Abnormal Growths on Micropropagated Elepidote Rhododendrons

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INTRODUCTION

The occurrence of abnormal growths or tissue proliferations (TP) on elepidote rhododendrons has been the subject of intense discussion at formal and informal meetings around the country and in nursery-related publications (Anonymous, 1992a; Anonymous, 1992b; Bayer, 1982; LaMondia et al., 1992; Rostan, 1992). Unfortunately, the information on the identity, significance, the mode of transmission, and cause of TP has been conflicting.

Experimental evidence is required to prove how damaging TP is to plant health. Observations in Ohio suggest the vascular system of stems with large TPs is disrupted, resulting in weak plants which cannot be sold. Plants with TPs on the upper trunk and branches are cosmetically unacceptable and may also be weakened. We will review some of the conflicting observations and speculate on possible causes of the TPs. References will also be made to reviews on topics covered here to provide those interested with a more comprehensive background.

CHARACTERISTICS OF TISSUE PROLIFERATION

The term "tissue proliferation" or TP is applied to the abnormal growths characterized as callus-like tissue with adventitious buds and/or shoots ("shooty") which are typically produced at the crown of plants. In Connecticut, efforts have been made to distinguish TP from crown gall whose diagnosis can lead to destruction or rejection of plant shipments into the state (LaMondia et al., 1992). This effort led to a preliminary conclusion that crown gall and TP could be distinguished by the characteristics summarized in Table 1. While these characteristics may apply in some cases, it is not yet certain they are clearly diagnostic as they rely on limited definitions of both TP and crown gall on rhododendrons.

Historically, crown gall has not been seen as a significant problem on rhododendrons and proofs that specific symptoms represent the disease are lacking. Well documented studies on crown gall of tobacco show that the symptoms of disease resulting from infection by *Agrobacterium tumefaciens* can vary from shooty to non-shooty tumors (Hooykaas et al., 1982; Landman, 1991; Morris, 1986; Turgeon, 1982). Genetic tumors in tobacco, which develop in the absence of pathogens, produce a similar range of symptoms, but tend to produce shoots ("shooty") from the tumor cells (Bayer, 1982; King, 1991). It has been demonstrated that *A. tumefaciens* induces tumor formation by inserting into host DNA the cytokinin biosynthetic isopentenyl transferase *ipt* gene (Ichikawa and Syono, 1991; Morris, 1986).

However, certain tobacco species contain a gene functionally equivalent to *ipt* in the absence of infection (Ichikawa and Syono, 1991). An analogous situation exists for the *roi* gene of *A. rhizogenes* which functions in auxin biosynthesis (Ichikawa and Syono, 1991) and induces tumors with root ("rooty") outgrowths, but a functionally equivalent gene can also be found in certain uninfected species of tobacco (Ichikawa and Syono, 1991).

Table 1. Characteristics initially used to distinguish tissue proliferation from crown gall on elepidote rhododendrons. These criteria are now in question.

Tissue proliferation	Crown gall
Adventitious shoots on tumors	No shoots on tumors
Organized vascular tissue	Unorganized internal tissues
Nodular, semiorganized, meristematic portions of tumors	No meristems or nodules on tumors
Tumors not seen on roots	Tumors present on roots

It is doubtful that degree of vascular differentiation can be easily used to distinguish TP and crown gall. Although the vascular tissues in crown gall proliferations are disorganized, similar disorganization has been observed in microscopic examinations of putative TPs in a range of sizes (David Leach, personal communication). Experimental evidence is also required to establish whether root tumors only occur in cases of crown gall. Root, crown, and aerial proliferations have been observed in plants putatively identified as having TP (personal observations and personal communication with David Leach). It is premature to exclude the possibility of TP symptoms on roots.

CAUSES OF TISSUE PROLIFERATION

If TP and crown gall are distinct, it is not surprising that a similar range in symptoms can result, since the biochemical and developmental processes producing cell proliferation may be similar (Bayer, 1982; Hooykaas et al., 1982; Ichikawa and Syono, 1991; Kung, 1991). To date, artificial inoculations with *Agrobacterium* isolated from elepidote rhododendrons and molecular probing for biovars of *Agrobacterium* have proven negative, or inconclusive. Similarly, in our lab and others, attempts to isolate bacteria from in vitro rhododendron cultures known to produce a high frequency of TP plants have proven negative. However, the striking similarity of symptoms produced in the case of crown gall and TP suggests if a strain of *Agrobacterium* is not involved, a similar disruption in normal metabolism has occurred.

If TP is not the result of crown gall, it may be induced by any of a number of other pathogens known to induce galling and brooming in ericaceous and other woody plants (Eck and Childers, 1966; Farr et al., 1989; Sinclair et al., 1987; Wicker, 1987). Observations in the northeastern U.S. suggest that TP is found almost exclusively in micropropagated plants. Plants produced by cuttings from non-

tissue culture stock plants, or seedlings, have not produced TP symptoms. However, not all tissue cultured rhododendrons develop TP. If biotic agents are involved in inducing TP, it is curious why tissue culture plants would be infected at a higher frequency than rooted cuttings or seedlings. There are contrasting claims that TP is produced on microporpagated plants, rooted cuttings, and seedlings of elepidote rhododendrons and that proliferations are similar to burls which are a natural mechanism of plant regeneration.

The comparative incidence of TP in a cultivar propagated by cuttings and through micropropagation needs to be established. Until the experimental results are known, we take the precaution of considering all micropropagated plants from batches showing TP as being suspect of having the capacity to develop and transmit the capacity to develop TP. This is only a precaution, but emphasizes the importance of research on the transmission of TP.

Observations made to date are in general agreement that TP is not highly contagious and is confined within a cultivar showing the problem with no spread to adjacent plants (Anonymous, 1992a). However, a nursery has reported that rooted cutting produced from stock plants with TP also develop TP. While there are also reports that TP is not transmitted through cuttings (Anonymous, 1992a), these results show it is necessary at present to know the propagation history of stock plants used for cuttings. Until experimental evidence clearly establishes the safety of using TP plants or non-TP plants from the same production, it is wise to only use stock plants that are (a) known to derive from cuttings tracing back to the original plant or (b) from micropropagated plant batches that have not shown TP. The need for careful records is essential until more is known.

TISSUE PROLIFERATION IN THE ABSENCE OF PATHOGENS

It is possible the expression of TP in the northeastern U.S. is enhanced by environmental stresses since some nurseries have had to discard plants due to their declining vigor. In contrast, nurseries in the Pacific Northwest have not seen a lethal decline in plant vigor. We observe that symptoms in Connecticut can vary among and between cultivars from being shooty, weakly shooty, to non-shooty or various combinations of these symptoms. Table 2 is a summary of characteristic symptoms observed in some cultivars. TP differs from burls in that the proliferations appear to lose their ability to organize normal meristems, buds, or shoots. Similarities can be drawn between such symptoms and the production of tumorous growth in tissue culture in the absence of pathogens (Gaspar et al., 1991). It has been observed that normal habituated callus (callus no longer dependent on presence of hormones for proliferation) is capable of organizing meristematic centers and organogenesis (totipotent); however, this ability can be lost progressively, passing next to a stage where totipotency is partly lost (eg., vitrified shoots), and finally to an irreversible stage in which totipotency is irreversibly lost (Gaspar et al., 1991).

If TP is not caused by an infectious agent, we speculate it requires a combination of (a) a genetic susceptibility to TP (a TP gene?) and (b) an alteration in normal genetic regulation in rhododendrons. It is known that the frequency and phenotypes of proliferations can vary among rhododendron cultivars which leads us to suspect a genetic basis in which some cultivars are highly susceptible and others relatively resistant. The genetic basis can be established by transmission genetics.

Table 2. A summary of characteristic symptoms observed in some rhododendron cultivars. This list is by no means all-inclusive and listed cultivars do not always develop tissue proliferation symptoms.

Rhododendron cultivars and tissue proliferation symptoms
'Album' Basal tumors accompanied by large numbers of small shoots, meristems or nodules.
'Scintillation' Basal tumors accompanied by a moderate to small number of short/compressed shoots, meristems or nodules. Occasionally shoots will be absent on tumors.
'Bessie Howells' Basal tumors usually without associated shoots or meristems. Occasionally shoots may be found on tumors.
'Solidarity' Tumors found at base of plant and on aerial portions of plants. Shoots on tumors have not been observed.

Epigenetic changes involve changes in gene expression due to altered genetic regulation. Habituation is an example of an epigenetic change in which cells grown in vitro on hormone-containing media (cytokinin and auxin) lose their requirements for the hormones (Ichikawa and Syono, 1991; Kung, 1991; Meins, 1989). The cells that once required exogenous hormone become hormone autotrophic. Tobacco cells transformed by *A. tumefaciens* behave as habituated cells, but cells that are not transformed can also become habituated. Studies on genetic tumors of tobacco show there are 3 steps in tumorigenesis: (1) gene for tumorigenesis must be present in the plant; (2) stress initiates additional cytokinin synthesis; and (3) the interaction between additional cytokinin and the tumorigenesis gene to induces abnormal growth (Kung, 1991). This scheme has been further refined to include regulation of the auxin metabolism in tumorigenesis (LaMondia et al, 1992).

If there are genetic tumors in rhododendrons, the mechanism may differ slightly from the model for tobacco. First, it appears that tissue culture propagation as well as the host genotype is important in making a cultivar highly susceptible. An epigenetic change or habituation (in extreme cases) may be induced during in vitro culture. Although the molecular mechanism of habituation is not known (Meins, 1989), it is known that tissue culture can activate transposable elements in maize which alter gene expression (Lee and Phillips, 1988). Similarly, other mobile (Landman, 1991; Weil et al. 1990) and nonmobile genes (Sachs and Ho, 1986) can alter the normal progression or regulation of gene expression. If an epigenetic change has occurred, a rhododendron may fully express its degree of susceptibility to TP. Environmental stresses (biotic or abiotic) or internal stresses (flowering) may then trigger the next stage (full habituation?) that would result in TP.

We have observed, along with others, the production of in vitro proliferations in the rhododendron cultivar 'Montego'. 'Montego' produces a high incidence of TP on plants in nursery production. 'Montego' will also produce shoots in vitro in the absence of auxin and cytokinin. One can speculate that this highly susceptible cultivar is fully habituated in vitro, a state that may not apply to most cultivars. TP may be an expression of the fully habituated state in rhododendrons containing TP susceptibility genes and genes which will effect epigenetic changes.

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