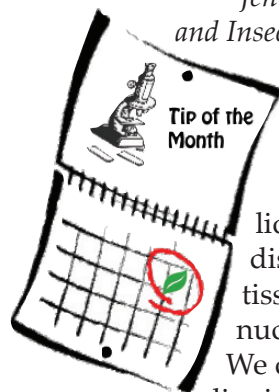


Diagnostic Updates

Tip for Using Liquid Nitrogen and a Mini-BeadBeater-1 for Tissue Disruption

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Our laboratory routinely uses liquid nitrogen to disrupt plant and insect tissues in preparation for nucleic acid extraction.

We do not have access to a liquid nitrogen tank in our building so we have developed a solution to running across campus to get liquid nitrogen for DNA and RNA extractions. It is also much safer than having an open vat of liquid nitrogen on the lab bench.

Several years ago we purchased a 20L dewar for liquid nitrogen storage (Fig. 1). We fill the dewar approximately every

four months with liquid nitrogen. The purchase of this tank has greatly reduced the amount of liquid nitrogen we waste and has saved us time since we no longer need to fill a small container several times a week. As an added advantage, the tank can be used for storage of a culture collection.

When we need to perform DNA or RNA extractions, we place plant or insect tissue inside a 2.0 ml plastic tube filled 25–50% with 2.5 mm glass beads (Fig. 2). It is important to try out different tubes because some plastic microcentrifuge tubes will break when immersed in liquid nitrogen. The beads may also damage the plastic during homogenization so you may need to try different size beads.

The tubes are clipped onto a “stick” that consists of three or four cryocanes squeezed together to make one long stick (Figs. 3, 4). The lid to the 20L dewar is opened and the stick is placed directly in the liquid nitrogen (Fig. 5). After 20–30 seconds, we remove the stick and unclip the frozen tube. Although you can wear



Fig. 1. 20L dewar for liquid nitrogen storage. Fig. 2. Plant tissue placed in 2.0 ml microcentrifuge tube with 2.5 mm glass beads. Fig. 3. Tube is clipped onto stick and ready for immersion in liquid nitrogen. Fig. 4. Four cryocanes are squeezed together to make one long stick.

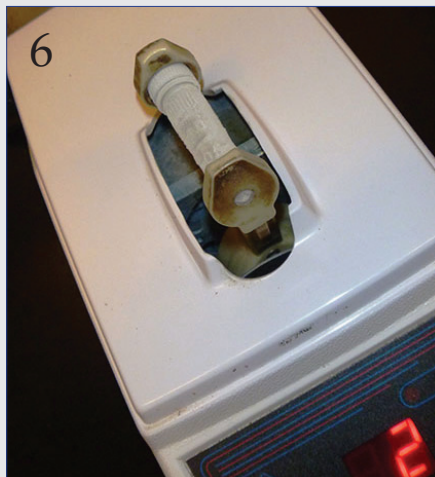


Fig. 5. Stick and tube are immersed in liquid nitrogen directly in the dewar. Fig. 6. Frozen tube is placed in Mini-BeadBeater-1 and ready for homogenization. Fig. 7. Microcentrifuge tube containing homogenized plant tissue.

special gloves to handle the tube, we find that nitrile gloves are adequate for the brief amount of time we handle the tubes. The frozen tube is placed in the Mini-BeadBeater-1 (BioSpec Products, Inc.) and processed for 20–30 seconds at 4200–4800 rpm (Fig. 6). We generally repeat the freezing and homogenizing process once to ensure that all tissue is disrupted. If there is a lot of tissue on the lid of the tube, we will briefly centrifuge the tube to concentrate the tissue at the bottom of the tube. The sample is ready for nucleic acid extraction (Fig 7). 🌿

Check out the Summer 2011 edition of the WPDN First Detector News on Invasive Horticultural Plants

Western Plant Diagnostic Network First Detector News

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In this Issue

Page 1: Editor's Notes: Invasive Horticultural Plants
Page 2-3: Introduction to Invasive Horticultural Plants
Page 4-6: Six examples of Invasive Horticultural Plants

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Arundo donax - Giant Reed

Western Plant Diagnostic Network News



Dear WPDN First Detectors, Have you ever planted a plant in your garden, landscape, or commercial fields and found that it very quickly spread and took over the area? **Yes**, certain **bamboos**, **Pink Jasmine**, **Mexican primrose**, **Vinca major** and **Tree of Heaven** (*Ailanthus altissima*) are some of the common invasives in horticulture. Most of the plants used in gardens and landscaping do not invade or harm wildland areas. But a few vigorous species can - and do - escape from cultivation into open landscapes where they cause a variety of ecological problems. They crowd out native plants, insects and animals, clog waterways, and can lead to increased flooding, fire and crop losses. Joseph [Joe] DiTomaso, University of California Cooperative Extension specialist for weedy species, has contributed his expertise to this edition. Joe DiTomaso and Evelyn Healy have authored two excellent volumes, entitled "Weeds of California and the Other Western States," 2007, and can be purchased at [ANR Catalog](#) and [Amazon](#). Please find links to the NPDN family of newsletters at: [Newsletters](#)



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