Plant Propagation Protocol for *ASAG2*

ESRM 412 – Native Plant Production

URL: https://courses.washington.edu/esrm412/protocols/*2024/ASAG2.pdf*

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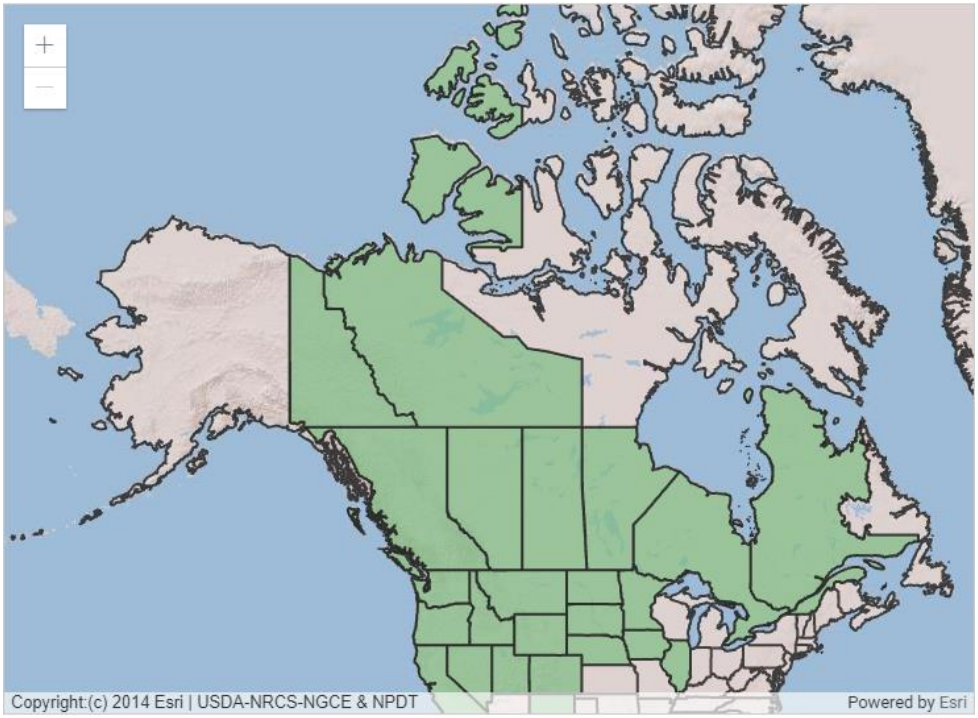
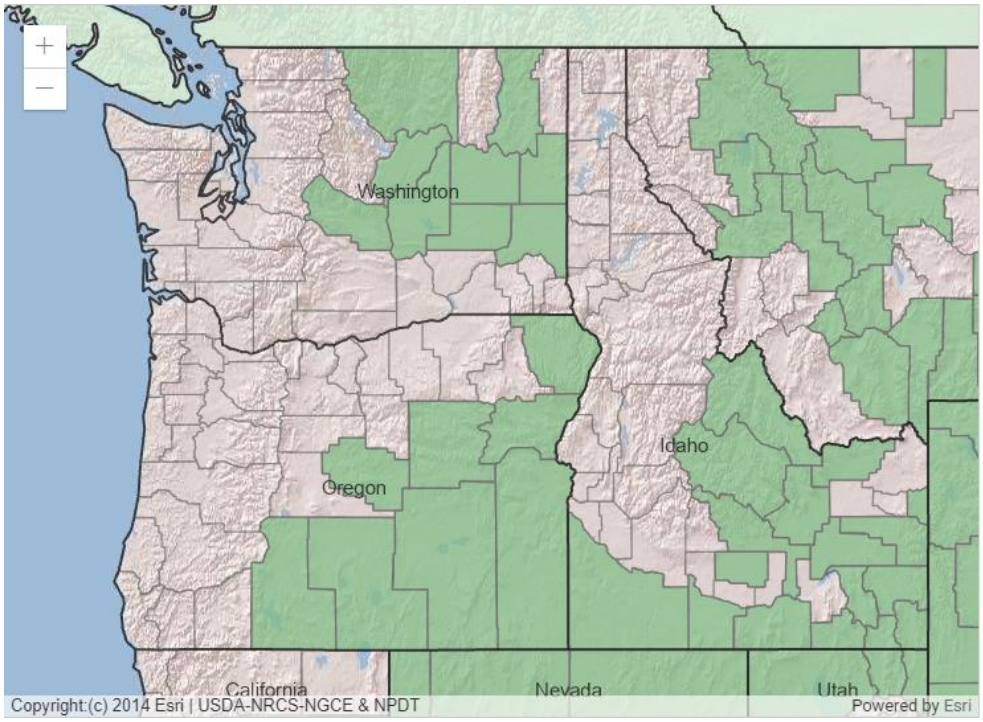
Purple coloration image: Al Schneider. United States, Colorado, NM, AZ, UT, Four Corners vicinity, within 150 miles of the corners. (Schneider)
White coloration image: Nevada Native Plant Society. Margaret Williams. Provided by Nevada Native Plant Society. United States, Nevada. (Williams)

TAXONOMY	
Plant Family	
Scientific Name	*Fabaceae Lindl.*
Common Name	Pea, Legumes Family
Species Scientific Name	
Scientific Name	*Astragalus agrestis Douglas ex G. Don*
Varieties	No varieties noted.
Sub-species	No sub-species noted.
Cultivar	No cultivars noted.
Common Synonym(s)	Astragalus goniatus Nutt. ex Torr. & A. Gray Astragalus hypoglottis Hook. Astragalus dasyglottis Fisch. ex DC. Astragalus danicus var. dasyglottis (Fisch. ex DC.) B. Boivin (*Astragalus agrestis Douglas ex G. Don*)

Common Name(s)	Purple Milkvetch, cock's-head, field milkvetch (*Astragalus agrestis Douglas ex G. Don*)
Species Code (as per USDA Plants database)	ASAG2
GENERAL INFORMATION	
Geographical range	USA: CA, CO, IA, ID, IL, KS, MN, MT, ND, NE, NM, NV, OR, SD, UT, WA, WY Canada: BC, SK Native Distribution: Across much of Canada and south to northeastern California, southern Utah, central New Mexico, Kansas, and Iowa. (*TWC Staff,* 2015) Note that this species also occurs in the UK and parts of Asia. Distribution is found across Britain and Ireland. Main population found in the eastern side of England, along the coastal grasslands. Absent in Northern Ireland. Has an isolated population on the Aran Islands. (*Natural England)* North America Distribution  *(Purple Milkvetch Distribution Data Map,* 2014)

	Northwest distribution.  *Purple Milkvetch Distribution Data Map,* 2014)
Ecological distribution	Commonly found in moist meadows and prairies and on cool brushy slopes. Spreads across much of Canada and south towards northeastern California, southern Utah, central New Mexico, Kanas, and Iowa. (*TWC Staff, 2015)* Canada: Occur in 3 different prairie types (Fescue, Mixed Grass, and Tall Grass). Also occur in open woodlands, sagebrush, and along streambanks in clayey, loamy, and silty soil. They are observed to be pollinated by bumble, honey, mason, sweat, halictid, leafcutter, and other bees, alongside skippers. (The Manitoba Museum, 2013) UK: In species-rich short, dry, and infertile calcareous grassland on limestone and chalk. Also found in coastal sand-dunes. Found to be generally grown on moderately acidic sands/gravels when competition is kept at a low. Reportedly found on old red sandstone sea cliffs and machair grasslands in Scotland. (*Natural England)*

	Montana: It's reported to be found in grasslands, meadows, sagebrush steppe, open forests, and thickets near streams. They are associated with ponderosa pine-juniper, woodlands, thickets and wooded coulees, mixed grass prairies, sagebrush steppe communities, and moist coulee bottoms and swales in Philips and Valley Counties in Montana. (Gucker & Shaw, 2023) North Dakota: Found to be associated with northern mixed-grass prairie which was dominated by Kentucky bluegrass (*Poa pratensis*), western wheatgrass (*Agropyron smithii*), green needlegrass (*Nassella viridula*), and sedge species (*Carex spp.).* (Gucker & Shaw, 2023) Northwestern Wyoming: Occurred in dry montane meadows, in northern mixed-grass prairie, in sagebrush, and along roadsides. (Gucker & Shaw, 2023) Colorado: Patches found on plains and mesas and well as in open ponderosa pine-Douglas-fir (*Pseudotsuga menziesii*) stands. (Gucker & Shaw, 2023) Utah: Found commonly in meadows and in the openings of sagebrush shrublands and quaking aspen (*Populus tremuloides*) woodlands. (Gucker & Shaw, 2023) California: Occurred in sagebrush vegetation. (Gucker & Shaw, 2023)
Climate and elevation range	They occur in moist and cool latitudes in low to mid elevations but can be found up to 10,500 ft or 3,200 m in the Rocky Mountains and 15,000 ft or 4,600 m in New Mexico. In California, due to its limited distribution, the plants occur around 5,500 ft or 1,700 m in elevation. In Utah, the elevation range of this species is 6,070 to 10,000 ft or 1,850-3,050 m, and in New Mexico it is 7,000 to 15,000 ft or 2,100-4,600 m in elevation. (Gucker & Shaw, 2023)
Local habitat and abundance	Frequently found in a variety of grasslands, shrublands, and vegetation types. Their often associated with poorly drained areas where water accumulates in in the early growing season. It prefers the moist shady sites such as meadows, water edges, and cool brushy slopes. (Gucker & Shaw, 2023) Did not find specific plants associations in terms of local habitat and abundance but in other states. Those can be found in listed in the Ecological distribution section. From those observations however, they can

	likely be found in areas where there is Sagebrush, open forests, fields, meadows, prairies, and any other like places that occur in the Pacific Northwest as well.
Plant strategy type / successional stage	Facultatively seral, thrives in different stages of succession. Colonizes disturbed areas via seed exposure and rhizomes in open bare areas. (Assumed from information derived from the overall research of this protocol.) Note that they are quite fire tolerant and emerge quickly after prescribed fires. It is noted to be more beneficial for them in terms of successful seedling emersion when emerging from burned prairies than mowed prairies. Hence making them stress tolerators. (Gucker & Shaw, 2023) They have been observed to increase the consumption of sagebrush and Sandberg bluegrass by elk which makes them competitors. (Gucker & Shaw, 2023)
Plant characteristics	Purple milkvetch are low growing perennial forbs that spread via rhizomes and seeds. Grow in patches, with weak stems often leaning on other vegetation. (*TWC Staff, 2015)* From images provided cited on the USDA page, it seems that the flowers can be white as well. The flowers are pea-like, (usually) purple in color that from in short clusters connected to the numerous slender hairy stems that grow 10 to 30 cm tall. The outermost flowers bloom first, with each of the flowers having one upper narrow banner petal, two sing petals, a pointed keel, and a toothed, hairy calyx. They have alternative leaves that are hairy and divided into 11 to 23 linear to oblong leaflets with notched tips. They also have fruits that are erect, oval-shaped, and hairy, which are pods that contain seeds that are about 1.5mm wide. (The Manitoba Museum, 2013), (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024) Note that this plant is potentially harmful to humans and animals if consumed, and some in the same genus are known to accumulate toxic levels of selenium. (The Manitoba Museum, 2013) They are also associated with nitrogen-fixing bacteria like many other rhizomatous plants. They spread quicky via their rhizomes under the right conditions. They are also pollinator friendly where flowers are often visited by butterflies and used as a host plant for many. (Gucker & Shaw, 2023)

PROPAGATION DETAILS: FROM SEED Note that this protocol is derived from a related species of Milkvetch. The Palouse Milkvetch.	
Ecotype	No ecotype was provided by this protocol, it was noted that this species is particularly found endemic to eastern Washington particularly Whitman County. (Skinner, David M, 2005) As seen in the map, the Purple Milkvetch also occur in this portion of eastern Washington.
Propagation Goal	Plants
Propagation Method	Seeds
Product Type	Container (plug)
Stock Type	10 cu. In. Ray Leach Super Cell conetainers
Time to Grow	4 months.
Target Specifications	Tight root plug in containers.
Propagule Collection Instructions	The collection of the fruit seed capsules is collected late July or early Auguet when the capsules brown and start to split. Note that the seeds should be hard and brown. Timing of collection is not as critical as they do not forcefully dehisce but note that the seeds will shatter and must be collected in a timely manner. (Skinner, David M, 2005)
Propagule Processing/Propagule Characteristics	Specific densities and longevity not specified.
Pre-Planting Propagule Treatments	Small amounts of the pods are crushed by hand to free the seeds then cleaned with an air column separator. While larger amounts are threshed with a hammer mill then cleaned with air screen equipment. The seeds should be able to withstand mechanical threshing. (Skinner, David M, 2005) The seed coat restricts water uptake, so scarification of the seeds is required to help them increase their chances of germination. Unpublished data shows that 58% of seeds germinate when untreated. In this same experiment, when seeds were hot water scarified, they had 78% while using mechanical scarification by rubbing between two pieces of sandpaper yielded 93% germination. (Skinner, David M, 2005) Specific storage and dormancy treatments not specified, but likely stored in a dry area before planting in January.
Growing Area Preparation / Annual Practices for Perennial Crops	The seeds in Skinner's protocol were inoculated with the proper Rhizobium species prior to their planting. They seeds were sown in January in a greenhouse in 10 cu. In. Ray Leach Super Cell conetainers (allowed for deep watering) with Sunshine #4 mix and lightly covered with a thin layer of pea gravel to prevent seeds from floating. (Skinner, David M, 2005)

Establishment Phase Details	The medium is kept moist until germination occurs which a few might within 7 to 8 days, but the bulk of them will not until 2 to 3 weeks later. A few seeds will also germinate a month or later after sowing. No non-scarified seeds will germinate after two weeks, overall, the total germination was much lower. (Skinner, David M, 2005)
Length of Establishment Phase	3 weeks
Active Growth Phase	The plants need to be watered deeply every other day and fertilized one a week. The fertilizer used was a water-soluble fertilizer that contained micronutrients. (Skinner, David M, 2005)
Length of Active Growth Phase	3 months
Hardening Phase	The plants are moved from the greenhouse into the cold frame for the hardening phase in April. They are then watered every other day if the weather is cool and every day when the weather is hot. (Skinner, David M, 2005)
Length of Hardening Phase	2 weeks
Harvesting, Storage and Shipping	No information was provided
Length of Storage	No information was provided
Guidelines for Outplanting / Performance on Typical Sites	No information was provided in the protocol.
Other Comments	It is classified as an Endangered plant in Great Britain in 2005. It's noted that very little is known about its seeds longevity but as been seen to reappear on a coniferous plantation after clearing. It suggested that they have quite a significant seed dormancy capacity. (*Natural England)* Likely to want the seeds and plants closed off in an area to prevent grazing, as it has been observed to have declined in areas where grazing occurs. Management is required with suggested low to moderate grazing during autumn and winter, but preferably no grazing and no grass fertilizer in areas where it grows as it increases shade in established sites. (*Natural England)* However, for germination, open bare ground and soil disturbance may be required to bring buried seeds to the surface alongside supplementation with seeds from the seed bank. (*Natural England)*

PROPAGATION DETAILS: FROM SEED Note that this is based on a Propagation Protocol from the Millennium Seed Bank in Wiltshire, UK.	
Ecotype	No specific ecotype listed but it's noted that the seeds tested were stored in the Millennium Seed Bank since July 2023 in Wiltshire. They provided a distribution map and areas the MSB has taken seeds from and conserved in their collection. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Propagation Goal	Not specified but likely plants, seeds, and cuttings for further additions into their collection.
Propagation Method	Seeds
Product Type	Plugs
Stock Type	Plug trays
Time to Grow	Laboratory method: Sown June 2014 grows for about a month and a half. Nursery method: Sown March 2021 grows for about 2 months and 12 days. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Target Specifications	Once several pairs of true leaves have grown seedlings are pricked out into plug trays and good for out planting in autumn. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Propagule Collection Instructions	The seeds are harvested in late July to early August. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024) Directions on how they were collected is lacking.
Propagule Processing/Propagule Characteristics	The approximate dry weight of 1000 seeds were noted to be 1.262g. Since the seeds used for testing were collected in July 2013, we can assume a significant seed longevity. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Pre-Planting Propagule Treatments	The seeds are stored alongside 15,000 other seeds in the Millennium Seed Bank where they are stored at 15% relative humidity in -20°C. The cleaning was not specified, but the seeds are physically dormant like many others in the Pea family with germination triggered by scarification. Laboratory method:

	They were placed in a seed coat chip for 3 weeks at 20°C. Nursery method: The seed coat was scarified with sandpaper. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Growing Area Preparation / Annual Practices for Perennial Crops	Laboratory method: 1 x 50 seed replicates. They were placed into 1% agar gel. Nursery method: 3 x 50 seed replicates. They were planted in seed trays filled with Petersfield Peat-free Supreme compost and Special Seed perlite in a 50:50 ratio along with sand, calcified seaweed and chalk mixed into the mixture. This helped replicate the varied habitats they are found in but was noted to possibly not be needed in terms of propagation. Note that 65% of the total germinated which was 40% of the total sown. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Establishment Phase Details	Laboratory method: They were placed into 20°C conditions. Nursery Method: In March 2021, the seed trays were placed outdoors in the cold frame. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Length of Establishment Phase	Laboratory method: After 7 days they germinate Their germination duration lasts for 14 days. Nursery Method: After 12 days they reach peak germination. Their germination duration lasts for 2 months. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Active Growth Phase	Laboratory method: n/a

	Nursery method: Placed into cold frame between March and May in Petersfield Peat-Free Supreme compost with Sinclair Special Seed perlite and a small amount of crushed chalk and feed. (Specific ratios not specified.) (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Length of Active Growth Phase	Laboratory method: n/a Nursery method: approximately 2-3 months. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Hardening Phase	Not listed.
Length of Hardening Phase	Not listed.
Harvesting, Storage and Shipping	Around 2 months after germination, seedlings are pricked out into plug trays once several pairs of true leaves have grown and planted into free draining peat-free compost with perlite and crushed chalk. They are then placed into a sunny site until plugs are ready to be out planted in the following Autumn. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Length of Storage	Not specified.
Guidelines for Outplanting / Performance on Typical Sites	Laboratory method: n/a Nursery method: The plugs can be out planted in the following Autumn. No other information was provided. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)
Other Comments	Note that the site for further information linked in the report is no longer running or accessible. Keep note of legalities in seed collection in the UK may be different from seed collection in the North Americas. Generally, want to consult site conditions and management in seed collection and out planting. (Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. 2024)

PROPAGATION DETAILS: VEGETATIVE Note that this was experimental and there were no out planting results.	
Ecotype	This was not specified, but it's noted that they are likely rescued plants.
Propagation Goal	Not specified, but the goal was to rescue the plants from the wetter growing seasons and grazing by wildlife. In result photos they are seen as bare root plants.
Propagation Method	Vegetative
Product Type	Not specified.
Stock Type	Not specified.
Time to Grow	4 weeks of propagation before root assessment. (Khanal et al., 2015) The plants were not noted to be outplanted.
Target Specifications	No specified size or characteristic, but the goal was to have high rooting success.
Propagule Collection Instructions	They conducted the study at the SPARC of AAFC near Swift Current, Saskatchewan in 2014-15. They collected and used hardwood, semi-hardwood, and top shoot cuttings. (Khanal et al., 2015)
Propagule Processing/Propagule Characteristics	Not provided.
Pre-Planting Propagule Treatments	Not provided.
Growing Area Preparation / Annual Practices for Perennial Crops	They were planted in various rooting treatments on a solid foam media in a growth chamber. The cuttings were established in potted soil medium that contained equal proportion of topsoil, vermiculite, and Professional Growing Mix (Sun Gro Horticulture). (Khanal et al., 2015) The authors decided to then look further into the top shoot cutting mainly in which then they promptly, tested them in five different rooting treatments. The cut ends were then dipped for 4 hours in the growth regulator r solutions including IAA 100mg L-1, NAA 100mg L-1 , IBA 100mg L-1 , mixture of equal strength solutions (100mg L-1) of IAA, NAA and IBA, and half-strength MHL solution. Each of the treatment contained 6 to 11 cuttings and were randomized in a complete block design each with 3 replicates. (Khanal et al., 2015)
Establishment Phase Details	The treated top shoot cuttings were then planted in sand medium in 3 x 3 size plastic containers that had shallow layers of vermiculite and calcined clay at the top and bottom of the containers. These were then placed into propagator tray and covered with a clear transparent dome-shaped cover that had 2 adjustable slits in them for air exchange. (Khanal et al., 2015)
Length of Establishment Phase	Not specified.

Active Growth Phase	Not applicable for some of parts the study since they come from hardwood and semi-hardwood cuttings, but the top shoot cuttings will require some active growth time which was not directly specified. However, they were placed into a greenhouse that comprised of 21°C/19°C Day/night temperature cycles with 300 µmol m-2 s -1 of photosynthetically active radiation (PAR) for 16 hours a day. This is while the sandy soils were kept moist by applying half-strength MHL solution. These conditions allowed for their roots to establish after 4 weeks of propagation. After 3 weeks of propagation the cover was removed to acclimate the plants under ambient humidity conditions. After this removal they were also kept under low light (100 µmol m-2 s -1 PAR) for 3 days to prevent young leaves from photo-oxidative damage. (Khanal et al., 2015)
Length of Active Growth Phase	n/a
Hardening Phase	Not applicable to the hardwood and semi-hardwood cuttings, but not specified for the top shoot cuttings.
Length of Hardening Phase	Not specified.
Harvesting, Storage and Shipping	After 4 weeks they are “harvested” to measure their survival and rooting percentages, but they are not noted to be stored or shipped afterwards. (Khanal et al., 2015)
Length of Storage	Not noted.
Guidelines for Outplanting / Performance on Typical Sites	Not noted.
Other Comments	After 4 weeks of propagation and after their assessment in survival and rooting percentage, root vigor, and longest root length, the authors came to a consensus that Purple Milkvetch can be propagated from hardwood, semi-hardwood, and top shoot cuttings, but at a variable degree of success. Initial measure shows better survival in semi-hardwood than hardwood and top-shoot cuttings. However, the semi-hardwood cuttings then suffered die backs from the top with top shoot cutting showing better persistence when transplanting, so they were further accessed than the other cutting types. In conclusion they found that they can be propagated in sand-based medium under greenhouse condition at a high degree of success using auxin like growth regulators and half strength MHL solution. (Showing high root vigor.) The authors note that further testing will be required to further support these results. (Khanal et al., 2015)

INFORMATION SOURCES	
References	1. *Astragalus agrestis Douglas ex G. Don. Retrieved [May, 17, 2024], from the Integrated Taxonomic Information System (ITIS) on-line database, www.itis.gov, CC0 https://doi.org/10.5066/F7KH0KBK* 2. *Astragalus agrestis Douglas ex G. Don. USDA, NRCS. 2024. The PLANTS Database (http://plants.usda.gov, 05/17/2024). National Plant Data Team, Greensboro, NC USA. https://plants.usda.gov/home/plantProfile?symbol=ASAG2* 3. *Gucker, C., & Shaw, N. (2023, November). Purple milkvetch (Astragalus agrestis). Western Forbs: Biology, ecology, and use in restoration. https://westernforbs.org/species/purple-milkvetch-astragalus-agrestis/* 4. *Khanal, N., Schellenberg, M. P., & Coulman, B. E. (2015, March 16). Standardizing vegetative propagation as a tool for polycross mating of ascending purple milkvetch (astragalus adsurgens pall.). HARVEST. https://harvest.usask.ca/items/fdee2b44-f3e0-4df2-be05-435b1d6dd5a4* 5. *Natural England. (n.d.). Purple Milk-vetch Astragalus danicus. Plantlife. https://naturebftb.co.uk/wp-content/uploads/2020/08/Purple_milk_vetch-Plantlife-factsheet.pdf* 6. *Purple Milkvetch Distribution Data Map*. (2014). USDA United States Department of Agriculture. Retrieved May 17, 2024, from *https://plants.usda.gov/home/plantProfile?symbol=ASAG2* 7. Royal Botanic Gardens, Kew. (2024, April 5). Millennium Seed Bank Partnership Propagation Protocol. Astragalus danicus Retz. Purple Milk-vetch. Royal Botanic Gardens, Kew. https://doi.org/10.34885/j4z3-zr20 8. Schneider, A. (n.d.). United States, Colorado, NM, AZ, UT, Four Corners vicinity, within 150 miles of the corners. USDA United States Department of Agriculture. Retrieved May 22, 2024, from https://plants.usda.gov/home/plantProfile?symbol=ASAG2. 9. Skinner, David M,. 2005. Propagation protocol for production of Container (plug) *Astragalus arrectus* Gray plants USDA NRCS - Pullman Plant Materials Center Pullman, Washington. In: Native Plant Network. URL: https://NativePlantNetwork.org (accessed 2024/05/21). US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. 10. The Manitoba Museum, (2013, October 3). Purple Milk-vetch. Prairie Pollination. https://prairiepollination.ca/plante-plant/astragale_rustique_ou_pourpre-purple_milk_vetch/ 11. *TWC Staff. (2015, March 16). Astragalus agrestis. Lady Bird Johnson Wildflower Center.*

	https://www.wildflower.org/plants/result.php?id_plant=ASAG2 (note that this source is listed as a source associated with the University of Texas and connected the USDA page for this plant) 12. *Williams, M. (n.d.). Nevada Native Plant Society. Margaret Williams. Provided by Nevada Native Plant Society. United States, Nevada. USDA United States Department of Agriculture. Retrieved May 22, 2024, from https://plants.usda.gov/home/plantProfile?symbol=ASAG2.*
Other Sources Consulted	None
Protocol Author	Ruihua Huang
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