of November. In December we lower the night time temperature to approximately 40F and let it go to 70F during the day. In the middle of March the nighttime temperature is raised to 55F and top growth starts. In May we will have a plant ready to go into a 3-gal container.

In conclusion, the point I wish to make today is that you don't have to have a high-tech facility to propagate successfully. A lot of money is not necessary to get started. All that is necessary to get started is spirit, determination, and the ability to observe what others have successfully done in the past.

Research Update on Tissue Proliferation

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INTRODUCTION

This paper is intended to update the I.P.P.S. membership on a condition, known as tissue proliferation (TP), which affects a number of rhododendron cultivars. It would be impossible to summarize all the information on TP in this brief forum. The reader is directed to have in hand any or all of the articles listed at the end of this paper, in particular that by Linderman (1993).

Tissue proliferation refers to a gall-like growth usually found at the base of the main stem on certain cultivars of*Rhododendron*, primarily, though not exclusively, when they are propagated from tissue culture. These galls range from 5 to 20 mm in diameter, usually are loosely attached, covered by a rough spongy rind, and may or may not produce small, spindly, short-lived shoots. TP typically shows up in the second or third growing season out of propagation (e.g., from a tissue culture microcutting) and galls may wither each winter only to regrow the following year. TP appears not to be contagious, and only rarely are all the plants in a block affected. Plants possessing TP may grow slower or be more disease-prone, but more often are healthy and vigorous.

Tissue proliferation was first observed in the 1980s, and attracted widespread attention in the early 1990s when growers started seeing large numbers of galled plants and some nurseries lost or destroyed a lot of plants. Adverse publicity brought the issue to the fore, and soon groups of scientists met in the northwest (1991), the northeast (1992), and Ohio (1993) to discuss TP.

CAUSES OF TISSUE PROLIFERATION

Is it a Disease? When first encountered, TP was thought to be crown gall, caused by *Agrobacterium tumefaciens*. Thankfully, early work detailed a number of differences between TP and crown gall, including shoot production on TP galls, the woody nature of the TP gall, and the inability to spread TP by co-cultivation, or inoculation of healthy plants with gall pieces or extracts. Numerous studies since have attempted to isolate pathogenic forms of*Agrobacterium* from TP tissues, to no avail. Indeed there is some doubt if rhododendrons ever get crown gall. Attempts to infect rhododendrons with pathogenic *Agrobacterium* from other plants has been unsuccessful, as have attempts to implicate other gall-forming diseases. As time

passes it seems increasingly unlikely that a pathogenic cause of TP will be found.

Other Causes of Tissue Proliferation. The most common factor linking observed cases of TP is propagation from tissue culture. Though in isolated cases TP has been found on seedlings, cuttings, and even grafted rhododendrons, the majority of TP-affected rhododendrons have come from tissue culture. Unfortunately, TP appeared just as many growers were buying tissue cultured rhododendrons for the first time. Some growers were quick to implicate commercial labs in TP. Yet, this actually may be a blessing in disguise—an opportunity for the industry to focus on how young tissue cultured plants should be handled in the conventional nursery.

The attention TP focused on tissue cultured rhododendrons came at the same time that the issue of tissue culture variability was being addressed in a number of trade magazines and journals. These reports defined several forms of variability turning up in tissue cultured plants, including genetic variation, epigenetic variation, and habituation.

Genetic variation is a stable change in the plant's DNA that can affect the plant's appearance dramatically (e.g. doubling, dwarfing, and sports). Epigenetic variation is a change in the way the plant's DNA is expressed, though the DNA itself is not changed. This switching of genes "on" and "off" occurs naturally in all living things as they develop and mature (e.g. changes in leaf shape and size, flowering, and growth habit). When propagating plants asexually (by cuttage, graftage, or tissue culture), we strive to avoid epigenetic variation as we pursue uniform shapes and colors. Yet it is remarkably easy to alter plants epigenetically . For example, the rejuvenation of plant material, often resulting in increased rooting capacity, is well documented in stooling, cutting propagation, and particularly in tissue culture. It is not surprising that tissue culture promotes epigenetic variation—in tissue culture individual cells are bathed in chemicals, nutrients, and light at much higher levels than normal. Habituation is an odd sort of epigenetic variation in which tissue cultured cells gain the ability to grow without some plant growth regulator they previously required. This usually develops after exposure to the chemical for weeks or months. Habituation to auxin and cytokinin is common in tissue culture. Cytokinin habituation may be responsible for some of the juvenile characteristics of tissue-cultured plants, including greater vigor, more basal branching, and darker leaf color. The effects of habituation can be lost once the plant is taken out of culture—the plant eventually reverts to normal. Variation can occur in all forms of asexual propagation, yet appears to be more common in tissue culture. The habituation of rhododendron cultures to cytokinin was one of the earliest hypotheses proposed for TP, and remains a distinct possibility (see "The Tissue Culture Link" below).

Another early hypothesis for TP arose from the appearance of TP as a swelling at the base of the stem. Ecologists familiar with Mediterranean plants noted that TP resembled lignotubers, swollen areas of the stem with multiple shoot primordia buried in a rind, that occur naturally on certain plant genera including, in the Ericaceae, *Arctostaphylos, Kalmia*, and *Rhododendron*. As with crown gall, differences in the characteristics of lignotubers and TP led some to discount a link between the two. These differences included the relative permanence of lignotubers, which develop slowly and are not so easily removed as the typical TP gall. Furthermore TP galls develop rapidly over a growing season, and even regrow the following season if they slough off over winter. Finally, lignotubers appear to function as a survival structure that sprouts new shoots after the main stem is damaged. TP galls, on the

other hand, form only short-lived shoots that are poorly attached to the stem. Research conducted since 1993 has shown that some cultivars exhibiting TP won't even produce shoots when the main stem is cut back to the gall, i.e. TP galls cannot function as normal lignotubers. This doesn't mean there is no relation of lignotubers and TP. Even lignotubers sometimes fail to sprout after the main stem is damaged or removed—factors such as gall age, root development, and plant health surely play a role in the regenerative capacity of lignotubers, and perhaps TP galls. TP may be a type of highly modified, rapidly growing, dysfunctional lignotuber.

A compelling argument for TP being a form of lignotuber is the observation that particular rhododendron species form lignotubers (including *R. griersonianum, R. maximum, R. occidentale,* and *R. ponticum*), and that some of these are represented in the parentage of TP-prone rhododendron cultivars. Some of the new techniques in molecular taxonomy might be valuable in elucidating common lignotuber-forming parents among TP-affected cultivars.

On another front, researchers are working to characterize the developmental anatomy of TP galls. This daunting task may be critical in reinforcing or undermining the link between lignotubers and TP.

An interesting hypothesis is that TP represents a partial epigenetic switching "on" of a lignotuber gene or complex of genes. For this to happen, TP plants first would have to possess genes for lignotuber formation. Secondly, an epigenetic change would be required, such as rejuvenation through tissue culture. These changes would "predispose" the plant material to form galls in response to some sort of environmental trigger, such as rapid growth or stress. In a predisposed plant, "pushing" growth in the nursery with heavy fertilization and pruning, application of pesticides, or the use of growth retardants, is a likely trigger for TP. The role of stress in natural lignotuber formation has been documented in damaged *Kalmia* seedlings. The differences seen between cultivars might be explained by the degree to which their lignotuber genes are "switched on". Likewise, cultivars lacking the gene would never develop TP. The dramatic differences in the incidence of TP between nurseries, even when growing rhododendrons from the same source, might reflect the need for an environmental trigger to set off the TP phenotype.

The Tissue Culture Link. How tissue culture leads to TP needs to be studied carefully. Using *Rhododendron* 'Montego' as a model system, one researcher has shown that plants with TP go into culture faster, multiply faster, and become cytokinin-habituated earlier than plants without TP. TP-negative plants also can be converted to TP-positive plants by long term exposure to cytokinin, or by selecting for adventitious shoots. In one study a five-fold increase in cytokinin led to a five-fold increase in the incidence of TP. Using leaves as the explant source (i.e. all shoots of adventitious origin) led to a 15% incidence of TP. Several labs have begun the arduous task of determining cytokinin levels in TP-positive and TP-negative tissues. No results are available at this time.

It is interesting to note that the work with 'Montego' shows a range of TP-positive morphological changes in leaf shape and size, and degree of tumor formation. This observation supports the above hypothesis that a number of genes control TP. The use of 'Montego' as a model system has been questioned, in part because this cultivar alone forms galls during tissue culture. This extreme behavior could reflect a greater degree of habituation than is seen with other tissue cultured rhododendron cultivars. On the other hand, this trait makes 'Montego' a useful tool because the TP-

positive phenotype can be detected earlier. The cultivar 'Solidarity' has been suggested as another model system because it too forms galls predictably, though not while in culture. 'Montego' should be kept as a model system, if only because it has been studied so long, while parallel studies are conducted with 'Solidarity'. Similar results in the two systems would lead to even stronger conclusions.

Cultural Triggers. An aspect of TP that remains most troubling is that if identical material is sent to two nurseries one may see a high percentage of TP while the other sees none at all. This fact lends the strongest support to the idea of culture triggering TP in predisposed plants. Yet, numerous attempts to link herbicide, pesticide, or growth regulator use to TP have failed. A consensus among those growing rhododendrons is that TP is more severe on container-grown plants. Apparently it is not difficult to produce quality plants in the field from TP-positive liners. Several commercial firms have grown TP plants in the field for long term evaluation and report they are doing fine. Consider too that container-grown plants usually are grown in lightweight media and receive more fertilizer, water, pesticides, and herbicides than field-grown plants. Container-grown plants also grow faster and may require more frequent pruning or the application of growth inhibitors. Introduce to this production system a plant that is predisposed to TP and you may wind up with TP. TP galls also have been shown to grow larger on faster growing, more vigorous stock.

Conventional wisdom tells us that TP will be less common if growers use less fertilizer, plant growth regulators, and pesticides. Furthermore, container mixes should include more soil, and crops should be grown a little "leaner and meaner".

TISSUE PROLIFERATION VERSUS QUALITY

To those who have been following the TP debate, the most dramatic change since 1993 has been a perceived decline in concern about the quality of TP plants. Perhaps the early consensus that TP is not a disease cooled things down. Perhaps commercial tissue culture firms are rouging more suspect plants. Apparently, some firms have stopped marketing the more TP-prone cultivars. In addition, though many growers still buy tissue cultured plants, a few have returned to cutting-propagated liners, at least when buying rhododendrons. Certainly, at the onset of TP more growers were unwilling to accept galled plants, and some experienced more severe problems, including increased disease and mortality, and slower growth. Also, the way rhododendrons are tissue cultured or grown may be changing in ways that will reduce the incidence of TP. And last but not least, more people now believe that TP does not reduce plant vigor or survival—the problem is only cosmetic. TP-positive plants in the landscape often grow normally, or nearly so, and some even lose TP with age (is this epigenetic reversal?). One study lined out TP-positive and TP-negative plants and found no increase in *Phytophthora* or blackvine weevil. Another saw only a slight increase in mortality, and actually documented a decline from 100% to 45% of the plants affected with TP over a 3-year period. Lower soil fertility in the field and landscape might be reducing the incidence of TP. Certainly, these plants should be tracked. Do they survive? Do they grow well? Do they form lignotubers as they age and mature? If they come from a lignotuber-competent lineage we might expect that they would. It's interesting that syndromes similar to TP have been observed on *Kalmia, Pieris*, and *Vaccinium* for years without serious consequences.

By and large the excitement over TP seems to have waned. Tissue cultured rhododendrons are still in demand, even though some growers are staying away. Only the continued perseverance of a few researchers, propagators, and growers will solve this mystery and, if possible, eradicate tissue proliferation from the nursery.

REDUCING THE INCIDENCE OF TISSUE PROLIFERATION

Based on scientific reports, and a consensus among growers, there are a number of steps that can be followed to avoid TP in your nursery.

- Keep a lookout for TP. If you find it, don't throw the plants away. Notify your source of the problem and work with them and your Cooperative Extension Service to determine why it appeared in your nursery. Screen plants for pathogens, experiment with soil fertility and container mixes, and evaluate performance in the landscape.
- Be prepared to educate your plant inspector if your crop is tagged for crown gall. Keep copies of the articles listed at the end of this paper. Most of them detail the differences between TP and crown gall.
- Experiment with your cultural methods to see if you can grow an acceptable crop using heavier soil mixes, less fertilizer, and fewer chemicals. Avoid "pushing" tissue-cultured rhododendrons.
- Do not take cuttings off production blocks. TP-positive plants yield TP-positive cuttings. Stick to TP-negative stock blocks or buy liners from a commercial source.
- Commercial labs should continue to use as little cytokinin as possible, avoid subculturing from basal- or callus-derived shoots and small-leaved or otherwise aberrant growth. Initiate cultures from shoot tips or axillary buds only. Restart cultures periodically, and store maintenance cultures in the refrigerator to slow growth. Grow plants to as large a size as possible before selling them—eg. sell liners rather than microcuttings—and rouge off-type plants. Learn how to recognize and test for habituation, and discard habituated material. Avoid tissue culturing TP-prone cultivars—leave those for cutting propagation. And finally, maintain mature specimens for display, reference, and as a source of explants.

TISSUE PROLIFERATION AND RELATED LITERATURE

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Shrub Rose Breeding and Evaluation at the Minnesota Landscape Arboretum

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INTRODUCTION

At the Minnesota Landscape Arboretum (MLA), where minimum winter temperatures of -25 to -30F are typical, the number of repeat-flowering shrub roses hardy enough to survive a winter without protection is limited. Those that show slight to moderate levels of cane injury after a Minnesota winter are typically from one of three groups: hybrid rugosas, and Explorer and Parkland roses from Agriculture Canada. The number of disease-tolerant, hardy repeat-flowering roses is smaller yet.

The Woody Ornamental Research Program at the MLA has taken a two-pronged approach to increasing the number of hardy, disease-tolerant shrub roses for gardeners in the northern tier of the U.S. Existing cultivars that have not yet been trialed in Minnesota are being planted and evaluated to identify those that will perform well. A hybridization program to develop new cultivars is also under way.

EVALUATION

Floral traits, rebloom, plant size and habit, disease incidence, insect incidence, and winter hardiness are monitored during evaluation studies. Roses are evaluated every 10 to 14 days over several growing seasons.

Floral Traits. Floral traits monitored are the color, form, diameter, and fragrance of mature, fully open blooms. Inflorescence size, or the number of blooms in a single cluster, is also measured.

To evaluate rebloom, the growing season is divided into three periods: June, July, and August/September periods. Bloom during each of these periods is recorded as slight, moderate, or heavy.

Plant Size and Habit. At the end of the growing season, each plant's height and width are measured and a plant form (dense, open, suckering, spreading, arching, rugosa, climbing, and groundcover) is assigned.