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* Present address: University of Colorado, School of Medicine, Denver.

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Toxic Effect of Pseudomonas tabaci on RNA Metabolism in Tobacco and Its Counteraction by Kinetin

Abstract. *Treatment of intact tobacco leaves with toxin-containing culture filtrates of* Pseudomonas tabaci *markedly reduced the ribonucleic acid content of the leaves. The decrease was counteracted by spraying the leaves with 10^{-4}M kinetin. Simultaneously with the decrease in RNA, the ribonuclease activity of extracts from tissues treated with toxin increased. Experiments on the effect of kinetin on the ribonuclease activity of toxin-treated tissues gave no consistent results.*

The toxin of *Pseudomonas tabaci* is a structural analogue of methionine (*1*). It has been shown by Braun in model experiments with *Chlorella vulgaris* that, as expected, the effect of the toxin can be counteracted by methionine. However, methionine failed to antagonize the toxin in tobacco (*2*). Therefore, further studies on the mode of action of the wildfire toxin seemed necessary. To this end, we investigated the effect of toxin-containing filtrates of *P. tabaci* cultures on the metabolism of the host. It has been found (*3*) that the amount of soluble proteins decreases upon treatment of the host tissues with the toxin.

Table 1. RNA content of tobacco half-leaves treated with toxin-containing culture filtrate of *Pseudomonas tabaci* and with nutrient medium, respectively.

Expt. No.	RNA content (mg/g fresh wt.)	
	Treated with medium	Treated with filtrate
1	1.54	0.83
2	1.20	0.69
3	1.35	0.95

Table 2. Effect of kinetin on the RNA content of tobacco half-leaves treated with toxin-containing culture filtrate of *Pseudomonas tabaci.*

Expt. No.	RNA content (mg/g fresh wt.)	
	Effect of water	Effect of $10^{-4}M$ kinetin
1	1.00	1.54
2	1.16	1.60
3	0.76	0.95

The protein concentration could be maintained at the level of the control by spraying toxin-treated leaves with kinetin (*3*). In view of the well known effect of kinetin on RNA metabolism (*4–6*) and because of the close connection between protein and RNA metabolism, we investigated the influence of toxin-containing culture filtrates on the RNA content of host tissues.

Intact half-leaves of *Nicotiana tabacum* "White Burley" plants were injected with toxin-containing filtrates of *P. tabaci* cultures. Control half-leaves were injected with Czapek's medium. After an incubation period of 4 to 5 days under ordinary greenhouse conditions disks were punched from the leaves for spectrophotometric determination of RNA content and ribonuclease activity. In the determination of RNA the procedure described by Osborne (*5*) was followed. The method adopted for the determination of ribonuclease activity was based on the principles described by McDonald (*7*) and Tuve and Anfinsen (*8*). Tissue extracts corresponding to 10 mg of fresh weight per milliliter were incubated in the presence of 0.1*M* acetate buffer, *p*H 5.0, with yeast RNA in a final concentration of 0.1 percent at 37°C for 30 minutes. The reaction was stopped by McFadyen's reagent, the reaction mixture was centrifuged, and after five-fold dilution of the supernatant the optical density was determined at 260 mμ. One enzyme unit corresponded to an increase in optical density of 0.010 over the zero time control (*8*).

Treatment of tobacco leaves with culture filtrates of the pathogen grown for 48 hours in Czapek's medium resulted in a decrease in RNA content (Table 1). This clearly indicates that the toxin damages the RNA metabolism of the host.

Kinetin counteracts the breakdown of RNA in detached leaves (*5*). Therefore, experiments were conducted to find out whether or not the toxin-induced damage to RNA metabolism can also be counteracted by kinetin. Intact whole leaves were injected with culture filtrates of *P. tabaci.* Half-leaves were sprayed three times in 24-hour intervals with $10^{-4}M$ kinetin; controls were sprayed with water. The toxin-induced decrease of RNA was inhibited in the kinetin-treated half-leaves (Table 2).

The regulation of the RNA content of plants is poorly understood. Although no conclusive evidence has been presented, it has been suggested repeatedly that ribonuclease may participate in the regulatory process. Therefore, the ribonuclease activity of extracts from half-leaves treated with toxin and from control half-leaves was compared. Ribonuclease activity was increased by about 100 percent in the tissues treated with culture filtrate (Table 3). Attempts to counteract by kinetin the toxin-induced increase in ribonuclease activity gave no consistent results (Table 4), in contrast to the highly reproducible effect of kinetin on the concentration of RNA. Thus, there is no clear-cut correlation between the ribonuclease activity of the tissue extracts and their RNA content.

Table 3. Ribonuclease activity in tobacco half-leaves treated with toxin-containing culture filtrate of *Pseudomonas tabaci* and with nutrient medium, respectively.

Expt. No.	Ribonuclease activity (enzyme units)	
	Treated with medium	Treated with filtrate
1	86	156
2	69	132
3	45	66

Table 4. Effect of kinetin on the ribonuclease activity of tobacco half-leaves treated with toxin-containing culture filtrate of *Pseudomonas tabaci.*

Expt. No.	Ribonuclease activity (enzyme units)	
	Effect of water	Effect of $10^{-4}M$ kinetin
1	63	50
2	33	29
3	52	55

The evidence presented above indicates that an important aspect of the mode of action of the wildfire toxin is damage to the RNA metabolism of the host. The effect is not based on the parasitically induced increase in ribonuclease activity.

L. Lovrekovich
Z. Klement
G. L. Farkas
Research Institute for Plant Protection, Budapest, Hungary

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