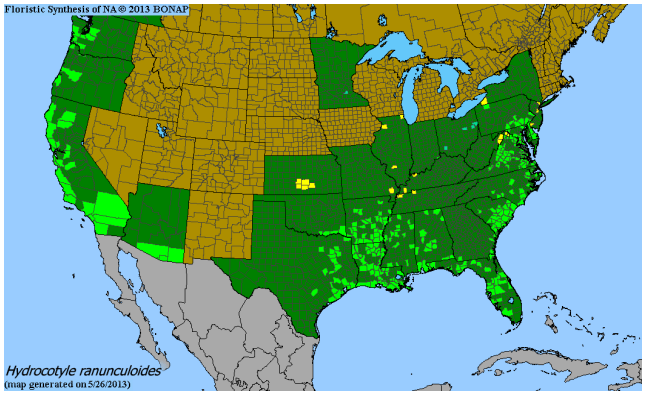
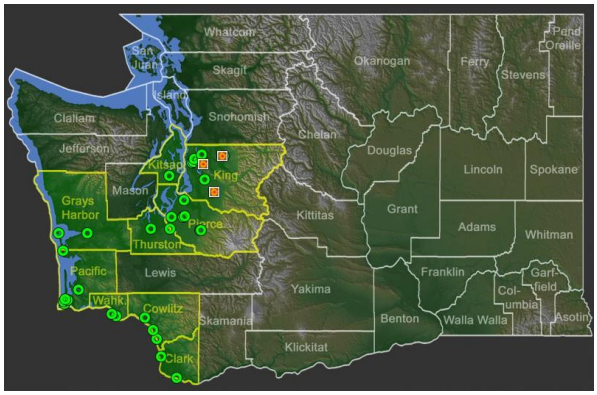
Plant Propagation Protocol for *Hydrocotyle ranunculoides*
ESRM 412 – Native Plant Production
URL: https://courses.washington.edu/esrm412/protocols/*2022*/HYRA.*pdf*

North America Distribution



Washington State Distribution



Source: BONAP (left), Burke Herbarium Image Collection (right)

TAXONOMY	
Plant Family	
Scientific Name	Araliaceae
Common Name	Ginseng
Species Scientific Name	
Scientific Name	*Hydrocotyle ranunculoides*
Varieties	*ranunculoides*
Sub-species	n/a
Cultivar	n/a
Common Synonym(s)	*Hydrocotyle adoënsis* Hochst. 1841 *Hydrocotyle americana* Walt. 1788 *Hydrocotyle batrachioides* DC 1830 *Hydrocotyle cymbalarifolia* Muhl. 1813 *Hydrocotyle natans* Cirillo 1788 *Hydrocotyle nutans* G. 1830 *Hydrocotyle ranunculoides* f. *minima* Kuntze 1898 *Hydrocotyle ranunculoides* var. *genuina* Urban 1879 *Hydrocotyle ranunculoides* var. *natans* (Cirillo) Urban 1879
Common Name(s)	floating pennywort
Species Code (as per USDA Plants database)	HYRA
GENERAL INFORMATION	
Geographical range	Native in North America. Considered an alien invasive species in Africa, Asia, Europe, and Australia that was introduced from the Aquarium hobby trade. See maps above for distribution in Washington and North America.[1]

Ecological distribution	Not found in open water, but next to ponds, ditches, streams, rivers, marshes, wetlands, lake margins, wet ground, fenland pools [3] Performs best in waterlogged conditions, but will adapt to a wide range of substrates. [10]
Climate and elevation range	Very adaptable species found in a wide range of humidity and temperatures. [2] Prefers high levels of nitrates and phosphates and is tolerant of a broad range of light. Elevation of under 1500m [3] Is not tolerant of frost and will go dormant. [10]
Local habitat and abundance	Locally common, found in ditches, shallow water, muddy wetlands, and boggy areas. Usually found with *Juncus* and *Carex* [4]
Plant strategy type / successional stage	Colonizer that outcompetes native flora in regions where it is not native through forming dense mats and regenerating from small root fragments [3]
Plant characteristics	Has round, peltate leaves that are ¾ to 3 inches wide, with 3 to 7 rounded toothed, shallow lobes around the edge. Leaf is attached to a smooth stalk that ranges from 2 to 12 inches long. Stems emerge out of the water, where they form dense mats with thick tangles of roots. [5]  Source: A. Hussner
PROPAGATION DETAILS - VEGETATIVE	
Ecotype	No literature found
Propagation Goal	Nodal segments between 2.5-3.0 cm (*Hydrocotyle asiatica*) [7] *H. ranunculoides* can even form shoots with nodes as small as 1 cm [6]
Propagation Method	Dispersal by seed has not yet been documented– only vegetative. [6]
Product Type	Node cuttings in tissue culture tubes (*Hydrocotyle asiatica*) [7]
Stock Type	Container stock

Time to Grow	10 days to induce buds, another 10 days to separate. Once shoots are rooted, they are then outplanted from flasks to vermiculite. (*Hydrocotyle asiatica*) [7]
Target Specifications	Rooted plantlets with shoots
Propagule Collection Instructions	In India in July, nodal segments were cut with sharp clippers, washed, cleaned with detergent, and sterilized. (*Hydrocotyle asiatica*) [7]
Propagule Processing/Propagule Characteristics	3-4 nodes per test tube. [7]
Pre-Planting Propagule Treatments	Tissue culture must be very clean, so collected nodes must first go through an intense cleaning with a labolene liquid detergent solution for 5 minutes, then pre-sterilized in fungicides for 15 minutes. Lastly, they are disinfected for 10 minutes in mercuric chloride before finally being rinsed in distilled water three times. (*Hydrocotyle asiatica*) [7]
Growing Area Preparation / Annual Practices for Perennial Crops	25x150mm glass test tubes are filled with 15ml of Murashige and Skoog (MS) agar growth medium that has 3% sucralose. Must be autoclaved for 15 minutes to sterilize. (*Hydrocotyle asiatica*) [7] Household sugar may be used in place of sucralose with no adverse effects. [9]
Establishment Phase Details	Test tubes with nodes are kept at 60% relative humidity with 16 hour photoperiods at 25-27 degrees celsius. [7]
Length of Establishment Phase	2 weeks [7] to 3 weeks [9]
Active Growth Phase	No literature found
Length of Active Growth Phase	No literature found
Hardening Phase	Developed plantlets would be transferred to vermiculite and MS growth media for two weeks in a culture room. Then, they are moved to soil, sand, and manure in a 1:1:1 ratio and a shade house for 1 week. [7] Can also transfer plantlets directly to sand with coco coir [9]
Length of Hardening Phase	3 weeks [7]
Harvesting, Storage and Shipping	No literature found
Length of Storage	No literature found
Guidelines for Outplanting / Performance on Typical Sites	90% of plantlets survived hardening and outplanting to the shade house. [7] 100% of hardened plants were successful after being established. [9]
Other Comments	*H. ranunculoides* is so invasive outside of the U.S. that it is illegal to grow in Europe without a permit. [8]
INFORMATION SOURCES	

Other sources consulted	Liu, R., Chen, Q., Dong, B. *et al.* Effects of vegetative propagule pressure on the establishment of an introduced clonal plant, *Hydrocotyle vulgaris*. *Sci Rep* 4, 5507 (2014). https://doi.org/10.1038/srep05507 Li-Min Zhang, Peter Alpert, Fei-Hai Yu, Nutrient foraging ability promotes intraspecific competitiveness in the clonal plant Hydrocotyle vulgaris, Ecological Indicators, Volume 138, 2022, 108862, https://doi.org/10.1016/j.ecolind.2022.108862. (https://www.sciencedirect.com/science/article/pii/S1470160X22003338)
References	1. "Hydrocotyle Ranunculoides (Floating Pennywort)." Edited by Djami Djeddour, *Hydrocotyle Ranunculoides (Floating Pennywort)*, https://www.cabi.org/isc/datasheet/28068. 2. "Eppo Global Database." *Hydrocotyle Ranunculoides (HYDRA)[Documents]| EPPO Global Database*, https://gd.eppo.int/taxon/HYDRA/documents. 3. European and Mediterranean Plant Protection Organization. 2006. Datasheets on quarantine pests: *Hydrocotyle ranunculoides*. OEPP/EPPO Bulletin 36:3-6. 4. WTU Herbarium, Burke Museum. "Hydrocotyle Ranunculoides." *Burke Herbarium Image Collection*, https://biology.burke.washington.edu/herbarium/imagecollection/taxonmap.php?Taxon=Hydrocotyle+ranunculoides&SourcePage=taxon. 5. "Hydrocotyle Ranunculoides (Floating Pennywort)." *Minnesota Wildflowers*, https://www.minnesotawildflowers.info/flower/floating-pennywort. 6. Hussner, A., Denys, L. and van Valkenburg, J. (2012): NOBANIS – Invasive Alien Species Fact Sheet – Hydrocotyle ranunculoides – From: Online Database of the European Network on Invasive Alien Species – NOBANIS *www.nobanis.org* Date of access: x/x/201x.

	7. Tiwari, Chandrakant, et al. “A Rapid Two Step Protocol of in Vitro Propagation of an Important Medicinal Herb Centella Asiatica Linn.” *African Journal of Biotechnology*, vol. 12, 6 Mar. 2013, pp. 1084–1090., https://doi.org/10.5897/AJB2012.2945. 8. Brundu, G., et al. “*Pistia Stratiotes*L. and*Eichhornia Crassipes*(Mart.) Solms.: Emerging Invasive Alien Hydrophytes in Campania and Sardinia (Italy).” *EPPO Bulletin*, vol. 42, no. 3, 2012, pp. 568–579., https://doi.org/10.1111/epp.12004. 9. Raghu, A.V., et al. “Low Cost Alternatives for the Micropropagation of Centella Asiatica.” *Journal of Plant Sciences*, vol. 2, no. 6, 2007, pp. 592–599., https://doi.org/10.3923/jps.2007.592.599. 10. Robert, H., Lafontaine, R.-M., Beudels-Jamar, R.C., Delsinne, T. (2013). Risk analysis of the Water Pennywort Hydrocotyle ranunculoides (L.F., 1781). - Risk analysis report of non-native organisms in Belgium from the Royal Belgian Institute of Natural Sciences for the Federal Public Service Health, Food chain safety and Environment. 59 p. 11. USDA (2004) Plant profile on *Hydrocotyle ranunculoides*. http://plants.usda.gov. 12. Washington State Department of Ecology (2004) *Hydrocotyle ranuncu- loides* L.F., water pennywort. http://www.ecy.wa.gov/programs/wq/plants/plantid2/descriptions/hydran.html.
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