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(Ohio Tissue Proliferation Meeting, February 1993)

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Tissue Proliferation and Tissue Culture

Given the murky and controversial relationship between tissue proliferation and tissue culture, do most researchers now believe there is a link? If so, what is occurring to predispose some micropropagated plants to express tissue proliferation?

At the Ohio meeting (see main story), most participants generally favored one hypothesis. According to this theory, tissue proliferation results from epigenetic changes that occur in certain cultivars during micropropagation. These changes predispose the plants to develop tissue proliferation later in response to certain triggering cultural practices.

The epigenetic variants that occur during micropropagation could be overly sensitive to growth-regulating substances. When microshoot plants with this increased sensitivity are rooted in nursery medium and grown on, they may later produce abnormally high levels of growth-regulating substances, such as cytokinins. (This is especially likely when some cultural practice spurs production of these chemicals.)

High cytokinin levels, or an increase in the ratio of cytokinin to auxin, can result in the calluslike growth seen in tissue proliferation. Researchers have already measured low auxin levels in tissue proliferation, but they have not yet measured cytokinin levels. Nevertheless, because shoots emerge from the calluslike growth, the tissues must either have naturally elevated cytokinin levels or they must be abnormally sensitive to cytokinin production triggered by nursery practices.

Several practices could increase cytokinin levels near the junction of roots and shoots. They include accelerated cultural conditions requiring severe pruning; applications of growth retardants to reduce the growth rate and encourage branching; applications of pesticides or herbicides that have some growth-regulating activity; and other stresses that might affect the distribution of carbon from photosynthesis.
Some five years ago, I learned of a problem on rhododendrons that resembled the disease known as crown gall. Affecting many plants, crown gall is a well-known bacterial malady caused by Agrobacterium tumefaciens. Calluslike growths are a primary symptom, and this pattern now seemed to be occurring on rhododendrons. But the plants I was seeing exhibited several features that made me wonder whether crown gall was really the problem.

First, the calluslike growths occurred only on a few cultivars — and then in scattered, random distribution within a production block. This suggested that, unlike crown gall, the new problem was not contagious.

Frequently, but not always, small shoots emerged from the calluslike growth, which occurred primarily on lower crown tissue. (Most people who had seen the syndrome believed the calluslike structures generally did not occur on roots.) Shoot proliferation is not common with crown gall; nevertheless, the problem resembled crown gall more than anything else, and other people who had seen the plants considered this the best tentative explanation.

To check this hypothesis, Dr. Larry Moore of Oregon State University, Corvallis, performed a series of tests on affected plants. To prove the presence of crown gall, a scientist must isolate bacteria (which are theoretically causing the problem) from the gall tissues, reinoculate healthy test plants (usually tomatoes) with the isolate, and reproduce gall symptoms. When Moore tried this procedure with affected rhododendrons, his results were negative. Still, the problem looked like crown gall to most people, including growers and nursery inspectors.

In January 1991, a group of interested parties gathered in Vancouver, WA, to evaluate the situation. They endorsed a proposal that the condition be referred to as “tissue proliferation” to distinguish it from crown gall. Participants reported the following observations:

- **Tissue proliferation**, often called TP for short, occurred only on a few rhododendron cultivars. Some were more uniformly affected than others. Most of the affected plants were at least two years old.
- Though many plants with TP were produced by tissue culture, the syndrome also occurred on plants propagated by cuttings.
- Vascular tissue differentiation was typical. Multiple buds and, often, small shoots emerged from the hard, calluslike structures. (By contrast, crown gall usually exhibits little or no such differentiation; all affected tissue remains calluslike.)
- Affected plants showed no loss of vigor.
- Some researchers had isolated Agrobacterium from the affected tissues. But none were able to reproduce the symptoms by inoculating test plants with the isolates. In addition, the isolates failed to react to DNA probes used to detect the crown-gall pathogen. Thus the Agrobacterium from affected plants was non-pathogenic — the same result one would expect of Agrobacterium isolated from healthy plants.
- Apparently, the syndrome did not spread from plant to plant during production.

After considering all these observations, the meeting participants concluded that tissue proliferation is not caused by a disease organism. Nevertheless, the syndrome represented a quality problem that was causing customers and inspectors to reject plants.

Subsequently, nurserymen and researchers convened in several additional meetings around the nation. Lacking definitive information about TP and its cause, growers, customers, researchers and inspectors remained concerned. Some nurseries experienced significant economic loss because they destroyed affected plants rather than sell material that might be diseased.

The most recent meeting was a workshop held this past February at the Holden Arboretum in Mentor, OH. I
Tissue Proliferation and Lignotubers

Participants at the Ohio meeting (see main story) spent a good deal of time trying to characterize the structural features of tissue proliferation. Without a clear anatomical analysis of the gall-like structures, it has been difficult for many people to distinguish tissue proliferation from other phenomena, such as crown gall and lignotubers (burrlike structures).

Participants had a lengthy discussion on the anatomical features of tissue proliferation. For example, only one observer has reported the syndrome on roots — but few have looked carefully in this area. (Some people once thought tissue proliferation must be unrelated to crown gall because crown gall occurs on roots and proliferation does not; however, if TP actually can occur on roots, that conclusion becomes suspect.)

There was also much speculation about the possible relationship between TP and lignotubers. Members of several genera, including Tilia, Sequoia, Ginkgo, Arctostaphylos, Kalimia and most Eucalyptus, produce lignotubers; so do many wild rhododendrons.

Experts indicate that lignotubers are part of the normal development of certain plants and are not caused by pathogens. Certain plants within a genus, or within a population of a species, show a genetic propensity to develop lignotubers; triggering stress seems unnecessary.

The ecological rationale behind lignotubers, which tend to form on plants that have a hard time reproducing themselves through seed, is as regenerative survival structures. Lignotubers are composed of many dormant buds and some small shoots (which may die back) within a large body of tissue at the base of a plant. This tissue has a high nutrient-storage capacity. When the normal branches of these plants are damaged or destroyed, regeneration occurs from the buds in the lignotubers.

Researchers who examined rhododendrons grown under nonaccelerated conditions discovered that the TP in these plants did indeed resemble lignotubers in Arctostaphylos. In both TP and Arctostaphylos lignotubers, there was a woody central core surrounded by a fleshy cortex in which numerous suppressed shoot buds were embedded. These shoot buds were connected via vascular traces to the vascular system of the main stem.

However, other examples of TP were unlike lignotubers. In these plants, the TP did not form a permanent extension of the stem. Instead, the gall-like growths were often loosely connected lateral appendages that were readily removed.

Lignotubers always contain numerous dormant buds; frequently, small shoots emerge but die back. The buds tend not to produce shoots as long as the primary stem is intact. When destruction or removal of the main stem prompts the buds to produce shoots, the shoots that develop are normal. Conversely, not all TP structures develop shoots. In plants that do develop shoots, the shoots sometimes emerge while the main stem is intact, although many of these shoots appear to be dwarfed or otherwise abnormal.

To distinguish tissue proliferation from lignotubers, observers must study how much cell differentiation appears in the gall-like structures. Specifically, they must look carefully to see whether there is a strong vascular link between the parent plant and the dormant buds or shoots emerging from the gall-like structures. The stronger the link, the greater the likelihood that the growth [sic] is a lignotuber rather than “classic” TP.

An anatomical comparison of TP and lignotubers also suggests that the numerous dormant buds that characterize lignotubers are often absent in TP. In addition, the tissue in TP structures grows much more quickly than normal lignotubers would grow over the same period. This rapid growth produces nonpersistent TP structures that often wither and slough off annually. Conversely, lignotubers are clearly permanent; some examples are known to be over 100 years old. (Perhaps tissue differentiation is relatively incomplete in TP structures simply because vascular connections have so little time to develop before the structures fall off.)

If TP does indeed occur on roots, this characteristic would also serve to distinguish it from lignotubers. Lignotubers invariably form on stems in association with suppressed development of axillary buds; they never occur on true roots. It is possible, however, that any gall-like growths that have been observed on roots are different from both TP and lignotubers. These root structures certainly merit separate and distinctive study.

The consensus of the workshop group was that, while TP is not simply a normal burl or lignotuber, the phenomena do appear to be related. The same theory of predisposing and triggering factors could apply to both. (In other words, certain genotypes may have a natural or induced tendency to form TP or lignotubers, and stress triggers their development.) Tissue proliferation could actually be an accelerated form of lignotuber development.

Careful long-term anatomical studies of tissue proliferation from roots, crowns and branches could confirm critical similarities and differences between TP and lignotubers.
If micropropagation favors the development of variants that are predisposed to exhibit tissue proliferation, the syndrome would naturally occur more and more frequently as an increasing number of micropropagated rhododendrons entered the trade.

... coordinated this session, which was attended by scientists selected for their interest in tissue proliferation and their expertise in plant physiology, ecological morphology, pathology, genetics and horticulture (sidebar, page 56). Our objectives were to discuss possible causes of TP and to identify research needed to pinpoint the cause and eliminate the problem. (For details, see “Progress, But No Answers, at Ohio Meeting,” April 1, 1993, page 15 — ED.)

The following article reflects the information and discussions developed at the workshop. While this information is still largely hypothetical, we did make progress toward understanding the causes and effects of tissue proliferation.

Attempts to diagnose the cause of tissue proliferation remain inconclusive, but the Ohio workshop produced the following possible explanations:

**Microbial pathogen.** The research findings mentioned earlier strongly indicate that TP is not caused by Agrobacterium tumefaciens. In addition, affected tissue lacks chemical constituents that would indicate the genetic transformation that occurs with crown gall.

Researchers have unsuccessfully tried to isolate other microbes that might cause galls-like growths, such as Erwinia carotovora var. herbicola, from affected tissue. Some feel that such bacteria, which are very difficult to isolate, may still be involved.

Assays to implicate mycoplasmalike organisms — such as the organism that causes elm yellows — as possible causes of TP have been negative. So have attempts to transmit some causal agent by grafting.

Because tissue proliferation occurs only on certain cultivars and seems unable to spread from plant to plant, the syndrome is probably not caused by a microbe. However, participants acknowledged that a microbial pathogen could not absolutely be ruled out on the basis of negative data. Until researchers can prove that TP is caused by something else, an unknown microbial pathogen remains a possibility.

**Genetic variation.** Observers have seen tissue proliferation primarily on rhododendron, but the syndrome occasionally appears on deciduous azalea, Kalmia and Fierzia as well. Based on current observations, it appears that only a few commercial rhododendron cultivars express tissue proliferation on a high percentage of plants. Most of those that do are in the elepidote (nonscaled) group. Elepidote (scaly-leaved) rhododendrons, with some noted exceptions, never seem to have tissue proliferation; when they do, the incidence and extent are much lower than on elepidote cultivars.

As mentioned earlier, the syndrome has appeared primarily, but not exclusively, on tissue culture plants. This suggests that micropropagation somehow induces genetic or epigenetic variation that predisposes plants to exhibit tissue proliferation (sidebar, page 56). (A genetic variation means the plant’s genetic composition has actually changed somehow, whereas an epigenetic variation changes the way a plant’s genes are expressed. Genes that are normally “switched off” may be “switched on” in an epigenetic variant, altering a plant’s growth or appearance.)

For some rhododendron cultivars, tissue proliferation seems to be a natural phenomenon. But only a few plants of a given cultivar are usually affected when plants are propagated conventionally. The percentage of affected plants may rise dramatically after the cultivar is propagated through tissue culture.

If micropropagation favors the development of variants that are predisposed to exhibit TP, the syndrome would occur more and more frequently as an increasing number of micropropagated rhododendrons entered the trade. These variants might simply have a greater responsiveness to triggering conditions that occur long after plants grow out of the tissue culture state.

Some observations indicate that normal vegetative cuttings taken from tis...
Researchers are still striving to identify factors that trigger tissue proliferation. If growers knew which practices caused plants to express the syndrome, they could reduce or eliminate the problem.

Sue culture parents retain an increased tendency to express tissue proliferation. But researchers have not verified this conclusion, and participants at the Ohio meeting identified this task as a priority.

Predisposing and triggering factors. Because relatively few of the several hundred commercial rhododendron cultivars strongly exhibit tissue proliferation, and the syndrome does not usually appear until plants are at least 2 years old, researchers have had a tough time figuring out where and when tissue proliferation begins.
Apparently, the syndrome has two phases: the predisposing phase (which probably occurs in propagation) and the triggering phase (which occurs in production). Clearly, plants predisposed to tissue proliferation during propagation must be triggered later by some nursery production practice or practices.

This must be so, since two different nurseries can grow liners of the same cultivar from the same tissue culture lab, and one nursery will have tissue proliferation while the other nursery does not. Unfortunately, researchers have not yet identified which cultural practices trigger TP.

Some researchers think common chemical pesticides or growth regulators may somehow cause proliferation-prone plants to express the syndrome. Tissue proliferation occurs primarily in container nurseries that use lightweight, soilless media. Growers who use heavy soil mixes, minimize fertilizer and pesticide applications, and avoid accelerated growth conditions and environmental stresses have observed less TP than those who "push" their plants. This remains true even when these growers start with micropropagated liners.

In one test, researchers applied high rates of several popular herbicides to proliferation-sensitive cultivars. Most of the chemicals did induce a significant increase in tissue proliferation. A repeat of the same experiment, however, failed to confirm the results.

At another location, a test using elevated rates of growth retardants resulted in severe growth retardation, and nearly every treated plant exhibited tissue proliferation, while untreated controls did not. But another trial that studied the effects of high levels of the growth regulator cytokinin (frequently used in tissue culture) failed to show any relationship between this chemical and TP.

Researchers are still striving to identify factors that trigger tissue proliferation. If growers knew which specific cultural practices caused plants to express the syndrome, they could reduce or eliminate the problem.

Nurserymen and their customers not only want to know what causes tissue proliferation; they also need to know whether the syndrome adversely affects plant growth.

Discussions at the workshop and with growers indicate that TP has been associated with reduced growth in some parts of the country and not in others.

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nots, but only those with tissue proliferation succumb to the disease. However, not all plants have Phytophthora, so this assumption is both invalid and misleading. The notion that plants weakened by TP could be more susceptible to Phytophthora infections could be true but remains unconfirmed. Furthermore, plants with TP but without Phytophthora root rot usually show no signs of reduced growth or higher mortality.

As a result of this discussion, meeting participants agreed that clarification of a possible link between tissue proliferation and Phytophthora root rot should be a research priority.

One final issue that meeting participants discussed was the mysterious and seemingly sudden appearance of tissue proliferation as a phenomenon. What happened to cause this syndrome to emerge at this time in the nursery industry?

This question has been debated in every discussion of TP I have attended. Perhaps tissue proliferation is simply a hidden fault that some rhododendron cultivars naturally express. Maybe more growers are now looking for the syndrome in their nurseries, so general...
Micropropagation may reveal more genetic diversity than was previously observed with cutting propagation, simply because tissue culture allows nurseries to grow larger numbers of many plants that were formerly unfeasible to produce.

The awareness of tissue proliferation has increased, making it appear as though the phenomenon is increasing.

On the other hand, recent changes in the industry may have caused tissue proliferation to surface. For example, within the past decade, many rhododendron growers have shifted from field growing to container production in lightweight, soilless media. Some use accelerated growth practices to produce larger plants faster, necessitating treatment with growth retardants or shear-
ing to induce branching and more flushes per year. Shearing may significantly stress plants and change their hormonal balance, triggering tissue proliferation.

Other common cultural practices include use of chemical fertilizers (often at excessive rates), pesticides (herbicides, fungicides and insecticides) and various growth-regulating chemicals. It is possible that plants with a genetic tendency toward TP are affected by some of these practices, triggering tissue proliferation. Surely some hormonal change is involved when this happens.

The rhododendron industry in the US and throughout the world has experienced tremendous growth in recent years. Furthermore, the number of commercial cultivars has greatly increased, partly because labs have been able to produce difficult-to-root cultivars using tissue culture. Micropropagation may reveal more genetic diversity than was previously observed with cuttings propagation, simply because tissue culture allows nurseries to grow larger numbers of many plants that were formerly unfeasible to produce commercially.

If tissue culture leads to genetic or epigenetic changes that result in increased sensitivity to growth regulators, the stage would be set for some cultural

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practices to trigger tissue proliferation.

While definitive answers remain elusive, researchers are definitely making progress in defining possible causes. However, readers should be careful not to misinterpret the hypotheses and observations reported in this article as facts. Only research can prove or disprove these theories.

Several studies supported by contributed funds (administered through the Holden Arboretum) are focusing on physiological, chemical, cultural and pathological factors involved in tissue proliferation. Scientists continue to emphasize the two phases of tissue-proliferation: predisposition (during propagation) and triggering (during production).

Answers to the critical questions raised in this article will lead us close to understanding, and eliminating tissue proliferation on rhododendrons in the nursery industry.

References

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