

Agriculture, and the State Department of Agriculture. This cooperative effort has resulted in the development of registration and certification programs for citrus, deciduous fruit trees, grapevine, and strawberry plant nursery stock.

There are now 120 nurserymen and growers participating in these programs. The term "California Certified" will identify to the agricultural industry nursery stock which is the best available from a pest cleanliness standpoint.

FRIDAY EVENING SESSION

Moderator: Dr. Dale Kester

PLANT PROPAGATION IN THE YEAR 2000

J. P. NITSCH

Laboratoire du Phytotron

Gif-sur-Yvette (S. et O.), France

Perhaps one does not often realize that the year 2000 is only thirty-six years ahead. It's not a very long time and, probably, that year will be no different than the year before and the year after. So we can look at the year 2000 as an ordinary year and can compare it with other ordinary years that have passed before. If we do this, we can look back in history and see what happened in the last thirty or forty years. We then observe that the discoveries which were made thirty or forty years ago are now being applied to the field of plant propagation.

Looking Back

For example yesterday, Dr. Snyder told you that plant growth hormones were discovered about thirty years ago. In fact, no one knew for sure what the substances were chemically until 1934, exactly 30 years ago, when Kogl, Haagen-Smit and Erxleben found that indoleacetic acid was active in plant growth. It was also in 1934 that Prof. Went found that they stimulate the rooting of pea stems. It was then shown very rapidly that, indeed, many auxins were very active in promoting the rooting of cuttings.

I can give you another example. Almost a hundred years ago Julius Sachs, a German plant physiologist, studied the nutrition of plants. He wanted to know what the soil furnishes the plant in order to make it grow. He tried to make solutions which would replace the soil. He and other workers, for example, Prof. Hoagland here in California, determined which chemical elements were essential for plant growth and which ones were unnecessary. The result of this work is that it is possible

now to devise solutions containing water and mineral salts which will enable one to raise whole plants without any soil whatsoever. This is illustrated in the first few slides. This white stuff here is our "soil". We don't use soil anymore. We grow all our plants on this white material which is glass wool. It is a special glass wool, which is very fine and doesn't hurt the fingers. It is produced in France under the name "Verrane". The next slide shows a tobacco plant which has been taken out of the pot; you can see the glass wool and the roots which have developed. The tobacco plant grew very well, taller than the man next to it, even though it had no soil. And so we now grow all our plants, strawberries, poplar, spruce, or what have you, on glass wool watered with a synthetic mineral solution which is prepared in a central place. We prepare about eight hundred gallons at a time, stored in a tank which contains two thousand gallons. We need about four thousand gallons per week of this solution which is piped to all greenhouses and experimental rooms of the French Phytotron at Gif, France. These pipes are all made of black plastic to prevent light from reaching the solution; otherwise algae would rapidly develop in the solution. Perhaps it will prove commercially interesting to have such a set-up in order to get away from soil altogether, thus eliminating damping-off and other troubles. Here we do not have to sterilize. Our glass wool doesn't have any nematodes or other bugs in it. We just plant our plants and water them with the nutrient solution. Watering is done automatically with the Chapin "Watermatic" system. We need very little manpower to take care of the plants.

Perhaps I can remind you of another discovery that was made by botanists about thirty or forty years ago and which has now found application in the field of plant propagation. This is a work of Prof. Knudson of Cornell on the germination of orchid seed. Before that time it was very hard to grow orchids. One had to get slips from plants which had been collected in the wild during expeditions in Central America or Asia. To multiply them was very slow. It was especially difficult to germinate the seed which are very tiny. People had developed theories that, in order to germinate, these seeds needed a symbiotic fungus. One had to isolate this fungus and then inoculate it in the medium on which the seeds were to germinate. Knudson showed that this was not necessary. Seeds could be germinated in a test tube without any fungus on a completely synthetic medium, containing mineral salts and sugar. Once this fact was established it opened the door for the production of a large number of orchids and new hybrids.

Thus some of the techniques which are used today in the field of plant propagation on an industrial scale spring from the careful work of scientists which was done thirty to forty years ago.

Looking Ahead

The same source which has been the origin of the fields we are exploiting today will continue to produce new ones in the

future. Thus, in order to have some idea of what possibilities will exist in the year two thousand one should look for the new things which plant physiologists are discovering at the present time.

Orchid Growing

I have mentioned the orchid field. In this connection I would like to say a few more words about orchids. First of all, even though it is now possible to germinate orchid seed rather easily, it is difficult to get uniform seedlings from seeds and it takes a long time—five to seven years between the time you sow the orchid seed and the time you see the first flower. It is also not easy to get very outstanding hybrids; growers say that, in the case of *Cymbidiums*, they may have to grow about a thousand seedlings in order to get two or three that are really outstanding. It would be very useful to be able to multiply these two or three outstanding plants very quickly and get rid of all the rest. Well, this has been rather difficult up to now because once you have such a plant, you can make one division every year or so, and get two or three plants every year. One doesn't build up a stock of excellent plants very quickly that way and, what is worse, during the course of this slow process, plants get infected with virus. This is the case, for example, with *Cymbidium Alexanderi* "Westonbirt", a choice variety which is completely infected.

In recent years pathologists and physiologists have developed means of getting rid of viruses. One of them is heat treatment. This can be tricky because one has to heat the plant enough to kill the virus but not too much, to avoid killing the plant. Thus, with certain species this technique is very difficult. Another means of getting rid of viruses in plants is to grow parts which are normally devoid of virus. Such a part is the apical meristem, the very tip of the shoot, which is enclosed in the small leaves and not larger than the head of a pin. One sterilizes the tip of the plant with something like "Chlorox" and then dissects carefully away all the young leaves to get to the growing point. The latter is excised under aseptic conditions using splinters of razor blades that have been welded onto a handle. It is planted in a test tube on a suitable medium like Knudson's number three medium. There the tip develops slowly and forms a rooted plant which is virus free. This technique is used for carnations, dahlias, etc. It can also be used for orchids. Dr. Morel, for example, has developed this technique and has been able to free many strains of orchid from virus. He also has been able to multiply these virus-free plants vegetatively. The growing point which has been regenerating in sterile culture to form a protocorm-like tissue can be cut into 4 pieces every month. If each piece is subcultured and again divided into four after a month, then the stock of choice plants can be raised to several millions per year in the form of protocorms which can then be grown to full-sized plants. This may take several years, but at least one

is sure that, in the end, he will have several millions of top choice specimens.

The rooting of cuttings

Synthetic auxins enhance very much the production of roots on many cuttings. It has been found, however, that species which root poorly naturally also root poorly (although slightly better) with hormone treatments. This is because compounds other than auxins are involved in the induction of root promordia. At the present time research is directed towards the study of the chemicals which enhance the auxin effect and which are called "synergists".

At least three groups of auxin synergists are known at the present time. One of these includes reducing substances with SH groups, such as glutathione. Another group is made of terpenoids, like vitamin E, vitamin K, vitamin H and some of the unknown natural terpenoids which Dr. Hess is working on right now. The third and important group is composed of some of the phenolic substances which are so abundant in plants. Phenolics in plants may be either synergists or inhibitors of auxin action. We already know what the chemical configuration of the "phenol" has to be for a compound to be synergistic. We know that it is necessary to have *ortho*-diphenols like chlorogenic acid or caffeic acid, and not monophenols like *para*-coumaric acid which would be inhibitory. We know that flavonoids like quercetin are very good synergists, whereas other flavonoids like naringenin are inhibitory. We also know that most of the anthocyanins are very good synergists of auxin action. Synergists may be used more and more in the future in order to improve rooting mixtures. Actually natural synergists exist already in plants, and their level and their identity is regulated by the environment. For example under long days more flavonoids of one type can be produced than in short days. Work in progress at the present time is centered along the effect of the red and the far-red light on the production of endogenous synergists and, also, the effect of temperature and other factors on the production of synergists by the plant itself. You know that the environment is very important for success in the rooting of cuttings. The next slide will indicate this by showing a result which was obtained by Dr. Waxman at Cornell some years ago with the rooting of red dogwoods. What was found was that under long days (18 to 24 hrs.) there were more roots produced than under short days (9 hrs.). This was true both when the stock plants had been kept under long days before the cuttings were rooted and the cuttings themselves were exposed to long days. Such results show why certain planting dates are important for success in plant propagation.

Control of the environment

The environment has also an important role in the *growth* of the woody plant. The next slide shows two seedlings of *Weigela florida* of the same age. Probably you don't even see one of them because it is so small. They were sown on the same soil,

given the same amount of sunlight (10 hours), yet one is about 2 feet tall after 2 months and the other less than 1/2 inch. The difference is not due to soil, nor photosynthesis nor temperature as these factors were the same for both plants. The only difference was that the large plant received an additional illumination of low intensity light (50 ft.c.) for 8 hrs. every day. The next slide shows that one can get similar results with seedlings of *Picea glauca* var. *albertiana*; on a fourteen-hour day growth quickly stops, but it keeps going on a twenty-four-hour day, so that one can get a Christmas tree much faster by giving extra light, provided that the temperature is high enough. All these effects are due to the fact that, under long days, the apical meristem produces leaf primordia which develop immediately into leaves. Under short days, on the contrary, they develop into scales instead, growth is arrested, and the plant becomes dormant. This knowledge is important not only if one wants to get quick growth, but also if one wants to prepare plants for winter. A plant which is growing actively is very tender and will freeze, whereas it will withstand cold weather if it has become dormant soon enough. Probably people will realize more and more the importance of having an adequate temperature and daylength control in their greenhouses. It may become a paying proposition, for example, to condition the stock plants in order to keep them in a state in which the cuttings will root easily. It may also become practical to induce dormancy in certain plants before shipping them. The next slide will show the Gif supergreenhouses. These are air-conditioned greenhouses with different day and night temperatures. In addition, they are equipped with metallic curtains which are rolled outside during the day and which automatically unfold at the proper time to cover the whole roof of each greenhouse compartment. At the same time, vertical partitions rise in order to close the sides, including doors. A completely light-tight box is thus produced. Genuine phytotrons also are developing in all parts of the world. In them one can even replace sunlight with artificial light. Under these conditions, one can grow all plant species at any time of the year. We have had spectacular results with very large sunflowers which flowered perfectly, even though they never saw any natural light, or with plants of South African deserts, such as *Stapelia flavirostris* which were grown from seed on our glass wool medium.

Bottom mist

Let us go back for a minute to the subject of rooting cuttings of woody plants. I would suggest that instead of having the overhead mist we change to bottom mist. The point which has always bothered me about overhead misting is that one gives a lot of water to the leaf. Now trees and shrubs are not aquatic plants. Their leaves like to grow in air, not in water. In fact, when too much water is given, it leaches nutrients out of the leaf. Actually what do we want? We want to prevent wilting of the leaves and also to provide a humid atmosphere at the

basal end of the cutting for roots to develop. If we could make some sort of perforated rubber sheet or embed cuttings in a plastic foam so that mist could be applied from the under side, to the bottom inch or so of the cuttings, I think this would give better results with species which take a long time to root. We could even heat the mist to the right temperature as bottom heat is very important. Thirdly, one could also add nutrients to this bottom mist. All these features might constitute an improvement over the overhead misting technique which is in use at the present time.

The use of cytokinins

Also, in addition to the basal hormone dip which we use for cuttings, we might have, in the future, dips for the tops. In order to produce a good rooting system, cuttings generally need to have healthy leaves on them. Thus it is important to keep these leaves healthy and green. We know now that there are substances which are capable of keeping leaves healthy and green. The next slide will illustrate this. It is a slide which Prof. A. C. Leopold of Purdue University kindly sent me and which shows very dramatically the effect of one such substance in keeping leaves green. One leaf of this cutting of French bean was treated with a synthetic cytokinin called benzyadenine, the other leaf being left untreated. The cutting was put in water and left there for a week or so. At the end of that time, the treated leaf was still healthy and green while the other had turned yellow. It is possible that cytokinins will become useful in keeping green the leaves on cuttings and allow them, therefore, to function in the manufacturing of whatever substances are needed for root formation.

While I am on this subject, may I also mention the possibility of keeping flowers from wilting by the application of cytokinins. They might make flowers last longer, especially in heated apartments.

Tower greenhouses

In order to industrialize the production of potted plants rather drastic changes will have to be made in greenhouses. In this connection, the tower greenhouse which has been devised by the firm Ruthner (Obere Donaustrasse 49, Vienna 2, Austria) may be a forerunner of a type of greenhouse of the future. As you can see from the slides which have been kindly lent by the Ruthner Co., a glass or plastic tower stretches the available space vertically into a third dimension. In this tower, a chain goes up and down, up and down again, and on that chain the pots hang. Looking into the tower upward, one gets the extraordinary sight of all these pots hanging in the air. The result is that the plants get maximum illumination from the sun. The chain moves slowly with the pots, so that the latter go through the whole volume of the greenhouse. As they go down and pass through a certain zone, they can be watered automatically. Such a system produces very uniform plants and eliminates the effect

of any pockets of dead air. It takes full advantage of the natural light, which is often limiting during winter in Northern climates.

Flower-inducing compounds

Turning to other things which may develop later on into practical applications, I must mention the work which is being done now in various laboratories about flower-inducing compounds. It would be nice to grow plants in greenhouses to the desired size, then spray some hormone and get everything into bloom. Actually, this dream has become true at least in one instance, that of the pineapple. The next slide shows a field of pineapple plants in Hawaii. This row has not been treated. This one has been treated with naphthaleneacetic acid. Pineapples are short-day plants which initiate flowers during the fall and winter. The fruits develop during the following spring all at the same time. A large labor force is needed to harvest them, but there is not enough work to do for all these people during the rest of the year. By applying naphthaleneacetic acid and similar compounds, it is now possible to induce flowering in one field after the other and thus to spread the harvest over the entire year.

The next slide will show an interesting result obtained in my laboratory by Dr. Harada with some Japanese chrysanthemums. These, I should hasten to say, are *not* short-day chrysanthemums, but they require a cold treatment in order to flower. It was found that when these chrysanthemums are subjected to cold, a new growth substance appears in the growing point. This new growth substance has been extracted and used to treat other chrysanthemums of the same variety: it caused their flowering under non-inductive conditions. This substance turned out to belong physiologically to the class of gibberellins. In fact, a treatment with gibberellic acid gave the same effect. There are several gibberellins known, which are active in triggering off flowering in a good number of long-day and of cold-requiring species. Other compounds, such as the new dwarfing chemicals, may stimulate flowering in azaleas or holly. Thus Dr. Marth at Beltsville has reported that a drenching spray of "Phosfon" (1.5 gram per liter) applied to rooted holly cuttings in July produced flowers and a crop of berries the following year.

Seeds with long-lasting germinating capabilities

You know very well that seeds, some of them at least, lose their germinating capacity after a short time. On the other hand physiologists have been working on the factors which stimulate or inhibit germination. Thus it was found that one variety, "Grand Rapids", germinates readily at 57°F. but not at 85°F., if maintained in total darkness. What is worse, after having been kept moist for a day or two at 85°F. in the dark and dried again, such seeds do not germinate at 57°F. anymore. The heat treatment in the dark has induced a dormant condition. Now it would be wonderful if one could induce a long-lasting dormancy in seeds. One could then store them for twenty years,

and they would still be as good as the first year. Incidentally, the trick to get Grand Rapids lettuce seeds to germinate after the 85°F. treatment is to give them a little light; they then germinate readily. Quite a few years ago when the Japanese were occupying Manchuria, they discovered in a lake which was drying up, seeds of *Nelumbo* (lotus) in a deep layer of earth. According to the geologists these seeds might have been a thousand years old, and yet they germinated perfectly. Therefore, longevity of seeds can be very long in certain species. In the soil many seeds keep their ability to germinate for a long time. When we will know all about the tricks a seed uses in order to stay viable and yet dormant, even though the soil becomes moist, then we will be able to devise powerful means of storing viable seeds for long periods of time.

Vegetative embryos

Talking about seeds, I would like to mention some exciting news in this field. In 1959 a German plant physiologist, Dr. Reinert, was growing in test tubes callus cultures derived from the root of a carrot. In the course of the experiment, he put these cultures on various media. He observed that some of the cultures behaved differently from the usual pattern and produced little carrot shoots with leaves. Investigating the matter further, he found that the culture mass was full of embryos. In the tissues were embedded what, under the microscope, looked exactly like carrot embryos with cotyledons, roots and buds. Yet there had been no flowers, no pollination. He tried to collect his notes and find out on what media these particular tissues had been grown, but apparently it was difficult to repeat the experiment and find exactly which factors had been responsible for the formation of the embryos. This year Halperin and Wetherell of the University of Connecticut published a paper showing that, in the case of the wild carrot, one could take any piece of the root, the stem, or even the petiole of a leaf and get embryos formed from it in tissue culture. The recipe is to grow the tissues first on a medium containing a strong auxin, such as 2-4-D, and a phytokinin, then to transfer them to a similar medium but without any auxin. The cultures produced hundreds of embryos. These embryos could be germinated, transplanted into soil and grown to maturity, forming normal carrot plants. Perhaps you don't fully realize the importance of this discovery but you will, if you think of animal instead of plant tissues, even human ones. It is possible, at the present time, to grow in the test tube pieces of human tissues, like pieces of skin and other parts. In fact, there are growing in several laboratories cultures of tissues from people who died years ago. Just think that these could now be stimulated to differentiate embryos, and you will have an idea of what has been achieved with carrots. For the plant propagator, this result could mean what I mentioned with the orchid at the beginning: these vegetative embryos are a means to produce plants exactly alike that from which they have been derived.

Bottled Flowers

In closing this presentation, which is not as much science fiction as it sounds, may I just mention a discovery which has been made at the Phytotron at Gif. It is the formation of flowers in test tubes and bottles, as shown in the next slide. If a piece of chicory root is planted in a test tube, under certain conditions which we are studying presently, it will produce a bud which gives rise to a flower. Such a production of flowers has also been obtained on pieces of tobacco stem grown in flasks on artificial nutrients. Other species will be grown in this way in the future, even roses perhaps. At that time then, one will be able, truly, to buy "Four Roses" in a bottle for Saturday night.

On this happy thought let me end my talk about the year 2000. Thank you very much.

DARA EMERY: What was the name of the cytokinin?

DR. NITSCH: The one I mentioned for keeping leaves green was benzyladenine. I think it is now produced commercially. You can get it from the Shell Company; ask Dr. J. Van Overbeek, Shell Agricultural Research Center, Modesto, California.

CHARLES LUGER: Would the cytokinin be of use right now in the spraying of cuttings?

DR. NITSCH: It is at your own risk, but you could try the chemicals now available, although better ones may be developed. A little word of warning: be sure to treat all the leaves that you want to keep on the cutting, because, if you treat only one leaf or two, these treated leaves not only will remain green, but will deplete the food from the other leaves, causing the untreated ones to become yellow faster.

CROCKER TEAGUE: I would like to know what concentration of gibberellic acid you use to produce flowering?

DR. NITSCH: Which flower are you referring to? Chrysanthemum? Well, actually 0.05 mg were applied to the growing point in a lanolin paste. You could obtain similar results with sprays containing about 100 milligrams per liter.

BREEDING NEW FRUITS AND NUT CROPS

DALE E. KESTER

University of California

Davis, California

The Department of Pomology in the California Agricultural Experiment Station has had an active program in fruit and nut breeding for many years. The various aspects of these breeding programs include:

a. Maintenance of collections of species, varieties and breeding materials although there is no attempt to maintain a complete collection.

b. Development and evaluation of new varieties with emphasis on commercial orcharding.