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# Tissue Proliferation on Rhododendron: A Current Perspective©

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## INTRODUCTION

Tissue proliferation (TP) was first found in the mid-1980s and became a significant topic for propagators and growers by the early 1990s. The disorder is characterized by the development of callus-like tissue, often accompanied by adventitious buds and/or shoots, typically produced at the crown of rhododendron plants. TP has been observed on large-leaf and small-leaf rhododendrons, azaleas, and *Kalmia latifolia*. The superficial similarity in appearance of the gall-like growths or tumors of TP to crown gall caused considerable concern and problems for growers and nursery inspectors.

No evidence has been generated demonstrating that TP is caused by *Agrobacterium tumefaciens* or any other pathogenic organism. Today, it is generally believed that TP is not crown gall of rhododendron. The occurrence of TP mostly on micropropagated plants, or those with a history of tissue culture, has focused attention on the tissue culture process as a trigger for the development of TP in rhododendrons. As a result, TP has made many growers unwilling to grow micropropagated rhododendrons due to a fear of TP development.

## OBJECTIVES

Our research has focused on answering two grower questions: (1) Can TP symptoms be transferred to new plants via stem cutting propagation? and (2) can tissue culture trigger development of TP? Moreover, can high-quality rhododendron plants be micropropagated that won't develop TP?

## EXPERIMENTS

**TP Transmission Through Stem Cuttings**. Stem cuttings from plants with TP symptoms [TP(+)] and without TP symptoms [TP(-)] were rooted and grown for two growing seasons before being evaluated for TP symptom development. TP(+) cuttings were collected from 4- to 6-year-old micropropagated plants exhibiting gall-like growths. TP(-) cuttings were collected from 20- to 30-year-old plants that had no history of tissue culture and were free of TP symptoms. The cultivars 'Besse Howells' (BH), 'Boule de Neige' (BDN), 'Calsap' (CS),'Catawbiense Album' (CA), 'Holden' (HO), 'Montego' (MON), and 'Scintillation' (SC) were used in this study.

In a second study, using only MON, plants were grown from microcuttings and stem cuttings from 3-year-old TP(+) plants, 6-year-old TP(+) plants, and TP(-) plants. Microcuttings were from tissue cultures known to produce TP(+) plants and TP(+) stem cuttings were from field-grown plants that were 3 or 6 years old, post-tissue culture, and exhibiting TP symptoms. All plants were grown for 2 years before being evaluated for TP symptom development.

**Induction of TP by Tissue Culture**. Tissue culture studies were conducted using 'Montego' only. TP(-) shoot tips (10 mm long), or excised leaves from maintenance tissue cultures were placed on Woody Plant medium (Lloyd and McCown, 1980) with either 10 or 50 µM 2iP (isopentenyladenine). Cultures were maintained on this medium for two, 4-week, subculture periods before being moved to basal medium for a third subculture period to encourage shoot extension and expression of TP characteristics in vitro.

The amount of apparent adventitious meristem formation was rated based on the width (mm) of the basal meristem mass that formed on stem explants: 0 = none; 1 = 0-2; 2 = 2.1-4; 3 = 4.1-8; 4 = 8.1-12; 5 = 12.1. At the end of the third subculture period, cultures were checked for the presence of tumors and compact, multi-branched shoot clumps that were capable of growth-regulator autonomous growth, indicating they were TP(+).

**Table 1.** Comparison of shoot and leaf characteristics of seven rhododendron cultivars two seasons after cutting propagation. Stem cuttings were taken from plants with tissue proliferation [TP(+)] or without [TP(-)] tumors or history of micropropagation. Cultivars were 'Besse Howells'(BH), 'Boule de Neige' (BDN), 'Calsap' (CS), 'Catawbiense Album' (CA), 'Holden' (HO), 'Montego' (MON), and 'Scintillation' (SC).

| Plant characteristic | Comparison | Cultivars |
|---|---|---|
| Tumors | None | All cultivars |
| Shoot length | TP(+) TP(-) | BDN, CA, MON |
| Number of leaves/shoot | TP(+) TP(-) | All cultivars |
| Leaf length/width ratio | TP(+) TP(-) | HO, MON, SC |
| Leaf area | TP(+) TP(-) | MN, SC |
| Plant size | TP(+) TP(-) | BDN, CS, MON, SC |

## RESULTS

**TP Transmission Through Stem Cuttings**. Tumors were not observed on any propagated plants, regardless of the TP status of the cutting stock plants. There were, however, morphological differences between plants from TP(+) and TP(-) stock plants (Table 1). Shoots of TP(+) plants were either similar in length to shoots of TP(-) plants, or were shorter, as was the case for BDN, CA, and MON. Plants grown from TP(+) cuttings of all cultivars had more leaves per growth flush than did plants from TP(-) cuttings. HO, MON, and SC TP(+) leaves were narrower than leaves from TP(-) shoots and had greater length to width ratios. Leaves from TP(+) MON and SC plants were shorter and smaller than leaves from their TP(-) counterparts.

In the second study, examining the effect of time out of tissue culture on TP transmission, cuttings from TP(+)-micropropagated plants less than 3 years old were more likely to develop tumors than were cuttings from older plants (Table 2). Eighty-three percent of plants frommicrocuttings and 74% of plants from 3-year-old TP(+) plants formed tumors, whereas no plants grown from 6-year-old TP(+) or TP(-) cuttings did so.

**Induction of TP by Tissue Culture**. An increased level of 2iP (50 µM) stimulated significantly more adventitious shoot formation than the 10 µM 2iP treatment for both stem bases and leaves (Table 3). The higher 2iP level also induced more explants to develop TP(+) symptoms in vitro. The expression of TP(+) symptoms varied among TP(+) cultures. Some induced TP(+) cultures were highly branched, very compact, produced small leaves, and had numerous large tumors. Others were less branched, with larger leaves, some shoot elongation, and small tumors present.

TP induction in tissue culture appears to occur in MON as a result of adventitious shoot formation and high cytokinin levels. TP(-) MON cultures have been maintained in my lab by axillary proliferation for 11 years and produce no in vitro TP(+) morphology. Furthermore, these 11-year-old, TP(-) cultures produce plants that are free of TP(+) symptoms and are true-to-type. This demonstrates that it is possible to maintain rhododendron cultures for extended periods without developing off phenotypes.

**Table 2.** Incidence and severity of tissue proliferation tumors on *Rhododendron* 'Montego' plants grown from stem cuttings collected from plants of varying ages postmicropropagation.

| Degree of tumorization | Micro-cuttings(%) | 3-yr-old TP(%) | 6-yr-old TP(%) | TP(-)(%) |
|---|---|---|---|---|
| Small individual tumors | 11 | 13 | 0 | 0 |
| One-quarter of stem | 20 | 23 | 0 | 0 |
| One-half of stem | 22 | 28 | 0 | 0 |
| Stem surrounded | 29 | 10 | 0 | 0 |
| No tumor | 17 | 26 | 100 | 100 |
| Tumor of any size | 83 | 74 | 0 | 0 |
| | n=117 | n=39 | n=42 | n=59 |

**Table 3.** The development of adventitious shoots and tissue proliferation morphology on *Rhododendron* 'Montego' shoot bases and excised leaves in response to 2iP concentrations.

| Development on shoot bases | | | |
|---|---|---|---|
| 2iP (µM) | With adventitious (%) | Rating | TP[+](%) |
| 10 | 83b | 1.5b | 10b |
| 50 | 100a | 3.8a | 50a |
| **Development on excised leaves** | | | |
| 2iP (µM) | With adventitious (%) | TP[+](%) | |
| 10 | 40b | 26b | |
| 50 | 90a | 42a | |

## CONCLUSIONS

This work indicates that TP symptoms can be transmitted when stem cuttings are collected from TP(+) plants. Propagators should refrain from this practice. However, it does appear that with the passage of time, distal plant portions begin to lose their TP(+) nature as the growing points become more distant from their original TP(+) bases. TP can be induced by adventitious events during the tissue culture of rhododendron, but not all adventitious events lead to development of TP. Higher cytokinin levels in vitro lead to more adventitious shoot formation and a greater incidence of TP. So, can tissue culture be used to produce high quality rhododendrons that are free of TP? The answer is yes, provided that care is taken to only proliferate shoots through axillary bud release. If adventitious shoot development is prevented or if adventitious shoots are discarded when subculturing is done, TP should not be a problem.