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2 August 1957

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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15 July 1957

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