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6 March 1978

Agglutination of Plant Protoplasts by Fungal Cell Wall Glucans

Abstract. *Glucans, called elicitors, isolated from cell walls of* Phytophthora infestans, *caused rapid agglutination and death of protoplasts isolated from potato leaf tissue. Cells incubated with high concentrations of elicitor were rapidly killed, but did not agglutinate. Agglutination and cell death did not occur with any of several commercial polysaccharides including laminarin, but laminarin did inhibit elicitor-mediated agglutination. The results are consistent with the existence of specific elicitor receptor sites on the outer surface of potato leaf plasma membranes.*

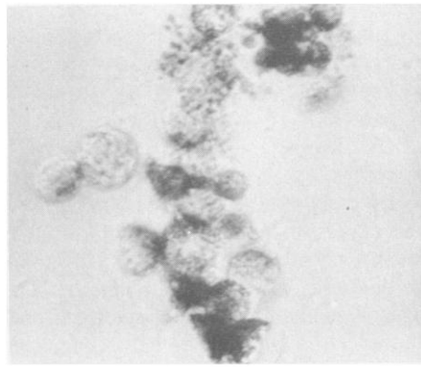
Elicitors are polysaccharides or glycoproteins of fungal origin that are toxic to a wide range of higher plants (*1*). The name of these chemicals comes from the fact that they elicit phytoalexin synthesis when applied to various plant tissues (*2*). Phytoalexins are fungistatic compounds that are synthesized by a plant in response to invasion by a microorganism. The accumulation of these chemicals is suspected to be a mechanism of disease resistance (*3*). Elicitors may thus be initiators of plant disease resistance. Mancarella (*4*) and Kota and Stelzig (*5*) have obtained evidence based on the electrophysiology of elicitor-treated potato petiole tissue that elicitor-mediated responses may be initiated at the outer surface of plant plasma membranes.

Our research was based on two premises: (i) that elicitor receptors exist on the outer surface of potato leaf plasma membranes and (ii) that elicitors are multivalent and therefore should be able to agglutinate isolated potato leaf protoplasts.

Cell walls of *Phytophthora infestans* (Mont.) de Bary were isolated by the method of Ayers *et al.* (*6*) and homogenized with laminaranase, a commercial preparation of β-1,3-glucanase (*7*). The homogenate was dialyzed (Spectrapor, 3500-MW cutoff) at 37°C against 6 liters of the homogenization buffer. The dialysis tubing was changed at 24-hour intervals, and after 72 hours the dialyzate was subjected to sequential cation and anion exchange or Bio-Gel P-2 chromatography (*8*).

Kennebec potato plants were grown (*9*) and protoplasts were isolated from well-expanded leaflets (*10*).

In initial experiments, 1 ml of protoplasts containing 2×10^5 cells were mixed with 1 ml of osmotically balanced salt solution or a similar solution containing elicitor isolated by ion exchange or a commercial polysaccharide. The protoplasts incubated with elicitor agglutinated within 10 minutes, whereas there was no agglutination with salt solution or the commercial polysaccharides even after 60 minutes (Table 1). The protoplasts incubated with elicitor agglutinated in about 3 minutes, and by 10 minutes debris had accumulated in the mass of agglutinated cells (Fig. 1). The nature of this debris is not known, but it is pos-



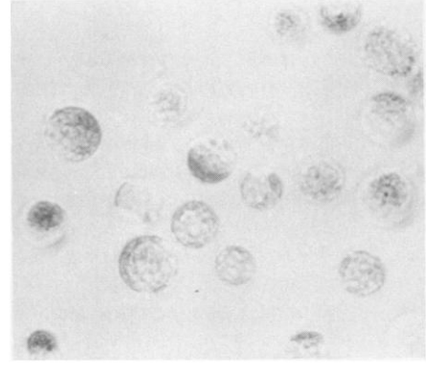


Fig. 1 (left). Potato leaf protoplasts that were incubated for 10 minutes with an elicitor from *P. infestans* at a final concentration of 250 μg of D-glucose equivalents per milliliter (× 800). Fig. 2 (right). Potato leaf protoplasts that were incubated for 1 hour with laminarin at a final concentration of 1000 μg/ml (× 800).

Table 1. Laminarin inhibition of elicitor-mediated agglutination of potato protoplasts.

Laminarin (μg/ml)	Agglutination	
	10 minutes	1 hour
0	+ + + +	+ + + + +
8	+ + + +	+ + + + +
16	+ +	+ + + +
31	+ +	+ + + +
62	+	+ + + +
125	+	+ + +
250	±	+ + +
500	±	+ +
1000	±	+ +
2000	±	+ +
4000	±	+

Table 2. Chemicals tested for their ability to agglutinate potato protoplasts.

Compound	Final concentration (μg/ml)	Agglutination	
		10 minutes	1 hour
Elicitor	250	+	+
Laminarin	8 to 1000	−	−
Soluble carboxymethylcellulose (high viscosity)	250 to 1000	−	−
Soluble carboxymethylcellulose (low viscosity)	250	−	−
Glycogen	250 to 1000	−	−
Gum arabic	250 to 1000	−	−
Inulin	250 to 500	−	−
Pectin (four preparations)	250 to 1000	−	−
Soluble starch	250 to 1000	−	−
Isotonic salt		−	−

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sible that it is cytoplasmic material from the protoplasts. The only evidence of this is that nearly all of the agglutinated cells were dead 10 minutes after the addition of elicitor, as shown by staining with fluorescein diacetate (*11*). In contrast, protoplasts incubated with salt solution or any of the commercial polysaccharides at the concentrations shown in Table 1 did not agglutinate the protoplasts, did not cause debris accumulation (Fig. 2), and did not result in cell death.

The remainder of the studies were done with elicitor isolated by Bio-Gel chromatography because substantially lower concentrations of elicitor were required to cause agglutination (21 versus 250 μg of D-glucose equivalents per milliliter). Agglutination occurred when protoplasts (1×10^5 cells per milliliter) were incubated for 10 minutes with this elicitor preparation at a concentration of 21 μg of D-glucose equivalents per milliliter, but not at concentrations lower than 10 μg or higher than 60 μg of D-glucose equivalents per milliliter. The agglutinated cells were dead and had the same appearance as those shown in Fig. 1. The cells incubated with elicitor at high concentrations were killed even though they did not agglutinate.

Laminarin did not agglutinate protoplasts, but it did inhibit elicitor-mediated agglutination when the laminarin was incubated with the protoplasts for 10 minutes prior to the addition of elicitor at a final concentration of 21 μg of D-glucose equivalents per milliliter (Table 2). Laminarin did not reverse agglutination once it had occurred.

The data presented are consistent with the existence of elicitor receptor sites on the outer surface of potato leaf plasma membranes. If this is the explanation of elicitor-mediated agglutination, it is evident that the elicitors isolated by our procedure are multivalent. The proposed receptors are capable of being saturated, as shown by the lack of agglutination with high elicitor concentrations. They are probably specific for β-1,3-glucan portions of the elicitor molecule because laminarin inhibits the agglutination.

The elicitor used in our studies was isolated from a race of *P. infestans* that can easily infect Kennebec potato plants. Thus, our research does not elucidate the mechanism of race specificity. It is possible that race-specific elicitors do exist (*12*), but this character is lost during the isolation of cell walls or elicitor. It is also possible that race specificity is due to other chemicals working in concert with elicitors (*6*), or that elicitors are released from the cell walls of an invading fungus only if the plant being penetrated has a substantial amount of resistance to that race of the fungus.

BRIAN M. PETERS
DAVID H. CRIBBS
DAVID A. STELZIG
Plant Sciences Division, West Virginia University, Morgantown 26506

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7. A Teflon pestle attached to a motorized drive was used to homogenize (at room temperature) 500 mg of cell wall and 50 ml of 0.05*M* sodium acetate buffer, *p*H 5.0, in a tight-fitting glass tube. When the slurry was homogeneous, 50 mg of laminaranase was added, and the homogenization was continued for 30 seconds.
8. The dialyzate was passed through a column (2 by 55 cm) of Dowex 50W-X8 in the H^+ form, and the column was washed with water until the eluate was nearly neutral. This eluate was then passed through a column (2.5 by 37 cm) of Amberlite IR-45 in the OH^- form, and the column was washed with three bed volumes of water. The combined eluate was then concentrated to 60 ml by flash evaporation at 40°C. In the second method, the dialyzate was first concentrated to 60 ml, and a portion containing 5 mg of carbohydrate was placed on a column (0.9 by 40 cm) of Bio-Gel P-2. The eluate was collected in 2-ml fractions, with water as the eluant. The fractions in the single peak of elicitor held up by the column that contained at least 60 μg of carbohydrate were then pooled. The elicitors isolated by both of these methods caused necrosis and phytoalexin synthesis when added to slices of potato tubers.
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Rabies: Decimation of a Wolf Pack in Arctic Alaska

Abstract. *In a pack of ten wolves, one wolf behaved atypically and fought with several packmates. This wolf was shot when it approached the author. Within 4 weeks at least six other members of the pack were dead. Rabies was confirmed in the wolf that was shot and in two others that had not decomposed. Most of the wolves infected with rabies had sought or remained at familiar areas in the core area of their territory, which implies that they were not contacting neighboring packs. This was confirmed with an aerial survey. Arctic foxes, experiencing a regionwide rabies epizootic, were suspected vectors.*

Rabies, a viral disease affecting the nervous system, causes altered behavior, paralysis, and death in most mammals. It is transmitted by introduction of virus-laden saliva into a bite wound, by ingestion of infected material (*1*), or by inhalation of contaminated air (*2*). Knowledge of the effect of rabies on family groups, behavior of normal animals in contact with rabid conspecifics, and behavior and movements of rabid animals would lead to better understanding of the persistence of rabies in wildlife populations (*3*). In this report I describe the behavior of rabid wolves in the wild.

Reports of rabies in wolves (*Canis lupus*) have dealt almost exclusively with attacks on man (*4*). Rabies has rarely been recorded in wolves in North America. In Alaska, for example, there were only six laboratory-confirmed cases before 1977 (*5–7*). Most rabid wolves in North America were reported during rabies outbreaks (epizootics) in arctic foxes (*Alopex lagopus*) or red foxes (*Vulpes vulpes*) (*5, 8–10*). Wolves seem to be an important reservoir or vector of rabies only in the eastern Mediterranean (*11*).

Rabies is enzootic in fox populations in tundra regions of Alaska (*6, 12, 13*), and in 1976 an epizootic began in arctic foxes along the Arctic Coast (*7*). In the summer of 1977 I documented an outbreak of rabies in a pack of wolves in the upper Hulahula River valley on the north slope of the Brooks Range in northeastern Alaska (Fig. 1). In the summer of 1976 I had studied the effects of human disturbance on this pack (*14*) and in 1977 had begun a study of its behavior. I was in the Hulahula valley from 5 June to 18 July, 2 to 10 August, and 12 August to 12 September 1977. Homesites (*15*) were observed for wolf activity from approximately 1.0 km with a spotting scope for a total of 160 hours between 8 June and 18 July. In June, two wolves were captured, immobilized, fitted with radio-transmitting collars, and released at their cap-

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