

number of other strontium phosphates (12) that have a reflection corresponding to a particular *d*-value calculated from the fragment of strontium-rich enamel.

The  $\text{Sr}_6\text{H}_3(\text{PO}_4)_5 \cdot 2\text{H}_2\text{O}$  contains many *d*-values common to an apatite, in addition to reflections, corresponding to the unique lines of the pattern of the intact fragment, with *d*-values of 6.48, 4.36, 3.35, 2.98, and 2.87 Å (Table 1). These results strongly suggest that  $\text{Sr}_6\text{H}_3(\text{PO}_4)_5 \cdot 2\text{H}_2\text{O}$  was present in the incisor enamel. This view is supported by precipitation studies (13) showing that  $\text{Sr}_6\text{H}_3(\text{PO}_4)_5 \cdot 2\text{H}_2\text{O}$  immediately precedes the formation of strontium hydroxyapatite.

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Table 1. Estimated amounts (micrograms per milliliter of culture medium) of gibberellin from cultures of fungi. Responses were compared to a dosage response curve for GA<sub>3</sub> with the dwarf-5 *Zea mays* assay. Assay response is the average of the sums of the length of the first and second leaf sheaths (*N* = 5).

Strains	Assay response (mm)	GA <sub>3</sub> equivalents (μg/ml)
	<i>Parental</i>	
2794a	*74 ± 4	2.40
2900A	89 ± 8	0.05
	<i>Progeny, ascus 1</i>	
1	*81 ± 3	3.75
2	*73 ± 4	2.16
3	66 ± 6	0.01
4	79 ± 6	0.03
	<i>Progeny, ascus 2</i>	
1	*71 ± 4	1.56
2	*84 ± 4	4.50
3	48 ± 3	0.00
4	68 ± 5	0.01
	<i>Progeny, ascus 3</i>	
1	*67 ± 5	1.05
2	*71 ± 9	1.56
3	86 ± 5	0.05
4	83 ± 4	0.04
	<i>Progeny, ascus 4</i>	
1	*87 ± 6	5.40
2	*81 ± 5	3.75
3	66 ± 4	0.01
4	77 ± 1	0.03
Controls	49 ± 3	

\* Extract diluted 100-fold before assay.

## Gibberellin Production: Genetic Control in the Fungus *Gibberella fujikuroi*

Abstract. *The fungus Gibberella fujikuroi* (Saw.) Wr. can be used in genetic studies of the production of gibberellins. A gene has been identified which controls a step in the biosynthetic pathway of gibberellin production. Apparently this step is early in the pathway for it affects the accumulation of all of the gibberellins produced by the fungus.

The gibberellins are a class of naturally occurring compounds that regulate a variety of growth and developmental processes in plants (1). While there is no evidence to indicate that gibberellins affect the growth of fungi, they are accumulated in large numbers and amounts by the fungus *Gibberella fujikuroi* (Saw.) Wr. (imperfect stage, *Fusarium moniliforme* Sheldon) (2). This report will show that *Gibberella fujikuroi* can be used to investigate the genetic control of gibberellin production. These studies provide evidence for a gene that controls the amount and kinds of gibberellins produced by the fungus.

Genetic studies with *G. fujikuroi* have been difficult in the past because of the inability to consistently obtain the sexual stage in the laboratory. However, perithecial production was recently induced when strains of opposite mating type were grown on a *Citrus* stem medium (3, 4).

Crosses were made by the simultaneous inoculation of this medium with strains of opposite mating type (5). Ma-

ture perithecia developed in 3 to 6 weeks. They were removed and ruptured in water, and the asci were placed on a medium of 2 percent agar. The ascospores from an individual ascus were separated with a micromanipulator and transferred individually to slants of potato-dextrose agar (6).

Preliminary studies were made to determine whether ascus development was normal, that is, whether the ascospores from one ascus represented all four meiotic products (a tetrad). The strains mated in this study varied with respect to pigments that diffused into the medium, pigmentation of mycelium, and texture of the mycelial pad. On the basis of these visual phenotypes, cultures derived from the eight ascospores of one ascus could always be grouped into four pairs. This was interpreted as indicating that all four meiotic products were recovered from each ascus. Since the spore pair derived from each meiotic product could be identified, it was necessary to test only one member of each pair. Fifty-eight tetrads were also tested for mating type (5); in each tetrad

there was the expected 2 : 2 segregation for mating type. Cultures classified as being derived from the same meiotic product always had the same mating type. We concluded that meiosis and ascus development in this fungus follow a conventional pattern.

The amounts and kinds of gibberellins produced by a strain were determined from extracts of the medium on which the fungus was grown. Cultures were grown for 1 week in 50 ml of liquid potato-dextrose medium in 125-ml erlenmeyer flasks. The cultures were maintained at 23°C on a rotary shaker (180 rev/min). After the incubation period, each culture was centrifuged to remove the mycelium. The supernatant medium was then adjusted to pH 2.5 and extracted three times with one-half volumes of ethyl acetate. The ethyl acetate fractions were combined, air dried, and assayed. The total amount of gibberellin-like materials in these extracts was estimated by the dwarf-5 *Zea mays* bioassay (4, 7). Individual gibberellins were separated from each other by several thin-layer chromatographic systems (8); their presence and position on the chromatograms were determined by bioassay of eluates from the chromatograms and by fluorescence on the chro-

matographic plates when viewed under ultraviolet light. The gibberellins, GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub>, GA<sub>7</sub>, and GA<sub>9</sub>, were identified by their chromatographic similarity to authentic samples (9). Other gibberellins were not identified because standards were not available.

A cross was made between two strains of *G. fujikuroi* (2794a × 2900A) that differed in their gibberellin phenotypes (Table 1). The high-producing strain, 2794a, produced GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub>, GA<sub>7</sub>, and GA<sub>9</sub>, plus other unidentified gibberellin-like materials. The low-producing strain, 2900A, produced only trace amounts of GA<sub>4</sub> and GA<sub>7</sub>; GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>9</sub>, and other gibberellin-like materials were not detected. Four asci from this cross were dissected. In each case, there was a 2:2 segregation for gibberellin production (Table 1); cultures derived from two of the tetrad of spores produced gibberellin, whereas cultures derived from the remaining two spores produced little or no gibberellin. The response differences between the high-producing and the low-producing strains were of the order of 100-fold. The low-producing progeny had either no detectable gibberellin or only trace amounts of GA<sub>4</sub> and GA<sub>7</sub>. The high-producing progeny, with one exception, had relatively large amounts of GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub>, GA<sub>7</sub>, and GA<sub>9</sub>, plus other unidentified gibberellin-like materials.

A second cross was made with the progeny obtained from the first mating. One of these progeny strains repeatedly failed to produce any detectable gibberellins or gibberellin-like substances, whereas the other was indistinguishable from the high-producing parent (strain 2794a). From this cross 91 asci were dissected. In each case there was again a 2:2 segregation for gibberellin production. These results suggest that total gibberellin production is under the control of a single pair of alleles.

Among the four tetrads studied in the first cross, one was of special interest. Although there was a 2:2 segregation for gibberellin production, qualitative differences were observed among the producing members of the tetrad. One strain produced relatively large amounts of GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub>, GA<sub>7</sub>, and GA<sub>9</sub>, plus other unidentified gibberellin-like materials; the other strain also produced relatively large amounts of GA<sub>4</sub>, GA<sub>7</sub>, and GA<sub>9</sub>, plus unidentified gibberellin-like materials, but no GA<sub>1</sub> or GA<sub>3</sub>. One explanation for the latter phenotype may be the presence of a second mutant gene which affects the

production only of GA<sub>1</sub> and GA<sub>3</sub>.

Our results indicate that *Gibberella fujikuroi* can be used for genetic investigations of gibberellin production. The analysis of 95 asci provides evidence that a single pair of alleles controls total gibberellin production. This gene may exert its effect early in the gibberellin biosynthetic pathway, since it controls the production of all the gibberellins and gibberellin-like materials assayed for in this study.

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3. The crossing medium was prepared by autoclaving young stems of *Citrus medica* L. in 125-ml erlenmeyer flasks which contained 10 ml of water. The medium was autoclaved for 15 minutes. Inoculations were made the same day as the medium was autoclaved.
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## Metacontrast: Its Relation to Evoked Potentials

*Abstract. Electrophysiological correlates of metacontrast were studied by means of averaged evoked potentials recorded from the scalp in man. Under conditions in which the brightness of the first of two successive stimuli appears diminished there is no accompanying attenuation of the evoked potentials to that stimulus. The results suggest that the amplitude and latency of evoked potentials correlate with stimulus intensity but not with brightness.*

When two equally intense visual stimuli with adjacent contours are presented in rapid succession, the brightness of the first stimulus appears greatly reduced. This type of brightness suppression, generally referred to as metacontrast (1), is one of several visual phenomena showing that brightness can be modified by a temporal interaction between stimuli.

Metacontrast has been extensively studied by psychophysical methods (2). It is readily observed under these conditions: A disk is presented very briefly and is followed, after a variable interval, by a surrounding ring of equal area, intensity, and duration. When the interval between disk and ring is short (0 to 10 msec), both are clearly seen. As the interval is increased, the brightness of the disk diminishes. At interstimulus intervals between 40 and 100 msec, metacontrast suppression becomes maximal and the disk virtually disappears. With further increases in the interstimulus interval the disk becomes progressively brighter again. When the two stimuli are separated by 200 to 250 msec, the disk appears to have

regained its original brightness. Throughout a sequence of such presentations, the appearance of the ring remains relatively unchanged.

Several different theories have been proposed to explain metacontrast suppression in terms of retinal (3), subcortical (4), and cortical (5) interactions between neural responses to the two stimuli. In order to evaluate such interpretations, one should be able to specify the neural correlates of brightness perception. This is not yet possible, but recent work with evoked potentials recorded from the scalp in man has shown that evoked potential amplitude increases and latency decreases as stimulus intensity (and therefore brightness) is increased (6). Are these covariations due to the altered stimulus intensity, or to the change in brightness, or both? In attempting to answer this question we wished to know whether the brightness reduction observed under metacontrast conditions (where brightness changes but intensity does not) is accompanied by evoked potential changes comparable to those that normally occur when stimulus intensity