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research is also needed to determine if Surflan exacerbates the development of TP in susceptible plants.

The results from the landscape performance experiment suggest the need for additional experimentation to verify or refute the possibility that TP does play a role in rhododendron mortality or poor performance in the early transitional period from nursery to landscape. Furthermore, absence of TP stem galls at the time of landscape planting does not guarantee that the plants will remain gall free. Given the uncertainty about the mechanism by which micropropagation appears to predispose plants to TP, the safest recommendation to minimize TP is to avoid use of micropropagated plants.

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Chromosomal Rearrangements Not Detected in Rhododendrons with Tissue Proliferation Disorder

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Tissue proliferation (TP) is a condition found on some cultivars of rhododendron (*Rhododendron* spp.), azalea (*Rhododendron* spp.), and mountain laurel (*Kalmia latifolia* L.). In rhododendron, TP is characterized by a mass of callus-like woody tissue that forms on the lower stem, crown, or roots. Although it superficially resembles crown gall, TP galls may have numerous shoots, unlike the galls associated with crown gall disease. Inoculation of plants with bacterial isolates from TP galls does not incite crown gall, either. Furthermore, the distribution of TP is usually random within production blocks, suggesting that the disorder is not contagious (Linderman, 1993).

TP can occur on plants derived from cuttings, tissue culture, seeds, or grafts, although according to claims from the industry, it occurs most often on plants from tissue culture. TP does not affect all cultivars, suggesting that some cultivars may be genetically predisposed to developing TP, while others are not. Various theories have been suggested to explain the occurrence of TP. Most researchers, however, tend to favor one hypothesis, that of epigenetic variation. According to this hypothesis, TP is a result of epigenetic changes that occur in some cultivars during micropropagation. These changes would predispose plants to develop TP if exposed to the right set of conditions. These conditions might include 1) excessive use of chemicals, such as growth regulators, or pesticides or herbicides with growth regulating activity, and 2) growing techniques that stress plants, such as severe pruning (Linderman, 1993).

Although epigenetic variation is probably the favored hypothesis, genetic mutation has not been ruled out as the cause of TP. We have been testing the hypothesis that some type of gross chromosomal rearrangement might be occurring at a higher frequency in tissue-cultured plants and be the cause of TP disorder in rhododendron. For this, we have analyzed DNA markers known as RAPD (random amplified polymorphic DNA) (Welsh and McClelland, 1990; Williams et al., 1990). For identification of RAPD markers, 10-base primers of arbitrary sequence but with GC contents of 50% or higher are used to amplify regions of genomic DNA. The DNA profiles generated are the result of the number and the size of fragments

amplified, which, in turn, depend on the nucleotide sequence of the primer and on the source of template DNA. Polymorphisms between samples arise from the absence of the homologous primer sequence in regions of one template DNA (absence of amplified fragments) or from differences in the distance between two adjacent primer annealing sites (amplified fragments of differing sizes). We have compared RAPD marker profiles from DNA of original or cutting-propagated plants of 'Montego' and 'Lee's Dark Purple' to profiles from tissue-cultured lines of these same cultivars. Plants derived from these tissue-cultured lines are known to exhibit symptoms of TP (Kenneth Mudge, personal communication).

EXPERIMENTS CONDUCTED

Plant material. Leaf material from the original hybrid 'Montego' and cutting-propagated 'Lee's Dark Purple' rhododendrons was provided by Stephen Krebs of the Holden Arboretum (Kirtland, Ohio). Tissue cultures of 'Montego' and 'Lee's Dark Purple' in the shoot proliferation stage were obtained from Kenneth Mudge of Cornell Univ., who had originally obtained these cultures from a commercial tissue culture laboratory (Herman Losely and Son, Perry, Ohio). For each cultivar, two lines identified as "old" and "new" were used. Those indicated as "old" had been in culture for more than 9 years when received in late 1993, whereas those designated "new" had been in culture for slightly more than 2 years. At this time, the "old" culture of 'Montego' was habituated and did not require a cytokinin in the medium for continued shoot proliferation and growth. Furthermore, the "old" culture of 'Montego' displayed visible swellings or nodules in vitro. The other three cultures ('Montego' "new," 'Lee's Dark Purple' "old," and 'Lee's Dark Purple' "new") were grown on medium containing 2-isopentenyladenine (2iP) at 2.5 mg·L⁻¹. The medium used for all four types was woody plant medium (WPM) (Lloyd and McCown, 1980) with (g·L⁻¹) 25 sucrose, 1 Gelrite, and 0.6 Sigma agar with 100 mL dispensed per Magenta GA-7 container. Cultures were grown at 25 ± 2 °C on a 16-h photoperiod with a photosynthetic photon flux of ≈80 μmol·m⁻²·s⁻¹.

RAPD reactions. DNA was extracted on two separate occasions from leaf tissue collected from the original hybrid known as 'Montego' and cutting-propagated 'Lee's Dark Purple', and from leaf and stem tissue collected from tissue-cultured lines of 'Montego' and 'Lee's Dark Purple'. DNA was isolated using a CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle and Doyle, 1987), as modified by Rowland and Nguyen (1993), but

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with the omission of the polyethylene glycol precipitation. Amplification reactions were performed as described previously (Levi et al., 1993; Rowland and Levi, 1994) using 10-base primers [Biotechnology Laboratory, Univ. of British Columbia (UBC), and Operon Technologies, Alameda, Calif.]. Amplification products were separated by electrophoresis through 1.4% agarose gels in 0.5× Tris-borate electrophoresis buffer (Sambrook et al., 1989). DNA fragments were visualized by ultraviolet irradiation after staining with ethidium bromide at 0.5 µg·mL⁻¹.

FINDINGS AND INTERPRETATION

RAPD marker profiles generated from DNA from the original hybrid 'Montego' and cutting-propagated 'Lee's Dark Purple' were compared to profiles generated from DNA from tissue-cultured lines (designated "new" and "old") of these same cultivars. Plants derived from these tissue-cultured lines were known to exhibit symptoms of TP.

Profiles were generated using 105 differing 10-base primers. Using our standard RAPD protocol, 80 of these resulted in good amplification of several DNA fragments. The other 25 primers resulted in poor or no amplification. RAPD marker profiles that could easily distinguish between 'Montego' and 'Lee's Dark Purple' were generated from 64 of the 80 primers (for profiles from one such primer, see Fig. 1). These results suggest that RAPD markers would be very useful for identification of rhododendron cultivars.

On first examination, profiles from five of the 80 primers appeared to distinguish the original hybrid 'Montego' or cutting-propagated 'Lee's Dark Purple' from their respective tissue-cultured lines. In all cases, the differences detected involved one or more amplified fragments present in the original 'Montego' or cutting-propagated 'Lee's Dark Purple', but absent in one or both ("new" and "old") of the respective tissue-cultured lines. These results appeared reproducible when profiles from separate RAPD reactions using the same stock DNAs were examined. However, we attempted to verify these results using DNA samples extracted for a second time from the original hybrid 'Montego', the cutting-propagated 'Lee's Dark Purple', and tissue-cultured lines (Table 1). None of the differences originally noted appeared reproducible, with the exception of one amplified fragment (using primer 399, from UBC) present in the original 'Montego' and absent in the tissue-cultured 'Montego' lines (Fig. 2). However, amplification of a similar-sized fragment, originally noted in cutting-propagated 'Lee's Dark Purple' and absent in tissue-cultured lines of 'Lee's Dark Purple', was not reproducible using DNA from the second set of extractions.

These results suggest that the minor differences observed, using the five primers noted (Table 1), are either not reproducible or may not be associated with the TP disorder. Further testing, however, is still required with primer 399 and plants of other cultivars with and without TP. Our best explanation for the discrepancies in RAPD marker profiles using DNA from separate extractions is that our original batch of plant material from original 'Montego' and from cutting-propagated 'Lee's Dark Purple' may have been contaminated with pollen or some type of microorganisms. Plant material from tissue-cultured lines would not be expected to be contaminated in this way. Thus, DNA extracted from original or cutting-propagated material but not from tissue-cultured material could be contaminated with DNA from pollen or microorganisms, and this DNA could, with some primers, result in amplification of DNA fragments. This explanation, however, is not completely satisfactory because in the case of primer P10 (from Operon), a DNA fragment appeared in samples from cutting-propagated 'Lee's Dark Purple' as well as the tissue-cultured "new" line of 'Lee's Dark Purple'; it was only absent in samples from tissue-cultured "old" line of 'Lee's Dark Purple'.

In conclusion, we have compared RAPD marker profiles from DNA of original or cutting-propagated cultivars of 'Montego' and 'Lee's Dark Purple' to profiles from DNA of tissue-cultured lines of these same cultivars. Plants derived from these tissue-cultured lines are known to exhibit symptoms of the TP disorder. Only five of 80 primers that resulted in amplification detected minor differences between original or cutting-propagated cultivars and their respective

Fig. 1. RAPD reactions using primer CCCGCCTTCC (#23 from the Univ. of British Columbia) and rhododendron DNA from the first set of extractions as template. From left to right are shown RAPD-PCR using DNA from original hybrid of 'Montego' (M₁), tissue-cultured line "new" of 'Montego' (M₂), cutting propagated 'Lee's Dark Purple' (L₁), tissue-cultured line "new" of 'Lee's Dark Purple' (L₂), and tissue-cultured line "old" of 'Lee's Dark Purple' (L₃).

Fig. 2. RAPD reactions using primer TTGCTGGGCG (#399 from the Univ. of British Columbia) and rhododendron DNA from first (A) and second (B) set of extractions as template. Labels M₁, M₂, M₃, L₁, L₂, and L₃ refer to DNA from original hybrid of 'Montego', tissue-cultured line "new" of 'Montego', tissue-cultured line "old" of 'Montego', cutting-propagated 'Lee's Dark Purple', tissue-cultured line "new" of 'Lee's Dark Purple', and tissue-cultured line "old" of 'Lee's Dark Purple', respectively. The arrow points to the fragment amplified from both sets of extractions of original hybrid 'Montego' but not from any extractions of tissue-cultured lines of 'Montego'. A similar-sized fragment was amplified from DNA from the first extraction of cutting-propagated 'Lee's Dark Purple' but not from the second extraction. Note: DNA was extracted only once from tissue-cultured line "old" of 'Montego'.

Table 1. Differences in RAPD marker profiles noted between original or cutting-propagated 'Montego' and 'Lees Dark Purple' and their respective tissue-cultured lines.

Primer name and (source)	Sequence	Type of difference noted	Reproducible
295 (UBC) ^a	CGCGTTCCTG	Multiple fragments present in original and cutting-propagated 'Montego' and 'Lee's Dark Purple' that were absent in tissue-cultured lines	No
388 (UBC)	CGGTCGCGTC	One fragment present in original and cutting-propagated 'Montego' and 'Lee's Dark Purple' that was absent in tissue-cultured lines	No
399 (UBC)	TTGCTGGGCG	Multiple fragments present in original and cutting-propagated 'Montego' and 'Lee's Dark Purple' that were absent in tissue-cultured lines	One fragment present in original 'Montego' and absent in tissue-cultured lines was reproducible
P2 (Operon)	TCGGCACGCA	One fragment present in original and cutting-propagated 'Montego' and 'Lee's Dark Purple' that was absent in tissue-cultured lines	No
P10 (Operon)	TCCCGCCTAC	One fragment present in cutting-propagated 'Lee's Dark Purple' and "new" 'Lee's Dark Purple' tissue-cultured line that was absent in "old" 'Lee's Dark Purple' tissue-cultured line	No

^aUniv. of British Columbia.

tissue-cultured lines. Furthermore, these differences were either not reproducible using DNA from a second set of extractions or probably not associated with TP. If a dramatic change in chromosome structure, such as loss of a chromosome arm, were responsible for TP, we would expect to have detected a reproducible difference in RAPD marker profiles in both cultivars, considering the large number of primers examined. Therefore, our results suggest that TP is caused either by epigenetic variation or by a genetic mutation affecting a relatively small region of DNA. Consequently, although very effective at distinguishing cultivars, RAPD marker profiles may not be useful for identifying tissue-cultured lines that will yield plants with TP.

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