MODERATOR LANCASTER. Thank you Our program this morning, as you have all noted from your bulletin, is broken into three categories. First, A Study of Plant Growth Substances in Easy and Difficult-to-root Cuttings; second, a panel discussion on the quick-dip method of using growth substances, and finally a discussion of the Budding of Dogwood in the Field.

We will hear from our speakers and then immediately following each speaker or panel we will have a short question and answer period.

As I walked into the hotel yesterday evening, one of the first gentlemen I bumped into was my good friend Hugh Steavenson. In the conversation we eventually talked about the wintering of container stock. He said, "We have the answer down in St Louis." I knew they were much, much colder than we are I became very much interested. He went on and explained that "What we are doing, we are selling all of our plants." I thought I would pass that along to you gentlemen.

Without any more ado, may I present Dr Charles E. Hess, Department of Horticulture, Purdue University, who will report to us on "A Study of Plant Growth Substances in Easy and Difficult-to-root Cuttings." Dr. Charles E. Hess! (Applause)

DR CHARLES E HESS: Thank you very much, Art

It is interesting that in the history given by President Nordine that the Propagators Society sort of died out at the time it did. A couple of years later, in 1935, the first identification of the natural hormones in plants was realized. This marked the beginning of the use of hormones to stimulate root initiation, and there have been thousands and thousands of papers dealing with the use of hormones to stimulate root formation. This morning I would like to spend some time on these, and then discuss some new aspects that we are getting into, finding that plant hormones themselves are not the entire answer.

A STUDY OF PLANT GROWTH SUBSTANCES IN EASY AND DIFFICULT-TO-ROOT CUTTINGS

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Since 1935, the use of plant hormones to induce roots on cuttings has received a tremendous amount of attention. However, 1935 did not mark the beginning of the use of plant hormones, since Dutch propagators used a form of root inducing hormone over 100 years ago.

The Dutch propagators split the base of a difficult-to-root cutting and inserted a wheat grain. The "prepared cutting" was stuck into a medium, and rooted faster and in higher percentages. Today, we know that the reason for this response was that as a wheat grain germinates it releases auxins or plant hormones. As the auxins were released by the germinating grain they were absorbed by the cutting and rooting was stimulated.

Auxin is another term for the natural hormone produced in plants. This natural hormone produced in the young leaves and in the buds of

the plant, moves down the cutting to the base. If the cutting was left on the parent plant, the auxins would just keep moving down the stem. However, as soon as we take the cutting, the normal pathway of the auxins is blocked and so they start accumulating at the base of the cutting. After the auxins reach an active concentration, roots will be initiated. Also, as you remember from some of the work I presented at a previous meeting (December, 1954) we know that sugars and other food materials also move down the stem and accumulate at the base of the cutting.

Now you may ask, if a plant is going to manufacture its own auxins or hormones, why go to the trouble of applying more? Well, one practical reason is that even though a cutting is easy to root, we can still get an increased rooting response by adding a synthetic auxin. In other words, in many cases the rooting response that occurs normally is not as great or as fast as the propagator would like. We want to speed up the reaction and get more roots on the cutting so we will be sure that we will have a uniform stand and one which will quickly reestablish its root system when potted up.

An example of this can be seen with cuttings of *Hedera helix*, the English ivy. The juvenile form, which is used as a ground cover, roots very easily. With no treatment at all, we get an average of four roots per cutting. However, if we treat them with napthaleneacetic acid, a root promoting substance, we increase the number of roots to 20 per cutting.

A second reason why we treat cuttings with synthetic materials is that besides being able to manufacture hormones, plants have the ability to destroy them This is a safety mechanism plants have, so that if some of the buds or leaves start to produce an excessive amount of hormone, the plant can maintain a proper hormone balance by destroying the excess. In contrast, synthetic compounds are different from the ones that are made within the plant, and the plant does not have the ability to destroy them. As a result the synthetics can get into the plant and do their work without being destroyed Therefore, we have a greater response from using synthetic root promoters in comparison to the use of a natural hormone

Auxins, however, are only part of the problem. I think you all realize that the harder a cutting is to root the less it responds to the application of a root promoter The cuttings with which we really have trouble are the ones which give the least response when we apply our root promoters. An example of this can be seen with cuttings of the mature, flowering form of *Hedera helix*. 'About the best rooting we can obtain with this mature wood is 7 to 16 per cent with an average of 1 or 2 roots on those that do root Treating the mature cuttings with 50 ppm napthaleneacetic acid did not increase the per cent rooting and the number of roots was increased only from 2 to 4 roots per rooted cutting.

Apparently, there is something else involved in root initiation besides auxin and food materials since if only these two factors were involved, we should be able to increase the rooting response of mature

cuttings up to that obtained with the juvenile cuttings. I feel we can say it is not food materials which are lacking because you can propagate the cuttings under mist where you have just about ideal conditions as far as food manufacture is concerned, and still the cuttings remain difficult to root In fact, the 16 per cent rooting we did get with mature *Hedera* cuttings was under mist.

What else, then, can be involved in the formation of roots other than auxins and food materials? The first lead to a solution of this problem was in a paper that was presented by Spiegel at the 14th International Horticultural Congress in the Netherlands

Working with easy and difficult-to-root cuttings of grape he found that the difficult-to-root cuttings contained an inhibitor or a substance which would block rooting rather than promote it.

To demonstrate this he took difficult-to-root cuttings and put them in a water bath and soaked them at room temperature for various periods of time ranging from 24 to 96 hours. After the cuttings were soaked, he planted them in the medium and found that they were much easier to root. It seemed that during this period of soaking something which was in the cutting was leached out, and then the cutting became easy to root.

To check this a little further, he took easy-to-root grape cuttings and soaked them in the same water in which the difficult-to-root cuttings had been soaked, and sure enough, the easy-to-root cuttings became difficult-to-root.

We decided to see if other difficult-to-root plants would respond in the same manner. We chose the English ivy as a plant to study, not only because we had a great difference in rooting between juvenile and mature forms, but also because we could get both forms of cuttings on the same plant In this way we were assured that the material was at least genetically similar, which may not be the case when you are using varieties.

The first thing we did was to make cuttings of the mature form and soak them in water They were still difficult to root. The next step was to make extracts of the mature and juvenile tissue to see if the extracts had inhibitors. As it turned out, we found inhibitors in both the mature and the juvenile wood With these results we could not explain the great difference in rooting between the juvenile and the mature form on the basis of inhibitor content.

About this time we began working on a rooting test which made use of etiolated Mung bean cuttings. The beans are germinated in complete darkness in a room which is kept at 78 degrees Fahrenheit and 80 per cent relative humidity. Five days after germination the beans are decapitated, that is we cut off the seed leaves or cotyledons as well as the primary leaves This removes a rich source of root promoting substances Cuttings, seven centimeters long, are made from the portion of the seedling which remains and then are placed in vials, with the extracts we are testing They remain in this vial for four days, and are then transferred into water and allowed to form roots. Root formation takes about five days from the time the cuttings were first made.

The most important thing we found with this test is that these cuttings do not respond to auxin or hormones. The cuttings form a certain number of roots, which is not increased by the addition of auxin. The Mung bean cutting, then, is very similar to the mature ivy cutting, which does not respond to the addition of root-promoting substances. We now have a test with which we can find substances that promote rooting other than auxins.

We first used this test to determine the response of the cuttings to extracts of the juvenile and mature forms of ivy. Both extracts alone did not increase the number of roots produced in the Mung bean cuttings Mixing the mature extract with auxin also, gave little increased rooting However, when we mixed the juvenile extract with auxin, we obtained a very large increase in the rooting response. From this and similar experiments we concluded that in the juvenile cutting, which is easy to root, there are substances which promote rooting only if they are in the presence of the natural plant hormone or a synthetic, like indolebutyric acid. We call the substances we find in the juvenile cuttings, "cofactors." They won't work alone, but when you apply them in combination with auxin, you get an increased rooting response. In contrast, the "cofactors" are lacking in the mature cuttings.

This can also be shown in an experiment in which a scion of juvenile ivy is grafted onto a mature ivy cutting. The mature portion of the cutting-graft combination, treated with an auxin, will now root! From this experiment we assume that the cofactors that are produced in the juvenile scion move through the graft union into the mature cutting. When the cofactors combine with the auxin, roots are initiated.

We have also worked with the red and white flowering forms of *Hibiscus Rosa sinensis*. The white form is fairly hard to root while the red flowering form is fairly easy to root. There is also a white variety which is intermediate in its ability to root.

We have made extracts of the tissues from the *Hibiscus* to see if we would isolate the rooting cofactors Incidentally, in the easy-to-root red variety, we had 100 per cent rooting, and averaged 15 roots per cutting. In the intermediate-to-root variety we had approximately 80 per cent rooting with an average number of five roots per cutting, and with the difficult-to-root we had approximately five per cent rooting with an average of three roots per rooted cutting From the extracts we found that the easy-to-root cutting had four cofactors or four substances which promote rooting in combination with auxin, the intermediate had three, and the difficult-to-root had one. Perhaps, with an "impossible-to-root" variety we would not find any cofactors Apparently, we can correlate the number of cofactors that are present with the ability of a cutting to root. That is, if the cutting is very easy to root it will have at least four cofactors present, if it is difficult-to-root it may have only one

We are now trying to purify these cofactors so that we can identify them. If this could be done, we could then extract them from the easy-to-root forms and apply them to the difficult-to-root cuttings and obtain good rooting. So far we have been able to do this through a graft union, but have not been able to do this with total extracts. What we

would like to do now is find the chemical nature of these substances and apply them to the difficult-to-root types. It may be necessary to have a synthetic form of these cofactors so the plant cannot inactivate them, as it does its natural auxins.

It is going to be a long drawn-out effort, but we eventually hope to be able to make a difficult-to-root cutting, easy to root by feeding it with the cofactors that are missing. We hope that it will be possible to supply these substances in a concentrated dip or talc at the same time we apply the root promoters which are presently being used.

To summarize, then, although auxin or hormones play a very important role in rooting, they are not the complete answer. The more difficult a cutting is to root, the less it responds to auxin alone. In some difficult-to-root cuttings such as the grape, there apparently are inhibitors which can be leached out, making rooting easy. In other cuttings which are difficult-to-root, the difficulty seems to be due to the lack of certain substances or cofactors. The cofactors are present in the easy-to-root forms, but are absent in the difficult-to-root forms. We hope to identify these substances and be able to apply them to the difficult-to-root forms in order to render them easy to root. Thank you.

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MR. RICHARD FILLMORE (Durham, N.C.): I would like to ask whether or not those grape cuttings were dormant, leafless cuttings or whether they were actively growing, leafy ones.

DR. HESS: These were dormant cuttings at the time they were tested. This brings up a point. As a plant becomes dormant the inhibitors that are present increase. This is a safety mechanism on the part of the plant in that these inhibitors keep it from growing during occasional warm periods. You might expect, on the basis of Spiegel's experiment, that you would find inhibitors present, since they were dormant.

Another example are hardwood cuttings which might be hard to root, and show very little response to root promoters. However, you might take softwood cuttings of the same plant when it is in full leaf, and it becomes rather easy to root, in addition to showing a great response to the application of auxins. Our interpretation of this is that in the hardwood cutting the cofactor level is quite low and in the leafy softwood cutting it is fairly high. The reason we can say this is that we know the cofactors are produced in the leaves of the cutting, so if the leaves are absent you would expect the cutting to be low in the cofactors and, therefore, low in their response to an application of auxin.

MR. GERALD VERKADE (New London, Conn.): Charlie, would you say that the same thing is happening when you graft Blue spruce on Norway?

DR. HESS: Exactly. When you graft Blue spruce onto the Norway you may get up to about 25 per cent of these grafts to strike roots

from the scion when they are in the grafting case. If you take just cuttings of Blue spruce and stick them under the same conditions, you may get one per cent, which is doing pretty good. Evergreens also have juvenile and mature stages of growth. When they are in the seedling stage they are usually in the juvenile condition; with continued growth they become mature. This is another reason why you can take cuttings from a small seedling of an evergreen and get it to root fairly easily. When it becomes old, it becomes more difficult to root. The Blue spruce is a clone of a mature spruce. I feel what is happening in the case of the Blue spruce is that there is a transmission of the cofactors from the juvenile Norway spruce seedling into the mature Blue spruce scion, and that is why you get more rooting when you have this graft combination than if you take an individual cutting.

MR. JIM WELLS (Red Bank, N.J.): First of all, how do you determine that there are four cofactors? Are these clearly separable?

DR. HESS: To answer that, Jim, and if it is all right, I will show you our procedure of extraction which is necessary to answer your question.

The leaves are the source of rooting cofactors because we can take isolated leaves of the juvenile form and root them without any trouble at all, and we have not been able to root the mature. So we can go back as far as the leaves and get the cofactors present in the juvenile form. These, of course, have been treated. When we make the extract, we use primarily the leaves. If we threw stems and buds in there we would have a lot more difficulty in determining what the actual sources are.

We bring the cuttings in, take the leaves off, and dry freeze them in a process called "freeze drying." The reason for this is to prevent any chemical reactions going on in the tissue during the extraction. After it has been freeze dried or lyophillized, we take a sample and extract it with alcohol. After a couple of hours of extraction we evaporate the alcohol extract to just about a dry condition, and we then add a small amount of alcohol to take the mixture back up into solution. The extract in a hypodermic needle is spotted on a piece of filter paper. This is called technically a chromatogram, which is hung in an economy sized test tube. We allow this filter paper to hang above the solvent for 18 hours and then lower it into the solvent. The solvent moves up the filter paper and after it reaches a certain point we take the chromatogram out.

In this spot are substances which have different solubilities in the solvent we use. As the solvent moves up this piece of filter paper, those substances which are most soluble will stay right in the original spot.

What we have done simply, then, is to take a mixture and separate it into several substances, by means of a chomatogram. This is cut into strips and placed in vials with Mung bean stem segments. This tells us where on this strip we find the substances stimulating rooting.

These Mung bean cuttings produce a number of roots without any treatment at all, and therefore we can also measure inhibition. In

other words, there is something in the extracts that would block rooting, it will also show up.

In extracts from the red, or easy-to-root variety we had four areas in the chromatogram which promoted rooting. In extracts from the difficult-to-root white variety there were three segments which inhibited rooting and one which promoted it.

MR. WELLS: Thank you very much, Charlie. That is very complete and clear.

Is it possible to take leaves of a plant which will root easily as a vigorous soft-growing shoot and to extract from those leaves in a simple manner and freeze that material and use it on a dormant cutting? Have you done that?

DR. HESS: No. You have to first separate these components and purify them. If you use the total extract you will have both inhibitors and promoters present. Whatever effect you will get will be the net between the activity of inhibition and the activity of promotion. So far in all lyophillized extracts we have been successful in getting the stimulating effect only after we have purified and separated promoters and inhibitors.

I doubt if it will be possible to take an extract of an easy-to-root plant and apply it to hard-to-root one until it has gone through some steps of purification. This would be a nice direct application but it needs a little more purification before it is possible.

MR. WELLS: Cannot a balance of cofactors be transmitted to the same type of cutting but at a different stage in its growth?

DR. HESS: It may be possible with a combination of leaching to remove the inhibitors from a hardwood cutting and then the application of an extract from a leafy softwood cutting to get promotion. I still say I am afraid you will have to do some purification of the extract from the softwood cutting before you will get the desired effect.

MODERATOR LANCASTER: Gentlemen, if any more of you have questions, keep them in mind for the question box.

We will carry on with our program, going on to a panel discussion on quick dip application methods. Dr. Hess is going to give us some preliminary results on some work he has done. (Applause)

A COMPARISON BETWEEN THE QUICK DIP AND POWDER METHODS OF GROWTH SUBSTANCE APPLICATION TO CUTTINGS

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Last year, as you will remember, we had a briel discussion as to whether the concentrated dip or the talc method would be better for the application of root promoting chemicals. As a result of this discussion we decided to see if there were any differences. We ran two experiments, one with *Taxus* and Pfitzer junipers and another one with *Rosa manetti*. In these tests we used talc and a concentrated dip at a con-