AFTER THE FIRST FORTY, WHAT IS NEXT?

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It is a tremendous pleasure to be back in Cleveland, and to see many friends, including several charter members. What I'd like to do this morning is to take us back through the first 40 years and trace some of the major developments that took place in the area of plant propagation and then finish up with a glimpse of the future.

The big challenge for propagators—in particular, propagation by cuttings—is to control moisture loss from the cuttings during the period of rooting. One of the early approaches to controlling water loss from cuttings was the bell jar. Although it works very well, you have conflicting demands of trying to keep the moisture contained, but at the same time not trapping too much heat from sunlight. As a result, you have to work with shading to keep the temperature under control, but the reduction of light reaching the cuttings reduces photosynthesis, which is essential for the production of carbohydrates and other substances used in root initiation and growth. This is a "Catch 22" situation if there ever was one. The bell jar technology was scaled up to the commercial level in the form of the Wardian case. Although more efficient in terms of handling large numbers of cuttings, the same problems were there. That is, the need to retain moisture and the liberal use of shade to prevent overheating. This combination of requirements restricted the range of plant materials that could be propagated by cuttings. During the evolution of the Plant Propagators Society, plastic films were introduced facilitating cutting propagation. Although plastic film gave us some new conveniences, we were still battling the basic problem of reducing moisture loss and maintaining reasonable temperatures through the use of shade.

There were some novel approaches that people used to try to address this challenge. Guy Neering in New Jersey developed the "Neering Frame." Neering built a frame which was exposed to the north with a large reflector on the south side. By this design, the cuttings received reflected light which provided reasonable light intensity without the heat. An even more novel approach was Leslie Hancock's operation in Canada, called the "burlap cloud" method of propagation. After the cuttings were stuck in outdoor frames, burlap was stretched over the cuttings. The burlap was frequently sprayed with water during the day. The evaporating moisture from the burlap kept the humidity high which reduced moisture loss from the cuttings, the process of evaporation

provided cooling which also reduced moisture loss from the cuttings, and burlap also provided shade. At the end of the day, Hancock removed the burlap and the cuttings were allowed to "breathe."

During this period, extensive use of root promoting substances such as indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) emerged. I remember one preparation called "Chloromone" that created a lot of interest. The manufacturer said he used the "two by four" method of extracting the preparation from young alfalfa shoots. Our tests indicated that it contained a high concentration of a substance that looked very much like NAA. However, the combination of NAA with a plant extract may have had a synergistic effect.

But even with root promoting substances, propagators were continuing to seek better ways to root cuttings and to extend the range of materials propagated. A number of researchers found that by adding humidity to the air, such as by the Binks humidifying system, the space around the cuttings could be larger and higher light intensities could be used. The space in the bell jar or Wardian case was kept small because it was desirable to have a small air volume which took less moisture to build high levels of humidity. This was important because the source of the humidity was moisture evaporating from the cuttings and the medium until an equilibrium was reached inside and outside the cutting. The disadvantage was that the small space would also heat up quickly when exposed to direct sunlight. By adding moisture from an external source, such as the Binks system, less moisture was lost from the cuttings and they remained turgid, and the space around the cuttings did not have to be confined. Cuttings could also be taken earlier (softer) after the new growth in the spring. Cell division is more active in the soft or less mature cuttings, and they are easier to root.

Investigators then discovered that even better results could be obtained if the cuttings were sprayed with a fine mist. When a film of water was maintained directly on the leaves of the cutting, there was no longer a need to confine the air surrounding the cuttings. Cuttings could be propagated in full light intensity and even out of doors. Originally, oil burner nozzles were used to provide the mist. But they frequently clogged and self-cleaning nozzles were introduced, followed by impact nozzles. The latter design, in which a fine stream of water is directed against a flat surface, became the industry standard.

Refinements on the mist system of plant propagation continued to be made. We were able to show that an intermittent form of mist was better than constant mist. The advantages were that when leaf temperatures were not lowered below optimum, there was less leaching of nutrients from the cuttings, and the medium was better

aerated and less likely to be water logged. Other advantages were that less water was used and drainage problems were reduced. A variety of control systems that provided intermittent mist during daylight hours evolved, including timers, and "electronic leaves" and similar devices based on the principle of evaporation. As the film of water from the "leaf" evaporated, the circuit was broken and a relay turned the mist on to reestablish the water film. Once the film was reestablished, the mist was shut off. Other systems used the weight of the water film to "trip" an on/off switch. The advantage of the latter systems of controlling the mist applications was that they were "weather conscious" and applied more mist during bright sunny weather and less on cloudy days.

Mist propagation represents one of the major breakthroughs that occurred in the formative years of the Society. We asked the question, why was it that we could get such high levels of rooting under mist compared to the conventional approaches to rooting softwood cuttings? In our work we measured the temperature, the rate of photosynthesis, the rate of respiration, and the carbohydrate content of cuttings under mist and compared them to cuttings in the standard Wardian or grafting case. As you would expect, the leaf temperature under mist was much lower, an average of 75 ° F, as compared to 86 ° F in the Wardian case. Full light intensity could be used with cuttings under mist. In the greenhouse at Ithaca, New York, light intensity averaged 7000 ft-c in the summertime. In the Wardian case, with the necessary shade, the light intensity was 240 ft-c. The combination of higher light intensity and lower leaf temperature (and a corresponding lower respiration rate), resulted in net accumulation of carbohydrates in the cuttings under mist of 138 mg per cuttings. There was little or no increase of carbohydrates in the cuttings in the Wardian case. Dogwood cuttings (*Cornus florida* 'Rubra') were used in our experiment. The rooting percentage under mist was 96% compared to 22% in the Wardian case. Because the environmental conditions under mist provided a net gain in carbohydrate production, it was possible to use very immature or soft cuttings. Even though soft cuttings may have a very low level of carbohydrate reserves at the time of taking the cuttings, the mist conditions provided the environment in which the carbohydrates and other substances essential for rooting could be synthesized and used in root initiation and growth.

In addition to the internal factors which appear to help explain the success experienced with mist propagation, there was much less disease experienced under mist conditions. One explanation was the fact that the cuttings were turgid and able to manufacture carbohydrates, and the cells were less susceptible to invasion by pathogens. Another factor is the fact that the intermittent

application of mist may have washed fungal spores or bacteria from the leaf surfaces. Also, the presence of a film of water and cool temperatures may have inhibited the germination of some spores. In contrast, the environment of the Wardian case with high humidity and high temperatures was almost ideal for the development of molds.

Propagators continued to experiment. One of the leaders of innovation was Harvey Templeton. Harvey developed the phytotector method of plant propagation. He combined some of the advantages of mist and some of the older techniques of using a polyethylene tent with some shade. The cuttings were rooted directly in the soil. The combination of mist and a plastic tent resulted in the cutting tissue being kept turgid by the application of the mist, with reasonably high temperatures that stimulated root initiation.

Another innovator is Bruce Briggs from the State of Washington and a frequent attender of the Eastern Region meetings. Bruce decided he would do away with the rooting medium and used styrofoam sheets to support the cuttings with the base in the dark, but in the air rather than in a rooting medium. The concept of rooting and growing plants with the rooting in the air continues to be explored, including developing systems of growing plants in space stations. The examples I have just given are an indication of the tremendous amount of creative activity that was shared at these early meetings. Everyone left with a whole new set of ideas to try in their own operations. We went from a group of professionals who guarded our practices and actually padlocked our propagation units, to an open sharing of ideas. The result was that almost everyone gained new ideas and tremendous progress was made in the field of plant propagation.

Although mist propagation was a major advance in the field, there were still many challenging questions to be answered. For example, why were there a lot of plants which just could not be propagated by cuttings, even when mist propagation was used? We began asking questions about what was going on inside of cuttings that made some easy to root and others difficult. We used *Hedera helix* as our experimental material. You are most familiar with it in its juvenile form as a very easy to root ground cover. The mature form, which is found on the tops of walls or trees, is very difficult to root. When you do get a cutting of the mature form to root, it will grow into an upright shrub. Juvenile cuttings form roots very well without any root promoting substance, but they also show a significant increase in the number of roots when NAA is applied. In contrast, cuttings of the mature forms root poorly and also show little response to the NAA treatment. This is one of the common observations in propagation—the more difficult to root a cutting

is, the less its response will be to auxin-type root promoting substances, such as IBA and NAA. So the question is, why? What is going on or not going on within the cutting to make the big difference in rooting ability? One important observation to make here, and I will refer to it later in my presentation, is that the juvenile and mature forms of *Hedera* are found on the same plant. Therefore, we are working with the same genetic material, even though there is a tremendous difference in the rooting ability of the two forms. Something within the ivy plant is turning some genes on to express juvenility—horizontal growth, lobed leaves, anthocyanin production and easy rooting—or to express maturity—upright growth, entire leaves, a lack of anthocyanin, the ability to flower and being difficult to root. This makes *Hedera* a particularly valuable plant in which to study root initiation. There are great physiological differences between the two growth forms, including rooting ability, but the genetic makeup is the same.

Other investigators studying difficult to root grape cuttings found that if hardwood grape cuttings were soaked in water for a period of 24 hours, the cuttings became easier to root. They suggested that an inhibitor had been leached out of the cuttings. To support their hypothesis, the researchers applied the leachate to easy to root grape cuttings and they became more difficult to root. Another approach is to look for evidence of root promoting substances. For example, we grafted scions of juvenile ivy on cuttings of mature ivy and found that the presence of the juvenile tissue enhanced the rooting response of the mature cutting. So now we have evidence of the presence of both root inhibiting and root promoting substances.

In the 1940's and 1950's, procedures had been developed to extract and biologically assay growth promoting and inhibiting substances in plants. So we applied these techniques to juvenile and mature *Hedera* tissue. The tissue was lyophilized (freeze dried) and extracted with ethanol. The extract was concentrated and spotted on a paper chromatogram—a strip of filter paper. The paper was dipped into a solvent which, as it ascended, separated the mixture of substances into individual components. The paper was dried and cut into sections for biological assay to determine if growth promoting or growth inhibiting substances were present. The biological assay was cylinders of tissue from oat coleoptiles whose growth in length was proportional to the amount of growth promoting substances present. Since there was some elongation of the coleoptile sections even without growth promoting substances, it was possible to also measure the presence of inhibitors. We used the biological assays on dormant and growing *Hedera* tissue.

We found that the *Hedera* tissue extracts contained both growth promoting and growth inhibiting substances. Growing tissue

contained more growth promoting and less growth inhibiting substances, as would be expected. However, although there were slightly higher levels of growth promoting substances in the juvenile tissue, the differential from the mature tissue did not seem substantial enough to account for the great difference in rooting ability. Also, there was not enough difference in the amount of inhibitors present to account for the difference in rooting. In fact, in growing *Hedera* tissue, the inhibitor content of the juvenile tissue was slightly higher than that of the mature tissue.

We then decided on another approach. We developed a new biological assay which was based on root initiation rather than cell elongation. In retrospect, this makes sense since we are interested in substances which regulate root initiation. Our bioassay was based on the rooting of cuttings made from mung bean seedlings. The mung bean germinates quickly, and the cuttings were small enough that you could place ten cuttings in a shell vial with the chromatogram section much in the way we had done with the oat coleoptile test. The cuttings rooted in about six days. The number of roots on each cutting was counted, and an average for the ten cuttings in each vial was determined. The results were expressed in the number of roots per chromatogram section. Len Stoltz, Charlie Heuser, and Dale Herman, all here today as members of the Eastern Region, and who worked on the root initiation studies as graduate students, had a lot of experience in counting roots on mung bean cuttings.

Using the mung bean bioassay, we did find differences between the extracts from juvenile and mature *Hedera* tissues. There were four major peaks of activity in the juvenile tissue with a couple of smaller peaks in the mature tissue. Since the substances appear to react synergistically with indoleacetic acid (IAA), we called these peaks of activity "rooting cofactors." Using the data from the *Hedera* experiments, we developed a working hypothesis about the rooting ability of cuttings. If a cutting was easy to root, then all four rooting cofactors would be present, along with an adequate supply of IAA, carbohydrate, and nitrogenous substances. If a cutting was difficult to root, it may lack enough IAA and this could be supplied with a synthetic root promoter such as IBA or NAA. But as I have pointed out, the more difficult to root cuttings do not respond to IBA or NAA. In this case, one or more of the cofactors may be missing and the degree of difficulty would be an expression of how many of the cofactors were not present in adequate levels.

One of the rooting cofactors seems to be a phenolic compound. Phenolic compounds are active in the mung bean bioassay and work synergistically with IAA. We also found that the structure of the phenolic compounds determined their ability to stimulate root initiation. Root initiation was stimulated only when the hydroxyl

groups on the benzene ring were next to each other in what is known as the ortho position.

While studies on the physiology of rooting were going on, there were also a lot of accomplishments being made in the field of plant tissue culture, which is another major breakthrough that benefitted the field of plant propagation. Initially, propagators cultured the growing points of plants to produce virus free plants. In using this technique for orchids, propagators observed that the growing points proliferated and produced what they called protocorms, each of which produced an individual plant. Plant scientists then found that it was possible to generate plants from callus tissue. Subsequently, other investigators found that the callus could be separated into single cells, each of which could produce a whole plant. When Sid Waxman and I were graduate students at Cornell in the early 1950s, the famous English plant physiologist, F.C. Steward, introduced the term "totipotency" to describe the fact that each cell in a plant contained all the genetic information that was required to form another whole plant. We now know that the information is included in the DNA located primarily in the nucleus. Another variation of this theme was discovered by J.P. Nitsch, a member of the Society and the major professor of Sid Waxman and myself. Not only could you develop a plant from a cell, you could also do it with a pollen grain. But here there was a difference. Since the pollen grain is a product of meiosis, it contains only one set of chromosomes rather than two sets. Therefore, the plants generated from pollen grains were haploid. But they could be treated with colchicine which would double the chromosomes and return the plant to the diploid status. The resulting plant would be homozygous, an advantage for plant breeders looking for the expression of recessive traits.

While horticulturists were doing all this good work with tissue cultures, scientists in medical schools were looking at the chemical structure of the chromosomes and the genes located on them. They discovered deoxyribonucleic acid (DNA) and that they were able to snip out pieces of DNA from the chromosome and in this way isolate a single gene. They found that it was also possible to reinsert that gene into a plasmid, a circular piece of DNA. The plasmid, with the new gene, could be put into a cell and, using the horticultural techniques of tissue culture, regenerated back into a whole plant. So we have seen during the past forty years the whole concept of genetic engineering and molecular biology evolve. It is now possible to take a single gene from one organism and put it into another.

A couple of examples will give you an idea of what we can expect in the not too distant future from this new technology. Scientists were looking for resistance to the broad spectrum herbicide, Roundup. They found resistance by growing bacteria in a solution

of Roundup. A few survived, and the survivors differed from the non-survivors by a single gene. The gene was isolated from the resistant bacteria and, using a plasmid, it was inserted into a plant. The plant now had resistance to Roundup and that resistance was passed on to future generations of the plant. This showed that the new information was incorporated into the genetic information in the plant.

A similar approach was used to develop insect resistance. The tomato hornworm can defoliate a tomato plant overnight. As you may know, you can use biological control for tomato hornworm. The product is called Dipel, and it is a culture of bacteria that produces a highly specific protein that is toxic only to the tomato hornworm. The disadvantage to using Dipel is that you have to reapply it frequently because the active material is destroyed by sunlight. It turns out that the toxic protein is produced by a single gene in the bacteria. That gene has been removed from the bacteria and inserted into tomato plants. The tomato plants can now produce their own protein which is specifically toxic for the tomato hornworm and is safe over humans and even other insects. Given the public concern for impact of agricultural chemicals on the environment, this approach to speed the development of genetic resistance to insect pests has great economic and environmental potential.

In addition to adding resistance to herbicides and insects, it is also possible to regulate growth and development processes such as ripening. The genes regulating the process of fruit ripening are being identified. In one case the gene has been removed, turned around, and reinserted into the plant. The result is that the gene produces less of the substance involved in ripening and the ripening process is slowed. The research is being conducted with tomatoes. Conceivably, it will be possible to pick a tomato when it is "vine ripened" and by slowing the balance of the ripening process, it can be shipped to the consumer firm rather than in the form of a puree.

Now, what does the future hold? It turns out that there are many hundreds of thousands of genes in every plant and scientists are beginning to map them. Some genes are called housekeeping genes. That is, they are in every part of the plant and they are turned on all the time. They are being expressed. There are genes in other parts of the plant that only get turned on at certain times; so there are genes that are turned on when flower initiation takes place, or turned on for pollen formation. In fact, one of the latest accomplishments is the identification of the gene that regulates pollen formation. By regulating this gene, it has been possible to turn that gene off and make male sterile plants. This is a great advantage when producing hybrids where you want to avoid self pollination.

Well, what has all this to do with plant propagation? Let us return to our story of juvenility in *Hedera*. As I mentioned earlier, we have an example in *Hedera* in which the same genetic material behaves very differently at different stages of growth, including the ability to root. What is happening is that different genes are being turned on or off to produce the juvenile and mature stages of growth. For the future then, our challenge is to identify those genes that are turned on to provide the juvenile stage, and more specifically the genes that are turned on to give easy rooting. By knowing the genes, it will be possible to also identify the specific substances that are involved in the process of root initiation and growth.

We have been through a tremendously exciting 40 years with the Society. We have seen the discovery and use of plant growth substances and their application in root promotion, substances like IBA, NAA and others; we have seen mist propagation expand the range of plant material propagated from cuttings as well as increasing the efficiency of propagation; we have gotten a better understanding of the complex nature of root initiation; we have watched the development of plant tissue culture and the growth of plants from single cells; and finally, we have entered the world of molecular biology or genetic engineering in which we can move genetic information, one gene at a time, among totally unrelated organisms. It has been an extraordinary 40 years, but we are really just at the beginning of the biological revolution. We now have the tools to ask questions and get answers in a way we have never been able to do before. Even the question "What makes a cutting difficult to root?" should be finally answered in the next forty years!

PETER ORUM: It is my pleasure to next introduce our program chairman for this meeting. He is a hardworking plant propagator, and a very good friend, Clayton Fuller.

CLAYTON FULLER: Thank you, Peter. I am very pleased to be here with you and I would like to thank all the speakers and moderators for their willingness to seek and share. Our moderator for this morning session is Robert E. Schutzki.