GROWTH REGULATION: THEN, NOW AND HENCE

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I think plant propagators are among the most fortunate of men. In the first place, the work that they do doesn't add to the world's burden of pollution and misery; it works in the opposite direction.

More importantly, however, plant propagators work in the presence of the great mysteries of biology because certain aspects of plant propagation involve some of the most mysterious and remarkable phenomena in the whole world of biology. Of these we know very little. I am not referring to the processes of budding and grafting, because when you take a piece of a plant and apply it to another plant it is essentially tissue culture, except that instead of hiring a technician to make up the nutrient medium you hire a stock plant to do it for you. On the other hand, seed germination is an extraordinarily complex and beguiling phenomenon. Some seeds need only water and they germinate, but some seeds, as you know, go through a period of dormancy which is a term behind which we hide our almost total ignorance of the phenomenon. Such seeds require some sort of a triggering mechanism which in itself is mysterious and can be different in different seeds.

Some years ago I studied one of the seed types which requires light, the black-seeded lettuces like the variety 'Grand Rapids.' They require fantastically small amounts of light. If you soak the seeds and keep them in the dark about 30 seconds exposure to an ordinary red bulb at a distance of several feet is enough to give 100% germination. Without this treatment germination is about 5%. The seed are so sensitive that some lots which may germinate to the extent of 20% or more in the dark do so (I think) because on the day they were harvested it was either moist or raining and they received a small amount of moisture and light during this time; thus they were able to get a small fraction of the necessary light stimulation and give a higher percentage germination. The remarkable thing about this process is that if instead of exposing them to red light you dip them briefly in gibberellic acid solution it has exactly the same effect as light and you get 100% germination in total darkness; that is, you do not need any red light. We thought, perhaps naively, that this meant that red light stimulated the plant to produce gibberellic acid. Upon extracting the seed, however, it turned out that they produce no more gibberellic acid than normal and that while both light and gibberellic acid act on the germination process, they evidently act on different stages of it.

Gibberellic acid in seeds stimulates the production of enzymes which hydrolyze the endosperm. By breaking down the endosperm tissue it makes food available to the embryo and thus stimulates germination. One can do the same thing by taking a very fine needle and injecting a small amount of enzyme into the seed; this starts the processes and you get very good germination. This proved what we were looking for,namely that the triggering phenomenon which starts germination is the making of soluble material available to the embryo. Since this can be done then by the application of either gibberellic acid or red light, it follows that the effect of giving the seed light is, in principle, the same as that of giving it gibberellic acid in that it enables the embryo to push its way through the outside coating. If with a very fine needle you prick or break the seed coat in exactly the right place in total darkness you can get nearly 100% germination.

There are, however, a number of seeds which are just the opposite of lettuce in that they are inhibited by light. We studied several of these and curiously enough found the same thing, namely that gibberellic acid stimulates them to germinate; here gibberellic acid acts like darkness rather than like light. Once again we found that if you make little holes in them at just the right places, they will germinate in light without any other treatment. So in all these cases, we have to deal with this mysterious triggering reaction whereby either soluble material is made available to the embryo, increasing the tendency of water to enter, or else the seed coat is softened by enzymes so that the embryo can find its way out of the seed. It is these first reactions which are the most difficult to study.

The rooting of cuttings, I believe, is even more obscure because here we must deal with something whose very basis we have no way of understanding. Cells within the stem are perfectly happy to be stem cells — if they are near the epidermis they would make chloroplasts and turn green, if they are near the cambium they might participate in the formation of extra cortex — but in any case they are stem cells and they know that it is their job to elongate with the stem and be a part of the cortex. But then along comes some plant propagator who cuts off this stem and gives it some stimulus, whatever it may be, and these cells entirely change their whole habit of life and instead of being stem cells they no longer know how to make chloroplasts, they don't elongate with the stem, instead they divide like mad, perpendicular to the axis. They then grow out at a characteristic angle to the stem instead of being aligned with the long axis of the stem. They now have their own characteristic angle at which they grow out or even straight down, and they are now root cells instead of stem cells. Their genetics, of course, is unchanged; they have the same chromosomes they have always had, but there has to be some triggering reaction to set off this series of events. They may be set off either by wounding, by treatment with auxin, or by some other stimulus which acts as the triggering mechanism. We do know something about the triggering reactions but only in an experimental way.

The other day I was visiting a nurseryman in Santa Cruz and I noticed he was dipping cuttings into IBA (indolebutyric acid) to stimulate rooting. I asked him if he knew about the IBA and its basis for action; he said he did know a little bit about it, but not very much, and so it occurred to me that perhaps I should take this opportunity to share memories with you in regard to the work on hormone control of rooting which took place so long ago.

The scientific study began with a very energetic Dutchman, van der Lek, who studied the rooting of cuttings of grapes, willows, and some berries. Although he did not discover very much, he made one very striking observation, and that was that if the cuttings had an active leaf or bud, roots would almost invariably form directly beneath this active leaf or bud and on the same side. He deduced from this that some stimulus must come from the bud or leaf to make the stem cells develop roots. This phenomenon is not dependent upon light because similar experiments were run in the dark using willow cuttings and the same results were obtained. Two deductions were made by van der Lek: *first that the stimulus moves from the bud or young leaf to the* stem tissue to make it form roots, and secondly that the stimulus moves downward. That is, roots are never formed above the leaf or bud but always directly below it. It was this second deduction which put us on to the right track because one often sees the same phenomenon in trees, and particularly in plants in the tropics; for example, the pandanus or screw-pine puts out large numbers of prop-roots much as does corn. All of these are located towards the base and they grow out at a characteristic angle to the stem; this angle does not change very much, all of the roots coming out at about the same angle. In the *Ficus* group it is quite striking to observe the large number of roots which grow out from the base of the tree; these increase in number as the trees get older and in some *Ficus* species they grow out from the lower side of branches and form a forest of little pillars; people often congregate for meetings under these large *Ficus* trees.

It so happened that Dr. F. W. Went, who had spent a lot of time in the tropics, came to join us at Cal Tech. While he was in Java he had been working on this same phenomenon using *Acalypha*, an herbaceous plant, and he found that cuttings of *Acalypha* behaved much as the grape cuttings of van der Lek in that they formed roots in an obvious relationship to the growing buds. While still in Java he made extracts of rice polishings, which are rich in thiamine, and he tested these and found that they promoted the formation of roots on his *Acalypha* cuttings. When he came to Cal Tech, I had been working on the postulated auxins which were supposed to be the growth substances of plants, and we had derived from *Rhizopus*, a fungus, some very concentrated, nearly pure extracts of these auxins. At this point we decided that we would make a parallel study of the postulated rooting substance. The thing which was very interesting was that the auxins had the peculiar character of moving morphologically downward in the plant, that is, they move from the upper to the lower tissues irrespective of the placement of the plant in space. If the plant is placed upside-down the auxin still moves from the tip toward the base, indicating that it is a morphological movement, not a gravitational one. All of the work on the rooting substance indicated that it moved in the same way and so we thought that there might be some parallelism with the auxins. We soon found that our most purified extracts of auxins also caused rooting. We worked up a very simple little assay using pea seedlings because they root very quickly. With this bioassay system we could show that the auxins did make the pea seedlings root. Finally we isolated a pure crystalline material and found that it promoted both growth and rooting (i.e. it was both an auxin and a root forming substance), and subsequently we identified it as indoleacetic acid. We next synthesized this compound and found that it also caused rooting. So we knew that it was not a question of a contaminant or an impurity but that this one substance had two quite different functions. This seemed difficult to believe at the time, that the same compound could act to promote elongation of stem cells and also to turn stem cells into root cells and finally roots.

It so happened that I obtained some synthetic compounds other than indoleacetic acid which were near enough related chemically that I thought it would be interesting to see if they functioned as did the indoleacetic acid. As it turned out, they did indeed induce rooting showing that it was not a property of one special compound. About this time Dr. Went visited the Boyce Thompson Institute at Yonkers, New York, and told them about our studies and I sent them a small sample of indoleacetic acid. Subsequently they synthesized and patented some related substances, one of which, indolebutyric acid, has been very widely used in rooting ever since.

We made a series of rooting studies and it soon became obvious that there were a number of plants which would not form roots in response to auxin. These plants were of special interest because we deduced that perhaps rooting required more than just one substance. We knew the process required sugar since with etiolated cuttings one must supply sugar in order to obtain roots, but we also felt that other materials may be needed, and that if we studied these plants which would not root in response to auxin by supplying them with optimum amounts of auxin we might find out what else was controlling rooting.

As is so often the case in research, it did not turn out that way. But we did find some rather curious things. One of these was that among the most difficult to root plants, such as the spruces and pines, it always turned out that the dwarf forms were able to root though the normally growing plants were not. We had a rather large selection of horticultural varieties of these plants and there was never an exception to this rule; in general the ability to root varies inversely with the rate of growth of these plants. We also found a plantation of spruces which had been overgrown by hardwoods so that the spruces had become dwarfed for a number of years. The hardwoods had recently been cut down and we found that cuttings from these spruce plants, though they were not dwarf forms, but simply normal trees which had been physiologically dwarfed, would root readily, just as readily as regular dwarfs.

The problem of what the compound is in such trees which is necessary for rooting, has never been solved. But there is now a more specific problem in that if the dwarf forms can readily root, perhaps there is an inhibitor in the normal forms which prevents rooting. I was interested to learn that gibberellic acid applied to cuttings tends to inhibit rooting. Gibberellin does promote the rapid stem elongation of a number of plants and it is possible that a substance of this sort is involved; that is, that the material necessary for rapid growth acts as an inhibitor of root formation. I think, however, that it is probably not gibberellin, because most of the conifers do not respond appreciably to applied gibberellin, but they nevertheless may have some other compound which promotes their rapid stem elongation and yet inhibits their root formation. An interesting side aspect of this is that in normal, rapidly growing trees there are always some slow growing shoots. In spruces, for instance, the lower branches have often almost stopped elongating, and these can often be made to root. This is curious because when one looks at the tree one sees that these branches are heavily overshadowed, bear very few needles and generally do not look very healthy, yet if there is any rooting of cuttings it is quite apt to be these branches which will root. A classical example of this is shown by the short shoots of pine, which do not elongate very much at all. We found that the short shoots, in some pine species at least, will root fairly well while normal shoots will not.

One of the strange characteristics of the rooting of evergreens is their extreme slowness to root. We made some experiments with *Abies alba* (Syn. A. *pectinata*) in which the cuttings showed no rooting for nine months, even though they were treated with auxin and were supplied bottom heat. After nine months those cuttings which were treated with auxin began to put out roots; the controls, however, did not. This is one of the most striking examples I know of, of a physiological effect which had obviously been initiated but shows nothing of its effects for a very long time.

Incidentally, I came across a record for the opposite response; that is - instantaneous rooting - when I was on holiday in Italy a while ago. I don't know how many of you may be familiar with the story, but there was a young lady named Daphne who attracted the attention of Apollo, the son of Zeus. Apollo was a great girl-chaser in his day and he was much taken by Daphne. Daphne did not, apparently, care for Apollo and she fled into the mountains. Apollo chased after her and when he was about to catch her, Daphne called upon her father to save her. Daphne was the daughter of the River-God, Peneus, in Thessaly. Peneus, hearing his daughter's plea and not wishing Apollo to have her, turned her into a laurel tree. In the art gallery in Rome there is a statue showing the moment at which Daphne is being changed into a laurel tree, with the young Apollo about to catch her, and you can see her toes already rooting. This legend may be the first historical reference to vegetative propagation. This further suggests to me that since plants are so intelligent (that is, we hear about their responding to music and other things) that perhaps propagators should have a little statue of Daphne in each of their greenhouses and show it to the cuttings so that they would know what is expected of them.

I am supposed to talk not only about the past but also about the future and, of course, it is not easy to say much about the future of propagation, any more than it is easy to say anything about the future of any human activity. Also my own direction of work has gone almost in the opposite direction, in that I am getting interested in the area of senescence, and it turns out that senescence is also a mechanism which is under at least partial hormonal control. It is rather easy to study, because if one removes leaves from young plants and puts them in the dark they very rapidly senesce and turn quite yellow in a few days. This gives us a beautiful biological assay for experiments. Using this method we can measure either the amount of chlorophyll left or the amount of amino acids which are set free as a result of the breakdown of protein. The interesting thing that we found is that senescence, just like seed germination, depends upon a triggering mechanism. Something must happen in the leaf which starts the proteins breaking down, and as the proteins break down, the chloroplasts break down, and the leaf turns yellow. This initial reaction is very much like the initial reaction in seeds whereby the proteins must break down and furnish nutrition to the young embryo. Thus there is a surprising parallelism between senescence— the end of the story — and seed germination — the beginning of the story. It remains to be seen how this will turn out, but it may be that some of these triggering mechanisms will be the result of the synthesis of one particular enzyme which attacks one particular material in the plant; from that all the other things which follow as normal consequences ensue. The triggering mechanism may require only a few molecules to get started.

I suppose the only thing I can say about the future of plant propagation arises from my observations at this meeting, which is the first time I have ever attended a plant propagators' meeting, and the one thing that is very striking is the great enthusiasm everyone has for their work. So I think I can predict that the future of plant propagation will be very active and lively.

CHARLEY HESS: On behalf of the entire Society I want to thank you, Dr. Thimann, for your fine talk, which gave us an insight into the development and use of indoleacetic acid and other growth regulators in the rooting process. It was a real thrill to hear this story first-hand as well as other aspects of plant growth development. Thank you once again, Dr. Thimann. This concludes the program for this evening.