ther studies. As is noted in a preceding paragraph, the action of red light is reversible by subsequent irradiation of seeds with far red light. The effect of gibberellin has not been reversed by a period of exposure to far red light that is sufficient to reverse fully the effect of red light. However, since gibberellin that has entered the seeds cannot be removed, the lack of reversal of the gibberellin effect by far red light cannot be considered definitive.

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6. This work was supported in part by research grant RG-3939 from the National Institutes of Health, U.S. Public Health Service (principal investigators, A. Lang and S. G. Wildman). A description of the details of these experiments and the results of further work now in progress is in preparation.
7. Part of the results reported here were presented at the annual meeting of the American Society of Plant Physiologists, Storrs, Conn., Aug. 1956; abstract in *Plant Physiol.* 31, Suppl., 37 (1956).
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## Survey of Fungi and Actinomycetes for Compounds Possessing Gibberellinlike Activity

Culture filtrates of the fungus *Gibberella fujikuroi* contain the plant growth-promoting compounds gibberellins A and B (*1*) and gibberellic acid (*2*). Recently, it has been shown that extracts of higher plants contain gibberellinlike compounds capable of stimulating growth in plants (*3*). This report describes an attempt to demonstrate plant growth-promoting activity similar to that of the gibberellins in the culture filtrates of various fungi and actinomycetes. Approximately 1000 fungus and 500 actinomycete culture filtrates were tested for the presence of these compounds.

The methods used to obtain culture filtrates of the various microorganisms were described in an earlier paper (*4*). In brief, the fungi were cultured in 500-ml erlenmeyer flasks containing 100 ml of corn steep-cerelose medium (Staley's corn steep liquor, 40.0 g; cerelose, 40.0 g; $CaCO_3$, 3.5 g; $NaNO_3$, 3.0 g; $K_2HPO_4$, 0.5 g; $MgSO_4$, 0.25 g; and deionized water, 1000 ml). Following inoculation, the flasks were placed on a reciprocating shaker (99 to 100 cy/min with 3-inch strokes) at 28°C for 7 days. The mycelial growth in each flask was removed by filtering through Whatman No. 1 filter paper and discarded. One drop of Tween 80 was added to 100 ml (approximate) of culture filtrate, which was adjusted to *p*H 5.0. The culture filtrates were tested without dilution or concentration.

The methods used to obtain the actinomycete culture filtrates were similar to those described in the preceding paragraph. The shake-flask medium (*p*H 7.0) was made up as follows: bacto-peptone, 5.0 g; glucose, 10.0 g; molasses (Brer Rabbit Green Label), 20 ml; $FeSO_4 \cdot 7H_2O$, 0.01 g; and distilled water, 1000 ml. The cultures were incubated on a reciprocating shaker (114 cy/min with 2-inch strokes) for 5 days at 30°C. The culture filtrates were treated as described for fungi and frozen until they were needed.

Corn seedlings (the single cross WF9 × 38-11) were grown in soil in 6-inch pots (six plants per pot). When the seedlings were 6 to 8 cm in height, they were treated by filling the whorls with the culture filtrates on each of two alternate days. The treated plants were allowed to grow for 10 to 12 days, when their heights were measured. Water and the uninoculated culture medium (without carbon source) served as the controls. Twelve to 18 plants were used for each treatment.

By use of the methods described, it was readily demonstrated that the heights of plants that were treated with the culture filtrate of *Gibberella fujikuroi* were 50 to 75 percent greater than those of the controls. Although some 1500 culture filtrates from other sources were used in treating corn plants, in no case was growth stimulation observed. The majority of the culture filtrates tested were obtained from unidentified fungi and actinomycetes that were obtained from soil by routine plating-out procedures. However, 258 filtrates were obtained from organisms which were identified as to genus or species. Table 1 summarizes briefly the major groups of organisms that were tested and the number of genera and species included in each group (*5*).

The number of organisms tested in these studies is admittedly only a small fraction of the total number of identified microorganisms. Furthermore, they were grown on only one medium and tested at only one concentration. In spite of these limitations, it appears that the production of the gibberellins by fungi and actinomycetes is not widespread (*6*).

Table 1. Summary of identified fungi and actinomycetes tested for growth stimulation in corn.

| Major groups included in experiments | No. of genera tested | No. of species tested |
|---|---|---|
| Schizomycetes | | |
| Actinomycetales | 2 | 7 |
| Phycomycetes | | |
| Peronosporales | 2 | 7 |
| Mucorales | 16 | 36 |
| Entomophthorales | 1 | 1 |
| Ascomycetes | | |
| Endomycetales | 5 | 8 |
| Eurotiales | 9 | 31 |
| Sphaeriales | 7 | 11 |
| Hypocreales | 2 | 2 |
| Pezizales | 3 | 3 |
| Basidiomycetes | | |
| Ustilaginales | 1 | 1 |
| Agaricales | 3 | 3 |
| Fungi Imperfecti | | |
| Sphaeropsidales | 3 | 3 |
| Melanconiales | 1 | 1 |
| Moniliales | 42 | 141 |
| Mycelia-Sterilia | 3 | 3 |

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## Occurrence of Iron, Copper, Calcium, and Magnesium in Tobacco Mosaic Virus

Cooper and Loring reported the finding of small amounts of acid-soluble materials when ultracentrifugally purified tobacco mosaic virus was treated with cold trichloroacetic acid (*1*). Examination of a concentrate of this fraction by paper chromatography (70 percent *tert*-butyl alcohol, 0.8*N* HCl) showed that

a component was present which behaved like ferrous iron when the chromatogram was sprayed with the perchloric acid-molybdate reagent of Hanes-Isherwood (*2*). A study of the emission spectra of a sample of virus ash confirmed the occurrence of iron in the virus and provided evidence for copper, calcium, and magnesium as well.

In order to establish the amounts of the four metals present, quantitative chemical analyses (*3*) were performed on 0.1*N* HCl solutions of virus ash prepared from different virus samples that had been purified by three or four ultracentrifugal purification cycles (*4*). The results confirmed the data obtained by paper chromatography and by emission spectroscopy and provided average values (Table 1) of about 30 μg of iron, 20 μg of copper, 300 μg of calcium, and 600 μg of magnesium per gram of virus.

In order to obtain evidence regarding the type of binding involved between the metallic components and the virus, various types of dialysis and ultracentrifugation experiments which would be expected to remove adsorbed metallic impurities were carried out. The types of treatment included equilibration in and ultracentrifugation from 0.5-percent ethylenediaminetetraacetic acid (EDTA) solution (*5*) at *p*H 7 and *p*H 8 and dialysis (*6*) at 4°C against water and 0.5 percent EDTA at *p*H 7. In each case, the virus was recovered by ultracentrifugation, lyophilized, and ashed, and aliquots of the 0.1*N* HCl solution of ash were analyzed.

The results of these experiments are summarized in Table 1. They show decreases particularly in calcium and magnesium content after treatment with or dialysis against EDTA, but iron and copper were relatively unaffected. After dialysis against EDTA for 7 days, further decreases appeared to occur, but significant amounts of all four components were still present. Similarly, dialysis against distilled water for 24 hours showed a significant decrease in magnesium content, but the concentrations of the other three metallic components were largely unchanged.

The recovered virus in each of the experiments shown was tested for activity by the local-lesion method (*7*) against the same concentration of untreated, purified virus. The half-leaf method was used on five or six of the largest leaves of the Holmes necrotic-type *Nicotiana tabacum* plants (*8*) 8 to 14 inches tall. From the activity measurements shown in Table 2, it may be seen that highly active virus comparable to the original was recovered in all cases with the possible exception of the sample that was dialyzed against EDTA at *p*H 7 for 7 days. In this instance, the dialyzed virus was probably slightly less active than the untreated preparation that was stored in the refrigerator over the same period of time.

Table 1. Metal content of purified tobacco mosaic virus before and after various treatments. The values are averages for at least two analyses, usually on two samples of ash.

| Treatment | Metal content (μg/g of virus) | | | |
|---|---|---|---|---|
| | Fe | Cu | Ca | Mg |
| Virus after three purification cycles* | 29 | 23 | 330 | 670 |
| Equilibration with 0.5 percent EDTA at *p*H 7 | 27 | 13 | 84 | 48 |
| Equilibration with 0.5 percent EDTA at *p*H 8 | 54 | 21 | 95 | 50 |
| Dialysis against 5 percent EDTA at *p*H 7 for 24 hours | 47 | 16 | 200 | 100 |
| Dialysis against water for 24 hours | 42 | 22 | 350 | 200 |
| Dialysis against 0.5 percent EDTA at *p*H 7 for 7 days | 22 | 4 | 42 | 22 |

* Average values for two different samples of virus.

It was of interest to determine whether the metallic components found in the virus were associated with the virus protein, with its ribonucleic acid, or with both. Analyses of a sample of the nucleic acid prepared by the method of Johnson and Harkins (*9*) from purified virus showed that all four metallic components were present and that the iron and magnesium occurred in about 20 times the concentration present in the virus itself. The amounts found in micrograms per gram of nucleic acid were as follows: iron, 640; copper, 31; calcium, 210; magnesium, 1900. Dialysis of a 2.5-percent solution of the virus nucleic acid at 4°C for 24 hours against 0.1*M* phosphate at *p*H 7 eliminated the calcium completely and decreased the magnesium concentration to about one-tenth of the original value but had only a slight effect on iron and copper. Dialysis against a mixture of 0.1*M* phosphate and 0.5-percent EDTA under the condition mentioned reduced the iron content to approximately one-fourth of the original value, eliminated copper and calcium completely, and decreased the magnesium content only slightly beyond that found after phosphate dialysis.

In agreement with earlier reports of Zittle (*10*) and Jungner (*11*), copper and magnesium were also found in two samples of commercial yeast sodium ribonucleate. No calcium was found, but iron was present in amounts comparable to that mentioned for the virus nucleic acid. The respective average values in micrograms per gram for the two samples were as follows: iron, 590 and 380; copper, 340 and 500; and magnesium, 900 and 800.

Although various lines of evidence have shown that the tobacco mosaic virus consists essentially of protein and nucleic acid (*12*, p. 18), the virus has not been examined previously for the metallic components mentioned as far as we are aware. The data presented here show that small amounts of relatively firmly bound iron, copper, calcium, and magnesium also occur in this virus. These results extend the earlier findings of trace metals in certain animal viruses (*13*) to a plant virus of a high degree of homogeneity and of relatively simple biochemical complexity. Strong confirmation has thus been provided that iron, copper, magnesium, and probably calcium are integral viral components. In the present experiments it has not been possible to determine precisely the lower metal concentration compatible with full virus activity. An approximation of this, however, appears to be the concentra-

Table 2. Infectivity of tobacco mosaic virus after various treatments.

| Treatment | Virus concn.* (g/ml) | No. of half-leaves | Avg. No. of lesions per half-leaf† | |
|---|---|---|---|---|
| | | | Treated | Control |
| Equilibration with EDTA at *p*H 8 | | | | |
| First treatment | $10^{-4}$ | 10 | 24 | 24 |
| Second treatment | $10^{-5}$ | 9 | 8 | 10 |
| Dialysis against 0.5 percent EDTA at *p*H 7 for 24 hours at 4°C | $10^{-6}$ | 11 | 10 | 9 |
| Dialysis against 0.5 percent EDTA at *p*H 7 for 7 days at 4°C | | | | |
| First test | $10^{-5}$ | 15 | 12 | 15 |
| Second test | $5 \times 10^{-5}$ | 38 | 17 | 24 |
| Dialysis against water for 24 hours at 4°C | $10^{-5}$ | 10 | 93 | 50 |

* Dilutions prepared in 0.1*M* potassium phosphate buffer at *p*H 7.
† Differences, with possible exceptions of the samples dialyzed against EDTA for 7 days and against water, are not of statistical significance.

tions found in the virus dialyzed for 7 days against EDTA. Assuming a molecular weight for the virus of $50 \times 10^6$, calculation of the number of atoms of iron, copper, calcium, and magnesium per virus particle gave values of 20, 3, 52, and 45, respectively.

The fact that iron and magnesium were concentrated approximately 20-fold in the virus ribonucleic acid compared with the occurrence of about 5 percent nucleic acid in the virus indicates that these metals are located in the nucleic acid rather than in the protein. That their occurrence in the virus nucleic acid in the concentrations found may be of more general significance in relation to nucleic acid chemistry is indicated by the comparable amounts found in yeast ribonucleic acid. The relatively strong binding of the virus ribonucleic acid for iron and magnesium as shown by the dialysis experiments against EDTA (*5*) indicates the existence of metal chelates or relatively stable metal complexes in ribonucleic acid structure. While the function of the individual metals in the virus or in its nucleic acid is not clear at present, some of them may be involved in the binding of smaller nucleic acid subunits into the larger asymmetric particles characteristic of "native" and infectious nucleic acid (*14*). Since the tobacco mosaic virus undergoes a disintegration into relatively small units soon after inoculation (*15*), it appears that the larger nucleic acid may undergo a subdivision into smaller genetic subunits during the infection process in a manner similar to that shown for bacteriophage by Doermann (*16*) and by Visconti and Delbrück (*17*). A further logical hypothesis is that the genetic subunits, the "vegetative phase" of Visconti and Delbrück, are joined into the larger asymmetric particles, the "genetic recombinants" characteristic of "native" and infectious ribonucleic acid by metal chelate bonds. Although no analyses for the metallic components considered in this paper have yet been made on "native" deoxyribonucleic acid, it is known that magnesium is present (*11*). The occurrence of smaller deoxyribonucletic acid units joined by metal chelate bonds into the highly asymmetric particles characteristic of "native" deoxyribonucleic acid would appear to provide a rational explanation for many of the physical (*18*) and biological (*19*) properties of this nucleic acid also (*20*).

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## Enzymatic Conversion of D-Glucose to D-Fructose

Xylose isomerase, which catalyzes the interconversion of D-xylose and D-xylulose, has been demonstrated in extracts of xylose-grown cells of *Pseudomonas hydrophila* (*1*), *Lactobacillus pentosus* (*2*), and *Pasteurella pestis* (*3*). Although it has been reported that other aldose-ketose isomerases (*4, 5*) isomerize series of structurally related aldoses, xylose isomerase has been described as unable to act on aldoses other than D-xylose. However, experiments in these laboratories have shown that sonic extracts and washed, lyophilized cells of xylose-grown *Pseudomonas hydrophila* (N.R.C. 491 and 492) (*1*) do in fact convert D-glucose to D-fructose. Similarly, 6-deoxy-D-glucose was converted to a sugar that readily reacted in the cysteine-carbazole test (*6*) and exhibited an $R_f$ in paper chromatography substantially greater than that of 6-deoxy-D-glucose. It seems reasonable to assume that the sugar formed is 6-deoxy-D-fructose (*5*).

The ability of the enzyme preparations to isomerize D-xylose or D-glucose is a concomitant of growth in the presence of D-xylose as the major carbon source. Growth on D-glucose, D-fructose, or maltose gives rise to cells which are essentially devoid of isomerase activity for either D-xylose or D-glucose.

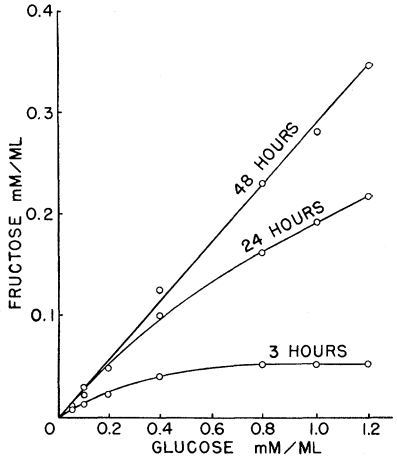


Fig. 1. Formation of D-fructose as a function of incubation time and initial D-glucose concentration. The final concentrations of the components of the incubation mixtures were as follows: arsenate buffer (*p*H 8.0), 0.05*M*; $MgCl_2$, 0.01*M*; washed lyophilized cells of *Pseudomonas hydrophila* (N.R.C. 492), 10 mg/ml; and D-glucose as indicated. Final volume was 2.0 ml, and the incubation temperature was 40°C. The reaction was stopped by withdrawing 0.25-ml aliquots into 4.75 ml of 0.5*M* $HClO_4$. After centrifugation and suitable dilution, fructose was estimated by a modification (*9*) of the cysteine-carbazole test. All values were corrected for the color contributed by D-glucose. The color contributed by the enzyme preparation was negligible.