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THE ROLE OF BIOCHEMICAL MEASUREMENTS IN EVALUATING VIGOR

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ABSTRACT- Vigor is defined as the ability of a plant to survive and grow when planted in a standard environment. Plant growth regulators (hormones) are unlikely to prove effective indicators of vigor because they are difficult to measure and their role in physiological processes is poorly understood. Abscisic acid is the only plant growth regulator that currently shows promise for assessing vigor. Enzymes and proteins are also unlikely to prove effective. Starch holds some promise. although the details of its relationship to vigor remain unknown. Sugars are probably too complicated to be useful for vigor assessment. Biochemical "fingerprints" of a number of compounds within a seedling might be effective in the future, but this technique is not presently available. Functional tests such as measurement of photosynthesis or dark respiration might be incorporated into a series of tests. Future tests will probably be more complicated, more expensive, more accurate. and more useful than current ones.

11.1 INTRODUCTION

Ever since Wakeley (1948) identified the need to examine the physiological status rather than the morphological characteristics of planting stock. foresters have been aware of the need to measure seedling vigor. Through the years a number of measurement techniques have been devised, several of which are adequately described elsewhere in these proceedings. But the fact that this workshop is being held underscores the need to develop more satisfactory methods of evaluating seedling vigor.

11.2 WHAT IS A VIGOR TEST?

For this paper, a vigor test can be defined as a method of differentiating plants that are capable of surviving and growing satisfactorily when outplanted under given conditions (standard) from those that are not. It is not necessary to define "growing satisfactorily" or "standard conditions" because these terms vary according to species or geographic location.

11.3 CHARACTERISTICS OF AN IDEAL VIGOR TEST

In practical terms, we would like to be able to measure some physical or chemical property of a plant that would indicate its ability to grow when planted in the field. If an ideal test of plant vigor could be devised, it would be

1. Rapid, yielding final results immediately

2. Simple to understand and use at all levels of operation

3. Cheap, accessible to all potential users 4.

Reliable, so that it "works every time"

5. Non-destructive, enabling tested plants to be outplanted

6. Quantitative, not subjective, allowing probability values to be assigned to results

7. Diagnostic, so that the cause of any seedling damage would be indicated.

There is an old saying that goes: "Good, cheap, fast--pick any two but you cannot have all three." This saying applies to vigor testing. To hope for a vigor test that meets all of the characteristics outlined above is not realistic; any test devised would probably compromise one or more of them. For example, a test that is cheap and simple is not likely to be highly reliable. And a test with diagnostic value will probably be expensive and is not likely to be simple. But these characteristics can serve as goals for developing new methods and as benchmarks for judging existing techniques.

11.4 APPROACH TO VIGOR TESTING

Ritchie (1984) has identified two approaches to assessing seedling quality. The first is measurement of physiological or morphological characteristics that correlate with subsequent plant growth under certain conditions. Examples are measurement of starch or electrical resistance. The second approach is measurement of the response of a seedling to a given environment or treatment. A certain period of time is usually required for the plant to respond. One example of this kind of test is the measurement of root growth potential as developed by Stone et al. (1967). Our workshop is organized around these two main approaches to assessing vigor, and we have seen several examples of each. This paper deals primarily with the correlation of physical characteristics with growth, although response to treatment or environment also plays a part in biochemical measurements and will be discussed briefly later.

11.5 BIOCHEMICAL INDICATORS OF PLANT VIGOR

11.5.1 Plant Growth Regulators (PGRs)

Plant growth regulators (PGRs), sometimes called "hormones." are chemicals produced in the plant in small quantities; they probably control its growth and development (Thimann 1977). The suggestion is often made that PGRs be measured to assess plant vigor. Whenever we observe growth phenomena that cannot be readily explained in terms of known processes, we tend to blame PGRs. However, although they are apparently involved in many physiological processes. PGRs probably control specific biochemical reactions rather than act as general growth promoters or inhibitors as we often imagine. Even the scientific community cannot agree on exactly what PGRs do or even if they actually regulate physiological processes (Weyers 1984). Because of this lack of understanding, relating PGR levels to vigor is difficult on a theoretical as well as a practical basis.

Perhaps the most striking characteristic of PGRs is their extremely low concentrations. They occur in plants in quantities almost too small to imagine, much less measure. Not only are they very difficult to measure, but also they are very easy to lose. Plants, being sensitive to PGRs, are often used to detect or "bioassay" them. In fact, the most common method for measuring PGRs has been by bioassay. Bioassays, however, can be deceiving. Plants used in bioassays usually respond similarly to a number of chemicals, many of which are found in plants. Consequently, it is never certain just which chemical the plant is detecting. Chemical assays may be more specific, but they are usually less sensitive and therefore cannot be used to measure PGRs in the small quantities of plant tissue usually available. Fortunately, recent advances in chemical assays enable them to approach the sensitivity of bioassays.

From what we now know, much of the older work based on bioassays is probably not to be trusted. On the other hand, modern PGR analysis has become so specialized that the necessary equipment is not commonly available. With these limitations in mind, let us now consider some of the known PGRs.

11.5.1.1 Auxin

Auxin or indole-3-acetic acid (IAA) has been implicated at one time or another with almost every physiological process known (Jacobs 1979). It is probably involved with cell wall extension, and it seems to be necessary for growth of certain meristems. The traditional bioassays for IAA were easy to conduct, but results were often confusing. With modern chemical procedures, we can now measure IAA easily and quickly in plant samples as small as one-tenth of a gram or even less.

Even though techniques for measuring IAA are available. are such measurements likely to tell us anything about plant vigor? Probably not. Physiological processes with which IAA is associated would not limit plant growth at the time vigor measurements are made. Indeed, current evidence indicates that IAA level is not directly related to plant vigor. Thus, the use of IAA in vigor assessment appears unlikely, at least in the near future.

11.5.1.2 Cytokinins

Four cytokinins are known to occur in plants: zeatin, zeatin riboside, isopentyneladenine, and isopentyneladenosine. Other cytokinins are undoubtedly present in plants. For example, dihydrozeatin probably occurs in Douglas-fir. and several conjugates of the known cytokinins (i.e., cytokinins combined with other compounds such as glucose) will undoubtedly be identified in this species in the near future.

Cytokinins are most notably associated with cell division, but they have also been implicated with root formation. And they may be active in the initiation of reproductive structures in conifers. Recent advances in genetic engineering have elucidated the role of cytokinins in organ differentiation. The specific enzyme that controls cytokinin synthesis in certain plant systems is known, and it may be possible to modify higher plants to produce more or less of a specific cytokinin. Even though the cytokinins are the most recently discovered of the PGRs, more is known about their operation than about that of the other PGRs. Cytokinins are easily analyzed with modern methods, but *very* few laboratories in this country are equipped to do so.

Although it is possible to measure the cytokinin content in less than a gram of tissue of Douglas-fir. such measurements will probably not be useful in assessing seedling vigor. Cytokinin levels may be correlated with dormancy during certain portions of the dormancy cycle. but vigor includes other physiological characteristics besides dormancy. Furthermore, if a measurement of dormancy status were desired, techniques other than measuring cytokinin levels would be easier to use. The extremely low quantities of cytokinins present in seedlings (typically 10^{-10} to 10^{-12} grams per gram of tissue) and the highly specialized techniques required for their measurement augur against their use in vigor measurements in the near future.

11.5.1.3 Gibberellins

Over 60 gibberellins are known to occur in plants. These substances are usually associated with shoot elongation and flowering, but they may also be involved in the dormancy cycle. There are several bioassays available for their measurement, but no single bioassay is sensitive to all of the known gibberellins. Because of their wide range in chemical properties. gibberellins are difficult to purify without losing some of them. Techniques in chemical analysis are becoming available, but at present one cannot expect to measure all of the gibberellins in a plant sample.

A crude measure of gibberellin concentration can be made by bioassay, and such measurements are useful for research purposes. The role gibberellins play in dormancy is not known. however, and how they are related to vigor has not yet been examined. Gibberellins may rise and fall with the dormancy cycle, but their measurement would provide at best an indirect and imprecise assessment of dormancy. These substances may someday be useful in understanding seedling vigor. but because of their complexity and low concentrations in plants. they will probably not be used for such assessment in the near future.

11.5.1.4 Abscisic acid

The function of abscisic acid (ABA) in plants has not been well-defined. ABA was first recognized as playing a part in the dormancy cycle. It was then implicated in the process of leaf abscission (hence the name). ABA occurs in Douglas-fir and changes in concentration throughout the year. It may be correlated with dormancy, but it is probably not the controlling factor. On the other hand, there is good evidence that it is involved in controlling the stomata.

Bell (1983) recently suggested that ABA might serve as an indicator of seedling vigor. Although no theory has been advanced to explain such a correlation, the possibility exists that ABA can be used to identify certain types of seedling damage. If so, such identification would contribute to the evaluation of seedling vigor. This hypothesis is currently being tested in detail by Professor Lavender at Oregon State University.

ABA can be measured by bioassay, but it can also be measured easily by chemical means. Of all the known PGRs, it is the most likely to be a direct indicator of plant vigor.

11.5.1.5 Other PGRs

Ethylene, the only PGR occurring as a gas, is known to function in fruit maturation and cell elongation. It is produced by seedlings during storage (Johnson and Stumpff 1984) and is easy to measure with a gas chromatograph. There is little likelihood that ethylene could be used to indicate vigor, however. Being a gas, ethylene disappears rapidly, and its concentration is difficult to assess. Furthermore, its

Undoubtedly, other PGRs are yet to be discovered. When they are, we should be prepared to investigate their role in physiological processes and determine their correlation with seedling vigor.

11.5.2 <u>The Future of PGRs in Vigor Evaluation</u>

PGRs are not useful in vigor measurements at present, but they may prove so in the future. As previously mentioned, ABA is now being assessed for use as a portion of a vigor test. New techniques such as immunoaffinity purification (the use of antibodies directed against specific PGRs) make possible the measurement of PGRs at extremely low concentrations. Such measurements, however, require sophisticated, expensive equipment. Those interested in seedling vigor will not be measuring PGRs extensively unless new correlations between vigor and PGR concentration are discovered.

11.5.3 <u>Enzyme Systems and Proteins</u>

Enzymes and proteins are like PGRs in that they are involved in specific biochemical reactions. Certain enzymes might serve as assays of a plant's ability to carry out a given metabolic process, or the occurrence of a certain protein might signal the ability of the plant to activate a certain enzyme. And the presence of certain classes of enzymes such as polyphenoloxidases might indicate damage to plant cells or membranes.

Enzymes are easier to measure than PGRs, but their assessment requires specialized laboratory equipment such as low-temperature workrooms. They may be useful in assessing certain discrete portions of a plant's physiological apparatus, but a single enzyme or even a few enzymes are incapable of providing an integrated assessment of a plant's physiological status. However, like ABA, enzymes might be used as part of a broad-spectrum assay of plant vigor.

11.5.4 <u>Other Compounds</u>

11.5.4.1 Starch

Krueger and Trappe (1967) looked at starch as an indicator of dormancy and found that it does not vary enough throughout the year to permit differentiation between dormant and non-dormant plants. Furthermore, the variation in starch concentration among plants is fairly high. But it seems likely that "food reserves" play a role in a plant's ability to grow when outplanted. Even if starch is just one component of vigor, it may turn out to be an important component and worth measuring, as suggested earlier by John Marshall in this workshop. If such is the case, starch measurements can be simplified. Because starch occurs in fairly high concentrations in plants, it could eventually be measured by field personnel as part of a vigor test.

11.5.4.2 Sugars

Although sugars, like starch, seem to be a "food reserve" in plant roots and stems, there are at present no convincing data to suggest that seedling vigor can be predicted by sugar concentrations within the plant. Sugars are difficult to measure. Furthermore, the definition of "food reserves" is ambiguous. Some sugars clearly serve as metabolic substrate, while others may be partially structural in nature. Plants metabolize different sugars at different rates, and some sugars are not utilized even when the plant is "starved." Thus, sugar concentrations will probably not be used as predictors of seedling vigor in the foreseeable future.

11.5.4.3 Other plant compounds

A case could be made for measuring many compounds other than those mentioned above. The presence of amino acids. the building blocks for proteins. may indicate that plants are able to synthesize proteins rapidly. The presence of certain phenols or polyphenols may indicate specific kinds of damage. Fats, fatty acids, and waxes may be correlated with the ability to grow rapidly or may indicate damage. The presence of terpenes may indicate a metabolic malfunction. Although there are thousands of compounds that might be measured. their use to indicate vigor is highly speculative at this time. We simply do not know enough about the physiological processes involved in what we term "vigor" to know which compounds are correlated with it and which are not.

11.5.5 <u>Biochemical</u> <u>"Fingerprints"</u>

Another approach is to measure many compounds simultaneously and thereby obtain a biochemical "fingerprint" of a seedling. Sufficient data could be generated with a plant sample as small as 1 or 2 grams. These data could be processed on a computer and used to assign seedlings to vigor categories. Such a system would require expensive equipment at first. but the process would become simpler as more data were accumulated. Only those compounds that vary with physiological quality would be analyzed.

One disadvantage of this approach is the large amount of data that would have to be collected initially in order to calibrate the readings. An advantage is the possibility of identifying metabolic processes critical to vigor evaluation. By concentrating on those processes, we could devise a simplified measure of vigor. Thus, this technique holds promise for the future.

11.5.6 <u>Functional Tests</u>

Tests of the functioning of a seedling or its organs might be useful in assessing its vigor. especially if such measurements were conducted in conjunction with other tests. Among the functions that might be tested are photosynthesis. dark respiration, and metabolic rate as indicated by plant temperature.

11.5.6.1 Photosynthesis

Photosynthesis is a critical component of a plant's ability to grow rapidly. One might therefore suppose that a simple measurement of the rate of net photosynthesis would indicate a plant's ability to grow quickly. Such a theory is untenable, however. because the photosynthetic system is relatively immune to many of the hazards (such as chemical pollutants or frost) that can injure or even kill a plant. Thus, although a low rate of photosynthesis may indicate low vigor, a high rate does not necessarily indicate strong vigor: the photosynthetic apparatus can remain functional even when other systems have failed.

11.5.6.2 Dark Respiration

Another function that might be measured is dark respiration--the seedling's ability to metabolize substrate (food) in the dark and thus produce carbon dioxide, water, and energy. A test of this function would be subject to the same problems as the one for photosynthesis and for the same reasons: respiration could continue even when other physiological processes failed. Thus, the enzymes involved in the Krebs cycle could be intact so that energy was being produced, but if other metabolic functions were disabled. the seedling would soon die.

11.5.6.3 Temperature

Plant temperature, a subject treated elsewhere in these proceedings, could serve as an indicator of metabolic rate, or it could indicate malfunction of the plant's hydraulic system. Temperature measurements may eventually be used as part of a battery of vigor tests.

11.6 FUTURE TRENDS

Current developments in vigor evaluation suggest the following trends for the future:

1. More tests to evaluate specific physiological functions. Such tests form an essential foundation upon which more comprehensive tests can be devised.

2. A battery of tests rather than a single test.

3. Improved statistical methods for evaluating vigor data, including identification of the cause of damage in some situations.

4. More rapid vigor tests.

5. Wider use of vigor tests and greater acceptance of test results. Nurseries can expect foresters to rely heavily on vigor tests as a cross-check on nursery management.

6. More sophisticated testing methods. Although more complicated tests mean that fewer people will be able to conduct them, the application of advanced technology to vigor evaluation is necessary if progress is to be made. The corollary to this trend is that vigor tests will be more expensive.

REFERENCES

Bell, T. E. 1983. A procedure for the purification and quantitation of abscisic acid and its application to a study of abscisic acid concentration in needles of cold stored Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings. M.S. thesis. Oregon State University, Corvallis. 80 p.

Jacobs. W. P. 1979. Plant hormones and plant development. Cambridge University Press. Cambridge. Mass. 339 p.

Johnson. J. D., and N. J. Stumpff. 1984. The influence of ethylene on root growth capacity and field performance of loblolly pine. Page 190 in Proceedings of the 8th North American Forest Biology Workshop, Logan, Utah (R. M. Lonner, ed.). July 30-Aug. 1. 1984.

Krueger, K. W., and J. M. Trappe. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. Forest Science 13:192-202.

Ritchie. G. A. 1984. Assessing seedling quality. Pages 243-259 in Production of bareroot seedlings (M. L. Duryea and T. D. Landis. eds.). Martinus Nijhoff/Dr W. Junk Publishers, The Hague/Boston/Lancaster. for Forest Research Laboratory, Oregon State University. Corvallis.

Stone. E. C., J. L. Jenkinson, and S. L. Krugman. 1967. Root-regenerating potential of Douglas-fir seedlings lifted at different times of the year. Forest Science 8(3):288-297.

Thimann, K. V. 1977. Hormone action in the whole life of plants. University of Massachusetts Press, Amherst. 448 p.

Wakeley. P. C. 1948. Physiological grades of southern pine nursery stock. Pages 311-322 in Proceedings of the Society of American Foresters 1948.

Weyers. J. 1984. Do plants really have hormones? New Scientist 17 May:9-13.