10. W. Kalow, *J. Pharmacol. Exptl. Therap.* **104**, 122 (1952).
11. R. Ammon, *Arch. ges. Physiol. Pflüger's* **233**, 486 (1934).
12. E. G. Erdös *et al.*, Abstracts of Communications, XX International Physiological Congress (1956), p. 274.
13. J. B. S. Haldane, *Enzymes* (Longmans, Green, London, 1930), p. 84; see also D. R. P. Murray, *Biochem. J.* **24**, 1890 (1930).

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Role of Polyphenolase in Streptomycin-Induced Resistance to Phytophthora in Potato

Some information has been obtained about the biochemical mechanisms of resistance to plant diseases from study of the potato, *Phytophthora infestans* de By. complex. Rubin and co-workers (*1*) and the Göttingen school (*2*) succeeded in demonstrating that the activation of polyphenolases (tyrosinase) at the site of infection may lead to the accumulation of polyphenol derivatives. The increase in these highly fungitoxic substances being more intense in resistant than in susceptible varieties, their accumulation may be regarded as an important factor contributing to disease resistance.

Recently Müller *et al.* (*3*) were able to show that, if streptomycin is absorbed by potato or tomato plants through their roots, these plants become resistant to *Phytophthora*. The effect is indirect, for the fungus is known to be highly insensitive to streptomycin *in vitro*. Similar data that pertain to other host-parasite complexes have been described (*4*).

This study was undertaken to shed some light on the mechanism of streptomycin action. As is shown, streptomycin absorbed by the potato tissues greatly enhances their polyphenolase activity. It seems, therefore, that both the natural and streptomycin-induced resistance of potato depend on the same biochemical mechanism.

Whole potato sprouts or detached leaves, or both, were placed in streptomycin sulfate solutions (100 ppm in tap water). Controls were treated similarly but were placed in pure tap water. Samples were taken for the assays every two days for a week. The leaves used for the experiments were cut into halves. One half was used for the determination of polyphenolase activity and the other for the assay of the streptomycin content. Enzyme activity was measured in homogenates by the use of conventional manometric procedures. Catalytic amounts of catechol were used as substrate, and hydroquinone was chosen as a suitable reductant (*5*). Streptomycin was assayed according to the method of Pramer (*6*), with *Bacillus subtilis* as a test organism.

In several consecutive experiments, 20 assays of polyphenolase activity were carried out; as a result of treatment with streptomycin, strong stimulation was found in each case (30 to 110 percent). Slight stimulation was found in the early stages of treatment, when streptomycin was present only in traces in the tissues. Higher streptomycin content was generally correlated with higher polyphenolase activity. Representative data are shown in Table 1. Autooxidation and trace-element catalysis of substrates was estimated by use of boiled controls. The data in the tables have been corrected for autooxidation values.

Table 1. Polyphenolase activity and streptomycin content in potato leaves treated with streptomycin. Enzyme activity is expressed as the increase in oxygen uptake upon addition of substrates (0.02 percent catechol and 0.6 percent hydroquinone) in cubic millimeters of oxygen per milligram (fresh weight) of tissue homogenate, per hour.

Hours after treatment	Polyphenolase activity		Streptomycin content (μg/g fresh wt.)
	Control	Treated	
24	0.81	1.02	Traces
72	0.90	1.61	40

Table 2. Respiratory rate and polyphenolase activity in potato disks treated with streptomycin. Respiratory rate is expressed as cubic millimeters of oxygen per gram (fresh weight) per hour. Enzyme activity is expressed as increase in oxygen uptake in cubic millimeters under identical conditions upon addition of substrates.

Hours after treatment	Respiratory rate		Polyphenolase activity	
	Control	Treated	Control	Treated
3	68	65	28	30
24	64	40	32	102

The effect of streptomycin on the tissues of tubers is very similar. Small disks (5 mm in diameter) of cortex tissues were placed in streptomycin solution or in water. The polyphenolase activity was measured by adding the phenolic substrates from the side bulb to the disks that were suspended in buffer solution in the main compartment of Warburg vessels. As may be seen (Table 2), the respiration of streptomycin-treated disks was strongly decreased. Simultaneously, the polyphenolase activity was considerably enhanced. The activation of polyphenolases was shown also by the quick blackening of the treated disks, in contrast to the modest discoloration of the controls.

The effect of streptomycin is indirect, for the antibiotic was shown to be totally inactive when tried directly as a "substrate" in the assay of polyphenolase activity.

The results reported provide strong evidence for the idea that streptomycin exerts its protective effect via the polyphenol-polyphenolase system of the host plant. Further support for the validity of this suggestion is delivered by the recent observation of McNew (*7*) which indicated a synergistic effect of copper and streptomycin. The relation of this finding to our results is evident: polyphenolases are copper enzymes, and their activity is greatly dependent on the copper supply of the plant.

Results similar to those described above were obtained with tomato plants.

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References

1. B. A. Rubin, E. V. Arzichowskaja, T. A. Proskurnikova, *Biokhimiya* **12**, 141 (1947); B. A. Rubin and B. A. Axenova, *ibid.* **22**, 202 (1957).
2. W. H. Fuchs and E. Kotte, *Naturwissenschaften* 41, 169 (1954); E. Christiansen-Weniger, *Phytopathol. Z.* **25**, 153 (1955).
3. O. K. Müller, J. H. E. Mackay, J. N. Friend, *Nature* 174, 878 (1954).
4. J. J. Grosso, *Plant Disease Reptr.* 38, 333 (1954); D. M. Coe, *ibid.* 39, 729 (1955).
5. D. R. Goddard and C. Holden, *Arch. Biochem.* 52, 380 (1950).
6. D. Pramer, *Ann. Appl. Biol.* 40, 617 (1953).
7. G. L. McNew, *Proc. Intern. Conf. Use of Antibiotics in Agr., Ist Conf., Washington* (1956), pp. 190–191.

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Effect of Gibberellic Acid on Breaking of Rest Period in Elberta Peach

Gibberellic acid, which is produced from the fungus *Gibberella fujikuroi,* has been reported to exhibit profound growth-regulating properties when applied to plants. Rappaport found a 60-percent increase in fresh weight 10 days after an application of this material to the first expanded leaf of the young tomato plant (*1*). Kahn reported that gibberellic acid replaces the red light required for proper germination of lettuce seed (*2*). Harrington's investigations revealed that gibberellic acid induces flowering in nonvernalized endive plants (*3*).

The multitude of effects that gibberellic acid has induced in a number of plants led to our investigation, in which gibberellic acid was used as a chemical activator for breaking the rest period of the peach. The rest period, as referred to in this report, is a state of dormancy during which a plant will not produce visible growth even though environmental conditions are favorable. In order to overcome or "break" the rest period in peaches, a period of chilling is necessary. According to Weinberger, the chilling

period necessary for activation of vegetative growth in the Elberta peach is 950 hours of temperature below 45°F (*4*).

Two-year-old Elberta peach trees which had "normally" dropped their leaves in the fall and had been exposed to temperatures below 45°F for a maximum of 164 hours were transplanted from the orchard to large cans and placed in a storage room kept at 65°F. United States weather records, taken in the area, were used in calculating the number of hours in which the temperature was below 45°F. Lights were on in the room from 8 A.M. to 5 P.M. daily, and the trees were watered as necessary.

On 23 February 1957, 95 days after the trees had been placed in the storage room, 14 trees were placed in the greenhouse for treatment with gibberellic acid (*5*). The following concentrations of gibberellic acid were used: 0, 50, 100, 200, 500, 1000, and 4000 ppm. Solutions of each concentration were sprayed on two trees. Fourteen days later, at the time of the second application, it was noted that a large percentage of the buds on the trees that had received the 1000- and 4000-ppm applications had grown and produced small green leaves. Trees that had been sprayed with the lower concentrations (0, 50, 100, 200, and 500 ppm) did not show any growth at that time. On 29 March, after the trees had received two applications of gibberellic acid (23 February and 8 March), the trees that had been sprayed with 1000 and 40000 ppm were growing rapidly. The trees that had received 200 and 500 ppm were growing some, but trees that had been sprayed with concentrations lower than 200 ppm were still dormant (Table 1). The growth response of trees that had received the same treatment was uniform.

In another experiment, three trees that had been exposed for 433 hours to temperatures below 45°F, nearly half of the number of hours necessary to break the rest period, were sprayed with 0, 100, and 200 ppm of gibberellic acid, respectively. The trees were sprayed four times at 10-day intervals. Four days after the first application it was noted that one or two buds on the tree that had received the 200 ppm spray were opening and exhibiting small leaves. Fifty days after the first application, the tree that had been treated with 200 ppm of gibberellic acid had an average of 14 inches of new terminal growth and large, "normal" green leaves. At the same time, buds on the tree that had been sprayed with 100 ppm had 3 to 4 inches of new growth, but there were still a few completely dormant buds. Two or three buds on the untreated control were just starting to "break" dormancy. Trees that have not been exposed to the necessary chilling period commonly have a few open terminal buds after a long period of favorable growing temperatures, but the growth is usually somewhat abnormal.

Table 1. Effect of gibberellic acid on the breaking of dormancy of buds of 2-year-old Elberta peach trees. The trees had been exposed to temperatures below 45°F for 164 hours before treatment. Data were recorded 29 March 1957, 36 days after the initial application of spray.

Gibberellic acid concn. (ppm)	Leaf buds growing (No.)	Leaf buds growing (%)	Av. growth per shoot (in.)
0	0	0	0
50	0	0	0
100	0	0	0
200	17	40	0.25
500	17	50	0.5
1000	28	85	2
4000	37	98	4

Table 2. Effect of gibberellic acid on breaking the rest period in dormant peach seeds. Seeds received approximately one-half of normal stratification period before treatment. Data were recorded 16 days after the gibberellic acid treatment.

Gibberellic acid concn. (ppm)	Germination (%)
0	30
20	50
100	80
200	70
500	40
1000	30

Tests were also conducted with peach seed that had received 35 days of stratification—that is, the seed were placed in a moist medium at a temperature near freezing. The standard time for stratification of peach seed is between 60 and 100 days at 41°F, according to Kains and McQuesten (*6*). In this experiment ten seeds were soaked for 24 hours in each of the following concentrations of gibberellic acid: 0, 20, 100, 200, 500, and 1000 ppm. The seeds were then planted in a flat of sand, and after 16 days the percentage germination was recorded (Table 2).

The percentage germination of seed that had been soaked in 100- or 200-ppm concentrations of gibberellic acid was greater than that of seed that had been soaked in other concentrations. Concentrations higher than 200 ppm may have been toxic for optimum germination and growth. Twenty days after the treated seed had been planted, the plants were measured. There was little root growth and no top growth of the plants grown from seed that had been treated with concentrations in excess of 200 ppm. The root and top growth of plants grown from seed that had been soaked in 20-ppm concentration was greater than that of the untreated controls but smaller than that of plants grown from seed that had been soaked in the 100-ppm concentration. Plants grown from seed that had been soaked in the 100- and 200-ppm treatments averaged the same length and weight, although there was considerable variation within a treatment. The plants grown from seed that had been treated with 100 ppm were much larger, averaging 48 percent more top growth than the untreated control plants. Three plants that had received the same treatment and that had the same amount of top growth were selected for comparison of their root systems. The roots of plants grown from seed that had been treated with 100- and 200-ppm concentrations of gibberellic acid were much larger than the roots of the untreated controls. Seed that had been soaked in the 100 ppm solutions produced plants with root systems of 56 percent greater length and 80 percent greater weight (on a fresh-weight basis) than did the untreated seed.

Gibberellic acid apparently activates the metabolic processes or nullifies the effect of an inhibitor of growth of young Elberta peach trees. Thus gibberellic acid "replaced" the cold requirement for the breaking of the rest period in the young trees. If mature trees responded similarly, without a detrimental effect on other plant processes, southern peach growers would be able to grow varieties that required a longer chilling period. There also exists the possibility of extending the peach industry further south to new areas. Further investigations of the effect of gibberellic acid on the biochemical processes may help in understanding the rest period of plants.

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References and Notes

1. L. Rappaport, *California Agr.* 10, No. 12, 4 (1956).
2. A. Kahn *et al.*, *Science* 125, 601 (1957).
3. J. F. Harrington *et al.*, *Science* 125, 45 (1957).
4. J. H. Weinberger, *Proc. Am. Soc. Hort. Sci.* 67, 107 (1956).
5. Gibberellic acid was kindly supplied by Merck and Co., Inc., Rahway, N.J.
6. M. G. Kains and L. M. McQuesten, *Propagation of Plants* (Orange Judd, New York, 1950).

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Protection of Guinea Pigs Against Radiation Death by Cell-Free Mouse Spleen Extract

In previous studies it has been demonstrated that cell-free saline extracts of mouse spleens obtained from donor animals 6 days after their exposure to an LD 30/15 days contained a factor which protects mice against radiation-induced