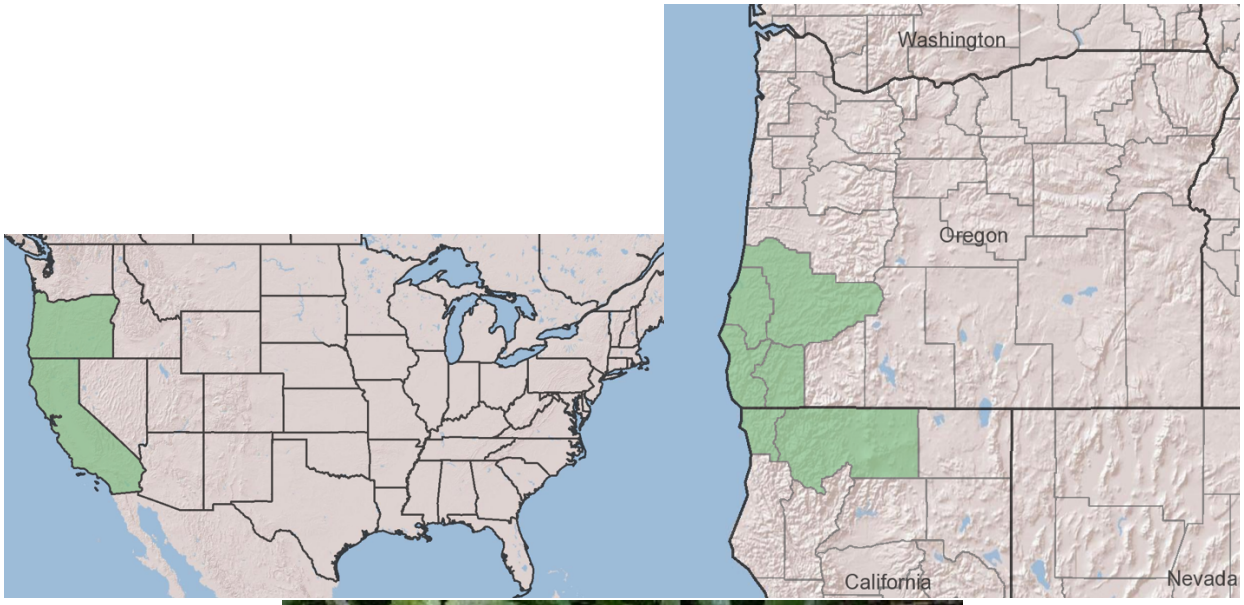
Plant Propagation Protocol for *Trillium rivale*
ESRM 412 – Native Plant Production
URL: https://courses.washington.edu/esrm412/protocols/2021/TRRI2.pdf



State- and county-level range of *T. rivale*; Image of *T. rivale*; courtesy of USDA PLANTS Database and J.S. Peterson.[1]

TAXONOMY	
Plant Family	
Scientific Name	Liliaceae
Common Name	Lily family
Species-Scientific Name	
Scientific Name	*Trillium rivale* S. Watson
Varieties	
Sub-species	
Cultivar	

Common Synonym(s)	*Pseudotrillium rivale* (S. Watson) S.B. Farmer (Species code PSRI3)[1]
Common Name(s)	Brook wakerobin; stream trillium; Siskiyou wakerobin
Species Code (as per USDA Plants database)	TRRI2
GENERAL INFORMATION	
Geographical range	Endemic to Siskiyou Mountains in southwestern Oregon and some of northern California.[2]
Ecological distribution	
Climate and elevation range	This plant is often found in climatic conditions such as these: annual precipitation 80.3-145.8"; summer precipitation 2.05-5.58"; coldest month temperatures 39.4-45.6; hottest month temperatures 58.8-68.0, humidity 0.32-16.68, and elevation 356-4607 ft.[7]
Local habitat and abundance	Prefers wetter areas and serpentine soils in areas such as streamsides and wet seepages.[2]
Plant strategy type / successional stage	Forest understory wildflower[3]
Plant characteristics	The plant is a small (4-6") perennial forb.[1,2] Wide white petals have purple spotting and the leaves have variable silver veining. Blooms March-June depending on elevation.[2] In general, trilliums grow from knobby brown rhizomes in the soil. In the spring, the sun warms the forest soil and a bud at the rhizome's apex elongates. Dormant axillary buds can develop into secondary rhizomes that eventually become flowering stems (this is how clumps of blooms aboveground can develop). Eventually whorls of 3 leaves unfurl and 3-petalled flowers bloom. Leaves are large and parallel to the ground to maximize sunlight capture as part of the understory.[3]
PROPAGATION DETAILS ***Seed Collection and Germination***	
Ecotype	
Propagation Goal	Plants
Propagation Method	Seed (rhizome division and tissue culture are possible, but are less feasible)[3]
Product Type	Container
Stock Type	1 gallon containers
Time to Grow	5-7 years[2]

Target Specifications	
Propagule Collection Instructions	Collect seed from stock plants in early July, when capsules are about to split (seed coat turns from green to tan or brown).[2,3] Collecting slightly underripe seeds to prevent seed loss has not shown any effects on germinaton.[2]
Propagule Processing/Propagule Characteristics	Each seed capsule holds 10-40 seeds.[3]
Pre-Planting Propagule Treatments	Seeds are hydrophilic – do not allow to get very dry during storage. Ideally, sow seeds immediately after cleaning, but storage is possible for up to 3 years if kept in dampened vermiculite in a sealed refrigerated container (eg. sealed plastic bag in a lidded jar).[3] If mold forms, rinse with a strong stream of water in a sieve and store with a small amount of vermiculite to prevent more mold.[4] Cleaning seeds is easiest with slightly unripe seeds (just turning tan). Peel off the calyx, pinch capsule walls and squeeze out seeds.[3] Another method is to allow the capsule to decay in a plastic bag in the fridge, then run through a sieve with a strong stream of water and pat dry before sowing. Do not use mechanical means as this can damage the delicate seed coat.[4] Much more research is needed, but gibberellin application can improve germination and subsequent development.
Growing Area Preparation / Annual Practices for Perennial Crops	Sprinkle seeds from capsules into a pot with a mature plant or into a 72-cell plug tray: (4.2 X 4.2 X 5.6 cm deep [1.7 X 1.7 X 2.25 in]; Land Mark Plastics Inc, Akron, Ohio). Leave seeds uncovered.[2] Cullina uses flats in 9x12x3" flats filled with Metro-mix, filled with about 200 seeds that are then covered with some more Metro-mix and 6mm of #1 filter sand. Flats are outdoors in shaded cold frames open to wind and rain, with Reemay floating row cover fabric topped with a half-inch hardware cloth lid (to keep weeds and pests out and to slow evaporation). [3] Media consists of 1:1 ratio of sphagnum peat moss and perlite, by volume.[2] Solt recommends that seed be sown twice as deep as the seed diameter – err on the deeper side. Good drainage and water holding capacity are essential for media.[4]
Establishment Phase Details	Water daily throughout the summer; house in a greenhouse with 80% shade cloth. Move to unheated shade house in the fall. Germination rates are

	usually 90-100% and cotyledons are usually visible by late February, though can be fickle.[2] Germination occurs in 2 stages. A root grows first and gets to 1-2" (usually emerging the first fall post-planting), then after cold stratification for 80-100 days, the cotyledon emerges (spring). Avoid excessive sun, heat over 85 F (be careful of greenhouses for this reason), drought, and rain to prevent early dormancy. Overwinter under Microfoam winter blankets and white 6mm polyethylene greenhouse film.[3]
Length of Establishment Phase	July-late February[2]
Active Growth Phase	Once cotyledons emerge, treat with Peters Professional Peat-Lite fertilizer (15N:16P_2O_5:17K_2O; The Scotts Company, Marysville, Ohio) at about 180 ppm N (0.5 tbsp/gal). After true leaves emerge, water with this treatment once every 2-3 weeks.[2] Once first nickel-sized oval leaf is produced the spring of the second year, transplant seedlings from flats to 2.25x2.25x3" pots with 2:1 Metro-mix 360:coarse perlite media.[3] Solt transfers second-year seedlings to 4x4x4 pots; Klest waits until the fourth year.[3,4] First whorl of 3 leaves arrives by third year. By the fourth year, move to 4.25x4.25x5" quart pots when plants are just emerging. Topdress with controlled release fertilizer (Osmocote Plus 15N:9P_2O_5:12K_2O). [4]
Length of Active Growth Phase	
Hardening Phase	After 3-4 springs, pot into 3.7-1gal containers with Rexius potting mix and top dress with Apex controlled-release fertilizer (19N:8P_2O_5:12K_2O; 10 to 12 mo release rate at 15.5℃ [60℉]; Simplot Turf & Horticulture, Lathrup, California) once annually each spring. Overwinter outside covered with Reemay fabric and white plastic.[2]
Length of Hardening Phase	
Harvesting, Storage and Shipping	
Length of Storage	

Guidelines for Outplanting / Performance on Typical Sites	*T. rivale* will bloom about 5 years post-planting of initial seed.[2]
Other Comments	Some difficulties with *T. rivale* include sowbugs and slugs eating seedlings or flowers and mice eating rhizomes during winter.[2] For trilliums in general, take care to avoid rhizome rot.[3] Because flowering occurs when energy reserves are accumulated enough, optimization of water and nutrient via fertilizers can perhaps speed up time to flowering.[4]
PROPAGATION DETAILS ***In-vitro propagation of other species in the Trillium genus***	
Ecotype	
Propagation Goal	Plants
Propagation Method	Sterile seeds germinated in culture; *in vitro* vegetative propagation[6]
Product Type	Plant
Stock Type	
Time to Grow	
Target Specifications	
Propagule Collection Instructions	
Propagule Processing/Propagule Characteristics	
Pre-Planting Propagule Treatments	Establishing Sterile Cultures: Rinse freshly collected seeds in a sieve with cold water for 15 mins, then transfer 20 seeds at a time to 50 mL of 10% volume commercial bleach with 2 drops of dishwashing liquid. Agitate on a rotary shaker at low speed for 20-30 min. Sterlize the seed surfaces by rinsing with 70% isopropyl alcohol followed by several rinses of sterile tap water. Place in sterile medium (modification of Murashige and Skoog Salt Base (Sigma Chemical Company, catalog number 6899; Murashige and Skoog 1962) prepared by diluting the commercially available preparation by 50% and adding back inositol (3.3 ml of 100 mg/ml stock solution per l of media) and thiamine hydrochloride (HCl) (0.1 ml of 1 mg/ml stock solution per l)) for germination in a small glass container. Incubate in a dark room at 25 C until germination.[6]

Growing Area Preparation / Annual Practices for Perennial Crops	
Establishment Phase Details	Propagation via Explants: Wash media away from the rhizome of a plant and remove the shoot and a 0.5 cm piece of rhizome with a scalpel. Cut any unfurled leaves in half and place the cutting in a 10% bleach and surfactant mixture for 20 min. Rinse in sterile water and set in a sterile Petri dish. Trim a couple of mms off the edges of both shoot and rhizome, then transfer to a new dish and cut into 1-2cm sections. Place onto sterile medium with the basal part inserted below the medium (which consists of the same medium as before with supplemental BAP and NAA). Incubate under 12-hour photoperiod. Callus begins to form after 6 weeks; when it reaches 2cm diameter, transfer again to medium without BAP and NAA. Shoots form after about 12-20 weeks. After 8-12 more weeks, small rhizomes form that can be separated and subcultured to multiply further. It is important to note that removing dead leaves or decaying rhizome sections improved shoot production in new cultures.[6]
Length of Establishment Phase	
Active Growth Phase	Establishing Sterile Cultures: Once radicle emerges, transfer to light conditions with a 12 hour photoperiod. Transfer to fresh medium 2-3 times a year and supplement with BAP and NAA.[6]
Length of Active Growth Phase	
Hardening Phase	
Length of Hardening Phase	
Harvesting, Storage and Shipping	
Length of Storage	
Guidelines for Outplanting /	

Performance on Typical Sites	
Other Comments	This information is from work done on *T. reliquum* and *T. persistens*; some information may not therefore be optimized for *T. rivale.*
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