 g plot⁻¹), Af-76 (84 g plot⁻¹), Af-5 (83 g plot⁻¹), Af-70 (78 g plot⁻¹), Af-32 (65 g plot⁻¹), Af-30.1 (60 g plot⁻¹), Af-75 (55 g plot⁻¹), and Af-33 (46 g plot⁻¹) were the top ten accessions for August biomass in 2005 (Table A-2). Af-48 had the least August biomass (4 g plot⁻¹). Af-48 did not differ from 34 accessions for August biomass.

Basalt milkvetch accessions differed significantly for October regrowth in 2005 (Table 3-4) at Millville and ranged from 0 to 17 g plot⁻¹. Af-75 and Af-5 exhibited the greatest October regrowth (17 g plot⁻¹). Af-75 and Af-5 had significantly higher October regrowth than all accessions, except Af-30 (17 g plot⁻¹), Af-70 (14 g plot⁻¹), Af-31 (14 g plot⁻¹), Af-76 (10 g plot⁻¹), Af-29 (10 g plot⁻¹), and Af-30.1 (7 g plot⁻¹) (Table A-2). Af-

48 had no harvestable October regrowth, which was not significantly different from 47 other accessions. High correlations were observed between the June and Oct. harvests in 2005 ($r = 0.85$, $P < 0.0001$), June 2005 and June 2006 ($r = 0.98$, $P < 0.0001$), June 2005 and Oct. 2006 ($r = 0.99$, $P < 0.0001$), Oct. 2005 and June 2006 ($r = 0.80$, $P < 0.0001$), Oct. 2005 and Oct. 2006 ($r = 0.87$, $P < 0.0001$), and June 2006 and Oct. 2006 ($r = 0.97$, $P < 0.0001$).

At Millville, accessions differed significantly for August biomass in 2006 (Table 3-4) and ranged from 5 to 147 g plot⁻¹ (Table A-2). Af-31 had the highest amount of August biomass (147 g plot⁻¹), but did not differ significantly from 24 other accessions. Af-5 (61 g plot⁻¹) had a significantly lower August biomass than Af-31 and Af-30; and Af-75 had a significantly lower August biomass than Af-31, Af-30, Af-70, and Af-29. Af-55 had the lowest August biomass yield with 5 g plot⁻¹, which did not differ from 33 other accessions. August biomass differed significantly between years with greater biomass produced in 2006 than 2005 (Tables 3-4, 3-5). In addition, no significant insecticide effect was observed for biomass yield at Millville.

Forage Quality

Accessions differed significantly for crude protein concentration and crude protein pool in both 2005 and 2006 (Table 3-4). Crude protein concentration ranged from 95 to 179 g kg⁻¹ with a mean of 140 g kg⁻¹ in 2005, and ranged from 118 to 202 g kg⁻¹ with a mean of 147 g kg⁻¹ in 2006 (Table A-3). Accessions with low biomass yield had high crude protein concentration. Crude protein concentration was greatest for Af-52

(179 g kg⁻¹ DM) in 2005 and 2006; however, Af-52 did not differ from 57 other accessions in 2005 and 53 other accessions in 2006. Accessions with high biomass yields (Af-75, Af-30, and Af-5) had low crude protein concentrations in 2005. Similarly, crude protein pool for 2005 varied from 0.1 to 1.0 g plant⁻¹ with a mean of 0.4 g plant⁻¹. For 2006, crude protein pool varied from 0.3 to 3.5 g plant⁻¹ with a mean of 1.4 g plant⁻¹. Af-31 (1.0 g plant⁻¹) and Af-5 (0.9 g plant⁻¹) had the highest crude protein pool per plant in 2005, and they significantly differed from all accessions except 8 other accessions. Af-48, Af-49, and Af-50 had the lowest crude protein pool (0.1 g plant⁻¹). In 2006, Af-5 had the greatest crude protein pool (3.5 g plant⁻¹), but was not significantly different from 16 other accessions. Af-48 had the lowest crude protein pool (0.3 g plant⁻¹). Crude protein concentration did not differ significantly between 2005 and 2006 (Table 3-4), but crude protein pool was greater in 2006 than 2005 (Tables 3-4, 3-5).

Accessions varied significantly for both NDF and ADF (Table 3-4). NDF ranged from 361 to 461 g kg⁻¹ with a mean of 420 g kg⁻¹, and ADF ranged from 308 to 380 g kg⁻¹ with a mean of 348 g kg⁻¹ (Table 3-5). Af-75 and Af-76 had greater ADF than Af-20, Af-54, and Af-69. Af-20 had a significantly lower ADF than Af-18, Af-30, Af-70, Af-75, and Af-76. Af-5, Af-7, Af-13, Af-29, Af-30.1, Af-31, Af-33, Af-39, and Af-67 did not differ for ADF (Table 3-6). Af-75 and Af-76 had the highest NDF, and significantly differed from Af-20, Af-54, and Af-69. Af-20 had a significantly lower NDF than Af-18, Af-30, Af-31, Af-39, Af-70, Af-75, and Af-76. Af-5, Af-7, Af-13, Af-29, Af-30.1, Af-33, and Af-67 did not differ from other accessions for NDF. Positive correlations were observed between biomass and ADF ($r = 0.42$, $P < 0.0001$) and between biomass and NDF ($r = 0.57$, $P < 0.0001$).

Table 3-6. Mean comparisons of acid detergent fiber (ADF) and neutral detergent fiber (NDF) for the June biomass harvest of basalt milkvetch accessions in 2006 at Evans Farm.

Accession	ADF	NDF
	-----g kg ⁻¹ DM-----	
Af-5	340 abc ¹	413 abcd
Af-7	342 abc	422 abcd
Af-13	342 abc	403 abcd
Af-18	371 ab	442 abc
Af-20	308 c	361 d
Af-29	336 abc	406 abcd
Af-30	369 ab	446 ab
Af-30.1	355 abc	432 abcd
Af-31	368 abc	442 abc
Af-33	344 abc	428 abcd
Af-39	368 abc	440 abc
Af-54	311 bc	380 bcd
Af-67	324 abc	390 abcd
Af-69	311 bc	367 cd
Af-70	370 ab	448 ab
Af-75	380 a	461 a
Af-76	377 a	457 a

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Seed Yield and Seed Weight

Accessions differed significantly in seed yield at Millville, and no interaction effect was observed between insecticide treatment and accessions in both years. In 2005, the accession effect was significant, but neither the treatment effect nor treatments by accession interaction were significant (Table 3-7). The range of seed yield was 0.0 to 9.5 g plot⁻¹ in 2005. Af-5 had the highest seed yield (9.5 g plot⁻¹), which was statistically greater than 40 other accessions. Af-30 and Af-76 had the next highest seed yield with 7.0 and 6.1 g plot⁻¹, respectively (Table A-4). Other accessions with high seed yields were Af-30.1 (3.5 g plot⁻¹), Af-31 (2.9 g plot⁻¹), Af-18 (2.5 g plot⁻¹), Af-26 (2.4 g plot⁻¹),

Table 3-7. Analyses of variance of basalt milkvetch accessions for seed yield in 2005 and 2006 for accession (A), insecticide treatment (T), and interaction between accession and insecticide treatment (A X T), and contrast between years 2005 and 2006 (contrast) for accession (A) at Millville.

Source	2005		2006		Contrast	
	d. f.	F	d. f.	F	d. f.	F
A	66, 260	3.03**	66, 258	3.44**	1, 5.1	31.78**
T	1, 260	0.27 ^{ns}	1, 258	23.42**	-	-
A X T	66, 260	1.12 ^{ns}	66, 258	1.04 ^{ns}	-	-

ns = not significant, ** = $P < 0.01$

Af-75 (2.4 g plot⁻¹), and Af-70 (2.0 g plot⁻¹). Af-7, which was among the accessions with highest biomass yield, was the lowest seed producer along with Af-51. However, Af-7 and Af-51 did not differ statistically from 60 other accessions for seed yield. The accession effect and treatment effect were significant for seed yield in 2006; however, the treatment by accession effect was not significant (Table 3-7). Seed yield among accessions ranged from 0.0 to 7.5 g plot⁻¹. The insecticide treatment resulted in significantly higher seed yield than no insecticide treatment in 2006. Accessions differed in their ranking for seed yield in 2006 compared to 2005. Af-36 (6.6 g plot⁻¹) and Af-70 (5.8 g plot⁻¹) produced statistically greater seed yields. Other accessions with high seed yields were Af-31 (7.5 g plot⁻¹), Af-60 (6.9 g plot⁻¹), Af-32 (6.5 g plot⁻¹), Af-30 (7.4 g plot⁻¹), Af-8 (6.2 g plot⁻¹), Af-45.1 (5.7 g plot⁻¹), Af-37 (5.6 g plot⁻¹), and Af-5 (4.7 g plot⁻¹); however, these accessions did not statistically differ from each other. Accessions Af-31, Af-30, Af-60, Af-32, and Af-8 were statistically lower than Af-36 and Af-70. Af-36 and Af-70 did not differ significantly from 61 other accessions (Table A-4); however, they did exhibit significantly greater seed yields than Af-55, Af-46, Af-50, and Af-49.

Accessions exhibited high variation in seed yield with significantly greater seed yields in 2006 than 2005 (Tables 3-5, 3-7).

Accessions differed significantly for seed weight (weight of 100 seeds) in both 2005 and 2006 (Table 3-4) with a range from 0.30 to 0.62 g 100⁻¹ seeds in 2005 and 0.29 to 0.62 g 100⁻¹ seeds in 2006 (Table 3-5). Despite their statistical significance and their range of seed weight, most accessions did not differ statistically from each other (Table A-4). Seed weight was more closely correlated with biomass in 2005 ($r = 0.49$, $P < 0.0001$) and 2006 ($r = 0.60$, $P < 0.0001$) than with seed yield in 2005 ($r = 0.30$, $P < 0.05$) and in 2006 ($r = 0.51$, $P < 0.05$) (Table 3-8).

Plant Mortality

Considerable variation in plant mortality over winter was detected among accessions at both study sites (Fig. 3-1A, 3-1B). At Evans Farm, winter mortality in 2005 ranged from 0 to 30 % with a mean of 9 %. In 2006, winter mortality ranged from 0 to 73 % with a mean of 23 %. At Millville in 2005, winter mortality ranged from 7 to 53 % with a mean of 25 %, and in 2006 winter mortality ranged from 13 to 83 % with a mean of 35 %. Af-31 had the least mortality (no mortality in 2005 or 2006 at Evans Farm, and only 13 % mortality by the end of 2006 at Millville). Among the basalt milkvetch accessions with high biomass yields at Evans Farm, Af-30.1 had high mortality (40 %). Af-5 had 27 % mortality at Evans Farm and 30 % mortality at Millville in 2005 and 2006. Also, Af-75 had 20 % mortality at Evans Farm and 50 % mortality at Millville in 2005 and 2006. This mortality was reflected in low biomass yields, especially in 2006.

Table 3-8. Pearson correlation coefficients (r) and their associated significance levels for Millville in 2005 and 2006. Variables in the matrix include seed weight (Sw), seed yield (Sy), and August biomass (Ab).

2005			2006		
	Sw	Sy		Sw	Sy
Sw			Sw		
Sy	0.304*		Sy	0.509*	
Ab	0.488**	0.751**	Ab	0.597**	0.714**

* = $P < 0.05$, ** = $P < 0.01$

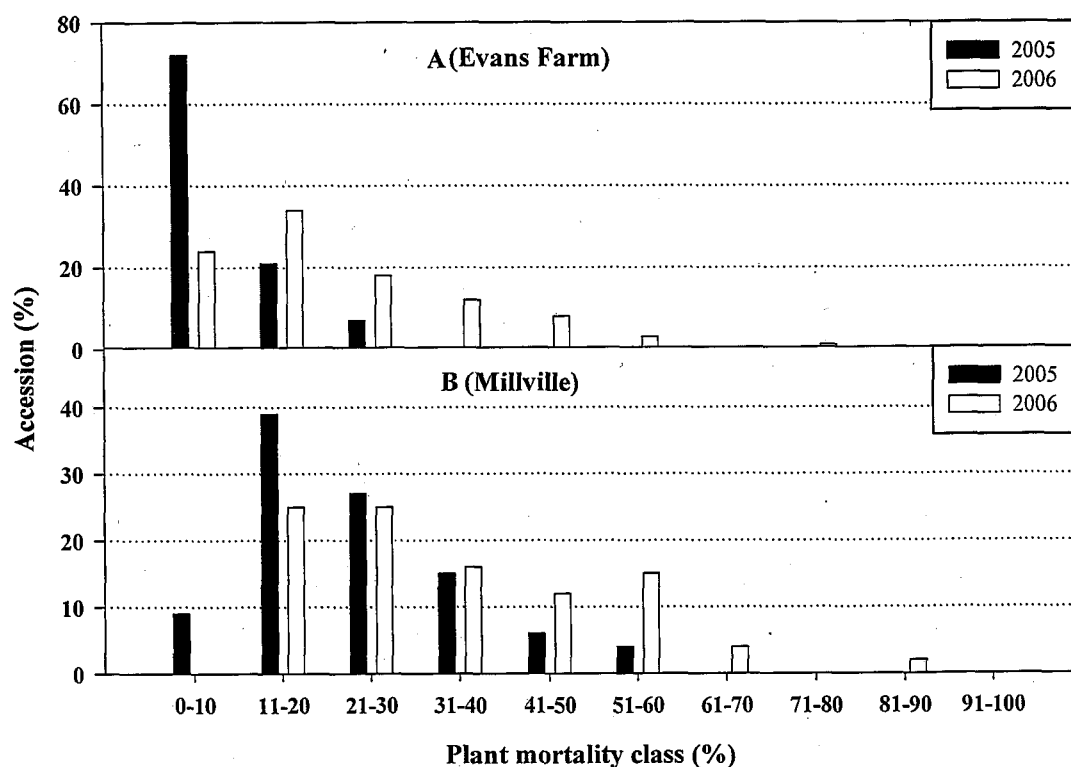


Figure 3-1. Plant mortality percentage of basalt milkvetch accessions in various plant mortality classes (% mortality of individual accession) at Evans Farm (A) and Millville (B).

Morphological Characteristics

Significant variation for plant height, number of stems, number of inflorescences, and plant vigor was detected among basalt milkvetch accessions. Plant height varied significantly at Evans Farm in both 2005 and 2006 (Table 3-4) and ranged from 16 to 37 cm in 2005 and from 16 to 34 cm in 2006 (Tables A-5, A-6). Similarly at Millville, plant height varied significantly in both years and ranged from 19 to 37 cm in 2005, and from 18 to 36 cm in 2006 (Tables 3-5, A-7, A-8). Number of stems differed significantly among accessions at Evans Farm in 2005 (range: 2 to 10) and 2006 (range: 4 to 32) (Tables 2-4, A-5, A-6). Similarly, number of stems differed significantly among accessions at Millville in 2005 (range: 2 to 13) and 2006 (range: 6 to 37) (Tables 3-4, A-7, A-8). In addition, number of inflorescences differed significantly among accessions at Evans Farm in 2005 (range: 0 to 17) and 2006 (range: 1 to 82) (Tables 3-4, A-5, A-6). Similar differences in number of inflorescences were detected at Millville in 2005 (range: 0 to 30) and 2006 (range: 2 to 79) (Tables 3-4, A-7, A-8). Accessions also differed significantly for plant vigor at Evans Farm with a range from 3.0 to 6.7 in 2005 and a range from 2.2 to 5.4 in 2006 (Tables A-5, A-6). Plant vigor also differed significantly at Millville with a range from 3.4 to 6.9 in 2005 and a range from 2.3 to 6.8 in 2006 (Tables 3-4, A-7, A-8).

At Evans Farm, Pearson's correlation coefficients between number of stems and biomass were significant in 2005 ($r = 0.87$, $P < 0.001$) and 2006 ($r = 0.86$, $P < 0.001$) (Table 3-9). Correlations between plant height and biomass were significant in 2005 ($r = 0.56$, $P < 0.001$) and 2006 ($r = 0.65$, $P < 0.001$). Also, October regrowth was correlated with number of stems in 2005 ($r = 0.53$, $P < 0.001$) and 2006 ($r = 0.70$, $P < 0.001$).

Table 3-9. Pearson correlation coefficients (r) and their associated significance levels for Evans Farm in 2005 and 2006. Variables in the matrix include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), June biomass (Jb), and October regrowth (Or).

	Ht	St	If	Vs
2005				
Ht				
St	0.474**			
If	0.510**	0.837**		
Vs	0.907**	0.618**	0.559**	
Jb	0.559**	0.865**	0.859**	0.602**
Or	0.361**	0.520**	0.528**	0.378**
2006				
Ht				
St	0.609**			
If	0.607**	0.775**		
Vs	0.880**	0.751**	0.660**	
Jb	0.651**	0.859**	0.803**	0.769**
Rb	0.521**	0.709**	0.676**	0.636**

** = $P < 0.01$

Similarly, October regrowth was correlated with plant height in 2005 ($r = 0.36$, $P < 0.001$) and 2006 ($r = 0.52$, $P < 0.001$). Number of inflorescences at Evans Farm was highly correlated with June biomass in 2005 ($r = 0.86$, $P < 0.001$) and 2006 ($r = 0.66$, $P < 0.001$). At Evans Farm, plant vigor was more strongly correlated with plant height in 2005 ($r = 0.91$, $P < 0.001$) and 2006 ($r = 0.88$, $P < 0.001$) than any other plant characteristic.

At Millville, number of stems was more strongly correlated with August biomass than plant height, seed yield, and number of inflorescence (Table 3-10). Seed yield was correlated with number of inflorescences in 2005 ($r = 0.88$, $P < 0.001$) and 2006 ($r = 0.53$, $P < 0.001$). Number of stems was correlated with seed yield in 2005 ($r = 0.70$, $P < 0.001$) and 2006 ($r = 0.46$, $P < 0.001$). Significant differences were observed between

Table 3-10. Pearson correlation coefficients (r) and their associated significant levels for Millville in 2005 and 2006. Variables in the matrix include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), August biomass (Ab), October regrowth (Or), and seed yield (Sy).

	Ht	St	If	Vs	Ab	Or
2005						
Ht						
St	0.411**					
If	0.438**	0.742**				
Vs	0.785**	0.595**	0.555**			
Ab	0.439**	0.677**	0.572**	0.496**		
Or	0.131**	0.419**	0.358**	0.183**	0.557**	
Sy	0.327**	0.67**	0.876**	0.45**	0.751**	0.317**
2006						
Ht						
St	0.558**					
If	0.634**	0.772**				
Vs	0.465**	0.554**	0.488**			
Ab	0.542**	0.732**	0.562**	0.451**		
Or	-	-	-	-		
Sy	0.359**	0.461**	0.525**	0.262**	0.714**	-

** = $P < 0.01$

2005 and 2006 for number of stems, number of inflorescences, and plant vigor at both field sites, but not for plant height (Table 3-4). Number of stems and number of inflorescences were significantly greater in 2006, but plant vigor score decreased.

Cluster Analysis

Cluster analysis was conducted to determine if accessions could be grouped based on the results of the measured variables from Evans Farm and Millville. Based on plant height, number of stems, number of inflorescences, plant vigor, June biomass, October regrowth, crude protein concentration, crude protein pool and winter mortality at Evans Farm, and seed yield, seed weight, August biomass, October regrowth, and winter

mortality at Millville, basalt milkvetch accessions clustered into 4 groups (Fig. 3-2). Group 1 had 14 accessions, Group 2 was composed of 19 basalt milkvetch accessions, Group 3 was the smallest group with 7 accessions, and Group 4 consisted of 27 accessions. Groups 1 and 2 were more similar to each other than Groups 3 and 4. Group 1 consisted of basalt milkvetch accessions from Nevada, Oregon, and Utah. Group 2 consisted of most of the accessions (70 %) from Nevada and all accessions from Washington. Group 3 was the smallest group comprising 7 accessions from north central Oregon. Group 4 consisted of all accessions from California, and some accessions from Idaho and Oregon. Five of 9 accessions from Idaho also clustered in this group. However, site characteristics of these 4 groups did not show any specific pattern of differences for elevation, mean annual maximum and minimum temperature, and precipitation (Table 3-11).

Accessions from Group 3 had the highest magnitude for all measured variables, except crude protein concentration and winter mortality (Tables A-9, A-10). Groups 1 and 2, which comprised accessions from Nevada, Washington, Utah, and some from Oregon and Idaho, had low magnitudes for all measured variables, except crude protein concentration and winter mortality.

DISCUSSION

Legumes have been shown to increase forage quality and quantity in pastures and rangelands (Rumbaugh et al. 1982, Posler et al. 1993, Cherney and Allen 1995, Aydin

Figure 3-2. Cluster dendrogram based on all measurements obtained at Evans Farm and Millville during 2005 and 2006 for 67 basalt milkvetch accessions.

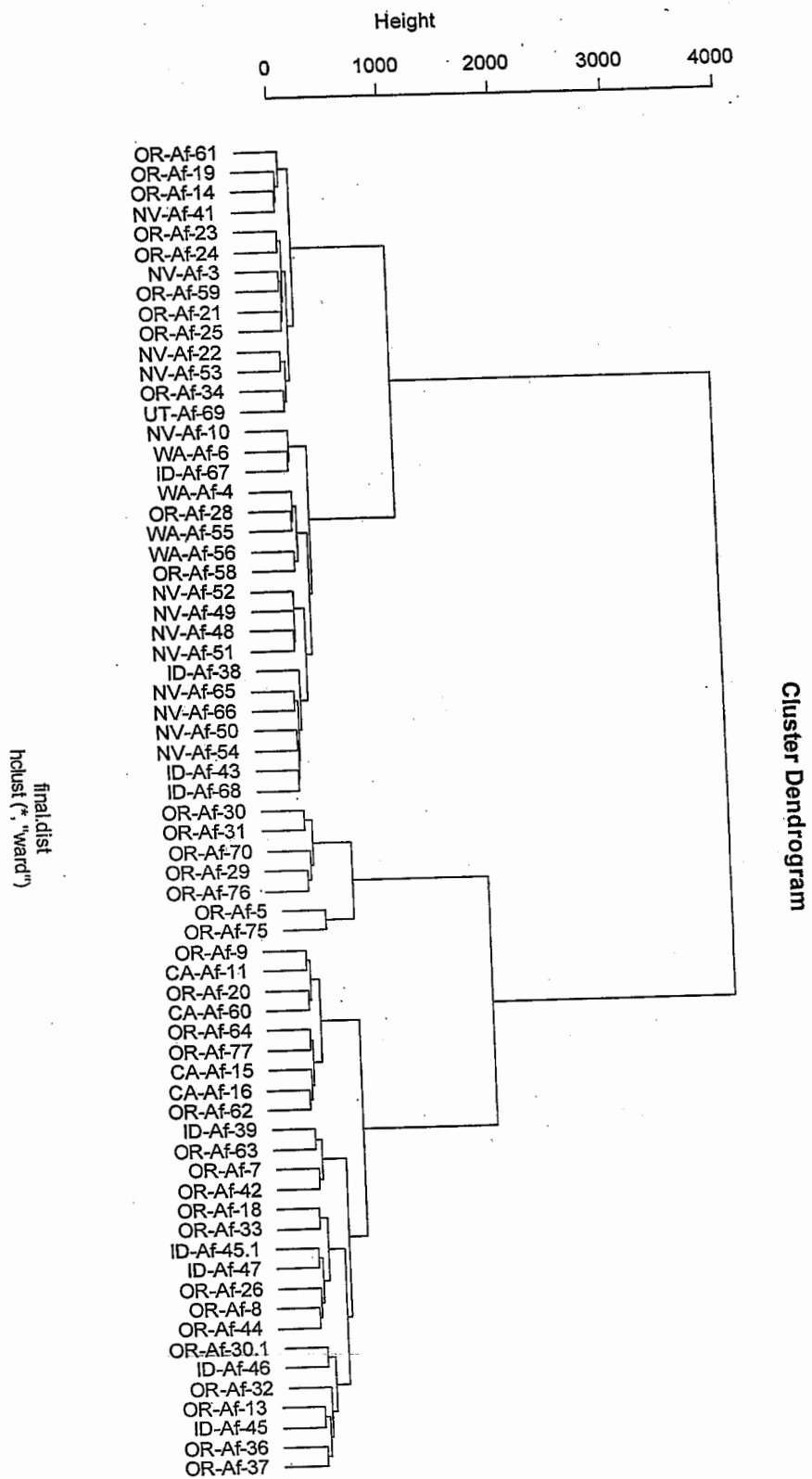


Table 3-11. Site characteristics for 4 groups of basalt milkvetch accessions separated based on cluster analysis of measured variables from Evans Farm and Millville in 2005 and 2006.

Group	Accession	Precip mm	Temperature		Elevation m
			Max	Min	
			-----°C-----		
1	Af-3, Af-14, Af-19, Af-21, Af-22, Af-23, Af-24, Af-25, Af-34, Af-41, Af-53, Af-59, Af-61, Af-69	213-741	10-17	-2-1	1,019-2,088
2	Af-4, Af-6, Af-10, Af-28, Af- 38, Af-43, Af-48, Af-49, Af- 50, Af-51, Af-52, Af-54, Af- 55, Af-56, Af-58, Af-65, Af- 66, Af-67, Af-68	196-741	10-19	-5-1	611-2,545
3	Af-5, Af-29, Af-30, Af-31, Af-70, Af-75, Af-76	258-463	13-16	1-3	202-687
4	Af-7, Af-8, Af-9, Af-11, Af- 13, Af-15, Af-16, Af-18, Af- 20, Af-26, Af-30.1, Af-32, Af- 33, Af-36, Af-37, Af-39, Af- 42, Af-44, Af-45, Af-45.1, Af- 46, Af-47, Af-60, Af-62, Af- 63, Af-64 Af-77	201-612	7-18	-4-3	201-612

and Uzun 2005). Basalt milkvetch, a native legume to the Intermountain West, may have been overlooked for revegetation/restoration programs because most *Astragalus* species are toxic to livestock and wildlife (Rumbaugh 1983; DiTomaso 2000), which would reduce its desirability for large-scale distribution and use in western North America. However, recent chemical analyses revealed that this plant is non-toxic (D. A. Johnson, unpublished data). Cheatgrass invasion in the Intermountain West has increased fire frequency (Whisenant 1990), so it is desirable to incorporate fire-tolerant native materials into land reclamation programs. From field observations, basalt milkvetch performs well

after fire, is an abundant seed producer, and competes well with cheatgrass (D. A. Johnson, unpublished data). Therefore, basalt milkvetch has many qualities that make it a good candidate species for restoration, revegetation, and reclamation programs in western North America.

Results of our experiments showed that 67 basalt milkvetch accessions collected from 6 states in the western U.S.A. varied for forage production, seed production, seed weight, forage quality, plant height, number of stems, number of inflorescences, crude protein concentration, crude protein content, acid detergent fiber, neutral detergent fiber, and plant vigor (Tables 3-4, 3-7) in the semiarid climatic conditions of northern Utah. A group of accessions from north central Oregon exhibited high biomass yields at both Evans Farm and Millville (Tables 3-1, A-1, A-2). Cluster analysis of accessions based on measured characteristics revealed 4 clusters or groups. Group 3 consisted of 7 accessions from north central Oregon, which had high biomass yields in both first and regrowth harvests in both years at both sites. These accessions also exhibited high plant height, number of stems, number of inflorescences, plant vigor, crude protein pool, and seed yield. They, however, had the lowest crude protein concentration because of their large plant size. Among these high yielding accessions, Af-75 had the highest total biomass yield across 2 years. In contrast, collections from Nevada, Washington, and Utah had lower biomass and seed yields.

Some basalt milkvetch accessions exhibited plant mortality from the October regrowth harvest to growth initiation the following spring. In our study, Af-31 exhibited no winter mortality at Evans Farm and 13 % mortality at Millville. Accessions generally exhibited greater mortality at Millville (35 %) than at Evans Farm (23 %) (Fig. 3-1). This

may be due to a shorter time interval between the harvests at Millville (about 2 months) compared to Evans Farm (4 months). Late harvest was reported as a cause of significant winter mortality in Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacMill. ex. B. L. Rob & Fernald) (DeHaan et al. 2003). This suggests that accessions such as Af-31 may be beneficial in plant improvement programs because of its low winter mortality, but high biomass and seed yields.

In our study, differences were detected between 2005 and 2006 for biomass production for the June and October harvests at Evans Farm and for the August harvest at Millville (Table 3-4). Basalt milkvetch accessions produced greater biomass in 2006 compared to 2005 (Table 3-5). Biomass was harvested later in the summer at Millville (August) than Evans Farm (June) because seed was harvested at Millville. This allowed late-season biomass comparisons after seed harvest. Results from Millville indicated that accessions with high biomass yields at the August harvest also exhibited high yields at the October harvest. The short (50-day) time period between the August and October harvests at Millville resulted in only negligible October regrowth for some accessions (such as Af-48), which exhibited the lowest biomass yield for both harvests in 2005 and 2006 at Evans Farm and for both harvests in 2005 at Millville.

Many variables such as temperature, drought, nutrient availability, growth stage, plant genetics, and herbivory influence forage quality (Buxton and Mertens 1995). Because all accessions were grown in a common garden, differences in forage quality among these accessions could be due to differences in plant maturity. Because plants were harvested at about 50 % bloom (Miller 1984), some accessions may have been at a different maturity stage than others. Our results confirmed that forage quality was

inversely correlated with biomass yield, which has been found in other studies (White and Wight 1984; van der Wal et al. 2000). In our study, crude protein concentration was negatively correlated with biomass yield in 2005 ($r = -0.23$, $P < 0.01$), but biomass yield was positively correlated with ADF ($r = 0.42$, $P < 0.0001$) and NDF ($r = 0.57$, $P < 0.0001$). High values of ADF and NDF in forage indicates low forage quality (Buxton and Mertens 1995). In 2005 at Evans Farm, crude protein concentration ranged from 98 to 179 g kg⁻¹ DM with a mean of 140 g kg⁻¹ DM for the June harvest. In 2006, crude protein concentration ranged from 118 to 202 g kg⁻¹ DM with a mean of 147 g kg⁻¹ DM. This difference may be due to more effective legume-*Rhizobium* symbiosis or greater effectiveness at scavenging N with a more expansive root system in 2006. When averaged across both years, crude protein concentration was 144 g kg⁻¹ DM, which is higher than many legumes native to north central U.S.A. (McGraw et al. 2004). For example, 13 of the 15 legumes had lower crude protein concentration (mean of 128 g kg⁻¹ DM) than basalt milkvetch (McGraw et al. 2004).

In our study, basalt milkvetch accessions varied for ADF and NDF, and accessions with low biomass generally had low ADF and NDF. Greater ADF indicates lower digestibility (Van Soest et al. 1991), and greater NDF indicates low dry matter intake (Van Soest 1994). Basalt milkvetch accessions with low NDF and ADF likely would provide forage with a high proportion of nutrients to herbivores. Higher ADF and NDF in plants with high biomass are quite common because as plants grow larger, the leaf to stem ratio typically decreases, and more structural tissues are formed to support the plant. DeHaan et al. (2003) reported that NDF and leaf fraction were negatively correlated in Illinois bundleflower in Minnesota. High ADF and NDF in Af-75 were

associated with high biomass in our study. In general, NDF was lower in basalt milkvetch (mean = 420 g kg⁻¹) than NDF for 15 native legumes from north central U.S.A. (mean = 571 g kg⁻¹ DM) (McGraw et al. 2004). Values of ADF for the 15 native legumes (mean = 416 g kg⁻¹) were higher than values for basalt milkvetch (mean = 348 g kg⁻¹). Consequently, our results indicated that basalt milkvetch produces a good quality forage. Basalt milkvetch accessions with high biomass yields had high crude protein pools and ADF and NDF values, but low crude protein concentrations. When the amount of forage and total available crude protein are critical, selection of accessions with high biomass yield would be beneficial (van der Wal et al. 2000) to provide greater amounts of total protein to herbivores than low yielding accessions with high crude protein content.

When plants produce low-weight seeds, a greater number of seeds can be produced for the given amount of carbon and nutrient resources compared to production of heavier seeds (Turnbull et al. 1999; Jakobsson and Eriksson 2000). However, heavier seeds within a species typically have high seedling vigor (Westoby et al. 2002), are able to emerge from a deep soil depth (Johnson 1985), and have a greater chance of successful establishment under severe environmental conditions (Asay and Johnson 1983; Milberg et al. 1998; Jakobsson and Eriksson 2000). Although seed weights varied significantly among basalt milkvetch accessions, most accessions did not differ significantly from each other. Among accessions with high seed yields, Af-30.1 had significantly heavier seeds than Af-5 and Af-75 in 2006, but not in 2005. Similar results were found with seed weight among ecotypes of sweetvetch (*Hedysarum boreale* Nutt.) (Johnson et al. 1989). In the present study, seed weight in basalt milkvetch accessions was positively correlated with biomass yield in 2005 ($r = 0.49$, $P < 0.0001$) and 2006 ($r = 0.60$, $P < 0.0001$), which

was similar to results for *Viola grypoceras* A. Gray (Sakai and Sakai 1996). Seed yields in our study varied significantly among basalt milkvetch accessions, and accessions with high seed yield generally had high biomass yield. In 2005, Af-5 had the highest seed yield per plot, but Af-5 produced less seed in 2006 than 2005. A similar trend was observed for Af-75. This could be due to high winter mortality with 50 % for Af-75 and 33 % for Af-5.

Pre-dispersal predation of seeds can significantly limit species recruitment by reducing seed production (Louda et al. 1990). A significant pre-dispersal seed predation of basalt milkvetch (more than 80 % in 1980 and 60 % in 1981) was documented in field sites in Oregon (Youtie and Miller 1986). Our study at Millville with Imidacloprid systemic insecticide, confirmed their findings and showed that plants treated with Imidacloprid produced significantly higher seed amounts than non-treated control treatments in 2006 (Tables 3-5, 3-7). However, a significant treatment effect was not detected in 2005. This could be because seed predators may not have colonized our field plots sufficiently high in 2005, given that no naturalized populations of basalt milkvetch occurred in nearby areas. Another reason may be the low seed yields that occurred in 2005 (38 % of the seed yield in 2006). Our study also showed that insect predation was independent of accessions, given a nonsignificant interaction between insecticide and accessions.

In our study, number of stems was closely related to biomass yield, which was closely correlated with seed yield. These correlations suggest that selecting plants with many stems would generally result in plants with high biomass and high seed yield in

basalt milkvetch. Numbers of stems would be a fast, easy way to select accessions with high biomass and seed yield.

We observed in our study that basalt milkvetch has the ability to develop new shoots from lateral roots, which is not typical of most *Astragalus* species (Barneby 1964). Creeping rootedness has been reported for alfalfa (*Medicago sativa* L.) and is believed to increase sward persistence under grazing (Pecetti and Piano 2002; Pecetti et al. 2004). Alfalfa accessions that exhibit creeping rootedness have latent stem apices on the roots (Pecetti and Piano 2002). This characteristic was not consistently observed in any particular accession of basalt milkvetch, but was noticed on several occasions. We also observed that when the main crown died in some basalt milkvetch accessions, plant shoots were able to regenerate by developing new crowns below the previous one. Further investigation of this characteristic in basalt milkvetch populations may be useful in plant improvement programs for this species.

MANAGEMENT IMPLICATIONS

Changes in rangeland botanical composition due to anthropogenic activities has increased fire frequency in the Great Basin (Whisenant 1990) and threatens the ecological and economical sustainability of the region (Miller et al. 1994; Pellant et al. 2004). Restoring native vegetation on degraded rangelands can restore ecosystem functionality (Roundy et al. 1995), and thus make rangeland ecosystem services sustainable. Arid and semiarid rangelands and pastures are usually N limited, and legumes can increase forage

production and forage quality on these lands (Posler et al. 1993). Only a few native legumes are available for restoring the Intermountain West (Johnson et al. 1989; Pyke et al. 2003). Basalt milkvetch is one North American legume that is widely distributed (Isely 1998) and may have potential for rangeland revegetation and restoration efforts. Although most *Astragalus* species are toxic to livestock and wildlife (Rumbaugh 1983; DiTomaso 2000), no instances of toxicity have been reported for basalt milkvetch. This species exhibits a flush of growth after fire, has good forage and seed production, has high forage quality, and is able to compete with cheatgrass. The results of my study indicated that accessions Af-5, Af-29, Af-30, Af-31, Af-70, Af-75, and Af-76 have considerable potential for use in plant improvement programs to provide plant materials for revegetation and restoration of degraded rangelands in areas similar to northern Utah in the Intermountain West. However, accessions from Nevada, Washington, Utah, and some accessions from Idaho and Oregon showed poor biomass and seed yield, suggesting that they may not be good to incorporate in plant improvement programs to maximize forage and seed yields.

LITERATURE CITED

- Andres, S., F. J. Giraldez, S. Lopez, A. R. Mantecon, and A. Calleja. 2005. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance

- spectroscopy. 1. Prediction of chemical composition and in vitro digestibility. *Journal of Science of Food and Agriculture* 85:1564-1571.
- AOAC. 1990. Official methods of analysis. Arlington, VA: Association of Official Analytical Chemists. 1141 p.
- Asay, K. H., and D. A. Johnson. 1983. Genetic variability for characters affecting stand establishment in crested wheatgrass. *Journal of Range Management* 36(6):703-706.
- Aydin, I., and F. Uzun. 2005. Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. *European Journal of Agronomy* 23(1):8-14.
- Barneby, R. C. 1964. Atlas of North American *Astragalus*. *Memoirs of the New York Botanical Garden* 13:1-1188.
- Becker, D. A., and J. J. Crockett. 1976. Nitrogen fixation in some prairie legumes. *American Midland Naturalist* 96:133-143.
- Buxton, D. R., and D. R. Mertens. 1995. Quality-related characteristics of forages. In: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: The science of grassland agriculture. Volume II. Ames, IA: Iowa State University Press. p 83-96.
- Cherney, J. H., and V.G. Allen. 1995. Forages in a livestock system. In: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: The science of grassland agriculture. Volume I. Ames, IA: Iowa State University Press. p 175-188.
- DeHaan, L. R., N. J. Ehlke, C. C. Sheaffer, R. L. DeHaan, and D. L. Wyse. 2003. Evaluation of diversity among and within accessions of Illinois bundleflower. *Crop Science* 43:1528-1537.

- DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. *Weed Science* 48(2):255-256.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Washington, DC: USDA-ARS Agric. Handbook 379. p 1-20.
- Harper, K.T. 1979. Some reproductive and life history characteristics of rare plants and implications for management. The endangered species: A symposium. *Great Basin Naturalist Memoirs* 3:129-137.
- Haynes, R. J. 1980. Competitive aspects of the grass-legume association. *Advances in Agronomy* 33:227-261.
- Haystead, A., N. Malajczuk, and T. S. Grove. 1988. Underground transfer of nitrogen between pasture plants infected with vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 108(4):417-423.
- Hazard, L., D. Barker, and S. Easton. 2001. Morphogenetic adaptation to defoliation and soil fertility in perennial ryegrass. *New Zealand Journal of Agricultural Research* 44:1-12.
- Hogh-Jensen, H., and J. K. Schjoerring. 2000. Below-ground nitrogen transfer between different grassland species: Direct quantification by N-15 leaf feeding compared with indirect dilution of soil N-15. *Plant and Soil* 227(1-2):171-183.
- Ihaka, R., and R. Gentleman. 1996. R: a language for data analysis and graphics. *Journals of Computational and Graphical Statistics* 5:299-314.
- Isely, D. 1998. Native and naturalized Leguminosae (Fabaceae) of the United States. Provo, UT: Brigham Young University. 1007 p.

- Jakobsson, A., and O. Eriksson. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos* 88:494-502.
- Johnson, D. A. 1985. Plant establishment characteristics for successful revegetation of semiarid rangeland: Crested wheatgrass, a case study. *Arid Zone Research* 3:149-200.
- Johnson, D. A., T. M. Ford, M. D. Rumbaugh, and B. L. Richardson. 1989. Morphological and physiological variation among ecotypes of sweetvetch (*Hedysarum boreale* Nutt.). *Journal of Range Management* 42(6):496-501.
- Jung, H. -J. G. 1997. Analysis of forage fiber and cell walls in ruminant nutrition. *Journal of Nutrition* 127:810S-813S.
- Lauault, F., V. D. Pillar, J. Aufrere, E. Garnier, and J. -F. Soussana. 2005. Plant traits and functional types in response to reduced disturbance in a semi-natural grassland. *Journal of Vegetation Science* 16:151-160.
- Lavorel, S., S. McIntyre, and K. Grigulis. 1999. Plant response to disturbance in a Mediterranean grassland: How many functional groups? *Journal of Vegetation Science* 10:661-672.
- Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27:237-277.
- Louda, S. M., M. A. Potvin, and S. K. Collinge. 1990. Predispersal seed predation, post dispersal seed predation and competition in the recruitment of seedlings of a native thistle in sandhills prairie. *American Naturalist* 124(1):105-113.

- Madison, L. A., and R. J. Robel. 2001. Energy characteristics and consumption of several seeds recommended for northern bobwhite food plantings. *Wildlife Society Bulletin* 29:1219-1227.
- McGraw, R. L., F. W. Shockley, J. F. Thompson, and C. A. Roberts. 2004. Evaluation of native legume species for forage yield, quality and seed production. *Native Plants Journal* 5(2):152-159.
- Mertens, D. R. 1992. Critical conditions in determining detergent fiber. Denver, CO: Proceedings of NFTA forage analysis workshop. p C1-C8.
- Milberg P., M. A. Perez-Fernandez, and B. B. Lamont. 1998. Seedling growth response to added nutrient depends on seed size in three woody genera. *Journal of Ecology* 86:624-632.
- Miller, D. A. 1984. Forage crops. New York, NY: McGraw-Hill Inc. 530 p.
- Miller, R. F., T. J. Svejcar, and N. E. West. 1994. Implications of livestock grazing in the Intermountain sagebrush region: Plant composition. *In*: M. Vavra, W. A. Laycock and R. D. Pieper [eds.]. Ecological implications of livestock herbivory in the west. Denver, CO: Society for Range Management. p 101-146.
- Painter, E. L., J. K. Detling, and D. A. Steingraeber. 1993. Plant morphology and grazing history-relationships between native grasses and herbivores. *Vegetatio* 106:37-62.
- Patra, D. D., M. S. Sachdev, and B. V. Subbiah. 1986. ^{15}N studies on the transfer of legume-fixed nitrogen to associated cereals in intercropping systems. *Biology and Fertility of Soils* 2(3):165-171.

- Pecetti, L., and E. Piano. 2002. Penetrance of creeping-rootedness in clonal progenies of lucerne and observations on underground morphology of plants differing for this character. *Euphytica* 128(1):35-45.
- Pecetti, L., E. Piano, and M. Romani. 2004. Effect of various plant factors on the expression of creeping-rootedness in lucerne (*Medicago sativa* L. complex). *Euphytica* 135(1):89-97.
- Pellant, M., B. Abbey, and S. Karl. 2004. Restoring the Great Basin Desert, U.S.A.: Integrating science, management, and people. *Environmental Monitoring and Assessment* 99:169-179.
- Pellant, M., and S. Monsen. 1993. Rehabilitation on public rangelands in Idaho, USA: A change in emphasis from grass monocultures. *In*: D. Eldridge and D. Freudenberger [eds.]. People and rangelands building the future. Queensland, Australia: Proceedings of the XVII International Rangeland Congress. p 778-779.
- 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.
- Pyke, D. A., T. O. McArthur, K. S. Harrison, and M. Pellant. 2003. Coordinated Intermountain restoration project-fire, decomposition and restoration. *In*: N. Allsopp, A. R. Palmer, S. J. Milton, K. P. Kirkman, G. I. H. Kerley, C. R. Hurt, and C. J. Brown [eds.]. Durban, South Africa: Proceedings of the VIIIth International Rangeland Congress. p 1116-1124.
- Richards, R. T., J. C. Chambers, and C. Ross. 1998. Use of native plants on Federal lands: Policy and practice. *Journal of Range Management* 51:625-632.

- Robinson, P. H. 1999. Neutral detergent fiber (NDF) and its role in alfalfa analysis. *In*: Proceedings, 29th California Alfalfa Symposium. Davis, CA: University of California. p 60-67.
- Romesburg, H. C. 2004. Cluster analysis for researchers. Lulu Press Inc., Morrisville, NC. p 129-135.
- Roundy, B. A., E. D. McArthur, J. S. Haley, and D. K. Mann. [eds.]. 1995. Proceedings of the wildland shrub and arid land restoration symposium. Ogden, UT: USDA Forest Service, Gen. Rep. INT-GTR-315. 384 p.
- Rumbaugh, M. D. 1983. Legumes- their use in wildland plantings. *In*: S. B. Monsen and N. Shaw (compilers). Managing Intermountain rangelands-Improvement of range and wildlife habitats. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-157. p 115-122.
- Rumbaugh, M. D., D. A. Johnson, and G. A. van Epps. 1982. Forage yield and quality in a great basin shrub, grass and legume pasture experiment. *Journal of Range Management* 35:604-609.
- Sakai, S., and A. Sakai. 1996. Why is there variation in mean seed size among plants within single populations? Test of the fertilization efficiency hypothesis. *American Journal of Botany* 83(11):1454-1457.
- Shaw, N., and S. B. Monsen. 1983. Nonleguminous forbs for rangeland sites. *In*: S. B. Monsen and N. Shaw (compilers). Managing Intermountain rangelands-Improvement of range and wildlife habitats. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-157. p 123-131.

- Smith, S. E., R. Mosher, and D. Fendenheim. 2000. Seed production in sideoats grama populations with different grazing histories. *Journal of Range Management* 53:550–555.
- Sollenberger, L. E., and D. J. R. Cherney. 1995. Evaluating forage production and quality. *In*: R. F. Barnes, D. A. Miller, and C. J. Nelson [eds.]. Forages: The science of grassland agriculture. Volume II. Ames, IA: Iowa State University Press. p 97-110.
- Tilman, D. 2000. Causes, consequences and ethics of biodiversity. *Nature* 405:208-211.
- Turnbull, L. A., M. Rees, and M. J. Crawley. 1999. Seed mass and the competition/colonization tradeoff: A sowing experiment. *Journal of Ecology* 87:899-912.
- van der Heijden, M. G. A., R. Bakker, J. Verwaal, T. R. Scheublin, M. Rutten, R. van Logtestijn, and C. Staehelin. 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiology Ecology* 56:178-187.
- van der Wal, R. S., C. van Lieshout, C. Dormann, R. Langvatn, and S. D. Albon. 2000. Trading forage quality for quality? Plant phenology and patch choice by Svalbard reindeer. *Oecologia* 123(1):108-115.
- Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Ithaca, NY: Cornell University Press. 528 p.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74:3583-3597.

- Volenc, J. J., A. Ourry, and B. C. Joern. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. *Physiologia Plantarum* 97:185-193.
- Westoby, M. 1989. Selective forces exerted by vertebrate herbivores on plants. *Trends in Ecology and Evolution* 4:115-117.
- Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33:125-129.
- Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River Plains: Ecological and management implications. In: E. D. McArthur, E. M. Romney, S. D. Smith, and P. T. Tueller [eds.]. Proceedings- Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-276. p 4-7.
- White, L. M., and J. R. Wight. 1984. Forage yield and quality of dryland grasses and legumes. *Journal of Range Management* 37(3):233-236.
- Wuff, R. D. 1986. Seed size variation in *Desmodium paniculatum*. II. Effects on seedling growth and physiological performance. *Journal of Ecology* 74:99-114.
- Youtie, B. A., and R. F. Miller. 1986. Insect predation on *Astragalus filipes* and *A. purshii* seeds. *Northwest Science* 60(1):42-46.

CHAPTER 4

SUMMARY

Thousands of hectares of rangelands need to be revegetated each year due to wildfire and to improve degraded rangelands in the Intermountain West of U.S.A. With preference increasing towards the use of native plants in revegetating degraded rangelands and thus regenerating ecosystem function, demand for native plants has increased. However, difficulty in purchasing commercially available seed, establishing many native plants (especially forbs), and livestock toxicity of many native legumes (Rumbaugh 1983; DiTomaso 2000) are major obstacles in using them in rangeland revegetation and restoration programs in the Intermountain West. Release of germplasm or cultivars of native plants with desirable characteristics can bridge the existing gap between supply and demand of native plant materials for use in improving degraded rangelands in the Intermountain West of U.S.A. Evaluation of various populations of plants in common gardens allows comparison of genotypic and phenotypic differences among plant populations. Identification of desirable characters is an important step for developing suitable germplasm or cultivars for revegetation, reclamation, and restoration purposes. My studies were conducted to evaluate and identify the best populations of a non-toxic North American legume (basalt milkvetch) for biomass and seed production. Because associated rhizobial strains are needed for maximizing N fixation, my studies also evaluated the infectiveness and effectiveness of rhizobial strains for N fixation in basalt milkvetch.

In Chapter 2, results from two greenhouse experiments were discussed that related to the evaluation of rhizobial strains and N fixation in basalt milkvetch. Greenhouse Experiment A showed no interaction between rhizobial treatments and basalt milkvetch accessions for N fixation, suggesting that the identified rhizobial strain would be best across basalt milkvetch accessions. In Greenhouse Experiment A, rhizobial strain I-49 was the best rhizobial strain that produced plants with the greatest nodule weights, and number of nodules. In Greenhouse Experiment B, I-49 was also the best rhizobial strain for shoot N pool, total N pool, and number of nodules, except that I-48 was not significantly different from I-49. As a result, I-49 was the most suitable rhizobial strain for basalt milkvetch. Also, nodule weight was a better indicator of N fixation than number of nodules because nodule weight was more closely related to total N pool and total biomass than number of nodules. A high positive correlation between total N pool and total biomass in Greenhouse Experiment B indicated that growth of basalt milkvetch can be increased by providing infective and effective rhizobial strains.

In Chapter 3, experiments were described that involved the evaluation of 67 basalt milkvetch accessions for forage and seed production and forage quality at two field sites (Evans Farm and Millville) in 2005 and 2006. The study at Evans Farm showed that accessions from north central Oregon exhibited high biomass yield in summer and fall in both years. Accessions from Nevada, Washington, and Utah, and some accessions from Oregon and Idaho showed poor biomass production in both summer and fall. Forage quality was measured by crude protein concentration, neutral detergent fiber (NDF), and acid detergent fiber (ADF). In general, basalt milkvetch accessions with low biomass had high crude protein concentration. ADF and NDF were positively correlated with biomass

yield. Accessions with high and low biomass differed little for crude protein concentration, ADF, and NDF. Accessions with high biomass yields (Af-5, Af-29, Af-33) did not differ from accessions with low biomass yields (Af-20, Af-54, Af-69) for ADF and NDF. Also, accessions with high biomass yield had a high crude protein pool. At Millville, accessions from north central Oregon also showed high biomass and seed yield. Seed weight per 100 seeds varied among basalt milkvetch accessions, and positive correlations of seed weight and biomass indicated that accessions with high biomass yields had a tendency to produce heavier seeds. Significant seed predation was observed in 2006 but not in 2005, which could be due to the high seed yields and a greater number of seed predators in 2006. No significant interactions between basalt milkvetch accessions and insecticide treatment indicated that seed predators did not prefer one basalt milkvetch accession over another. High correlations between number of stems and biomass and seed yield indicated that number of stems is a good predictor of high biomass and seed production. Greater winter mortality was observed at Millville than Evans Farm and may be due to a shorter regrowth interval at Millville than Evans Farm. Plant height did not vary between 2005 and 2006, but number of stems, number of inflorescences, biomass, and seed yields were greater in 2006 than 2005.

Basalt milkvetch is a promising native legume species for use in revegetation, reclamation, and restoration of rangelands in western North America. Greenhouse studies showed that I-49 rhizobial strain was the best strain for maximizing biological N fixation in basalt milkvetch. Field studies showed that accessions from north central Oregon produced the greatest biomass yields of all basalt milkvetch accessions. Consequently, I-

49 rhizobial strain and basalt milkvetch accessions from north central Oregon have the most promise for subsequent plant improvement efforts for this species.

LITERATURE CITED

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management.

Weed Science 48(2):255-256.

Rumbaugh, M.D. 1983. Legumes- their use in wildland plantings. *In*: S. B. Monsen and N. Shaw (compilers). Managing Intermountain rangelands-Improvement of range and wildlife habitats. Ogden, UT: USDA Forest Service, Gen. Tech. Rep. INT-157. p 115-122.

APPENDIXES

APPENDIX A: SUPPLEMENTAL TABLES

Table A-1. Mean comparisons of June biomass and October regrowth of basalt milkvetch accessions from Evans Farm in 2005 and 2006.

Accession	2005		2006	
	June	Oct.	June	Oct.
	-----g plot ⁻¹ -----			
Af-3	11.8 cdefghijklmno ¹	4.6 cdefghijklm	30.5 abcdefghijk	11.3 bcdefghijk
Af-4	3.3 no	1.6 lm	10.5 abcdefghijk	4.0 ghijkl
Af-5	43.8 a	13.0 bcdefgh	83.0 ab	19.7 abcdefghijk
Af-6	9.4 efghijklmno	5.1 cdefghijklm	29.1 abcdefghijk	10.7 efghijk
Af-7	19.9 abcdefgh	12.8 bcdefg	75.8 abcdefg	24.6 abcdefg
Af-8	17.0 abcdefghijk	5.9 bcdefghijkl	55.8 abcdefghij	18.7 abcdefghij
Af-9	9.0 efghijklmno	5.1 cdefghijklm	36.9 abcdefghijk	16.2 bcdefghijk
Af-10	8.0 efghijklmno	3.9 defghijklm	24.9 abcdefghijk	10.7 bcdefghijk
Af-11	9.1 efghijklmno	6.2 bcdefghijkl	27.9 abcdefghijk	12.6 bcdefghijk
Af-13	13.7 bcdefghijklm	4.5 bcdefghijklm	34.0 abcdefghijk	9.6 cdefghijk
Af-14	9.6 defghijklmno	2.8 efghijklm	24.3 abcdefghijk	6.5 efghijk
Af-15	10.6 defghijklmno	3.9 cdefghijklm	25.7 abcdefghijk	8.6 defghijk
Af-16	11.7 bcdefghijklmn	4.0 cdefghijklm	32.0 abcdefghijk	10.1 cdefghijk
Af-18	24.8 abcdef	12.3 bcdefghi	70.7 abcdefg	24.1 abcdefg
Af-19	9.5 defghijklmno	2.9 efghijklm	32.1 abcdefghijk	5.4 fghijkl
Af-20	9.8 defghijklmno	2.3 efghijklm	20.0 efghijk	5.9 efghijkl
Af-21	11.7 cdefghijklmno	3.1 efghijklm	31.3 abcdefghijk	6.5 fghijkl
Af-22	9.5 defghijklmno	5.1 bcdefghijklm	36.1 abcdefghijk	12.6 bcdefghijk
Af-23	8.9 efghijklmno	4.1 bcdefghijklm	19.3 defghijk	9.6 cdefghijk
Af-24	6.9 fghijklmno	4.3 cdefghijklm	21.3 fghijk	12.5 bcdefghijk
Af-25	6.0 ghijklmno	4.0 cdefghijklm	30.2 abcdefghijk	8.0 defghijk
Af-26	19.0 abcdefghi	8.4 bcdefghijk	41.5 abcdefghij	18.7 abcdefghij
Af-28	5.0 klmno	1.9 klm	6.4 ijk	4.4 ghijkl
Af-29	28.7 abcdef	23.2 ab	85.1 abcdef	59.0 a
Af-30	30.8 abcd	17.8 bcd	89.1 abcd	44.2 abc
Af-30.1	24.4 abcdefgh	14.4 bcdef	41.0 abcdef	33.1 abcdefg
Af-31	39.6 ab	19.1 abc	89.8 abcde	47.3 ab
Af-32	18.0 abcdefghijk	5.5 bcdefghijkl	35.4 abcdefghij	18.1 abcdefghij
Af-33	19.0 abcdefghij	9.5 bcdefghijk	73.3 abcdef	23.7 abcdefgh
Af-34	7.3 fghijklmno	6.0 bcdefghijklm	37.5 abcdefghijk	19.3 abcdefghijk
Af-36	20.3 abcdefghij	7.2 bcdefghijkl	40.4 abcdefghijk	12.8 bcdefghijk
Af-37	11.4 defghijklmno	6.6 bcdefghijkl	40.2 abcdefghij	17.2 abcdefghijk
Af-38	6.3 jklmno	2.4 jklm	18.6 defghijk	4.4 hijkl
Af-39	14.9 bcdefghijklm	10.0 bcdefghij	45.8 abcdefghi	17.9 abcdefghij
Af-41	9.0 efghijklmno	3.7 efghijklm	32.0 abcdefghijk	7.7 defghijk
Af-42	15.9 abcdefghijkl	10.0 bcdefghij	68.3 abcdefg	22.8 abcdefghi
Af-43	5.2 klmno	2.6 jklm	17.8 ghijk	2.6 kl
Af-44	19.9 abcdefghijk	7.4 bcdefghijkl	64.9 abcdefghi	13.0 bcdefghijk
Af-45	11.7 bcdefghijklmn	5.7 bcdefghijklm	35.4 abcdefghij	14.7 bcdefghijk
Af-45.1	18.1 abcdefghijk	12.0 bcdefghij	64.0 abcdefgh	19.5 abcdefghijk

Table A-1 (continued)

Accession	2005		2006	
	June		Oct.	
	-----g plot ⁻¹ -----			
Af-46	10.6 bcdefghijklmno	9.6 bcdefghijk	47.1 abcdefghij	25.6 abcdefghi
Af-47	15.2 bcdefghijklm	10.5 bcdefghij	67.0 abcdefghij	20.4 abcdefghijk
Af-48	2.2 o	0.5 m	2.4 k	0.5 l
Af-49	3.4 mno	0.9 lm	7.1 jk	6.0 ghijkl
Af-50	4.5 lmno	2.3 ijklm	14.9 fghijk	4.4 ghijkl
Af-51	5.5 ghijklmno	1.9 ghijklm	7.5 hijk	2.6 ijkl
Af-52	5.1 klmno	2.3 ijklm	13.0 fghijk	3.8 jkl
Af-53	10.3 defghijklmno	6.6 bcdefghijkl	34.6 abcdefghijk	12.5 bcdefghijk
Af-54	5.4 ijklmno	2.8 efghijklm	17.0 abcdefghijk	6.4 fghijkl
Af-55	6.0 jklmno	1.9 ijklm	11.2 fghijk	2.9 jkl
Af-56	8.4 efghijklmno	1.6 jklm	15.9 defghijk	5.0 fghijkl
Af-58	10.6 defghijklmno	3.0 efghijklm	13.0 fghijk	5.8 fghijkl
Af-59	11.1 efghijklmno	3.5 defghijklm	25.4 cdefghijk	9.0 defghijk
Af-60	12.8 bcdefghijklmn	2.8 ghijklm	23.0 defghijk	5.9 fghijkl
Af-61	9.9 defghijklmno	2.4 hijklm	22.3 bcdefghijk	7.0 efghijk
Af-62	10.5 defghijklmno	4.1 efghijklm	36.2 defghijk	11.9 defghijk
Af-63	12.7 bcdefghijklm	5.1 bcdefghijklm	42.6 abcdefghijk	14.1 abcdefghijk
Af-64	9.7 efghijklmno	5.2 bcdefghijklm	42.3 abcdefghijk	12.0 bcdefghijk
Af-65	4.7 jklmno	2.1 fghijklm	17.8 efghijk	7.0 efghijk
Af-66	5.1 klmno	1.9 jklm	15.7 fghijk	4.2 ghijkl
Af-67	16.3 abcdefghijk	4.3 cdefghijklm	47.6 abcdefghij	8.4 efghijk
Af-68	5.4 hijklmno	1.8 jklm	22.2 defghijk	5.8 ghijkl
Af-69	8.2 efghijklmno	4.2 cdefghijklm	37.2 abcdefghijk	15.7 abcdefghijk
Af-70	26.1 abcde	14.0 bcde	62.8 abcdefg	31.4 abcdef
Af-75	22.1 abcdefg	69.2 a	114.0 a	35.9 abcde
Af-76	34.6 abc	18.1abc	88.1 abc	37.1 abcd
Af-77	12.7 bcdefghijklm	5.3 bcdefghijkl	45.4 abcdefghij	11.4 bcdefghijk

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-2. Mean comparisons of August biomass and October regrowth of basalt milkvetch accessions from Millville in 2005 and 2006.

Accession	2005		2006
	August	Oct.	August
	-----g plot ⁻¹ -----		
Af-3	21.7 hijklmnopqr ¹	2.5 ghijklmn	46.9 efghijklmnopqrst
Af-4	14.4 jklmnopqr	0.9 klmn	22.0 nopqrst
Af-5	82.6 abcde	17.4 a	60.7 cdefghijklmnopqrs
Af-6	21.8 hijklmnopqr	2.1 ghijklmn	23.3 opqrst
Af-7	18.5 hijklmnopqr	1.2 hijklmn	42.5 fghijklmnopqrst
Af-8	40.4 bcdefghijklm	3.4 efghijklm	88.9 abcdefghij
Af-9	39.4 defghijklmno	3.1 efghijklm	88.2 abcdefghijk
Af-10	13.2 klmnopqr	1.2 jklmn	24.4 mnopqrst
Af-11	41.1 bcdefghijklm	2.4 fghijklmn	82.0 abcdefghijklmn
Af-13	39.6 bcdefghijklm	1.4 ghijklmn	84.0 abcdefghijkl
Af-14	26.3 fghijklmnopqr	1.0 jklmn	57.5 defghijklmnopqrst
Af-15	34.8 defghijklmno	2.2 ghijklmn	70.1 abcdefghijklmnopq
Af-16	32.8 efghijklmnopq	2.0 ghijklmn	73.0 abcdefghijklmnop
Af-18	40.0 cdefghijklmn	6.4 cdefghi	84.5 abcdefghijklm
Af-19	18.7 hijklmnopqr	2.4 ghijklmn	63.0 cdefghijklmnopqr
Af-20	41.7 bcdefghijkl	1.3 hijklmn	86.7 abcdefghijk
Af-21	17.7 hijklmnopqr	0.3 mn	38.5 fghijklmnopqrst
Af-22	15.8 jklmnopqr	1.5 ghijklmn	31.9 jklmnopqrst
Af-23	15.1 jklmnopqr	0.8 jklmn	35.5 ghijklmnopqrst
Af-24	21.8 hijklmnopqr	1.7 ghijklmn	44.4 efghijklmnopqrst
Af-25	16.0 jklmnopqr	0.7 klmn	35.0 hijklmnopqrst
Af-26	38.1 cdefghijklmn	3.9 efghijkl	87.2 abcdefghijk
Af-28	16.6 jklmnopqr	1.6 ghijklmn	15.0 rst
Af-29	84.9 abc	10.0 abcde	123.7 abcd
Af-30	97.7 a	16.9 ab	136.1 ab
Af-30.1	59.6 abcdefghi	7.0 abcdefg	85.6 abcdefghijkl
Af-31	93.1 ab	13.8 abcd	147.2 a
Af-32	64.5 abcdefg	4.0 efghijkl	107.3 abcde
Af-33	46.1 abcdefghij	3.0 efghijklmn	108.4 abcde
Af-34	14.1 jklmnopqr	2.1 ghijklmn	38.8 fghijklmnopqrst
Af-36	34.4 fghijklmnopq	3.2 fghijklmn	65.2 bcdefghijklmnopqr
Af-37	39.4 bcdefghijklm	5.6 cdefghij	68.2 bcdefghijklmnopqr
Af-38	16.1 ijklmnopqr	1.5 ghijklmn	23.9 mnopqrst
Af-39	25.3 fghijklmnopqr	3.7 efghijklm	44.4 efghijklmnopqrst
Af-41	22.5 hijklmnopqr	1.1 ijklmn	49.4 efghijklmnopqrst
Af-42	29.3 fghijklmnopq	2.4 ghijklmn	69.4 bcdefghijklmnopqr
Af-43	12.5 mnopqr	0.7 klmn	33.9 ijklmnopqrst
Af-44	42.4 bcdefghijkl	3.3 efghijklmn	88.4 abcdefghij
Af-45	39.1 cdefghijklmno	2.8 efghijklmn	82.8 abcdefghijklm
Af-45.1	39.9 cdefghijklmnop	4.3 efghijklm	94.1 abcdefghi

Table A-2 (continued)

Accession	2005		2006
	August	Oct.	August
	-----g plot ⁻¹ -----		
Af-46	46.3 abcdefghijk	7.4 bcdefgh	91.2 abcdefghij
Af-47	46.9 abcdefghij	4.5 defghijk	96.8 abcdefh
Af-48	3.6 r	0.0 n	13.1 pqrst
Af-49	7.7 pqr	0.3 lmn	15.4 qrst
Af-50	18.3 ijklmnopqr	1.2 jklmn	19.6 pqrst
Af-51	7.7qr	0.6 klmn	25.4 lmnopqrst
Af-52	5.6 r	0.3 mn	7.2 st
Af-53	18.9 hijklmnopqr	1.4 ghijklmn	32.4 jklmnopqrst
Af-54	7.6 pqr	0.3 lmn	13.7 qrst
Af-55	7.3 pqr	0.2 mn	5.4 t
Af-56	10.0 nopqr	0.6 klmn	16.4 qrst
Af-58	15.4 jklmnopqr	0.8 jklmn	16.5 qrst
Af-59	20.1 hijklmnopqr	1.0 jklmn	38.4 ghijklmnopqrst
Af-60	40.3 cdefghijklmn	3.5 efg hijklm	98.1 abcdefg
Af-61	33.0 fghijklmnopq	0.7 klmn	63.5 bcdefghijklmnopqr
Af-62	29.0 ghijklmnopq	0.9 jklmn	78.3 abcdefghijklmno
Af-63	22.7 hijklmnopqr	1.7 ghijklmn	55.8 efg hijklmnopqrs
Af-64	30.0 fghijklmnopq	1.4 ghijklmn	66.4 bcdefghijklmnopqr
Af-65	9.8 opqr	0.4 mn	23.4 mnopqrst
Af-66	8.3 mnopqr	0.1 mn	15.4 pqrst
Af-67	11.6 lmnopqr	0.7 jklmn	25.9 klmnopqrst
Af-68	10.6 nopqr	0.9 jklmn	22.5 opqrst
Af-69	17.5 hijklmnopqr	1.8 ghijklmn	48.6 efg hijklmnopqrst
Af-70	78.1 abcdef	13.9 abc	126.6 abc
Af-75	55.3 abcdefgh	17.4a	47.5 efg hijklmnopqrst
Af-76	83.8 abcd	10.2 abcdef	102.9 abcdef
Af-77	35.1 defghijklmno	2.6 efg hijklmn	74.8 abcdefghijklmnop

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-3. Mean comparisons of crude protein concentration (CPC) and crude protein pool (CPP) of basalt milkvetch accessions from Evans Farm in 2005 and 2006.

Accession	CPC		CPP	
	2005	2006	2005	2006
	-----g kg ⁻¹ -----		-----g plant ⁻¹ -----	
Af-3	141 abcdefgh ¹	152 abcdefgh	0.4 efghijklmno	1.45 efghijklmnopqrs
Af-4	138 abcdefgh	121 abcdefghij	0.1 vw	0.89 klmnopqrstuvw
Af-5	106 fgh	147 abcdefghij	0.9 a	3.36 a
Af-6	132 abcdefgh	120 abcdefghij	0.3 lmnopqrs	1.2 nopqrstuvw
Af-7	143 abcdefg	148 abcdefghij	0.6 abcde	2.56 abcde
Af-8	140 abcdefgh	145 abcdefghij	0.5 bcdefghi	1.72 bcdefghijklmn
Af-9	132 abcdefgh	142 abcdefghij	0.2 jklmnopqr	1.26 hijklmnopqrstu
Af-10	146 abcdefg	146 abcdefghij	0.3 jklmnopqr	1.18 fghijklmnopqrst
Af-11	148 abcdefg	160 abcdef	0.3 hijklmnopq	1.23 ghijklmnopqrstu
Af-13	153 abcdef	145 abcdefghij	0.5 bcdefghijk	1.40 defghijklmnopqr
Af-14	132 abcdefgh	144 abcdefghij	0.3 ghijklmnopq	0.82 lmnopqrstuvw
Af-15	143 abcdefgh	139 bcdefghij	0.3 fghijklmnopq	0.87 mnopqrstuvw
Af-16	144 abcdefg	141 bcdefghij	0.4 efghijklmn	1.09 ijklmnopqrstuv
Af-18	138 abcdefgh	139 bcdefghij	0.7 abcd	2.13 abcdefg
Af-19	165 abc	148 abcdefghij	0.3 efghijklmno	1.08 ghijklmnopqrstu
Af-20	141 abcdefgh	142 abcdefghij	0.3 fghijklmnopq	0.59 rstuvwxy
Af-21	127 abcdefgh	150 abcdefghi	0.3 fghijklmnopq	1.02 ijklmnopqrstuv
Af-22	146 abcdefg	170 abc	0.3 fghijklmnopq	1.42 bcdefghijklmnop
Af-23	138 abcdefgh	154 abcdefgh	0.2 ijklmnopq	0.76 opqrstuvw
Af-24	131 abcdefgh	147 abcdefghij	0.2 mnopnpqrst	0.67 stuvwxy
Af-25	138 abcdefgh	142 abcdefghij	0.2 nopnpqrstu	0.98 jklmnopqrstuv
Af-26	146 abcdefgh	128 efghij	0.6 abcde	1.35 cdefghijklmnop
Af-28	108 defgh	167 abcd	0.2 tuvw	0.44 wxy
Af-29	131 abcdefgh	156 abcdefg	0.8 abc	3.14 ab
Af-30	100 gh	123 ghij	0.6 abcd	2.67 abcd
Af-30.1	141 abcdefgh	140 abcdefghij	0.8 abcde	2.54 abcdef
Af-31	124 abcdefgh	118 j	1.0 a	2.13 abcdefg
Af-32	143 abcdefgh	136 cdefghij	0.6 bcdefg	1.35 bcdefghijklmnop
Af-33	114 bcdefgh	119 ij	0.5 bcdefghij	2.05 abcdefghij
Af-34	132 abcdefgh	146 abcdefghij	0.2 mnopnpqrst	1.33 ghijklmnopqrst
Af-36	124 abcdefgh	127 defghij	0.5 bcdefghi	1.05 ghijklmnopqrst
Af-37	135 abcdefgh	144 abcdefghij	0.4 ghijklmnopq	1.68 bcdefghijklmno
Af-38	167 abc	202 abc	0.3 opqrstuv	1.09 klmnopqrstuvw
Af-39	155 abcdef	165 abcd	0.5 bcdefghij	2.05 abcdefgh
Af-41	144 abcdefg	144 abcdefghij	0.3 hijklmnopq	1.03 lmnopqrstuvw
Af-42	151 abcdef	125 abcdefghij	0.5 bcdefgh	1.98 abcdefghi
Af-43	154 abcdef	145 abcdefghij	0.2 qrstuvw	0.73 uvwxy
Af-44	154 abcdef	134 cdefghij	0.6 bcdef	2.05 bcdefghijklm
Af-45	137 abcdefgh	134 defghij	0.5 efghijklmn	1.60 abcdefghij
Af-45.1	157 abcde	151 abcdefgh	0.6 bcdef	2.34 defghijklmnop

Table A-3 (continued)

Accession	CPC		CPP	
	2005	2006	2005	2006
	-----g kg ⁻¹ -----		-----g plant ⁻¹ -----	
Af-46	144 abcdefg	165 abcd	0.4 efghijklmnop	2.13 abcdef
Af-47	129 abcdefgh	147 abcdefghij	0.4 efghijklmn	2.04 abcdefghijkl
Af-48	157 abcde	160 abcde	0.1 uvw	0.28 y
Af-49	163 abc	156 abcdefg	0.1 stuvw	0.40 xy
Af-50	141 abcdefgh	153 abcdefgh	0.1 stuvw	0.70 qrstuvwxy
Af-51	142 abcdefgh	150 abcdefghij	0.2 opqrstuv	0.50 vwxy
Af-52	179 a	179 a	0.2 nopqrst	0.79 pqrstuvw
Af-53	147 abcdefg	179 abcdefgh	0.3 fghijklmnopq	1.45 defghijklmnopqrs
Af-54	153 abcdef	162 abcde	0.2 pqrstuv	1.13 klmnopqrstuvw
Af-55	122 bcdefgh	143 abcdefghij	0.2 rstuvw	0.61 tuvwxy
Af-56	119 bcdefgh	152 abcdefgh	0.2 mnopqrst	0.73 pqrstuvw
Af-58	113 cdefgh	152 abcdefgh	0.3 klmnopqrs	0.61 qrstuvwxy
Af-59	125 abcdefgh	155 abcdefg	0.3 klmnopqrs	0.89 mnopqrstuvw
Af-60	141 abcdefgh	147 abcdefghij	0.4 efghijklmn	0.83 pqrstuvw
Af-61	131 abcdefgh	153 abcdefgh	0.3 hijklmnopq	1.00 mnopqrstuvw
Af-62	170 ab	151 abcdefgh	0.4 defghijklm	1.54 ghijklmnopqrstu
Af-63	161 abcd	154 abcdefg	0.4 cdefghijkl	1.45 bcdefghijklmnop
Af-64	148 abcdefg	147 abcdefghij	0.3 ghijklmnopq	1.65 defghijklmnopq
Af-65	155 abcdef	153 abcdefgh	0.2 qrstuv	0.66 pqrstuvwxy
Af-66	179 abc	157 abcdef	0.1 w	0.79 qrstuvwxy
Af-67	144 abcdefg	159 abcdef	0.5 bcdefgh	1.95 abcdefghijk
Af-68	151 abcdef	174 ab	0.2 nopnpqrstu	1.00 lmnopqrstuvw
Af-69	171 ab	160 abcdef	0.3 fghijklmnopq	1.30 defghijklmnopqrs
Af-70	116 bcdefgh	126 fghij	0.7 abcd	2.04 abcdefghi
Af-75	95 h	142 abcdefghij	0.5 bcdefghijkl	2.7 abcde
Af-76	109 efgh	122 hij	0.8 ab	2.50 abc
Af-77	163 abcd	135 cdefghij	0.4 bcdefghijk	1.31 cdefghijklmnop

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-4. Mean comparisons of seed weight (per 100 seeds) and seed yield of basalt milkvetch accessions from Millville in 2005 and 2006.

Accession	2005		2006	
	Seed weight	Seed yield	Seed weight	Seed yield
	g 100 ⁻¹ seed	g plot ⁻¹	g 100 ⁻¹ seed	g plot ⁻¹
Af-3	0.33 def ^l	0.43 abcde	0.38 ijklmn	0.70 abcde
Af-4	0.35 cdef	0.06 cde	0.37 jklmn	0.84 abcde
Af-5	0.42 abcdef	9.51 a	0.44 bcdefghijklmn	4.71 abcd
Af-6	0.37 abcdef	0.15 cde	0.33 lmn	0.57 abcde
Af-7	0.36 bcdef	0.04 e	0.46 abcdefghijklm	1.18 abcde
Af-8	0.39 abcdef	0.05 cde	0.47 abcdefghijklm	6.22 abc
Af-9	0.35 cdef	1.05 bcd	0.42 bcdefghijklmn	2.28 abcde
Af-10	0.39 abcdef	0.27 bcde	0.45 bcdefghijklmn	1.32 abcde
Af-11	0.54 abcde	0.98 abcde	0.59 abc	2.89 abcde
Af-13	0.49 abcdef	2.32 abcde	0.50 abcdefghijkl	3.26 abcd
Af-14	0.45 abcdef	0.12 cde	0.49 abcdefghijkl	3.30 abcde
Af-15	0.47 abcdef	0.67 abcde	0.48 abcdefghijklm	2.20 abcde
Af-16	0.37 abcdef	0.16 bcde	0.43 bcdefghijklmn	2.11 abcde
Af-18	0.43 abcdef	2.48 abcde	0.50 abcdefghijkl	4.27 abcde
Af-19	0.39 cdef	0.21 bcde	0.46 abcdefghijklm	3.47 abcde
Af-20	0.41 abcdef	1.00 abcde	0.43 bcdefghijklmn	1.00 abcde
Af-21	0.33 def	0.50 abcde	0.39 ghijklmn	2.33 abcde
Af-22	0.34 cdef	0.76 abcde	0.40 lmn	1.19 abcde
Af-23	0.46 abcdef	0.33 bcde	0.43 bcdefghijklmn	0.87 abcde
Af-24	0.49 abcdef	0.27 bcde	0.55 abcdefghi	1.07 abcde
Af-25	0.43 abcdef	0.26 bcd	0.49 abcdefghijkl	2.81 abcde
Af-26	0.48 abcdef	2.43 abcde	0.55 abcdefgh	2.79 abcde
Af-28	0.37 abcdef	0.90 abcde	0.38 ijklmn	0.51 abcde
Af-29	0.50 abcdef	1.24 abcde	0.46 abcdefghijklm	1.88 abcde
Af-30	0.52 abcdef	7.03 ab	0.59 abc	7.38 abcd
Af-30.1	0.60 a	3.54 abcde	0.62 a	4.46 abcd
Af-31	0.59 ab	2.89 abcde	0.58 abcd	7.47 abcd
Af-32	0.47 abcdef	1.50 abcde	0.56 abcdefg	6.51 abcde
Af-33	0.55 abcd	1.02 abcde	0.57 abcdef	2.82 abcde
Af-34	0.34 cdef	0.04 de	0.43 bcdefghijklmn	1.07 abcde
Af-36	0.55 abcd	0.58 abcde	0.57 abcdef	6.63 a
Af-37	0.42 abcdef	0.75 bcde	0.45 bcdefghijklmn	5.63 ab
Af-38	0.42 abcdef	0.19 bcde	0.39 ghijklmn	2.04 abcde
Af-39	0.34 abcdef	0.47 abcde	0.34 lmn	3.39 abcde
Af-41	0.45 abcdef	0.59 bcde	0.46 abcdefghijklm	3.88 abcd
Af-42	0.45 abcdef	0.16 bcde	0.43 bcdefghijklmn	3.41 abcde
Af-43	0.49 abcdef	0.09 de	0.61 cdefghijklmn	0.45 abcde
Af-44	0.48 abcdef	0.57 bcde	0.47 abcdefghijklm	1.79 abcde
Af-45	0.40 abcdef	0.38 bcde	0.49 abcdefghijkl	1.84 abcde
Af-45.1	0.46 abcdef	0.33 bcde	0.46 abcdefghijklmn	5.65 abcd
Af-46	0.58 abc	0.30 de	0.55 abcdefgh	0.22 cde
Af-47	0.44 abcdef	1.99 abcde	0.40 efghijklmn	1.42 abcde

Table A-4 (continued)

Accession	2005		2006	
	Seed weight	Seed yield	Seed weight	Seed yield
	g 100 ⁻¹ seed	g plot ⁻¹	g 100 ⁻¹ seed	g plot ⁻¹
Af-48	0.38 abcdef	0.05 bcde	0.36 bcdefghijklmn	0.14 abcde
Af-49	0.41 abcdef	0.04 bcde	0.38 hijklmn	0.02 e
Af-50	0.42 abcdef	0.11 bcde	0.42 defghijklmn	0.33 de
Af-51	0.43 abcdef	0.01 e	0.48 abcdefghijklm	0.34 abcde
Af-52	0.50 abcdef	0.01 de	0.50 abcdefghijkl	1.91 abcde
Af-53	0.61 a	0.61 abcde	0.54 abcdefghijk	1.09 abcde
Af-54	0.56 abcd	0.12 bcde	0.54 abcdefghij	0.36 abcde
Af-55	0.30 f	0.12 de	0.31 mn	0.15 bcde
Af-56	0.30 ef	0.03 de	0.42 bcdefghijklmn	0.79 abcde
Af-58	0.32 def	0.34 bcde	0.37 jklmn	1.47 abcde
Af-59	0.47 abcdef	0.08 bcde	0.45 abcdefghijklmn	1.72 abcde
Af-60	0.44 abcdef	0.42 bcde	0.49 abcdefghijklm	6.88 abcd
Af-61	0.48 abcdef	1.29 abcde	0.44 bcdefghijklmn	3.59 abcde
Af-62	0.56 abcd	0.34 bcde	0.54 abcdefghijk	4.41 abcd
Af-63	0.34 def	0.32 abcde	0.41 defghijklmn	2.11 abcde
Af-64	0.41 abcdef	0.77 abcde	0.46 abcdefghijklmn	3.44 abcde
Af-65	0.40 abcdef	0.14 bcde	0.29 n	0.58 abcde
Af-66	0.40 abcdef	0.16 bcde	0.40 fghijklmn	0.53 abcde
Af-67	0.32 def	0.17 bcde	0.37 klmn	0.10 abcde
Af-68	0.37 abcdef	0.11 bcde	0.50 abcdefghijkl	0.51 abcde
Af-69	0.46 abcdef	0.26 bcde	0.47 abcdefghijklm	1.77 abcde
Af-70	0.53 abcdef	2.01 abcd	0.60 ab	5.81 a
Af-75	0.40 abcdef	2.35 abcde	0.42 bcdefghijklmn	2.79 abcde
Af-76	0.55 abcd	6.11 abc	0.52 abcde	3.04 abcde
Af-77	0.41 abcdef	0.17 bcde	0.49 abcdefghijkl	3.40 abcde

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-5. Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Evans Farm in 2005.

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-3	26.8 abcdefghijklm ¹	3.9 cdefghijklmno	4.4 abcdefg	5.4 abcdefghij
Af-4	24.4 abcdefghijklmn	2.3 lmno	0.9 fg	4.0 ghijkl
Af-5	34.4 ab	10.0 a	17.2 a	6.7 a
Af-6	25.8 abcdefghijklmn	4.8 bcdefghijklmn	2.7 cdefg	5.2 abcdefghij
Af-7	26.4 abcdefghijklm	6.7 abcdef	3.8 abcdefg	5.7 abcdefgh
Af-8	26.8 abcdefghijklm	4.9 abcdefghijklmn	2.6 bcdefg	5.4 abcdefghi
Af-9	21.4 defghijklmn	3.7 cdefghijklmno	1.2 defg	4.3 cdefghijkl
Af-10	23.3 bcdefghijklmn	2.7 hijklmno	0.7 defg	4.5 bcdefghijkl
Af-11	24.7 abcdefghijklmn	3.0 fghijklmno	1.5 defg	4.5 bcdefghijkl
Af-13	26.1 abcdefghijklmn	4.9 abcdefghijklmn	2.9 abcdefg	5.4 abcdefghij
Af-14	24.4 abcdefghijklmn	3.4 defghijklmno	2.0 bcdefg	5.0 abcdefghijkl
Af-15	29.3 abcdefghijk	3.8 cdefghijklmno	2.1 bcdefg	4.9 abcdefghijkl
Af-16	28.5 abcdefghijk	4.0 cdefghijklmno	1.4 cdefg	5.4 abcdefghij
Af-18	28.3 abcdefghijk	6.7 abcdefg	6.0 abcdefg	5.7 abcdefg
Af-19	26.2 abcdefghijklmn	3.3 efghijklmno	1.7 bcdefg	5.1 abcdefghijk
Af-20	22.3 bcdefghijklmn	4.0 cdefghijklmno	0.7 fg	4.6 bcdefghijkl
Af-21	24.2 abcdefghijklmn	4.8 abcdefghijklmno	4.4 abcdefg	5.0 abcdefghijkl
Af-22	25.1 abcdefghijklmn	3.0 fghijklmno	2.2 bcdefg	5.0 abcdefghijkl
Af-23	26.9 abcdefghijklm	2.8 hijklmno	2.5 bcdefg	5.2 abcdefghij
Af-24	25.1 abcdefghijklmn	2.7 hijklmno	1.5 bcdefg	4.8 abcdefghijkl
Af-25	20.4 fghijklmn	3.1 fghijklmno	2.6 abcdefg	4.0 ghijkl
Af-26	25.8 abcdefghijklmn	6.9 abcde	5.7 abcdefg	5.4 abcdefghij
Af-28	34.1 abc	2.7 no	2.9 abcdefg	6.1 abcdef
Af-29	32.4 abcdef	6.2 abcdefghi	7.1 abcdefg	6.0 abcdefg
Af-30	27.7 abcdefghijk	9.6 a	14.0 ab	6.0 abcdefg
Af-30.1	26.6 abcdefghijklm	7.4 abcde	6.6 abcdefg	5.6 abcdefgh
Af-31	30.5 abcdefghi	9.2 ab	11.7 abcd	6.2 abcd
Af-32	26.5 abcdefghijklm	5.9 abcdefghij	5.0 abcdefg	5.3 abcdefghij
Af-33	32.5 abcde	5.0 abcdefghijklm	6.7 abcdef	5.9 abcdefg
Af-34	21.9 cdefghijklmn	3.7 cdefghijklmno	2.6 bcdefg	4.5 bcdefghijkl
Af-36	32.4 abcde	5.9 abcdefgh	6.8 abcdefg	6.1 abcde
Af-37	30.1 abcdefghijk	4.0 cdefghijklmno	3.9 abcdefg	5.6 abcdefgh
Af-38	19.2 jklmn	3.3 efghijklmno	2.1 cdefg	3.3 ijkl
Af-39	30.8 abcdefgh	5.0 abcdefghijklmn	5.4 abcdefg	6.0 abcdefg
Af-41	22.3 cdefghijklmn	3.3 defghijklmno	2.3 cdefg	4.3 bcdefghijkl
Af-42	25.8 abcdefghijklmn	4.6 bcdefghijklmno	2.2 defg	5.2 abcdefghij
Af-43	18.6 klmn	2.9 ghijklmno	1.8 defg	3.7 hijkl
Af-44	25.8 abcdefghijklmn	5.4 abcdefghijk	2.7 bcdefg	5.3 abcdefghij
Af-45	27.4 abcdefghijkl	5.4 abcdefghijk	2.7 bcdefg	5.7 abcdefgh
Af-45.1	27.5 abcdefghijkl	5.2 abcdefghijkl	1.8 bcdefg	5.5 abcdefgh

Table A-5 (continued)

Accession	Plant height	Stems	Inflorescences	Vigor
	cm			
Af-46	25.6 abcdefghijklmn	4.5 bcdefghijklmno	0.7 fg	5.1 abcdefghijk
Af-47	26.6 abcdefghijklm	4.3 cdefghijklmno	3.0 bcdefg	5.3 abcdefghij
Af-48	16.0 n	1.9 o	0.6 defg	3.0 l
Af-49	16.7 lmn	2.3 klmnno	0.3 g	3.4 jkl
Af-50	20.0 hijklmn	2.3 no	1.4 cdefg	4.1 fghijkl
Af-51	21.1 defghijklmn	2.8 hijklmno	1.3 defg	4.3 bcdefghijkl
Af-52	19.1 ijklmn	2.7 hijklmno	0.7 efg	4.1 fghijkl
Af-53	26.5 abcdefghijklmn	3.1 fghijklmno	2.7 abcdefg	4.9 abcdefghijkl
Af-54	23.4 bcdefghijklmn	2.6 ijklmno	1.8 cdefg	4.5 bcdefghijkl
Af-55	33.0 abcd	2.8 hijklmno	2.2 bcdefg	5.5 abcdefgh
Af-56	31.6 abcdefg	3.2 efghijklmno	1.7 cdefg	5.6 abcdefgh
Af-58	36.7 a	2.3 mno	3.9 abcdefg	6.0 abcdefg
Af-59	26.2 abcdefghijklmn	2.8 ghijklmno	3.0 abcdefg	5.2 abcdefghijk
Af-60	24.9 abcdefghijklmn	5.4 abcdefghijkl	4.8 abcdefg	5.2 abcdefghij
Af-61	22.6 bcdefghijklmn	3.8 cdefghijklmno	2.2 cdefg	4.6 bcdefghijkl
Af-62	25.8 abcdefghijklmn	3.7 defghijklmno	1.6 cdefg	5.0 abcdefghijkl
Af-63	28.2 abcdefghijk	3.6 defghijklmno	2.8 abcdefg	5.4 abcdefghij
Af-64	21.9 cdefghijklmn	4.3 bcdefghijklmno	0.5 fg	4.6 bcdefghijkl
Af-65	20.7 efghijklmn	2.5 jklmno	0.5 fg	4.1 efghijkl
Af-66	16.4 mn	2.2 no	2.1 cdefg	3.2 kl
Af-67	23.9 abcdefghijklmn	4.8 abcdefghijklmn	4.8 abcdefg	4.9 abcdefghijkl
Af-68	20.7 efghijklmn	3.8 cdefghijklmno	1.7 defg	4.1 efghijkl
Af-69	20.2 ghijklmn	3.2 efghijklmno	0.8 defg	4.2 defghijkl
Af-70	29.8 abcdefghij	7.9 abc	11.2 abc	6.3 ab
Af-75	32.2 abcdef	7.5 abcd	8.8 abcde	6.2 abc
Af-76	30.9 abcdefgh	8.2 abc	9.2 abcdefg	6.1 abcde
Af-77	24.2 abcdefghijklmn	4.5 bcdefghijklmno	0.9 fg	5.0 abcdefghijkl

[†]Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-6. Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Evans Farm in 2006.

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-3	30.2 abcd ¹	10.2 efghijklmnop	15.3 bcdefgh	4.2 abc
Af-4	28.3 ab	7.9 fghijklmnop	8.8 cdefgh	3.6 abc
Af-5	32.7 ab	27.6 abc	72.3 ab	5.4 a
Af-6	20.9 abcd	10.8 efghijklmnop	8.1 cdefgh	3.1 abc
Af-7	29.8 abcd	26.6 abc	32.4 abcdefg	5.0 a
Af-8	26.5 abcd	17.8 abcdefghijkl	17.3 bcdefgh	4.3 abc
Af-9	25.1 abcd	13.2 bcdefghijklmnop	6.2 cdefgh	4.3 abc
Af-10	27.5 abcd	10.8 efghijklmnop	5.5 cdefgh	3.8 abc
Af-11	22.8 abcd	9.9 efghijklmnop	10.4 cdefgh	3.2 abc
Af-13	27.3 abcd	14.8 bcdefghijklmno	26.0 abcdefgh	4.1 abc
Af-14	24.7 abcd	13.3 bcdefghijklmnop	10.4 cdefgh	3.7 abc
Af-15	25.3 abcd	12.7 bcdefghijklmnop	7.9 cdefgh	3.5 abc
Af-16	28.2 abcd	13.6 bcdefghijklmno	9.2 cdefgh	3.8 abc
Af-18	27.1 abcd	23.0 abcdef	40.4 abcd	4.3 abc
Af-19	28.2 abcd	13.2 bcdefghijklmnop	19.5 abcdefgh	4.0 abc
Af-20	21.0 abcd	12.6 bcdefghijklmnop	1.2 gh	3.1 abc
Af-21	24.6 abcd	12.3 bcdefghijklmnop	15.4 bcdefgh	3.6 abc
Af-22	25.0 abcd	12.1 cdefghijklmnop	17.4 abcdefgh	3.7 abc
Af-23	26.2 abcd	6.6 jklmnop	14.8 bcdefgh	3.5 abc
Af-24	26.0 abcd	7.7 ijklmnop	12.4 cdefgh	3.7 abc
Af-25	27.9 abcd	10.7 efghijklmnop	24.7 abcdefgh	4.1 abc
Af-26	27.3 abcd	21.0 abcdefg	16.6 abcdefgh	4.4 abc
Af-28	24.2 abcd	4.2 op	6.7 cdefgh	2.8 abc
Af-29	32.6 ab	18.1 abcdefghijkl	39.6 abcde	5.1 a
Af-30	30.4 abcd	26.6 abcd	45.0 abc	5.1 a
Af-30.1	28.2 abcd	23.7 abcdef	36.1 abcdef	4.6 abc
Af-31	30.2 abcd	24.0 abcde	35.9 abcde	4.9 a
Af-32	25.9 abcd	16.8 bcdefghijklm	19.9 abcdefgh	4.2 abc
Af-33	33.9 a	21.5 abcdefgh	38.4 abcde	5.1 a
Af-34	25.7 abcd	18.2 abcdefghijkl	13.8 cdefgh	3.9 abc
Af-36	26.0 abcd	16.4 bcdefghijklm	23.9 abcdefgh	3.9 abc
Af-37	29.8 abcd	15.3 bcdefghijklmno	23.1 abcdefgh	4.3 abc
Af-38	22.2 abcd	9.4 fghijklmnop	18.2 cdefgh	3.3 abc
Af-39	31.8 abc	17.1 bcdefghijkl	30.2 abcdefgh	4.9 ab
Af-41	24.5 abcd	10.5 efghijklmnop	13.7 cdefgh	3.7 abc
Af-42	32.3 abc	20.8 abcdefg	25.8 abcdefgh	4.9 a
Af-43	21.3 abcd	9.9 efghijklmnop	11.1 cdefgh	2.9 abc
Af-44	29.2 abcd	20.1 abcdefghi	18.7 bcdefgh	4.5 abc
Af-45	26.5 abcd	18.6 abcdefghijkl	22.0 abcdefgh	4.2 abc
Af-45.1	28.6 abcd	19.2 abcdefghij	31.5 abcdefgh	4.3 abc

Table A-6 (continued)

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-46	29.2 abcd	18.5 abcdefghijk	16.8 cdefgh	4.4 abc
Af-47	29.0 abcd	15.9 bcdefghijklmn	21.2 abcdefgh	4.5 abc
Af-48	16.9 cd	3.5 p	1.9 fgh	2.3 c
Af-49	16.3 d	5.5 mnop	0.8 h	2.2 c
Af-50	21.8 abcd	6.3 lmnop	10.3 cdefgh	3.1 abc
Af-51	17.1 cd	4.4 op	3.1 efgh	2.4 bc
Af-52	18.0 bcd	7.0 ijklmnop	4.8 defgh	3.1 abc
Af-53	26.0 abcd	10.2 efghijklmnop	13.8 cdefgh	3.8 abc
Af-54	22.9 abcd	8.0 hijklmnop	7.4 cdefgh	3.4 abc
Af-55	33.7 a	7.1 jklmnop	13.5 cdefgh	3.7 abc
Af-56	26.2 abcd	10.9 efghijklmnop	9.7 cdefgh	3.5 abc
Af-58	29.9 abcd	5.0 nop	14.7 bcdefgh	3.4 abc
Af-59	29.5 abcd	8.6 ghijklmnop	17.0 bcdefgh	3.8 abc
Af-60	23.0 abcd	11.4 defghijklmnop	7.7 cdefgh	3.5 abc
Af-61	21.1 abcd	9.4 fghijklmnop	14.7 cdefgh	3.3 abc
Af-62	27.4 abcd	13.7 bcdefghijklmnop	15.6 cdefgh	3.9 abc
Af-63	28.0 abcd	15.6 bcdefghijklmn	21.3 abcdefgh	3.9 abc
Af-64	26.4 abcd	19.5 abcdefghijk	17.4 cdefgh	4.0 abc
Af-65	21.7 abcd	6.5 klmnop	5.8 cdefgh	2.9 abc
Af-66	21.4 abcd	5.4 mnop	9.3 cdefgh	3.1 abc
Af-67	25.5 abcd	14.4 bcdefghijklmno	12.8 cdefgh	4.1 abc
Af-68	20.1 abcd	8.3 ghijklmnop	14.6 cdefgh	3.1 abc
Af-69	27.4 abcd	13.6 bcdefghijklmno	18.4 bcdefgh	3.8 abc
Af-70	31.1 abcd	21.4 abcdefg	35.4 abcde	5.1 a
Af-75	30.7 abcd	31.9 a	82.0 a	5.2 a
Af-76	32.4 abc	27.6 ab	41.9 abc	5.2 a
Af-77	24.7 abcd	14.1 bcdefghijklmno	18.0 bcdefgh	3.9 abc

[†]Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-7. Mean comparisons of plant height, number of stems per plant (stems), number of inflorescence per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Millville in 2005.

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-3	26.5 abcdefghi ¹	3.9 fghijkl	3.9 bcde	4.9 bcdefg
Af-4	37.1 abcd	2.7 hijkl	1.5 bcde	5.8 abcde
Af-5	36.8 ab	12.5 a	30.1 a	6.9 a
Af-6	25.5 abcdefghi	4.3 efg hijkl	2.3 bcde	4.9 bcdefg
Af-7	25.5 abcdefghi	3.6 fghijkl	0.9 de	4.8 bcdefg
Af-8	28.1 abcdefghi	5.3 bcdefghijkl	1.5 cde	5.3 abcdefg
Af-9	29.3 abcdefghi	7.0 bcdefgh	5.3 bcde	5.6 abcdefg
Af-10	30.2 abcdefghi	5.0 cdefghijkl	4.1 bcde	5.7 abcdef
Af-11	37.1 a	5.0 bcdefghijkl	4.4 bcde	6.1 abcd
Af-13	33.1 abcdef	5.3 bcdefghijkl	5.9 bcde	6.1 abcd
Af-14	27.2 abcdefghi	3.9 fghijkl	1.9 cde	5.0 bcdefg
Af-15	30.9 abcdefghi	6.1 bcdefghij	6.3 bcde	5.8 abcde
Af-16	30.2 abcdefghi	3.8 fghijkl	1.3 cde	5.5 abcdefg
Af-18	28.7 abcdefghi	9.2 abcde	8.0 bcde	5.9 abcd
Af-19	28.9 abcdefghi	4.3 defghijkl	2.1 bcde	5.3 abcdefg
Af-20	33.4 abcde	8.0 bcdefg	7.2 bcde	6.1 abcd
Af-21	27.0 abcdefghi	5.3 bcdefghijkl	3.6 bcde	5.2 abcdefg
Af-22	24.1 cdefghi	3.9 fghijkl	3.9 bcde	6.6 bcdefg
Af-23	28.5 abcdefghi	2.9 hijkl	2.0 bcde	5.1 abcdefg
Af-24	35.5 abc	4.9 bcdefghijkl	4.5 bcde	6.2 abcd
Af-25	29.4 abcdefghi	4.0 efg hijkl	3.8 bcde	5.5 abcdefg
Af-26	28.0 abcdefghi	7.4 bcdefg	8.7 abcde	5.8 abcde
Af-28	35.6 abc	2.2 jkl	5.7 bcde	5.6 abcdefg
Af-29	31.2 abcdefgh	5.4 bcdefghijkl	5.8 bcde	5.7 abcdef
Af-30	31.0 abcdefghi	10.6 abc	16.3 ab	6.4 abc
Af-30.1	27.9 abcdefghi	6.7 bcdefghi	6.9 bcde	5.6 abcdef
Af-31	31.5 abcdefg	8.0 bcdef	7.7 bcde	6.1 abcd
Af-32	28.6 abcdefghi	5.8 bcdefghijk	6.0 bcde	5.6 abcdefg
Af-33	33.9 abcd	4.8 cdefghijkl	5.5 bcde	5.9 abcd
Af-34	23.9 cdefghi	2.8 hijkl	0.8 de	4.2 defg
Af-36	30.7 abcdefghi	5.2 bcdefghijkl	5.4 bcde	5.7 abcdef
Af-37	31.0 abcdefghi	4.9 bcdefghijkl	3.7 bcde	5.5 abcdefg
Af-38	26.5 abcdefghi	4.7 defghijkl	4.2 bcde	5.1 abcdefg
Af-39	32.8 abcdef	5.2 bcdefghijkl	5.0 bcde	6.0 abcd
Af-41	26.9 abcdefghi	4.0 efg hijkl	3.0 bcde	5.0 bcdefg
Af-42	27.9 abcdefghi	4.1 fghijkl	1.6 cde	5.3 abcdefg
Af-43	21.5 fghi	4.3 efg hijkl	0.5 e	4.2 defg
Af-44	32.5 abcdefghi	5.6 bcdefghijk	5.1 bcde	5.9 abcd
Af-45	28.1 abcdefghi	3.7 fghijkl	3.0 bcde	5.1 abcdefg
Af-45.1	28.5 abcdefghi	4.9 bcdefghijkl	2.6 bcde	5.6 abcdefg

Table A-7 (continued)

Accession	Plant height	Stems	Inflorescences	Vigor
	cm			
Af-46	27.3 abcdefghi	5.7 bcdefghijk	1.3 de	5.3 abcdefg
Af-47	32.3 abcdef	6.3 bcdefghi	6.8 bcde	6.0 abcd
Af-48	19.7 ghi	2.1 ijkl	0.7 cde	3.7 efg
Af-49	19.3 i	2.1 jkl	0.4 de	3.6 fg
Af-50	24.4 cdefghi	3.2 ghijkl	2.1 bcde	4.6 cdefg
Af-51	27.0 abcdefghi	2.4 ijkl	0.8 cde	5.0 bcdefg
Af-52	19.6 hi	1.8 l	0.4 e	3.4 g
Af-53	25.8 abcdefghi	3.3 fghijkl	2.7 bcde	4.9 bcdefg
Af-54	25.9 abcdefghi	2.4 ijkl	1.1 cde	4.7 cdefg
Af-55	31.2 abcdefgh	2.4 ijkl	2.4 bcde	5.2 abcdefg
Af-56	32.5 abcdef	3.1 ghijkl	1.2 cde	5.5 abcdefg
Af-58	34.4 abcd	2.0 kl	3.4 bcde	5.3 abcdefg
Af-59	36.5 ab	4.3 efghijkl	3.5 bcde	5.9 abcde
Af-60	27.8 abcdefghi	6.1 bcdefghij	3.1 bcde	5.3 abcdefg
Af-61	27.1 abcdefghi	6.4 bcdefghi	6.6 bcde	5.2 abcdefg
Af-62	31.0 abcdefghi	5.3 bcdefghijkl	2.9 bcde	5.7 abcdef
Af-63	29.7 abcdefghi	5.4 bcdefghijkl	5.0 bcde	5.8 abcde
Af-64	29.0 abcdefghi	5.4 bcdefghijk	3.2 bcde	5.6 abcdefg
Af-65	25.4 abcdefghi	2.5 ijkl	1.1 cde	4.7 cdefg
Af-66	22.0 defghi	2.2 ijkl	2.6 bcde	4.0 defg
Af-67	25.3 abcdefghi	4.6 defghijkl	4.2 bcde	5.2 abcdefg
Af-68	21.7 efghi	3.8 fghijkl	2.4 bcde	4.2 defg
Af-69	25.2 bcdefghi	3.3 ghijkl	2.5 bcde	4.8 bcdefg
Af-70	31.6 abcdefg	9.5 abcd	11.1 abc	6.1 abcd
Af-75	26.2 abcdefghi	8.3 bcdef	11.5 abcde	5.3 abcdefg
Af-76	33.0 abcdef	10.7 ab	11.4 abcd	6.7 ab
Af-77	27.1 abcdefghi	4.5 defghijkl	2.3b cde	5.3 abcdefg

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-8. Mean comparisons of plant height, number of stems per plant (stems), number of inflorescences per plant (inflorescences), and plant vigor of basalt milkvetch accessions from Millville in 2006.

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-3	27.1 abcd ¹	16.9 cdefghijklmnop	35.8 abcdefghij	4.0 abcdefghijkl
Af-4	29.8 abcd	11.3 ijklmnop	14.6 cdefghij	3.2 cdefghijkl
Af-5	27.0 abcd	36.7 abcd	65.8 abcde	4.4 abcdefghijk
Af-6	25.4 abcd	11.9 ijklmnop	15.2 cdefghij	2.9 fghijkl
Af-7	26.8 abcd	19.5 abcdefghijklmnop	32.4 abcdefghij	3.8 abcdefghijkl
Af-8	32.6 abc	34.3 abcde	93.0 a	5.7 abc
Af-9	30.4 abcd	32.5 abcdefg	64.4 abcdef	4.8 abcdefghij
Af-10	29.4 abcd	15.3 efghijklmnop	20.8 bcdefghij	3.9 abcdefghijkl
Af-11	36.2 a	17.8 cdefghijklmnop	41.1 abcdefghi	5.2 abcdefg
Af-13	35.4 a	24.4 abcdefghijkl	64.5 abcdefg	5.2 abcdefg
Af-14	30.6 abcd	24.0 abcdefghijklm	56.0 abcdef	4.7 abcdefghij
Af-15	32.1 abc	22.4 abcdefghijklmn	46.6 abcdef	4.8 abcdefghij
Af-16	34.8 ab	25.8 abcdefghijk	43.4 abcdef	5.0 abcdefgh
Af-18	29.8 abcd	39.8 ab	70.9 abcde	4.7 abcdefghij
Af-19	34.5 ab	29.6 abcdefghi	72.9 abcd	5.4 abcde
Af-20	31.7 abc	28.0 abcdefghij	36.0 abcdefghij	4.8 abcdefghij
Af-21	29.3 abcd	23.8 abcdefghijkl	42.8 abcdef	4.0 abcdefghijkl
Af-22	21.1 cd	13.3 ghijklmnop	12.8 cdefghij	2.9 ghijkl
Af-23	28.4 abcd	10.2 klmnop	26.4 abcdefghij	3.6 bcdefghijkl
Af-24	32.3 abc	13.5 ghijklmnop	36.2 abcdefghij	4.2 abcdefghijkl
Af-25	29.4 abcd	17.4 defghijklmnop	41.5 abcdefghi	4.3 abcdefghijkl
Af-26	28.5 abcd	33.2 abcde	61.1 abcdef	5.0 abcdefgh
Af-28	26.7 abcd	10.4 lmnop	21.8 abcdefghij	3.3 cdefghijkl
Af-29	31.3 abc	26.0 abcdefghijk	40.2 abcdefghi	4.9 abcdefghij
Af-30	30.1 abcd	42.5 a	65.7 abcdef	5.1 abcdefghi
Af-30.1	28.8 abcd	32.9 abcdef	50.9 abcdef	5.0 abcdefghi
Af-31	31.1 abc	38.3 abc	68.9 abcdefg	5.3 abcdef
Af-32	30.4 abcd	32.2 abcdefg	70.1 abcde	5.2 abcdef
Af-33	33.6 abc	27.3 abcdefghijk	63.3 abcdefgh	5.7 ab
Af-34	27.1 abcd	16.6 defghijklmnop	20.9 abcdefghij	3.9 abcdefghijkl
Af-36	32.1 abc	21.3 abcdefghijklmno	52.9 abcdef	4.8 abcdefghij
Af-37	35.7 a	28.2 abcdefghij	64.3 abcdef	5.0 abcdefghi
Af-38	25.7 abcd	15.4 efghijklmnop	44.8 abcdefghi	4.0 abcdefghijkl
Af-39	32.3 abc	24.9 abcdefghijkl	64.3 abcde	5.0 abcdefghi
Af-41	28.8 abcd	19.5 abcdefghijklmnop	43.2 abcdefghi	4.3 abcdefghijkl
Af-42	32.5 abc	28.4 abcdefghijklmnop	61.2 abcdef	4.8 abcdefghij
Af-43	24.9 abcd	20.0 abcdefghijklmnop	16.1 bcdefghij	3.6 bcdefghijkl
Af-44	29.5 abc	27.9 abcdefghij	49.4 abcdef	5.0 abcdefghij
Af-45	30.7 abcd	19.3 abcdefghijklmno	22.1 abcdefghij	4.2 abcdefghijkl
Af-45.1	31.9 abc	28.8 abcdefghi	51.5 abcdef	4.1 abcdefghij

Table A-8 (continued)

Accession	Plant height cm	Stems	Inflorescences	Vigor
Af-46	26.6 abcd	24.0 abcdefghijklm	11.1 defghij	4.1 abcdefghijkl
Af-47	32.0 abc	26.8 abcdefghijk	38.5 abcdefghi	4.8 abcdefghij
Af-48	18.8 cd	5.8 op	3.4 fghij	2.5 ijkl
Af-49	17.8 d	7.4 nop	1.8 ij	2.3 kl
Af-50	21.0 cd	11.2 klmnop	7.7 fghij	2.8 hijkl
Af-51	20.7 cd	7.9 mnop	8.4 efg hij	2.8 hijkl
Af-52	17.9 d	6.0 p	0.8 j	3.5 l
Af-53	23.7 abcd	11.9 ijklmnop	17.1 bcdefghij	3.2 defghijkl
Af-54	24.0 abcd	10.1 jklmnop	14.6 bcdefghij	3.4 bcdefghijkl
Af-55	23.4 abcd	6.2 p	9.4 cdefghij	2.5 jkl
Af-56	34.7 abc	13.6 defghijklmnop	27.6 abcdefghij	3.9 abcdefghijkl
Af-58	27.2 abcd	6.6 p	20.3 bcdefghij	3.1 efg hijkl
Af-59	33.6 abc	11.8 ijklmnop	38.2 abcdefghij	4.2 abcdefghijkl
Af-60	36.0 a	29.7 abcdefgh	56.6 abcdef	5.6 abc
Af-61	30.0 abcd	25.5 abcdefghijk	58.5 abcdef	4.7 abcdefghij
Af-62	35.7 a	30.8 abcdefgh	72.1 abcd	5.6 abcd
Af-63	30.6 abcd	24.8 abcdefghijkl	57.2 abcdef	4.4 abcdefghijk
Af-64	31.9 abc	25.9 abcdefghijk	40.9 abcdefghi	4.9 abcdefghij
Af-65	23.6 abcd	10.7 ijklmnop	6.4 fghij	3.0 efg hijkl
Af-66	22.4 abcd	6.8 mnop	20.4 abcdefghij	2.8 efg hijkl
Af-67	25.5 abcd	14.1 fghijklmnop	52.4 abcdefghi	3.7 abcdefghijkl
Af-68	21.7 bcd	13.2 hijklmnop	19.1 bcdefghij	3.0 fghijkl
Af-69	28.1 abcd	18.7 bcdefghijklmnop	31.6 abcdefghij	3.6 bcdefghijkl
Af-70	34.6 ab	38.7 ab	76.6 ab	6.1 a
Af-75	28.1 abcd	30.2 abcdefgh	78.8 abc	4.8 abcdefghij
Af-76	30.3 abcd	28.7 abcdefghi	34.1 abcdefghij	4.9 abcdefghij
Af-77	31.8 abc	24.4 abcdefghijkl	66.3 abcde	4.1 abcdefghij

¹Values followed by the same letter within a column are not statistically significant at $P = 0.05$.

Table A-9. Evans Farm data used to form cluster dendrogram. Variables included plant height (HT), number of stems (ST), number of inflorescences (IF), plant vigor scores (VS), June biomass (BM), Oct. biomass (RG), crude protein concentration (CPC), and crude protein pool (CPP) in 2005 and 2006, and winter mortality (MT) in 2006.

	2005								2006								MT
	HT	ST	IF	VS	BM	RG	CPC	CPP	HT	ST	IF	VS	BM	RG	CPC	CPP	
Af-3	27	4	4	5	12	5	141	1.7	30	10	15	4	30	11	152	4.6	27
Af-4	24	2	1	4	3	2	138	0.5	34	10	9	4	13	4	121	1.5	73
Af-5	34	10	17	7	44	13	106	4.6	33	28	72	5	83	20	147	12.2	27
Af-6	26	5	3	5	9	5	132	1.2	21	11	8	3	44	11	120	5.2	30
Af-7	26	7	4	6	20	13	143	2.9	30	27	32	5	76	25	148	11.2	13
Af-8	27	5	3	5	17	6	140	2.4	27	18	17	4	56	19	145	8.1	3
Af-9	21	4	1	4	9	5	132	1.2	25	13	6	3	37	16	142	5.2	23
Af-10	23	3	1	5	8	4	146	1.2	28	11	6	4	25	11	146	3.6	40
Af-11	25	3	1	5	9	6	148	1.3	23	10	10	3	28	13	160	4.5	20
Af-13	26	5	3	5	14	5	153	2.1	27	15	26	4	34	10	145	4.9	17
Af-14	24	3	2	5	10	3	132	1.3	25	13	10	4	24	6	144	3.5	10
Af-15	29	4	2	5	11	4	143	1.5	25	13	8	3	26	9	139	3.6	20
Af-16	28	4	1	5	12	4	144	1.7	28	14	9	4	32	10	141	4.5	17
Af-18	28	7	6	6	25	12	138	3.4	27	23	40	4	71	24	139	9.8	10
Af-19	26	3	2	5	10	3	165	1.6	28	13	20	4	32	5	148	4.8	10
Af-20	22	4	1	5	10	2	141	1.4	21	13	1	3	20	6	142	2.8	10
Af-21	24	5	4	5	12	3	127	1.5	25	12	15	4	31	6	150	4.7	17
Af-22	25	3	2	5	9	5	146	1.4	25	12	17	4	36	13	170	6.1	17
Af-23	27	3	3	5	9	4	138	1.2	26	7	15	3	19	10	154	3.0	13
Af-24	25	3	2	5	7	4	131	0.9	26	8	12	4	21	13	147	3.1	7
Af-25	20	3	3	4	6	4	138	0.8	28	11	25	4	30	8	142	4.3	13
Af-26	26	7	6	5	19	8	146	2.8	27	21	17	4	41	19	128	5.3	17
Af-28	34	2	3	6	5	2	108	0.5	24	4	7	3	6	4	167	1.1	57
Af-29	32	6	7	6	29	23	131	3.8	33	18	40	5	85	59	156	13.2	10

Table A-9 (Continued)

	2005								2006								
	HT	ST	IF	VS	BM	RG	CPC	CPP	HT	ST	IF	VS	BM	RG	CPC	CPP	MT
Af-30	28	10	14	6	31	18	100	3.1	30	27	45	5	89	44	123	11.0	13
Af-30.1	27	7	7	6	24	14	141	3.4	28	24	36	5	41	33	140	5.7	40
Af-31	30	9	12	6	40	19	124	4.9	30	24	36	5	90	47	118	10.6	0
Af-32	27	6	5	5	18	6	143	2.6	26	17	20	4	35	18	136	4.8	23
Af-33	32	5	7	6	19	9	114	2.2	34	21	38	5	73	24	119	8.8	10
Af-34	22	4	3	5	7	6	132	1.0	26	18	14	4	38	19	146	5.5	20
Af-36	32	6	7	6	20	7	124	2.5	26	16	24	4	40	13	127	5.2	10
Af-37	30	4	4	6	11	7	135	1.5	30	15	23	4	40	17	144	5.8	30
Af-38	19	3	2	3	6	2	167	1.1	22	9	18	3	19	4	202	3.8	50
Af-39	31	5	5	6	15	10	155	2.3	32	17	30	5	46	18	165	7.5	20
Af-41	22	3	2	4	9	4	144	1.3	25	11	14	4	32	8	144	4.6	10
Af-42	26	5	2	5	16	10	151	2.4	32	21	26	5	68	23	125	8.6	17
Af-43	19	3	2	4	5	3	154	0.8	21	10	11	3	18	3	145	2.6	33
Af-44	26	5	3	5	20	7	154	3.1	29	20	19	5	65	13	134	8.7	13
Af-45	27	5	3	6	12	6	137	1.6	27	19	22	4	35	15	134	4.7	43
Af-45.1	27	5	2	5	18	12	157	2.8	29	19	32	4	64	19	151	9.7	17
Af-46	26	5	1	5	11	10	144	1.5	29	19	17	4	47	26	165	7.8	40
Af-47	27	4	3	5	15	10	129	2.0	29	16	21	4	67	20	147	9.9	10
Af-48	16	2	1	3	2	0	157	0.3	17	4	2	2	2	1	160	0.4	47
Af-49	17	2	0	3	3	1	163	0.6	16	6	1	2	7	6	156	1.1	33
Af-50	20	2	1	4	5	2	141	0.6	22	6	10	3	15	4	153	2.3	43
Af-51	21	3	1	4	5	2	142	0.8	17	4	3	2	8	3	150	1.1	47
Af-52	19	3	1	4	5	2	179	0.9	18	7	5	3	13	4	179	2.3	37
Af-53	27	3	3	5	10	7	147	1.5	26	10	14	4	35	12	179	6.2	33
Af-54	23	3	2	4	5	3	153	0.8	23	8	7	3	17	6	162	2.8	37
Af-55	33	3	2	6	6	2	122	0.7	34	7	14	4	11	3	143	1.6	53
Af-56	32	3	2	6	8	2	119	1.0	26	11	10	3	16	5	152	2.4	30
Af-58	37	2	4	6	11	3	113	1.2	30	5	15	3	13	6	152	2.0	23
Af-59	26	3	3	5	11	4	125	1.4	29	9	17	4	25	9	155	3.9	13
Af-60	25	5	5	5	13	3	141	1.8	23	11	8	3	23	6	147	3.4	17

Table A-9 (Continued)

	2005								2006								
	HT	ST	IF	VS	BM	RG	CPC	CPP	HT	ST	IF	VS	BM	RG	CPC	CPP	MT
Af-61	23	4	2	5	10	2	131	1.3	21	9	15	3	22	7	153	3.4	23
Af-62	26	4	2	5	11	4	170	1.8	27	14	16	4	36	12	151	5.5	17
Af-63	28	4	3	5	13	5	161	2.1	28	16	21	4	43	14	154	6.5	7
Af-64	22	4	1	5	10	5	148	1.4	26	19	17	4	42	12	147	6.2	23
Af-65	21	3	0	4	5	2	155	0.7	22	7	6	3	18	7	153	2.7	17
Af-66	16	2	2	3	5	2	121	0.6	21	5	9	3	16	4	157	2.5	20
Af-67	24	5	5	5	16	4	144	2.3	25	14	13	4	48	8	159	7.6	23
Af-68	21	4	2	4	5	2	151	0.8	20	8	15	3	22	6	174	3.9	27
Af-69	20	3	1	4	8	4	171	1.4	27	14	18	4	37	16	160	6.0	10
Af-70	30	8	11	6	26	14	116	3.0	31	21	35	5	63	31	126	7.9	17
Af-75	32	7	9	6	22	69	95	2.1	31	37	82	5	114	36	142	16.2	20
Af-76	31	8	9	6	35	18	109	3.8	32	28	42	5	88	37	122	10.7	7
Af-77	24	4	1	5	13	5	163	2.1	25	14	18	4	45	11	135	6.1	7

Table A-10. Millville data used to construct cluster dendrogram. Variables included August biomass (BM), seed yield, and seed weight in 2005 and 2006, and Oct. biomass (RG) and winter mortality (MT) in 2006.

	2005				2006			
	BM	RG	SY	SW	BM	SY	SW	MT
Af-3	22	3	0.43	0.33	47	0.70	0.38	43
Af-4	14	1	0.06	0.35	22	1.01	0.37	63
Af-5	83	17	9.51	0.42	61	4.71	0.44	33
Af-6	22	2	0.15	0.37	23	0.57	0.33	57
Af-7	19	1	0.04	0.36	43	1.18	0.46	37
Af-8	40	3	0.05	0.39	89	6.22	0.47	27
Af-9	39	3	1.05	0.35	88	2.28	0.42	27
Af-10	13	1	0.27	0.39	24	1.32	0.45	60
Af-11	41	2	0.98	0.54	82	2.89	0.59	23
Af-13	40	1	2.32	0.49	84	3.26	0.50	13
Af-14	26	1	0.12	0.45	58	3.30	0.49	30
Af-15	35	2	0.67	0.47	70	2.20	0.48	13
Af-16	33	2	0.16	0.37	73	2.11	0.43	27
Af-18	40	6	2.48	0.43	85	4.27	0.50	27
Af-19	19	2	0.21	0.39	63	3.47	0.46	33
Af-20	42	1	1.00	0.41	87	1.00	0.43	23
Af-21	18	0	0.50	0.33	38	2.33	0.39	20
Af-22	16	2	0.76	0.34	32	1.19	0.40	30
Af-23	15	1	0.33	0.46	35	0.87	0.43	20
Af-24	22	2	0.27	0.49	44	1.07	0.55	30
Af-25	16	1	0.26	0.43	35	2.81	0.49	37
Af-26	38	4	2.43	0.48	87	2.79	0.55	20
Af-28	17	2	0.90	0.37	15	0.51	0.38	43
Af-29	85	10	1.24	0.50	124	1.88	0.46	23
Af-30	98	17	7.03	0.52	136	7.38	0.59	23
Af-30.1	60	7	3.54	0.60	86	4.46	0.62	20
Af-31	93	14	2.89	0.59	147	7.47	0.58	13
Af-32	64	4	1.50	0.47	107	6.51	0.56	13
Af-33	46	3	1.02	0.55	108	2.82	0.57	37
Af-34	14	2	0.04	0.34	39	1.07	0.43	43
Af-36	34	3	0.58	0.55	65	6.63	0.57	20
Af-37	39	6	0.75	0.42	68	5.63	0.45	20
Af-38	16	1	0.19	0.42	24	2.04	0.39	43
Af-39	25	4	0.47	0.34	44	3.39	0.34	30
Af-41	23	1	0.59	0.45	49	4.66	0.46	33
Af-42	29	2	0.16	0.45	69	3.41	0.43	33
Af-43	13	1	0.09	0.49	34	0.45	0.61	53
Af-44	42	3	0.57	0.48	88	1.79	0.47	30

Table A-10 (Continued)

	2005				2006			
	BM	RG	SY	SW	BM	SY	SW	MT
Af-45	39	3	0.38	0.40	83	1.84	0.49	13
Af-45.1	40	4	0.33	0.46	94	5.65	0.46	20
Af-46	46	7	0.30	0.58	91	0.22	0.55	17
Af-47	47	4	1.99	0.44	97	1.42	0.40	13
Af-48	4	0	0.06	0.38	13	0.17	0.36	63
Af-49	8	0	0.04	0.41	15	0.02	0.38	37
Af-50	18	1	0.11	0.42	20	0.33	0.42	50
Af-51	8	1	0.01	0.43	25	0.34	0.48	53
Af-52	6	0	0.01	0.50	7	1.91	0.50	67
Af-53	19	1	0.61	0.61	32	1.09	0.54	37
Af-54	8	0	0.12	0.56	14	0.44	0.54	53
Af-55	7	0	0.12	0.30	5	0.15	0.31	60
Af-56	10	1	0.03	0.30	16	0.94	0.42	83
Af-58	15	1	0.34	0.32	16	1.47	0.37	37
Af-59	20	1	0.08	0.47	38	1.72	0.45	43
Af-60	40	4	0.42	0.44	98	6.88	0.49	23
Af-61	33	1	1.29	0.48	64	3.59	0.44	40
Af-62	29	1	0.34	0.56	78	4.41	0.54	27
Af-63	23	2	0.32	0.34	56	2.11	0.41	30
Af-64	30	1	0.77	0.41	66	3.44	0.46	20
Af-65	10	0	0.14	0.40	23	0.70	0.29	57
Af-66	8	0	0.19	0.40	15	0.79	0.40	60
Af-67	12	1	0.20	0.32	26	0.12	0.37	60
Af-68	11	1	0.11	0.37	22	0.51	0.50	57
Af-69	17	2	0.26	0.46	49	1.77	0.47	43
Af-70	78	14	2.01	0.53	127	5.81	0.60	20
Af-75	55	17	2.35	0.40	48	2.79	0.42	50
Af-76	84	10	6.11	0.55	103	3.04	0.52	20
Af-77	35	3	0.17	0.41	75	3.40	0.49	23

APPENDIX B: PERMISSION-TO-USE LETTER



United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area

116

Forage and Range Research Lab
Utah State University
Logan, UT 84322-6300
Phone: 435-797-3082
Fax: 435-797-3075
Email: tomjones@cc.usu.edu

April 9, 2007

School of Graduate Studies
Utah State University
Logan, UT 84322-0900

To Whom It May Concern:

This letter serves as a permission-to-use letter for Chapter 3 of Kishor Bhattarai's thesis.

I anticipate being a co-author on a paper developed from this chapter and give Kishor Bhattari permission to use any material from our paper in his thesis.

Sincerely,


Thomas A. Jones
Research Geneticist