that follicular smooth muscle cells (found only in Amphibia) are necessary for the extrusion of ova. However, the observations that $10^{-4}M$ KCN does not inhibit the normal contractions of intact, excised ovaries and that autonomic blocking agents, antihistamine, and smooth muscle depressants do not inhibit pituitary-induced ovulation suggest that the energy-consuming process is not muscular contraction. Ovulation seems more likely to be related to proteolysis of the stalk membrane or to more obscure processes supporting increased intrafollicular pressure.

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Effect of Gibberellin on Germination of Lettuce Seed

The gibberellins are a group of plant regulators that were discovered nearly 20 years ago by Japanese scientists (*1*), and lately these compounds have attracted the attention of plant physiologists in many countries (*2*). The gibberellins which are known chemically are produced by strains of the fungus *Fusarium moniliforme* (Sheld.) Snyder and Hansen emend., the asexual stage of *Gibberella fujikuroi* (Saw.) Wr. Materials with identical physiological activity have been shown to occur also in flowering plants (*3*). The gibberellin effect that was observed first and has been studied in a wide variety of plants is promotion of stem elongation. More recently, however, it was found that the gibberellins also have profound morphogenetic effects; they induce bolting and flower formation in cold-requiring plants and in long-day plants under temperature and light conditions which usually do not permit flowering (*4*).

The finding that gibberellin can "replace" light (long days) in flower induction of long-day plants led to our study of the effects of gibberellin on the germination of light-requiring seeds, since the action spectra of the light control of flowering and of seed germination are similar (*5*). The purpose of this report is to summarize the findings that have been obtained to date (*6, 7*).

All our experiments have been performed with seeds of lettuce (*Lactuca sativa* L., variety Grand Rapids). Several types of light effects are known for lettuce seed. Some lots yield low germination in the dark, and germination may be increased by a brief exposure to light; they may be said to have a "primary" light requirement. Other lots exhibit equal or nearly equal germination percentages in light and in darkness, but a "secondary" light requirement can be induced in at least two different ways: (i) if such seeds are imbibed and stored at a temperature of 35°C, their percentage germination in darkness is greatly reduced and can be restored to the original level or higher by a brief exposure to light (*5*); (ii) the presence of an osmotically active material in the medium reduces germination in the dark in direct proportion to the osmotic pressure of the solution, and this "dark-osmotic inhibition" is also released by a small quantity of light (*7*). It is likely that the basic mechanism of these light actions is the same, red light having the greatest effect in promoting germination of lettuce seed, and its effect being reversible by subsequent irradiation with far red light.

We have worked with two lots of seeds, one (seed lot A) possessing the primary type of light requirement and the other (seed lot B) acquiring the secondary type during pretreatment with high temperature or in the presence of an osmotically active material such as mannitol in solution (*8*). The gibberellin preparation used in these experiments consisted of a mixture of gibberellin A_1 (gibberellin A) and gibberellin A_3 (gibberellic acid) and will henceforth be called "gibberellin" (*9*).

Table 1 shows that the primary light requirement of seed lot A is bypassed by the addition of gibberellin. In the presence of 100 mg of gibberellin per liter, germination in darkness is as high as germination after a brief exposure to red light. An identical result has been reported by Lona (*10*) for light-requiring seeds of a wild species of lettuce, *Lactuca scariola* L.

Table 2 shows that, when gibberellin is present during pretreatment of seed lot B with high temperature, no secondary light requirement becomes apparent. As is shown in Table 3, gibberellin also promotes germination in darkness after a dependency on red light has been established by pretreatment with high temperature; thus, it removes the secondary light requirement formed by the pretreatment. Table 4 shows that gibberellin also reduces or negates dark-osmotic inhibition when it is given simultaneously with the inhibitory solution or when it is supplied as a pretreatment.

Thus, in lettuce seed, gibberellin apparently can substitute for red light in all cases examined in which such light has a promotive effect on germination; it "replaces" the primary light requirement that is typical of certain seed lots, and it prevents or releases the secondary light requirement that can be created in other lots. Whether these effects are based on a common mechanism, and how they are related to the effect of red light, will have to be the subject of fur-

Table 1. Effect of gibberellin on germination in the dark of lettuce seed possessing a natural ("primary") requirement of red light for germination (seed lot A).

Treatment	Germination (%)
Water	24
Water; 3 min of red light	70
Gibberellin (100 mg/lit)	70

Table 2. Effect of gibberellin supply during treatment with high temperature on subsequent germination of lettuce seed in darkness at 21°C (seed lot B).

Treatment (5 days at 36°C)	Germination (%)
Water	20
Water; 10 min of red light following high-temperature period	94
Gibberellin (50 mg/lit)	92

Table 3. Effect of gibberellin supply after pretreatment with heat on germination of lettuce seed in darkness at 21°C (seed lot B). All seeds were given 5 days at 36°C on water and were completely dried following the pretreatment with heat.

Treatment	Germination (%)
Reimbibed on water	26
Reimbibed on water; then 10 min of red light	94
Reimbibed on gibberellin (50 mg/lit)	45
Reimbibed on gibberellin (100 mg/lit)	68

Table 4. Effect of gibberellin on dark-osmotic inhibition of lettuce seed (seed lot B); 0.15M mannitol was used.

Pretreatment (6 hr)	Solution	Gibberellin (mg/lit)	Germination (%)
None	Water	0	82
None	Mannitol	0	22
None	Mannitol	35	61
Water	Mannitol	0	23
Gibberellin (50 mg/lit)	Mannitol	0	87

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).
8. Seed lot A was kindly supplied by Carlos O. Miller of the department of botany, University of Wisconsin, Madison; seed lot B was obtained commercially from Ferry Morse Seed Co.
9. $[\alpha]_D^{25} + 62°$. We wish to express our gratitude to Frank H. Stodola, U.S. Department of Agriculture, for supplying the preparation.
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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