EXPERIENCES IN PLANT PROPAGATION

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I think I should probably start out by saying the title which reads, "Experiences in Plant Propagation", is really very misleading. I think it is probably put in this way as a trap to make sure that you came back after the recess before lunch.

What I would like to do is discuss with you some of the experiments that you have been continuing along the same lines that I was working on in 1958. I have been concerned with working on some of the factors that control rooting. A plant very easy to root is Lombardy Poplar. The first question to ask is, why is the Lombardy Poplar so easy to root? The answer is a very simple one - it is very easy to root because the roots are already present in the tree at the time that you go out to take your cuttings. The root primordia in the poplars and in many of the willows are laid down as a normal feature of development of these particular plants and so they are already in the tree. All we have to do, then, is concern ourselves with what regulates the growth of these new primordia.

What are the factors which control their emergence? A number of the speakers at this meeting talked about rooting in 4 to 6 weeks. This is, of course, the sort of thing you have to face if you are initiating primordia from cuttings. If you are starting from the cells they have to reorganize to form roots. In the poplars, all we have to do is make them grow. If we can make them grow, they will come out in 4 to 6 days instead of that many weeks.

I would like to review some of the things I talked about last time, to set the stage for some of the more recent experiments we have done since that time.

The roots of the Lombardy Poplar are formed in the phloem of the stem and they are arranged in rather systematic fashion, about one every centimeter along the line. You can't tell precisely where they are from the outside of the stem, but if you peel the bark you can see there are tiny dimples in the bark itself, and these mark the positions of the root primordia. They are generally laid down toward the end of the first year of growth and in all of the experiments that I will describe we worked therefore with cuttings that were at least two years old.

Apparently there are some varietal differences here. We had some material sent to us from Canada with the root primordia apparently formed very early in the first year and you don't have to wait to be able to study this.

The big question that attracted me to this problem was, if the root primordia are present in the poplars, why don't they grow out right on the tree? Why aren't poplars covered with roots all the time? There seemed to be a pretty obvious answer to this because if you take a cutting and stick it in the ground, it is the portion in the dark which roots and the portion which remains in the light does not root. So we put some containers around the branches, and wherever we darkened the stem, roots would emerge and they would emerge in 5 or 6 days. It certainly seemed as though light was a factor which normally keeps the roots from growing out on the poplar stems.

We wanted to find out something about the sensitivity of these roots to light and so we made some cuttings and gave them daily periods of light, low intensity light, the sort of light Dr. Waxman talked about with the photoperiod studies yesterday, and intermittent light experiments, and I will have something to say on how it relates to the Lombardy Poplar, in a few minutes.

We take ten centimeter cuttings, put them in a small fish tank, a little water in the bottom, and this very quickly becomes a saturated atmosphere with 100% relative humidity. A tank cutting of this sort will root in five or six days. If we keep them in the light, the roots will not come out.

The next slide shows what happens if we put them in the dark. You can see that the distribution of roots along the cuttings is rather uniform. The roots which emerge from the preformed primordia are peculiar in several respects. Notice the direction in which they are growing. They don't really grow down; they sort of grow out horizontally with a slight inclination toward growing down.

Here we have cuttings that have been given light on a daily schedule. We have plotted the number of roots that form per cutting on the different days. For example, in the dark, the first roots to appear, appeared on the fifth day. We got about 2-1/2 roots per cutting. On the sixth day we added another three roots. On the seventh day we had another two roots. By this time we had something like seven roots per cutting and it falls off on about the eighth day, and perhaps a small amount after that, but most of the roots emerged between day five and day eight.

If we give these cuttings one minute of light per day, of about 300 foot-candles, we inhibit root development by about 50%. If we give them four minutes of light per day we inhibit them almost completely. With fifteen minutes of light per day of one hundred foot-candle intensity, we cut our number of roots by about 50%, and with four hours of light we cut it down to practically zero.

We continued this kind of regime for 14 days and then we put all of the cuttings in complete darkness. We stopped the light-dark cycle. We expected the cuttings would recover from this once they were in complete darkness.

We found that green and blue gave us very, very little inhibition. But if we gave 30 minutes of red light we got just about complete inhibition. So red light apparently is the offender here, and the reports in the literature have indicated if you have a particular responseof a plant to red light you can reverse this effect by subsequent exposure to this far red light. This is a well known system.

If we supply far red light we find its effect can vary slightly and this may be a leakage effect. If we give 30 minutes of red light, which gives us almost complete inhibition, and follow it by 30 minutes of far red light, we have reversed the effect of the red light. So apparently in the poplar roots we are dealing with the same system that other people have worked out. They are stopped at this level of development and will not recover.

As far as commercial practice here, I really don't think it has any real importance, but certainly I would not leave poplar and willow cuttings around in the light before putting them in the ground.

This slide shows some experiments on the quality of the light. We wanted to know what part of light, which of course is made up of a mixture of all parts of the spectrum, is bringing about the inhibition. So we gave them darkness, green light, blue light, standard red light and far red light. We used these because of what people had published in the literature before on visible light effect.

Next we wanted to know how efficiently the stem is able to absorb and use this light. It is coming into it as light energy. Is it going to do anything with it? It has to convert to some sort of chemical energy, so we took our light cycle and broke it up as follows: We had what we called a long cycle in which we gave 24 minutes of light in 24 hours. We took the same 24 minutes of light per 24 hours, but broke it up into a series of very short bursts. We gave our short cycle - 6 seconds of light every 6 minutes. In 24 hours the cuttings received the same amount of light on the short cycle as on the long. What we find is when we interrupt light by darkness, our short cycle is a much more effective inhibitor. This is much the same kind of thing, I believe, that Dr. Waxman and others have found.

What this tells us is that during the light exposure this energy is trapped by some pigment present in the stem and this is the primary light reaction. Then this energy which is trapped is passed on to other chemical systems during the dark period which follows, and this light trapping system can obviously handle only so much light at a time. It is much more effective than when you give one long burst of energy.

Next we tried to find out something more particularly about this pre-emergence period. What happens during the five days before the roots come out? So we gave them 24 hours of light, but scattered it through this five day pre-emergence period. In other words, light on the first day followed by complete darkness.

With another set of cuttings we gave darkness on the first day and light on the second, followed by darkness.

If we give them light on the first day, it doesn't really recognize it. The same number of roots are the same as for total darkness. If you give them light on the second day, after one day of darkness, we have significant inhibition, but when the light is administered on the third day after two full days of darkness, it is most potent. If we give that light on the fourth day, after three days of darkness, it is less effective, and progressively less effective as this light period comes later and later in the pre-emergence period.

We used 1, 2, 3, 4, and 5 days of light and followed by complete darkness. When we had a solid block of light followed by darkness, it takes 5 days approximately of complete darkness from the time we shut off the light, before the roots appear. Then we gave them different numbers of days of darkness and then followed it by light, because if what I said before is true, ie., that something is happening in the dark, which promotes root emergence, which starts these roots to grow, then how much of this darkness is really required?

So, again, this supports the idea I mentioned before, that something happens very slowly in the dark and this something can be very rapidly destroyed by light, and once it is destroyed by light in the cutting, it is apparently not replaced. When we returned our cuttings to the complete darkness after different amounts of light, they were frozen at the level of roots they had. They do not recover from this.

So we have built up an idea which says this: That something forms very slowly in the dark, is inactivated very rapidly in the light, and this material is present in rather short supply in the cutting. Now if it is present in short supply in the cutting, how does it get into the cutting in the first place? And also, when the tree is growing in the field as an intact tree, why isn't this material destroyed by the light that the tree gets?

This puzzled us for quite a while and we set up the following experiment. After all, a cutting is not an intact plant, although I didn't mention earlier that we debud all our cuttings before we set them out; remove all the lateral buds to conserve the food supply in the cutting, and we began to wonder whether the source of this substance which is depleted in the dark period, is converted to something which is required for re-growth, whether perhaps this isn't supplied by the buds. We ran the same experiments with active buds on the cuttings with active growing leaves and it makes no difference.

Then it occurred that perhaps the root system of the plant itself was supplying this material, and so we did the following experiment to check this out: We took a tree and severed it at ground level and brought the whole tree into the laboratory, and set it in a bucket of water for about 10 days, so we illuminated the whole tree rather than the cutting, but it was rootless and we made cuttings of these and left them in the dark and the cuttings only saw darkness. But cuttings made in the dark and kept in the dark will form no roots.

So we began to suspect that whatever this substance is, it is coming from the roots. So we went out and made some large marks on some of our trees, about two feet in diameter. We wounded our stem and wrapped this whole thing in plastic and covered with black cloth and developed an aerial system of roots, a second system. We left this on the trees for almost a year. We now had a tree which had a root system about three feet off the ground and also a system of roots in the ground. The following year we cut such trees off, brought them into the laboratory, put them in a bucket of water in a light room; so now you see it had lost its original system but it had a second one.

We compared these with other trees that were in the water and in the light, but had no roots again. What we found again when we made the cuttings from these two; the ones with the aerial root system, the second system, could tolerate the exposure to light, and cuttings from them would root.

So we believe now that there is some substance in the poplar which is required for rooting, not for initiation, but to trigger these roots into growth which comes from and is constantly being replenished from the natural root system of the tree. We have tried to find out what this substance might be, but all we have is a long list of things that it is not. (Laughter) It is not auxin, for example. It does not behave that way.

Well, there is another natural step, and that is about where we stand with this. I hope that we will learn more about it in the next couple of years.

There is another natural phenomenon which controls rooting in the Lombardy Poplar, and this is seasonal. We ran into this unexpectedly, and if we could have the next slide (slide), I will show you something about this system. For many of our experiments we keep our cuttings only in the dark, and this shows you the number of roots which we got on experiments over, I think, a 1-1/2 year period, with all cuttings being in the dark. In November, December, January and February, we had high numbers of roots, between eight, and what some experimentors counted in March, April, June and July, in which the number was very low, and then it rose again in the fall and dropped again the following spring. We didn't quite know what this was due to, but it was very apparent that whereas the roots emerge on the cuttings during the winter months when the tree is dormant, all along the length of the cutting, as you would expect because they are from pre-formed root initials, that when we have our low number of roots in the summer months only, those roots at the base of the cutting will emerge. So the roots are polarized at a basal end at the number where they are distributed throughout the length of the cutting during the winter dormant months.

This is not a simple relationship with activity in dormancy on the part of the plant. At least, not so far as cambial activity or the setting of its terminal bud is concerned.

The next slide will show the way these polarized cuttings look. There are roots just at the base, no roots up above. Whenever you get a polarized condition like this in plants, especially if you get it with something that is involved in auxin, and we know auxin accumulates at the basal end of the cutting, we began to suspect this might be related to auxin and changes in auxin between the two seasons. If we invert such a cutting, the roots still know which end is up or down and the roots will emerge at the basal end with regard to its position with respect to gravitation. So it is not gravitation.

We followed a devious line of thinking and decided these cuttings which are polarized must have an auxin deficit. At first we were reluctant to accept this. We felt these were cuttings from trees in active growth when trees are forming auxins and it should have an abundance of these materials, but everything else led us to believe they must have a shortage. So we applied artificial auxin, indoleacetic or indolebutyric acid, and you see it in the controls.

Here we got the typical peeling that you get very often on callus formation, very pronounced here and completely removing this polarization of the roots. So it turns out that the indoleacetic acid is probably present in the cuttings from these active trees in insufficient quantity to meet the needs of the primordial that is buried in the bark. We can change summer cuttings that are polarized in the winter, and non-polarize cuttings by adding additional auxin. We know roots are very, very sensitive to auxin. Probably of all the parts of the plant, the root is most sensitive to auxins and low concentrations of auxin, usually inhibitive. The same concentration which will stimulate shoot growth will almost invariably inhibit root growth.

The next slide (slide) will show you some numbers. We weren't low at all of these. Let's take this series here in which we have auxin concentration in parts per million. The number of roots that develop, the number of roots in the apical half and the number of roots in the apical. With no auxin we have only three roots, only 10% of these were in the apical half, in other words, a strongly polarized, and were 2.6" in length. We went all the way up to 800 parts per million which is an enormous amount of auxin. All these concentrations broke the polarization - 47 to 48% of the roots in the upper half. The root length is inhibited only very slightly. We were never able to inhibit these by any amount of auxin we applied at the apical end, and we have an explanation for it, which is based upon the fact that we believe that once the root starts to grow it immediately precludes the possibility of an excessive auxin reaching the tip of that root. It has a mechanism which says the auxin in it will not go from the base to its apex. If the auxin has to reach it from the inside of the cutting, it has to move down to the root from the basal part to the apical part. We believe the root has ability in this mechanism which prevents it from happening once it starts to grow.

To test this, we took the roots once they emerged from the cutting, and dipped them in auxin solution, so now the auxin is bathing the bare tip of the root. When we do this we find concentrations not of this order but concentrations of one hundredths or one thousandths of a part per million, will stop their growth completely. So they are sensitive to auxin, but they have some kind of ability in this protection mechanism against being inhibited by the very high concentrations of auxin that might possibly reach it through the cutting itself.

I think that is the last slide for this. There is only one other thing I want to say. We wonder what happens to these roots as the tree gets older. After all, these root primordia are deposited at the end of the first year of growth, in a five or six year old tree - are these roots buried in the bark, or can you root a five or six year old poplar branch as easily as a two year old poplar branch? If the roots actually remain behind and are buried in the root, it should be much harder to root this material. It turns out that six year old or eight year old, or ten year old root not only as easily but as quickly as two year old material. The root primordia are still in the bark and we tested this idea in a number of ways and, for example, if we put a little black patch on just one section of the cutting, the root which is under that patch will emerge, but all other roots on the cutting will be inhibited.

MODERATOR COGGESHALL: Are there any questions in relation to that talk?

MR. RICHARD FILLMORE: I would like to ask if this system might not be used in relation to the appropriate time for taking such cuttings as grape and currant and gooseberry and so on; the grape being one of the very few plants, together with poplar and possibly the willow, which I know about, which will actually come better from dormant cuttings.

DR. SHAPIRO: I think it might be used where you have the root primordia present in grape.

MR. WALTER GRAHAM: Is there any study going on with a series of plants that have root primordia?

DR. SHAPIRO: We are not doing it. The Lombardy Poplar will take very, very small amounts of light. Some of the willows can tolerate quite a bit of light. I think most of you know pussy willow branches which have these primordia rooting will root in a clear glass vase at home, one time or another.

MR. JAMES WELLS: I would just like to clarify something and repeat something I think you said. That was that red light tended to inhibit rooting, and far light tended to reverse that effect. Now I seem to recall the data we were given at Beltsville, that red light triggered it and far red reversed. How do you explain that?

DR. SHAPIRO: I should have said something about this. Remember, whenever you have a light reaction, this light is only the first of many, many steps before the energy which is absorbed, whatever it is that it does, the light has to be captured. This is a physical thing. You have to have a pigment. The pigment picks up this light. It then puts it into any one of a number of different chemical pathways. In some plants it will turn out that the end result of this will be inhibition, and in other plants it will turn out that the end result will be stimulation, but the initial light trapping is so far removed from the final result, what the plant does with this energy, that it is very easy to see that this can happen. It isn't a direct action, by any means.

MR. HUROV (Cornell): Your experiments with the large cuttings are very interesting, but I see no reason why large cuttings should root where more difficult ones don't.

DR. SHAPIRO: My only feeling is that here the roots are formed at the end of the first year and if the subsequent growth of the plant buried these root primordia, they obviously could not grow through five years of wood deposited around. If that happens, they should root very poorly. When I found they rooted very easily, it gave me the clue that the roots must be in the bark and therefore must be keeping pace with the addition of wood.

MR. HUROV: There is another thing I want to ask you. Bare rooted liners - is there any reason to believe that if the roots on bare rooted liners are exposed to light they won't root?

DR. SHAPIRO: No, I have gone through a lot of literature on the effect of light on roots in general. It is impossible to generalize. There are plants which are inhibited by light, there are plants which are stimulated by light, as far as root growth is concerned. There are other plants which are totally indifferent to light. The only way you can find out whether something of that sort is happening is to try it with each individual species. You can't relate this even to members of the same family; some will be stimulated, some will be inhibited, and some indifferent.

DR. CHARLES HESS (Purdue): Seymour, do you ever guide advantitious root formation in poplar?

DR. SHAPIRO: Today, you can get new roots formed as a consequence of auxin applications in three and a half.

DR. HESS: Even though the pre-formed initials are growing, so there is no inhibition from roots being present?

DR. SHAPIRO: No. It is interesting when you get new roots formed as a result of auxin applications, anatomically they are identical but physically they are not. They are insensitive to light and they do not ignore gravity the way these do.

DR. HESS: A second question was - you felt the polarity effect was due to a lack of auxin which you could replace by an exogenous application. Have you measured the auxin to see if this would be correlated?

DR. SHAPIRO: Yes, we did some auxin extractions to find as far as the extractable auxins are concerned we can get as much out of polarized cuttings as depolarized cuttings, and this is a very perplexing thing. If we extract the auxins from these polarized ones that we say are auxin starved, from a single auxin starved cutting, we can extract enough auxin to break the polarity on five other cuttings, so obviously if the auxin is there, it is there in a form which is not available to the roots or else our extraction procedure is converting something else into auxin.

DR. HESS: There was a paper at AIBS where a chap was correlating extractable auxin with disfusible auxin and here he found in many systems he could extract a lot of auxin but there was no physiological response, but to correlate the physiological with the fusible.

DR. SHAPIRO: We have tried to do the fusible auxin study. We have run into a lot of technical difficulties with this, but we will be doing more of it.

MODERATOR COGGESHALL: Dr. Snyder, of Rutgers.

DR. SNYDER: Going over the question Charlie raised, he said if we have pre-formed initials and they grow, you can still get the other type of adventitious roots forming. Have you found the opposite situation where the presence of inactive pre-formed root primordia will have any effect on the primordial root growth?

DR. SHAPIRO: I don't know. I have no information.

MODERATOR COGGESHALL: Someone in the back of the room.

MR. WILLIAM FLEMER, III (Princeton): Have you a list of those poplars which do not have root primordia already there?

DR. SHAPIRO: I can't list them now. I just don't remember them, but I will see that the list is put into the proceedings.

MR. FLEMER: One other question - have you ever worked with very rare poplinonce seocarba, great big leaves, like catalpa?

DR. SHAPIRO: No. I worked with all the poplars you have at Princeton because you sent some of them, but I don't think that was one of them.

PRESIDENT VAN HOF: There is a question from a dirt farmer here. Did you ever try the hard-to-root plants like crabapple or beeches? Would that work the same way?

DR. SHAPIRO: No, I don't think it would.

PRESIDENT VAN HOF: Two years ago I set some crabapples during the summer with intermittent mist, too. There was a variety that had a root here and there but not of any significance.

DR. SHAPIRO: It would be very hard to say whether this was involved or not, but if there are pre-formed primordia you might well expect this kind of a thing.

QUESTION: What variety?

PRESIDENT VAN HOF: Red Silver.

MR. ROLAND DeWILDE: I have experimented with crabapples for three years and the results aren't always the same every year, but one variety roots rather easily, but none of them as fast as you say, in five or six days.

DR. SHAPIRO: I should certainly not leave you with the impression that I was the one that discovered these plants had primordial roots. This is something that has been in the literature probably for years and years, probably one hundred years. People have known that poplars and willows had these primordial. Their anatomy had been studied. We started at this point with this information.

MR. JAMES WELLS: Is the source of energy important? In other words, have you tried any other source of energy to effect these roots?

DR. SHAPIRO: We know that ionizing radiation, X-ray will inhibit these roots much the same way that visible light does. We know also that if we combine visible light with ionizing radiation there is a synergizing between them. If we select an intensity of light to give us 20% inhibition of root emergence and select those X-rays which will give us 20% inhibition, we would expect with no interaction between the two, that we would get no more than 40% inhibition, possibly less than 40%. When we do the experiments we get somewhat better than 80% inhibition. So the two can act together and by some means that we don't understand yet, give much more than the simple sum of the two alone.

DR. ANDREW T. LEISER (Clarksville, Md.): Dr. Shapiro, would Metcalfe and Chalk perhaps have a listing? That would be a source that people could go to to get listings of species that would have the pre-formed?

DR. SHAPIRO: I think if you really worked your way through you might find it there. We got some of our information from them but not a complete picture. We still don't have a really complete picture.

DR. L. C. CHADWICK (Ohio State): I wonder if you have done any work, or know of any work that has been done on the time of initiation of flower bud on the poplar?

DR. SHAPIRO: No.

DR. CHADWICK: Any correlation between flower bud formation and the development of roots?

DR. SHAPIRO: In our material we have, I have been growing Lombardies now for more than 10 years, probably closer to 15, what we do, we have a small thicket of these and we cut them back about every two or three years to use in experiments, so none of our stems generally are more than three years old. Our root system is quite old and we never get flowers on 3 or 4 year old material. We have seen a little bit of it. Some of our trees have gotten some, but I can't relate the two.

DR. STEVE O'ROURKE (Michigan State): I presume then that these shoots are epiformus, arising from the basis of plants. My question concerns the position of the cutting on the plant and if there is a difference in degree of rooting between those taken near the base and those further up?

DR. SHAPIRO: As long as it is two years or older, there is no difference. You cannot work with one year old material because the root initials are not well established. You can see them in some one year old material as a small maristomatic mask, but it is not formed into a root yet, and this has given some people interested in poplar breeding, a lot of trouble. They have been trying to get more easily rooting lines and talking with someone from Canada, for example, I learned that he screened some 500 or 600 selections for root-ability, and found they all rooted with very great difficulty, but he was only working with one year old material and this was his mistake. But once the material is two years old or older we get no difference with age.

MODERATOR COGGESHALL: Any other questions?

MR. CASE HOOGENDOORN: In other words, you are trying to imply two year old wood poplar will root easier than one year. Is that it?

DR. SHAPIRO: Exactly, and in Lombardy Poplar this is true.

MR. HOOGENDOORN: Would that hold true for other trees or shrubs?

DR. SHAPIRO: I will only say Lombardy Poplars. (Laughter) I think if you are to compare them not on the basis of when do you get your first roots, but if you were to say when do new roots form, truly new roots, it may well be that they will form earlier on one year than on two year old wood, but that will take at least three and a half weeks. I am talking only about these roots that come out in five or six days. Those roots will not come out on one year old cuttings, and from the experience of this man in Canada, he doesn't get very many of the other kind of roots even after six or seven or eight weeks, and everything I say is based upon this.

MR. HOOGENDOORN: One more question. As to tolerance to light and pussy willows, if they can't stand the light and root, why do they root in the house?

DR. SHAPIRO: They will root with the amount of light they find in a house, but they will not root with a much stronger sunlight.

MR. HOOGENDOORN: You mean in the house it is diffused light?

DR. SHAPIRO: The intensity is much lower and they will tolerate that and they will not if you give them strong light. I would certainly appreciate suggestions of plants you people know.

DR. CHADWICK: Are these root primordia always in the formed tissue?

DR. SHAPIRO: Yes.

DR. CHADWICK: Near the pericycle of the cortex?

DR. SHAPIRO: I have never seen them in all the anatomical work done.

DR. CHADWICK: Apparently they are not tissue cut off from proformus.

DR. WAXMAN: I was wondering, Seymour, if you had worked with photoperiod and initiation of these root initials since they root at the end of the season. Is it lack of daylight response or not?

DR. SHAPIRO: There is a long story. We have done photoperiodic work particularly with regard to the polarization and depolarization. We haven't done enough for me to be able to say with real confidence, but if you can imagine working a photoperiod with these trees, it is hard. They built a number of very attractive buildings, all brick and shaped like out-houses, and the size of the shape they had, and they moved them over the trees every night to control the period, because I thought we had a case of two different photoperiodic mechanisms operating in the same plant. In other words, the fact that the cuttings of poplar rise so late in the spring compared to when growth starts, made me feel it was not directly related to the onset of vegetative growth. Buds break. It followed this by too large a gap. Similarly, the fact that it followed the setting with terminal bud in the fall by such a long period. What we found was this: That if we kept the photoperiod in the spring from getting to be long, and I think it was 11 hours, that we did not interfere with vegetative growth but the roots did not polarize and I think it takes a photoperiod of about 12-1/2 hours before the roots will polarize, so I think it is photoperiodically controlled but it has a different photoperiod threshhold than the initial growth. We have to do a lot more with this.

DR. HESS: In relation to Dick's statement last year, Paul Wilms working with Steve O'Rourke gave a paper on the bubble cuttings. I think this is in the proceedings, and there I think he had some reference to the types of plants with which he worked in particular that you could select with these apparently pre-formed initials in the conifers. I think it was the thujas and junipers.

I would also like to make a comment. I feel on Seymour's very significant finding on a substance in the root system which apparently moves to the upper portion of the plant and affects its subsequent capacity to form or stimulate the growth of the roots, the reason I say this is that working with juvenility, and this is particularly in the ivy, we have always noticed that it seems as though the plant which is closest to the root system was the most juvenile, and you have all heard the relationship of the epicormics, the shoots that rise from the base of the near juvenile. Take cuttings from the base of the plant and you get juvenile material. We feel that there is a relationship between the capacity of a plant to be juvenile and its proximity to a root system. It is quite far out, perhaps a guess, and we are trying to establish it experimentally, but the fact that now you have found a substance which definitely does influence this response, I think is very significant.

One other comment along this line - where you can observe, if you have ivy in a transitional state and you can get in where you have juvenile, have high lobed leaves and have rapid rate of growth and you can get a source of quasi-mature where the leaves are more tired and the growth rate is slower, if this vine hits the ground and forms roots, then all the growth subsequent to that point will be very highly juvenile as the rejuvenated. I repeat once again I think this is really great that you have found the substance.

DR. SHAPIRO: Incidentally, I should say it is probably too late now. When I spoke last time I also showed you some pictures of the results of some of the radiation induced mutation work we were doing at Brookhaven. Some of you seemed interested in that at the time. I brought some pictures along to show a few of the mutations we obtained with radiation since that time. I suspect we probably haven't time to do this. We can slip through them in order in 5 or 6 minutes.

MR. CASE HOOGENDOORN: I have one question, rather one more puzzle. Does Euonymus vegetatus also have this root?

DR. SHAPIRO: I have often thought to look at it because in looking at the plant you see bumps around the node which I may well suspect may be root primordia.

MR. HOOGENDOORN: This is my biggest trouble - to take it beyond. You take the vegetatus cutting in sand under the fog line and in a week you have a wonderful root system and you pull the cutting up and you have nothing.

DR. SHAPIRO: They are at the node. Make sure your basal cut is right at the node. I will look into this.

MR. HUROV (Cornell): You studied the effect of light on cuttings which already have root initiation. What is the effect on the cuttings that haven't root initiation?

DR. SHAPIRO: It varies.

MR. HUROV: Correlation has been shown where you put a part of a tissue like marked cuttings, you form these.

DR. SHAPIRO: According to the literature you get very variable results depending upon species on the initiation of the roots, but there are reports of stimulation, also reports of inhibition.

(Slide) In summary, what happens when we radiate something? We damage the chromosomes in the cells and as they rearrange themselves we pick up this effect at the cellular level in a change of shape. These are normal chromosomes separating as the cell and here is the irradiated cell. You can see the orderliness of the process disrupted. Some of the chromosomes are sticky chromosomes, little pieces actually break off. They get lost. They don't go with either of the two daughter cells. This is the basic thing that happens with a lot of these changes.

The next slide will show what happens in a single cell. This is a petal showing a mutated cell which is changed from white to red. What happens here is very, very important. The time at which the mutation takes place in the development of the stem or flower is very important in whether you can get a coverable mutation or not, so here the mutation obviously took place too late in development of the flower that only a single cell shows mutation. If it took place earlier and this cell had a chance to divide one or more times, you would see what we have in the next slide.

(Slide) Here is mutation. You have a streak on the petal for color.

Now the next slide will show what happens if it takes place still earlier. Half the flower is now of a different color. This is the mutated area and this is the original. The distinction between mutated tissues and unmutated is very sharp. You can see down in this petal - half is darker than the other half and slightly longer. The darker mutated area of the petals are a little longer than the sharper demarcation part.

(Slide) This is Bericans rose. We have been able to get more support for both darker and lighter color. There is a graduation between the better types color and this very pale and this very velvety. We have about four integrates between this and we have all these propagated as separate plants and they continue to show the new color. Some of these, and this will be true for many of the things I show you, are things which have been seen and have been found in nature. In many regards we duplicate the sports which occur spontaneously. It is possible all the sports we get by radiation are only duplicates of what we get in nature. This point can be debated. One thing is certain, that we can bring out all the sports in better types of two years of research in the plant, that people pick up spontaneously in periods of 30 or 40 years. We accelerate the process.

Now we get this as a sport from one of the better types. (Slide) It maintains the completely speckled appearance, sometimes a little more white and sometimes not quite so much flecking. This is a variegated sport as far as I have been able to determine as not being picked up spontaneously, by not asking enough people.

QUESTION: When you revert back, are the chromosomes rearranged?

DR. SHAPIRO: What it means, you are selecting stems that were not damaged originally. They outgrow the other cells that were damaged. There is a competition between the cells changed by radiation and the cells which are not changed. If these things grow together, in fact have the same ratio, we have a perfectly stable situation. If one grows a little faster, it will in time outstrip the other. These are things you can control by propagation, knowing which buds to force can give you this or this or the variegated type.

MR. WILLIAM FLEMER: Variegated flowers always seem to be unstable. In azaleas they are always reverting back to the solid colors one way, or also in camellias.

DR. SHAPIRO: I certainly can't explain it on the basis of any simple chimera. I believe there are genes which control the maturation.

Now we come to the geraniums. We charted out Enchantress fiat at the suggestion of Professor Nelson of Connecticut. He supplied us with our earliest plant. This is what fiat would look like, and this is what the Enchantress would look like. We found we could change Enchantress to fiat very easily. It arose originally as a spontaneous mutate. It is probably a chimera. I also got a very pale color type and we got one the same size but which is much more pale in color than the original and as far as I can tell the geranium people have never seen these before. The small type can revert to it. We started with it being reverted to fiat. Whenever it is reverting to fiat it takes place with the petal increase in size.

Here is toning of reddish tissue on one petal. It overgrows. This is due to a difference in cell size. The mutant we have here is smaller cell size. If you incorporate the original tissue, the cells are larger.

You can put fringes on some of this Enchantress. This is stable. This (Slide) shows the pale pink mutant we got and in this case with a fringe on it associated with some of the fiat type petals which are not fringed.

Coming to Mungus we did a good deal with the Shoesmiths, starting with the white. From this we picked up on Schwartz which appeared to be identical with yellow Shoesmith, and also one you see here which is variegated, single petal, has some yellow streaks on it. This is perfectly stable. We propagated this any number of times.

We come now to the Masterpiece, this bronze master. There is a sector for bronze master and a Shoesmith sector, which is unknown to the people who have grown this. It is white.

Here is our Masterpiece - half of it has been changed to bronze Masterpiece. If you propagate from bud on this we can get all Masterpiece. If we propagate in the middle we will get some kind of thing.

(Slide) This was grown at too high a temperature for Masterpiece color to show up well. This is a larger sector from the white color that you saw earlier.

We also get a clear yellow out of a bronze master. People at Yoder Brothers tell me they have never seen a clear yellow, have tried to get it, and it holds up very well at higher temperatures, and also shows that we get streaked in the Masterpiece series also. This is one that came from the original Masterpiece. The yellow is streaked and the yellow runs and there are occasional small streaks of the white.

(Slide) There is a change in petal form. We also pick up spider tips from the Masterpiece series, and I think one from the shasta group. Some of these, Bill Duffy of Yoder Brothers, says he has never seen in his experience. It may be radiation can give not only an acceleration of what will take place in nature, but also some types which are distinctly new. (Applause)