

BEN DAVIS: Did you apply the Simazine-Diphenamid combination to newly transplanted materials?

FRED LANPHEAR: Yes.

BRUCE BRIGGS: How does peat moss or ground bark compare with charcoal in absorbing herbicides?

FRED LANPHEAR: Activated charcoal is by far one of the most effective absorbers of chemicals such as Simazine. Other organic materials such as peat moss, or ground bark, will tie up some herbicides but not nearly so effectively as charcoal. This brings up a point in relation to using herbicides in combination with a mulch. Some materials cannot be used because they are absorbed by the mulch to a point where they are not as effective as they should be. However, it turns out that Casoron is not absorbed by the peat moss and therefore can be used in combination with the mulch.

BRUCE BRIGGS: Have you tried the activated charcoal treatment on peat pots?

FRED LANPHEAR: Yes, we have tried activated charcoal on peat pots and it does work. We dipped the peat pots into a 10% slurry of activated charcoal. However, one problem that you may run into, if you are planting in peat pots when the soil temperatures are warm, is that the root growth out of the peat pots is so rapid that the protection of the carbon on the surface of the peat pot is left behind. Under these conditions it may be necessary to apply a drench of activated charcoal around the plants.

MODERATOR CANNON: Our next speaker will be Mr. Edward Hume who will speak on the subject of cytokinens.

### **CYTOKINENS, POWERFUL FORCES TO BE CONSIDERED IN PLANT PROPAGATION**

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Cytokinens, scarcely known in plant propagation are, along with the gibberellins, opening new horizons in plant manipulations that cannot be ignored by any serious student of the field. These compounds affect more and varied functions in the plant kingdom than most of us consider possible. The rate of increase in research in this field is most astonishing. This paper merely calls attention to some of the findings. Those who wish to investigate the field more thoroughly are referred to Letham (15) and Miller (17) for recent and earlier reviews of the field.

Kinetin was the first product identified in this group and has been the subject of much investigation in this field. Now all compounds of this type affecting plant response are classified as cytokinens. The list of these and the number of different

plants from which they have been extracted is increasing ever more rapidly. These compounds, along with the gibberellins and auxins are the regulators of all cell division, cell enlargement, cell differentiation and the formation of plant organs in developing plants. There is evidence that cytokinins are generated in roots. Rooted leaf cuttings and cuttings treated with cytokinins behave in the same way.

There is no generally accepted method of measuring the effect of cytokinins in plants, as the oat coleoptile method is used to measure auxin activity. Of the some eleven methods that have been used, none are free of complications. This is caused by many possible actions, over wide ranges of concentrations and the complex interactions with gibberellins and auxins.

Cytokinins have been found in at least 40 kinds of plants. Because they are in nature extremely diluted, it requires very large samples and careful concentration and purification to demonstrate their presence. Probably they exist in all living tissue. More actively growing tissue generally has greater concentration; while dormant tissue, such as seeds, have levels too low to be recognized with our present methods of testing. Most of the positive tests have dealt with roots, bleeding sap, germinating seeds and especially developing fruits or nuts.

There is an interaction between cytokinins and the phytochrome mechanism as shown by the germination of light sensitive lettuce seed. Within 24 hours kinetin can be detected in seeds subjected to red light if germination is not inactivated by far red light. This contrasts with the action of the gibberellins which do not interact.

Cytokinins play an important part in cell division. This may modify the size and shape of the resulting plants. This results from interactions of cytokinins with the RNA in the nucleus in a rather complex manner. Cytokinins also affect the movement and accumulation of proteins, amino acids, and phosphorus. They can control the development of storage organs and accumulation of food stored within them (18).

The presence of cytokinins is not restricted to higher plants. They have been found in mosses, fungi, algae and bacteria. In these lower forms kinetin control extends to nonnucleate cells, indicating a direct effect of cytokinins on the cytoplasm (26). Kinetin, which has been the chief material used in research, has the chemical composition of 6-furfuralaminopurine. Its molecular weight is 215, and it is available from Calbio, Box 54282, Los Angeles, California 90054.

One method of putting it into solutions is to float the desired amount of the crystals in a container of water. Then autoclave it until it dissolves at a pressure of 15 psi. It does not dissolve in such common solvents as alcohol, acetone or carbon tetrachloride. Other cytokinins have yet to become commercially available. The most active of these is Zeatin which is identified as 6-(4-hydroxy-3 methylbut-trans-2 enyl)-aminopur-

ine. In order to obtain 0.7 milligrams of the pure material, Miller (17) purified 60 kilograms of immature corn seeds. This compound is active at the tremendous dilution of  $5 \times 10^{-11}$ M. This is a hundred times more dilute than the maximum dilution of kinetin still showing action on plant tissue. To get a better idea of the action of Zeatin, think of dissolving one gram in about a hundred million liters of water.

When tissue culture was first successful, researchers used to add coconut milk to their media to supply some then unknown substance needed to organize callus tissue into new plants. Using kinetin Raghaven (23) and others have been able to develop miniature embryos from callus type tissue without the addition of coconut milk.

Flowering and reproduction can be modified in many ways by the use of cytokinins. Whittwer (24) found that kinetin inhibited the flowering of tomatoes, but accelerated it in peas. Furuta (9) showed that kinetin was synergetic with gibberellic acid in promoting the flowering of unchilled azalea. Ikuma (10) induced flowering of tobacco plants with kinetin and gibberellic acid at two light intensities.

The type of flower produced is also affected by the cytokinin level. Katsumi (11) found that both the diameter and the doubleness of a composite flower can be changed by the cytokinin concentration. Negi (19) converted a male flower into a perfect one. Cytokinins can stimulate fruit set or induce parthenocarpy (fruit formation without seed). Of special interest to plant propagators is the work of Kummerlow and Choffmann (14). They applied kinetin at 50 and 100 ppm/ to *Pinus radiata* seedlings which greatly stimulated meristematic activity, stretching normally short shoots into long ones; and stimulating renewed division of parenchyma cells of the wood. Caperson (3) also stimulated cambial activity by applying kinetin to the apical end of cotyledons.

Apical dominance in plants is controlled by the interaction of auxins, cytokinins and gibberellins. Davis (6) found that kinetin promoted the uptake of indoleacetic acid which maintained the apical dominance by inhibiting the growth of lateral buds. At the same time the IAA stimulated the downward movement of the kinetin. But Bauer (1) found that kinetin cancelled apical dominance in a moss. Panigraph and Audus (21) made similar observations on the cotyledonary buds of decapitated *Vicia Faba* seedlings.

Still another role discovered for cytokinins is their ability to overcome some of the lethal effects of nuclear radiation. Powell and Griffith (22) found that levels of beta radiation which were lethal to untreated bean leaf sections did not kill sections treated with kinetin. Cope (5) found similar results with *Lemna*, an aquatic, when they were exposed to glucose solutions containing varying levels of deuteration. Apparently kinetin can supply something de-activated by the radiation, but not far beyond the normal tolerance level.

A most extraordinary property discovered for these cytokinens is their ability to preserve the chlorophyll in a functioning condition under long periods of very low light intensity or even total darkness where the untreated leaves turn yellow and can no longer function. The mechanism for this still is not clear; but appears related to the higher nitrogen level, particularly the protein and amino acid in the kinetin treated tissue. The phosphorus is also higher which supplies a key link in the photosynthetic action. There is also a higher level of proplastids in kinetin treated plants. All these actions are, of course, involved with the various enzyme actions. The work on these is too long to consider at this time.

Kinetin also affects leaf retention. Burrows and Carr (2) placed kinetin on the pulvinar region, a hairy flattened place at the base of the petiole, or introduced it through the transpiration stream. It delayed abscission either way. These workers in another paper (4) found that lupine leaflets on treated leaves dropped their leaves faster if they were young and in the dark; but older leaves retained the leaflets longer. In the light they stayed on longer regardless of age.

Cytokinens have a peculiar role in relation to chromosomes and gene behavior. They not only interact with RNA in regular cell operation, but they may induce doubling of the number, or polyploidy. Or they may overcome the mutating action of colchicine (13).

Under some conditions cytokinens may act in controlling disease. Selmann (24) has shown that kinetin inhibits virus multiplication through an increase in the ribonuclease activity. In another experiment he found petunia leaf strips had fewer and smaller local lesions on the lower surface following mechanical inoculation of the upper with kinetin. Lovenkovich (16) indicates that kinetin is an antagonist to the toxic effect from the fungus *Pseudomonas tabacci*. Dekker (7) found that 20 ppm. of kinetin, while not suppressing the initial inoculation of cucumber leaf discs floating in the solution, did stop further growth of the disease.

There will probably be many more papers in the next few years helping us to understand more about these remarkable substances.

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MODERATOR CANNON: Our next paper will be on the subject of "Crossing Eastern Cottonwood in the Greenhouse" and will be presented by Robert Farmer, Jr.