Threatened plant gets boost from biotech lab

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Woodland agrimony is a rare and threatened plant and in New York state. Credit: Joyce Van Eck

Woodland agrimony isn't much to look at—the short plant with jagged leaves and tiny yellow flowers is likely to be overlooked on an afternoon

hike—but this rare, threatened plant got a high-tech hand from researchers at the Boyce Thompson Institute (BTI).

BTI scientist Joyce Van Eck and research assistant Patricia Keen developed a test tube tissue culture procedure that multiplies the plant's numbers, which they detailed in the journal *Native Plants*. They collaborated with Victoria Nuzzo, an ecologist and the founder of the company Natural Area Consultants, who transferred the lab-cultured plants to forests in New York state to study why this threatened species is in decline.

Nuzzo, and her colleagues Bernd Blossey, associate professor in Cornell University's Department of Natural Resources, and Andrea Dávalos, a research associate in his lab, reached out to Van Eck to see if she could help them with a research quandary.

"We were looking at the impacts of different stressors on native plants and particularly on four rare native plants in the northern U.S., and needed seeds and seedlings of each species for our experiments." said Nuzzo. "One of the rare plants we worked with was woodland agrimony. We found a single population and we simply couldn't collect enough seeds to use in our experiments, or to grow seedlings to transplant."

Van Eck and Keen began developing a procedure to propagate the plant, starting with common agrimony and then switching to the threatened woodland agrimony once they had worked out the details.

The first—and most troublesome—step was cleaning the plant segments used to start the in vitro multiplication process to remove contamination. Bacteria and fungi can take over a culture and kill the plants, while insects hatched from attached eggs can spread microbes from culture to culture. The agrimony proved especially difficult to decontaminate.

"We really struggled trying to get material that was clean for tissue culture. We tried all sorts of things," said Van Eck. "There's a fine line sometimes between getting rid of the contaminants and the plant," she laughed.

Ultimately, Keen overcame the contamination issue through a strenuous washing protocol including soap, alcohol, bleach and miconazole, an anti-fungal compound found in athlete's foot treatments, which she also added to the culture medium.

Keen cut apart the shoots every four weeks to make new plantlets—much like the magical broomstick in the movie Fantasia that forms new brooms when the original is cut into pieces—and cultivated them in test tubes containing salts, nutrients and growth regulators that encouraged root and shoot growth. The plantlets grew "true-to-type," meaning that they looked just like the original material. After a stay in the greenhouse to harden up, the researchers transplanted the agrimony into the forest field sites.

In six months, Keen had generated 1013 plants from a starting population of just 35.

"I think the tissue culture option is something people in the conservation field should consider, if they aren't already," said Van Eck. "When you have limited plant material and need a way to bulk up the numbers, tissue culture is a great way to do that."

Nuzzo and her colleagues planted the agrimony at 12 field sites where they could observe and control the effects of slugs, deer, earthworms, invasive plants and other environmental factors. Though interactions between different stressors are complex, grazing deer appear to be an important factor contributing to the decline of the remaining woodland agrimony populations.

Currently, there are no wide-scale agrimony replanting efforts in the works, but the study shows that replenishing the population through tissue culture is a viable option.

While Van Eck and Keen's role in the project is over, their test tube plants live on. In many of the test locations, the woodland agrimony is reproducing the old-fashioned way and establishing new populations.

More information: J. Van Eck et al. Development of an in vitro propagation method for the classified New York State-threatened native species Agrimonia rostellata, *Native Plants Journal* (2016). DOI: 10.3368/npj.16.3.227

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