

## Micropropagation of Trillium Species®

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### INTRODUCTION

I work on native herbaceous perennials that I believe are garden worthy. Garden worthy, for me, describes a plant that is easy to establish and maintain in a garden. I think trilliums are garden worthy. Based on personal experience with trilliums in my own garden, I think of trilliums as being very forgiving garden plants as they are easy to establish, easy to maintain, and are tolerant of neglect. Most of the trilliums that are presently on the market do not begin to tap the available diversity in foliage variation, flower color, flower form, or plant form found in the genus or the individual species. There is an abundance of variation present in *Trillium discolor* leaf variegation (Fig. 1) and in *T. grandiflorum* flower form (Fig. 2) and color (Fig. 3). This is one reason why I decided to work with trilliums a few years ago. I am interested in being able to develop micropropagation protocols so that superior trilliums can be made generally available. Tissue culture of trilliums allows for the generation of large amounts of clonal material and the subsequent development of reliable, repeatable protocols.

A second reason for me to work with trilliums is related to the University of Delaware being located close to Mt. Cuba Center where a good friend and colleague, Jeanne Frett, is employed. I am fortunate that Mt. Cuba Center is a ready source of trillium plant material, which is essential to my tissue culture research program.

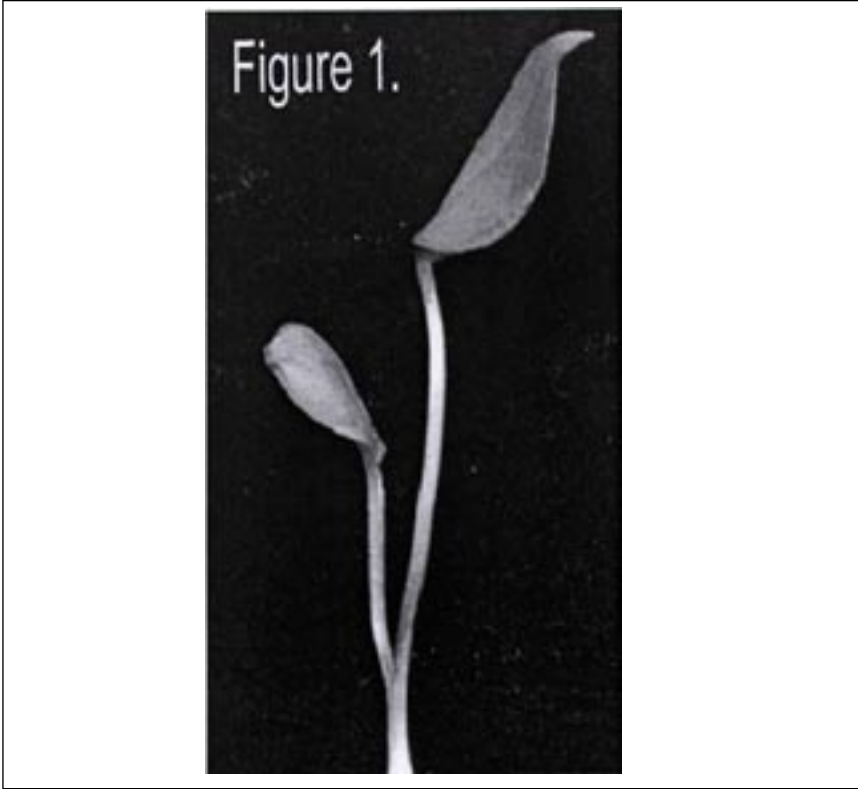
### MATERIALS AND METHODS

For the micropropagation experiments, the plants used include one seedling clone of *T. discolor* and two seedling clones of *T. grandiflorum*. Mother cultures were maintained on Murashige-Skoog (1962)-based media supplemented with sucrose, glycine, and the growth regulators, BA and 2,4-D for rhizome proliferation, and IBA for root generation experiments. Media were liquid or gelled with Phytagar.

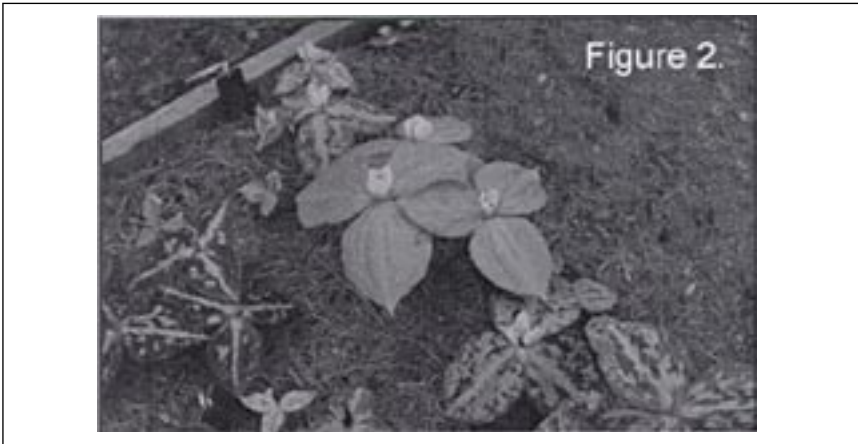
### RESULTS

Some species are easier to clean up and establish in sterile culture. We have not been able to establish *T. grandiflorum* 'Quick Silver' after 4 years compared to *T. maculatum*, *T. rugelii*, and *T. decumbens* that were established on the first try. Leaf, ovary, and stem tissue explants from early spring growth have been the most reliably responsive in vitro.

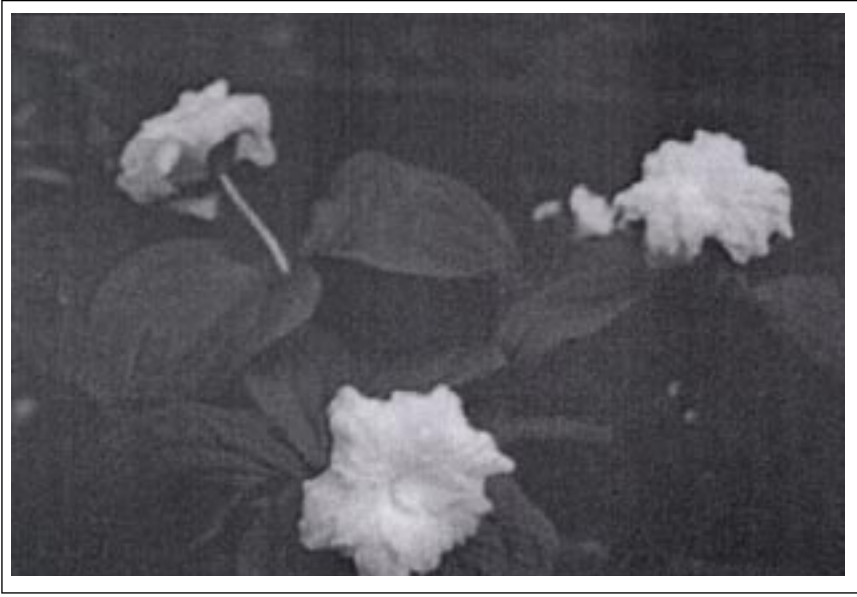
A new protocol for surface disinfecting plant material has improved the ease with which we have been able to establish trilliums in vitro. Dr. Alice Waegel, a microbiologist at Neumann College, Aston, Pennsylvania, who worked in my laboratory Fall 2001, developed this protocol. This protocol involves placing the plant material in a medium that will encourage the germination and growth of the microbial contaminants during a 12 to 24-h period followed by a bleach treatment. This cycle of microbial growth followed by a bleaching death can be repeated any number of times and has greatly facilitated my work with trilliums. We now know that we



**Figure 1.** In-vitro-generated trillium rhizome that has roots and leaves.



**Figure 2.** *Trillium discolor* plants growing at Mt. Cuba Center (photo courtesy of Mt. Cuba Center).



**Figure 3.** *Trillium grandiflorum* 'Pamela Copeland' plant displaying white double flowers (photo courtesy of Mt. Cuba Center).



**Figure 4.** A pink-flowered form of *Trillium grandiflorum* growing at Mt. Cuba Center (photo courtesy of Mt. Cuba Center).

can expect high rates of clean up which is important with plants where there are commonly only small amounts of plant tissue available during a few weeks in the early spring.

While we have been successful over the years in establishing trillium rhizomes in the field, success has been very inconsistent. We were interested in developing a protocol that could be used to improve the consistency of rhizome reestablishment. We decided to compare the starch status of rhizomes that were clonal, or identical, to each other but that were either maintained in vitro or in a field plot. We examined the starch content in 5-year-old field maintained *T. grandiflorum* clone 6 rhizomes, at dormant (29 Jan. 2002) and vegetative (30 March 2002) growth points, and compared it to the starch content of in vitro-maintained rhizomes. In vitro-maintained rhizomes had starch profiles similar to dormant, field-established rhizomes.

We also compared the starch content within *T. grandiflorum* clone 11 rhizomes that had been established in the field for less than 1 year or for more than 5 years. Rhizomes established in the field for less than 1 year had a starch profile similar to the starch profile found in the dormant 5-year-old field-maintained rhizomes.

Successful establishment of rhizomes in the greenhouse or field requires the production of roots. *Trillium discolor* rhizomes were cultured on proliferation or rooting media and placed in cold (4°C) or ambient laboratory (27°C) environments. Rhizomes were oriented either with the apical bud facing upright or sideways. Rhizomes oriented sideways rooted compared to upright-oriented rhizomes that did not root.

To determine the correct planting environment, rhizomes were placed either in tall black pots or deep seed flats. Rhizomes were potted using Metro Mix 510 and were cold treated at 4°C for 10 weeks after which they were potted in tall black pots and placed in a field shade house until the experiment was terminated at 42 weeks. There was no difference in weight gain, survival, rooting percentage, or root number during the course of the experiment.

We know that larger seedling-generated rhizomes flower earlier. We were interested in examining the effect of liquid culture on rhizome weight gain and subsequent effects on survival and rooting compared to gelled medium. In the laboratory, rhizomes were grown on either liquid or gelled medium for 12 weeks. Rhizomes in liquid gained significantly more weight. Fourteen of 18 rhizomes on liquid medium were vitrified and not planted out. However, the liquid-medium-treated rhizomes that were planted out survived and rooted better compared to the control rhizomes.

## CONCLUSIONS

In-vitro-maintained rhizomes had starch profiles similar to dormant field-established rhizomes. A horizontal rhizome orientation improved rooting. Rhizomes survived a cold treatment equally well in tall black pots and deep seed flats. Liquid medium may be a better pre-establishment treatment compared to gelled medium.

## LITERATURE CITED

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.