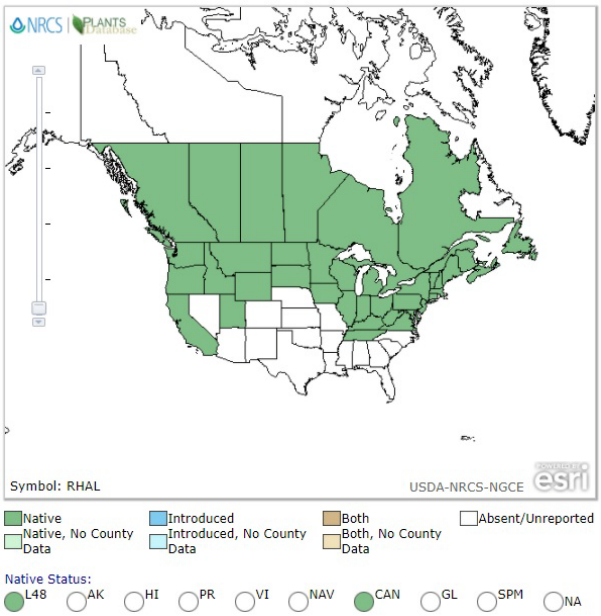
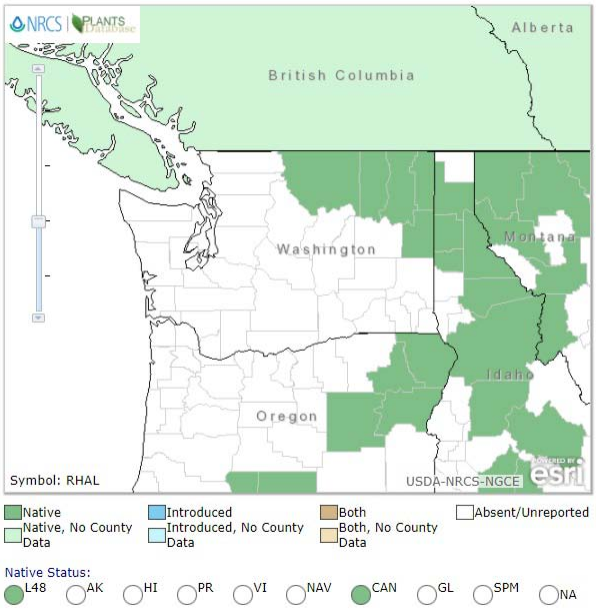
Plant Propagation Protocol for *Rhamnus alnifolia*
ESRM 412 – Native Plant Production
Spring, 2018

North America Distribution



Washington State Distribution



Source: https://plants.usda.gov/core/profile?symbol=RHAL (USDA Plant Database)

TAXONOMY	
Plant Family	
Scientific Name	Rhamnaceae (USDA Plant Database)
Common Name	Buckthorn family (USDA Plant Database)
Species Scientific Name	
Scientific Name	*Rhamnus alnifolia* L'Hér (USDA Plant Database)
Varieties	None
Sub-species	None
Cultivar	None
Common Synonym(s)	None
Common Name(s)	Alderleaf buckthorn (USDA Plant Database)
Species Code	RHAL (USDA Plant Database)
GENERAL INFORMATION	
Geographical range	1. CAN: Alta., B.C., Man., N.B., Nfld. and Labr. (Nfld.), N.S., Ont., P.E.I., Que., Sask. (Flora of North America). 2. USA: Calif., Conn., Idaho, Ill., Ind., Iowa, Maine, Md., Mass., Mich., Minn., Mont., N.H., N.J., N.Y., N.Dak., Ohio, Oreg., Pa., R.I., S.Dak., Tenn., Utah, Vt., Va., Wash., W.Va., Wis., Wyo. (Flora of North America). 3. See the detail map above. (USDA Plant Database).
Ecological	"Fens and swamps, generally calcareous, riparian thickets, interdunal

distribution	swales, shore lines, marshes and mats, wet meadow edges, outcrops, deciduous and coniferous forests." (Flora of North America)
Climate and elevation range	1. Found in moist place. (Sargent, 1894). 2. 10–2700 m. (Flora of North America).
Local habitat and abundance	Woodland, Swamp/Marsh, Bog/Fen, Lakeshores (Sharma and Graves, 2005).
Plant strategy type / successional stage	1. $CaCO_3$ Tolerance: High (Sharma and Graves, 2005). 2. Shade tolerance (Plant Database, Lady Bird Johnson Wildflower Center at The University of Texas at Austin). 3. *Rhamnus alnifolia* is a primary host for the soybean aphid (Voegtlin *et al.*, 2005). 4. Known to be an alternate host for *Puccinia coronata* Corda, the crown rust of oats (Dietz and Leach, 1930).
Plant characteristics	"Perennial shrub, 0.5–1(–1.5) m, unarmed. Branchlets gray to brown, glabrous or pubescent. Leaves deciduous, alternate; petiole 5–15 mm; blade dark green to olive green on both surfaces, lanceolate-oblong to elliptic or lanceolate-ovate, 4.5–11 cm, herbaceous, base cuneate to rounded, truncate, or subcordate, margins crenate to crenate-serrate, apex obtuse to acute or acuminate, abaxial surface glabrous or puberulent along veins, adaxial surface glabrous or glabrate; secondary veins (4–)5–7 pairs, all diverging at nearly same angle. Inflorescences fascicles or flowers solitary. Pedicels 2–10 mm. Sepals 5. Petals 0. Drupes black, globose or slightly elongate, 6–8 mm; stones 3. Flowering May to July." (Flora of North America)
PROPAGATION DETAILS **Propagation by Seed**	
Ecotype	1. Gravel bar of stream; adjacent to Pine/ Fir forest, Many Glacier, Glacier National Park, Glacier Co., MT. 1585m elevation (Luna *et al*., 2008). 2. Two- to three-seeded drupes (fruits) were collected from a natural population of *R. alnifolia* in eastern Vilas County, WI, on July 27, 2002 (Sharma and Graves, 2005). 3. Another collection was made on September 6, 2002, from plants growing in Marinette County, WI (Sharma and Graves, 2005).
Propagation Goal	Plants
Propagation Method	Seed
Product Type	Container (plug) (Luna *et al*., 2008).
Stock Type	1. 160 ml containers (Luna *et al*., 2008). 2. 11 cm^2 pot (Kord Products, Brampton, Ont., Canada; volume = 1327 cm^3) (Sharma and Graves, 2005).
Time to Grow	1. 11 Months (Luna *et al*., 2008). 2. 102 days (Sharma and Graves, 2004).
Target Specifications	1. Stock Type: Container seedling, Height: 5 cm, Caliper: 7 mm, Root System: Firm plug in containers (Luna *et al*., 2008). 2. Height: 20 cm (Sharma and Graves, 2004).
Propagule Collection	1. "Collect mature fruits when they turn black in September. Seeds are

Instructions	brown at maturity. Fruit is collected in plastic bags and kept under refrigeration prior to cleaning." (Luna *et al*., 2008) 2. "Although the outer tissues of fruits had changed from green to dark blue, it is possible the seeds had not ripened fully at the time of collection in late-July. Higher germination percentages often are obtained among seeds that are collected after they have dried while attached to the mother plant than when germination is attempted with immature seeds collected in mid-season." (Sharma and Graves, 2005)
Propagule Processing / Propagule Characteristics	1. 65,500 seeds per pound (USDA Plant Database). 2. Seeds/Kg: 5,000/kg, Purity: 100%, Germination: 75% (Luna *et al*., 2008).
Pre-Planting Propagule Treatments	1. "Seeds are cleaned by maceration using a Dybvig seed cleaner. Seed Storage is at least 5 to 7 years at 3 to 5℃ in sealed containers. Seed dormancy is classified physiological dormancy. 5 month outdoor cold, moist stratification." (Luna *et al*., 2008) 2. "Seeds were extracted before pretreatment by removing the surrounding fruit tissues by hand. While softening of outer tissues of seeds may benefit germination, scarification for 10 min in sulfuric acid likely damaged the embryos. It is conceivable that shorter scarification periods might be more beneficial for abrasing the seed coat without damaging the embryonic tissues. Scarification of seeds in hot water and subsequent moist-prechilling at 5℃ for 30 days inhibited germination. Summer germination: Highest germination percentage (13%; germination value = 0.15) was observed among *R. alnifolia* seeds moist-prechilled for 90 days. Fall germination: Germination percentage was 48% (germination value = 1.9) among fresh *R. alnifolia* seeds harvested in September and then moist-prechilled for 30 days." (Sharma and Graves, 2005) 3. "Cold stratification for up to 90 d resulted in 48% germination and germination value of 1.9, whereas only 7% germination occurred among seed stratified for 120 d. Seeds did not germinate if they were untreated or if they were scarified and stratified." (Sharma and Graves, 2004)
Growing Area Preparation / Annual Practices for Perennial Crops	1. "Outdoor nursery growing facility. Sowing Method: Direct seeding. Seeds are covered with medium. Growing medium used is 6:1:1 milled spaghnum peat, perlite, and vermiculite with Osmocote controlled release fertilizer (13N:13P_2O_5:13K_2O; 8 to 9 month release rate at 21℃) and Micromax fertilizer (12%S, 0.1%B, 0.5%Cu, 12%Fe, 2.5%Mn, 0.05%Mo, 1%Zn) at the rate of 1 gram of Osmocote and 0.20 gram of Micromax per 172 ml container. Containers are filled and sown in late fall and irrigated thoroughly prior to winter stratification. Seedlings germinate in spring under fluctuating outdoor temperatures and are grown under full sun exposure. Seedlings are irrigated with Rainbird automatic irrigation system in early morning until containers are thoroughly leached."

	(Luna *et al*., 2008) 2. Growing medium: LC-1; Sun Gro Horticulture Canada Ltd., Seba Beach, Alta., Canada. 11 cm^2 pot (Kord Products, Brampton, Ont., Canada; volume = 1327 cm^3) (Sharma and Graves, 2005).
Establishment Phase Details	1. "Germination began in early June and was very uniform. True leaves were evident 2 weeks after germination and were thinned at this stage." (Luna *et al*., 2008) 2. "Seeds were placed in a glasshouse where day/night temperatures averaged 22/20℃. Supplemental irradiance was not provided during this experiment. The substrate was kept moist with tap water." (Sharma and Graves, 2005) 3. Survival of germinants was 90 to 100% regardless of prior seed treatment (Sharma and Graves, 2004).
Length of Establishment Phase	4 weeks (Luna *et al*., 2008).
Active Growth Phase	"Shoot and root growth occurred rapidly after thinning. Plants were fertilized with 13-13-13 liquid NPK fertilizer during the growing season. 50% of seedlings were root tight 7 weeks after germination." (Luna *et al*., 2008)
Length of Active Growth Phase	10 weeks (Luna *et al*., 2008).
Hardening Phase	"Plants are fertilized with 10-20-20 liquid NPK at 200 ppm during August and September. Irrigation is gradually reduced in September and October. Plants were given one final irrigation prior to winterization." (Luna *et al*., 2008)
Length of Hardening Phase	4 weeks (Luna *et al*., 2008).
Harvesting, Storage and Shipping	"Total Time To Harvest: 11 months. Harvest Date: September. Storage Conditions: Overwinter in outdoor nursery under insulating foam and snow." (Luna *et al*., 2008)
Length of Storage	5 months (Luna *et al*., 2008).
Guidelines for Outplanting / Performance on Typical Sites	No information.
Other Comments	"Threatened and endangered information: endangered in Illinois and Tennessee States. Noxious weed information: primary noxious weed in Iowa State." (USDA Plant Database)
PROPAGATION DETAILS **Propagation by Vegetative**	
Ecotype	1. Gravel bar of stream; adjacent to Pine/ Fir forest, Many Glacier, Glacier National Park, Glacier Co., MT. 1585m elevation (Evans, 2001). 2. From plants of *Rhamnus alnifolia* growing on a slope along a stream

	in Clayton County, IA, after appropriate permits were obtained from The Nature Conservancy (Sharma and Graves, 2005).
Propagation Goal	Plants
Propagation Method	Vegetative
Product Type	Container (plug) (Evans, 2001).
Stock Type	1. 800 ml containers (Evans, 2001). 2. Plastic container [SR225, The Lerio Corp, Mobile, AL; vol = 14 in^3 (227 cm^3)] (Sharma and Graves, 2005).
Time to Grow	15 Months (Evans, 2001).
Target Specifications	Stock Type: Container cutting, Height: 15 cm, Caliper: 7 mm, Root System: Firm plug in containers (Evans, 2001).
Propagule Collection Instructions	1. Vegetative Propagation Method: Pre-Rooting, Type of Cutting: Summer softwood stem cutting collected in late June (Evans, 2001). 2. "Fifty, terminal, 25-cm-long (10 in), actively growing shoots were collected on June 7, 2003. Shoots were then cut mid-length to obtain two types of cuttings: 1) terminal cuttings and 2) subterminal cuttings." (Sharma and Graves, 2005)
Propagule Processing / Propagule Characteristics	1. Cuttings are kept moist and under refrigeration prior to pre treatment (Evans, 2001). 2. Stems were transported on ice to Iowa State University in Ames, IA, where they were stored at 5℃ (41°F) until they were processed the next day (Sharma and Graves, 2005).
Pre-Planting Propagule Treatments	1. "Cuttings were 17 cm in length and were taken in late June. Cuttings were treated with 8000 ppm Hormex rooting powder and struck in sand media under mist with bottom heat for 7 weeks. Cuttings produced well branched roots. Cuttings were potted in 800 ml pots and were root tight in 4 weeks. Rooting %: 50%." (Evans, 2001) 2. "Application of 3000 and 8000 mg/L IBA in talc led to 85% rooting. While 75% of untreated cuttings rooted, fewer roots formed without IBA." (Sharma and Graves, 2004) 3. "Bases of cuttings were dipped for 10 sec into the IBA solution and were then dried for 1 min to allow absorption of the solution. To apply the talc, cuttings were dipped in the powder (2-cm-deep) and then were tapped slightly to remove excess powder." (Sharma and Graves, 2005). Effect of IBA on cutting of *Rhamnus alnifolia* is showed in Table 1 (Sharma and Graves, 2005). **Table 1. Bases of cuttings of *Rhamnus alnifolia* were treated with IBA, and cuttings were kept under intermittent mist for 35 d. Because interactions among treatments were not detected, data for type of substrate and for position of stem cutting on ortet were pooled (n = 20).** **IBA g/kg (ppm) / Cuttings with leaves remaining green (%) / Callus (%) / Rooting (%) / Root no. / Length of longest root (cm)** 0 / 0a[x] / 80a / 75a / 7c / 6a 3 (3000) [talc][z] / 10a / 90a / 85a / 15b / 8a 8 (8000) [talc] / 30a / 85a / 85a / 30a / 5a 3 (3000) [solution][y] / 5a / 15b / 15b / 4c / 2b 8 (8000) [solution] / 0a / 0b / 0b / 0c / 0b [z]Rhizopon® # 2 talc and # 3 talc were used to apply 3 and 8 g/kg (3000 and 8000 ppm) IBA, respectively. [y]Liquid formulations of IBA were prepared by dissolving the chemical in a 1:3 mixture (by vol) of acetone (2-propanone [$(CH_3)_2$ CO]) and deionized water. [x]Means with the same letter within columns are not significantly different (Fisher's LSD test; $\alpha = 0.05$).

Growing Area Preparation / Annual Practices for Perennial Crops	1. "The outdoor mistbed has automatic intermittent mist that is applied at 6 second intervals every 6 minutes. Too frequent misting will result in leaf and stem rot. Misting frequency is increased or decreased according to daily outdoor temperature and wind. Bottom heat is maintained at 21℃ with heating cables 12 cm beneath rooting media. Rooting media is 50% perlite and 50% sand. Mistbed is covered with shadecloth during rooting. After cuttings are potted, they are moved to an outdoor shadehouse for 4 weeks. They are later moved to full sun exposure in the outdoor nursery and are irrigated with Rainbird automatic irrigation system in early morning until containers are thoroughly leached. Average growing season of nursery is from late April after snowmelt until October 15th. First average frost is September 5th, although freezing temperatures can be expected anytime in Glacier National Park." (Evans, 2001) 2. More roots developed in 100% vermiculite than in 1 vermiculite : 1 perlite (by volume), which also diminished the number and apparent health of leaves on cuttings during the rooting period (Sharma and Graves, 2004). 3. Effect of substrate on cutting of *Rhamnus alnifolia* is showed in Table 2 (Sharma and Graves, 2005). **Table 2. Bases of cuttings of *Rhamnus alnifolia* were treated with IBA, and cuttings were kept under intermittent mist for 35 d. Main effect of substrate (n = 50) is reported because interactions were not observed among substrate, position of cutting, and hormone treatment.** **Medium** / **Cuttings with leaves remaining green (%)** / **Callus (%)** / **Rooting (%)** / **Root no.** / **Length of longest root (cm)** Vermiculite: 17a[z] / 60a / 58a / 15a / 5a perlite:vermiculite (1:1 by vol): 3b / 53a / 52a / 10b / 3b [z]Means with the same letter within columns are not significantly different (Fisher's LSD test; $\alpha = 0.05$).
Establishment Phase Details	1. "Time to Transplant: 8 weeks. Cuttings that were prerooted were lifted out of mistbed after adequate root systems were formed. Roots generate from the nodes below the surface of the rooting media." (Evans, 2001) 2. "Potted cuttings were placed under a mist system controlled by an evaporative sensor (Mist-a-Matic™, E.C. Geiger, Harleysville, PA). Ambient temperature in the glasshouse was recorded every 20 min by using a HOBO datalogger (Onset Computer, Bourne, MA). The day/night temperature averaged 22/18℃ (72/64°F)." (Sharma and Graves, 2005)
Length of Establishment Phase	1. 7 weeks (Evans, 2001). 2. 35 days (Sharma and Graves, 2005).
Active Growth Phase	"After cuttings were lifted from the mistbed, they were potted into 800 ml containers. Growing media used is 70% 6:1:1 milled spaghnum peat, perlite, and vermiculite and 30% sand with Osmocote controlled release fertilizer (13N:13P_2O_5:13K_2O; 8 to 9 month release rate at 21℃) and Micromax fertilizer (12%S, 0.1%B, 0.5%Cu, 12%Fe, 2.5%Mn, 0.05%Mo, 1%Zn) at the rate of 5 grams of Osmocote and 2 grams of

	Micromax per conetainer. Cuttings were irrigated after potting and placed in the shadehouse for 4 weeks. After establishment in the shadehouse, plants were moved to full sun exposure in the outdoor nursery." (Evans, 2001).
Length of Active Growth Phase	8 weeks (Evans, 2001).
Hardening Phase	"Plants are fertilized with 10-20-20 liquid NPK at 200 ppm during August and September. Irrigation is gradually reduced in September and October. Plants were given one final irrigation prior to winterization." (Evans, 2001)
Length of Hardening Phase	4 weeks (Evans, 2001).
Harvesting, Storage and Shipping	Total Time To Harvest: 1.3 years from cuttings. Harvest Date: September. Storage Conditions: Overwinter in outdoor nursery under insulating foam and snow (Evans, 2001).
Length of Storage	5 months (Evans, 2001).
Guidelines for Outplanting / Performance on Typical Sites	No information.
Other Comments	
INFORMATION SOURCES	
References	Dietz, S.M. and Leach, L.D., 1930. Methods of eradicating Buckthorn (*Rhamnus*) susceptible to crown rust (*Puccinia coronata*) of Oats. Methods of eradicating Buckthorn (*Rhamnus*) susceptible to crown rust (*Puccinia coronata*) of Oats., (133). Evans, J. 2001. Propagation protocol for production of Container (plug) *Rhamnus alnifolia* L'Her plants 800 ml containers; USDI NPS - Glacier National Park West Glacier, Montana. In: Native Plant Network. URL: http://NativePlantNetwork.org (accessed 2018/04/13). US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. Flora of North America, Vol. 12. Retrieved from http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=250101340, accessed on April 5, 2018. Luna, T., Evans, J., Wick, D. 2008. Propagation protocol for production of Container (plug) *Rhamnus alnifolia* L'Her plants 160 ml containers; USDI NPS - Glacier National Park West Glacier, Montana. In: Native Plant Network. URL: http://NativePlantNetwork.org (accessed 2018/04/05). US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. Plant Database, Lady Bird Johnson Wildflower Center at The University of Texas at Austin. Retrieved from https://www.wildflower.org/plants/result.php?id_plant=RHA

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Other Sources Consulted	John O. Sawyer, Jr. 2012, *Rhamnus alnifolia*, in Jepson Flora Project (eds.) *Jepson eFlora*, http://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=41067, accessed on April 06, 2018. Pappani, K., Riparian Plant Reference Guide. Retrieved from https://scc.idaho.gov/media/17069/riparian-guide.pdf, accessed on April 5, 2018. Smith, M.S., Fridley, J.D., Goebel, M. and Bauerle, T.L., 2014. Links between belowground and aboveground resource-related traits reveal species growth strategies that promote invasive advantages. PloS one, 9(8), p.e104189.
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