Update on Micropropagation of Trillium Taxa

S.L. Kitto, David Opalka, and Jesse Sinanan
Department of Plant and Soil Sciences, University of Delaware, 531 S. College Ave., Newark, Delaware 19716-2170 U.S.A.

Our laboratory works on the development of micropropagation protocols for trilliums with the objective of making superior cultivars available to the gardening public. Superior trillium cultivars can have unique flower color, double flowers, or special leaf variegation or be fast multipliers. We can establish trilliums in culture from vegetative material. We can proliferate the rhizomes in culture and produce thousands of rhizomes in a much shorter time frame than under field conditions. The current stumbling block appears to be physiological and centered on the in vitro-proliferated trillium mini-rhizomes. We will report on three recent areas of research: (1) refinement of the surface disinfestation protocols, (2) visualization of mini-rhizome initiation and proliferation, and (3) qualification of carbohydrate reserves within in vitro-generated rhizomes with respect to in vitro and ex vitro treatments.

FRACTIONAL DISINFESTATION

A new protocol for surface disinfesting plant material has improved the ease with which we have been able to establish trilliums in vitro. This protocol was developed by Dr. Alice Waegel, a microbiologist, who worked in the laboratory fall 2001. We have refined this protocol, which involves placing the plant material in a medium that will encourage the germination and growth of the microbial contaminants for 4 h followed by a 10 min 10% bleach treatment and three 10 min water rinses. This cycle of vegetative microbial growth followed by a bleaching death can be repeated any number of times and has greatly facilitated our work with trilliums. We now know that we can expect high rates of clean up, which is important with plants that commonly have only small amounts of plant tissue available. Using this protocol we have been able to establish the double white *T. grandiflorum* 'Flore Pleno' in vitro.

VISUALIZATION OF PROLIFERATION IN VITRO

We wanted to document what happened to a mini-rhizome during culture. Trillium rhizomes proliferate slowly and, it seems, randomly in vitro. We can culture a singlet mini-rhizome and weeks later we have proliferative masses containing rhizomes of many different sizes. But how does it happen? When are secondary mini-rhizomes generated and where do they come from? We chose to study this proliferation phenomenon using *Trillium maculatum*, a relatively fast proliferator in vitro. Five singlet mini-rhizomes were individually cultured in Magenta G-5 boxes containing trillium proliferation medium. Rhizomes were then photographed one or two times a week for 15 weeks. Care was taken during monthly subcultures to maintain the identical orientation. The "movie" created from these photos using Microsoft Producer for PowerPoint 2003 demonstrated that *T. maculatum* rhizome growth in vitro is dynamic and cyclic, as green leaves turned brown, new mini-rhizomes were generated within the axils.

CARBOHYDRATE RESERVES IN TRILLIUM RHIZOMES

We had been chilling rhizomes prior to field establishment based on personal experience and the literature. Trillium and close relatives require a cold treatment either

of the seed for germination or (for lack of a better phrase) for maturation of the rhizome prior to flowering. We were looking for a treatment regime that would "trick" the in vitro trillium rhizomes. We were searching for a cold-warm cycling regime that the rhizomes could be subjected to that would result in "mature" rhizomes that would be ready to flower once planted/potted out. We had been chilling (4 ºC) the rhizomes for 10 to 12 weeks and were having very little success with establishing the rhizomes. We cut a rhizome that had been chilled for 10 weeks, stained it with iodine to visualize the presence of starch, and to our surprise there was very little stain response. An experiment was set up to examine starch level in rhizomes over time. Rhizomes that had been either cold treated (4 ºC) or warm treated (greenhouse) were harvested weekly and stained with iodine to visualize starch. While the iodine staining was very crude, making it difficult to draw any but the grossest conclusions, it appeared that the in vitro rhizomes contained starch but that the starch dissipated over an 8-week cold or warm treatment post culture.

Judge Them by Their Appearance: Trialing Landscape Shrubs at Longwood Gardens

Debbie Metrustry
Longwood Gardens, PO Box 501, Kennett Square, Pennsylvania 19348 U.S.A.

GENERAL INTRODUCTION

The shrub trials at Longwood Gardens were started in 1997 with the objective to provide information to the industry and amateur growers alike on what happens to a plant *after* it leaves the nursery — in other words, to answer the question: "how well does it do in a typical landscape situation?" The difference between our trials and others, and our strength as we see it, is that as well as being conducted over a long period of time, we use a great range of people to evaluate these plants. Our evaluators include students, staff, and volunteers, who between them demonstrate a variety of horticultural expertise and experience: they are our representative sample of the general gardening public. This makes for a reliable and exciting long-term study.

HISTORY

The construction of the site for the plant trials began in 1996 with the selection of four sites, designated fields A–D, comprising nearly 7 acres in the nursery area of the gardens. The fields were not tilled, grass was eliminated by herbicides to make the planting beds, and 3–4 inches of mulch were added. Planting began shortly afterwards, in 1997.

Each field was divided into numbered rows of planting beds 12 ft wide separated by 8-ft grass strips. A brass tag mounted on a fiberglass stake every 10 ft along the row indicates distance from the beginning of the row and an additional sign marks every 50 ft. Each shrub is assigned a specific location and a location number, which makes navigation in the fields logical and straightforward.

It had been decided that a large proportion of the plants would be commonly used and commercially available, a good number would be commercially available but less widely used, and a small proportion would originate from wild-collected seed, seed exchanges with other botannical gardens world-wide, or other noncom-