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ALTERATION IN THE SUSCEPTIBILITY OF *VICIA FABA* TO *BOTRYTIS FABAE* BY VARIOUS PRETREATMENTS OF THE LEAVES[1]

Verandering van de vatbaarheid van Vicia faba *planten voor* Botrytis fabae *door verschillende voorbehandelingen van de bladeren*

H. H. SOL

Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn

Infection of broad bean leaves by *Botrytis fabae* was influenced by various pretreatments of the leaves. The number of lesions developing on test leaflets increased after pretreatment of the leaflet for 24 hours with a sucrose, a potassium chloride or a lanthanum chloride solution; pretreatment of leaflets by keeping them in darkness had a similar effect. At least the first phase of the infection process, viz. germination of the conidia on the leaf, was stimulated by these pretreatments. This stimulation may have been caused by increased amounts of leached nutrients present on the pretreated leaflets in comparison with those on untreated ones.

INTRODUCTION

Several workers have shown that the germination of conidia on a leaf surface may be favoured by substances leached from the host. BROWN (1922) found that the conductivity of drops of distilled water increased during their stay on leaves of broad beans. When conidia of *Botrytis cinerea* were added to these drops the percentage that germinated increased with increase in conductivity of the drops. He concluded from his experiments that germination was stimulated by nutrients leached from the leaf. The identity of the substances was not established. KOSUGE & HEWITT (1964) demonstrated that glucose and fructose, and not amino acids, present in the exudates of grape berries, had a stimulating effect on the germination of conidia of *Botrytis cinerea*. It is possible that these substances have a similar influence on the germination of conidia present on leaf surfaces. LAST (1960), and also DEVERALL & WOOD (1961), working with *Botrytis fabae*, showed that sugars added to conidial suspensions when applied to the surface of broad bean leaves increased the number and size of lesions. KERLING (1964), studying the populations of fungi present on rye and strawberry leaves, found that the number of colonies of *Botrytis cinerea* on strawberry leaves increased rapidly as the leaves approached senescense and were maximal when they became necrotic. The reason might well have been that the older leaves produced much more nutrients than the younger ones because of an alteration in the permeability of the cell membranes. TUKEY, TUKEY & WITTWER (1958) sampled more leached material from an old leaf than from a younger one.

The permeability of the leaf cells can be altered by different factors, so that the uptake of water and salts can be stimulated or inhibited. DE HAAN (1933, 1935) investigated the influence of salts on the permeability of cells of the inner epidermis of bulb scales of *Allium cepa*. Salt solutions in certain low concentrations diminished water permeability, whereas at higher concentrations they

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raised it. SOL (1958) found that the rate of uptake of chloride ions by *Vallisneria* leaves was influenced by various pretreatments of the material. Pretreatment with sugar and salt solutions had initially an inhibiting influence on the rate of uptake, whereas light had a stimulating effect.

The influence of a pretreatment with salt solutions may be explained by assuming certain alterations in structure in the plasm or plasma membranes which may be brought about by cations (BOOY & BUNGENBERG DE JONG, 1956). Low concentrations of a trivalent cation with a monovalent anion, e.g. $LaCl_3$, influenced the uptake of chloride ions more strongly than a monovalent cation did with a monovalent anion, e.g. KCl (SOL, 1958).

Little is known about the mechanism of the transport of substances from the inside of a cell to the outside. Possibly this process, also, may be influenced by a pretreatment, following which the total amount of leachates might be increased or decreased when compared with that from an untreated leaf.

As leached material seems to be important in relation to the behaviour of a fungus on the leaf surface, the question was raised whether certain pretreatments of broad bean leaves could induce a change in their susceptibility to *Botrytis fabae*. The influence of the following factors was examined: sugar, salts, light an darkness.

MATERIAL AND METHODS

Broad bean plants, *Vicia faba* var. 'Vroege brede witkiem', were grown in a glasshouse, where all experiments were carried out. Usually the plants developed five or six bifoliate leaves, followed by tri- or quadrifoliate ones. The plants were used for the experiments when the oldest trifoliate leaf had fully developed. The leaflets of the bifoliate leaf just below that trifoliate one were used for the various pretreatments. Pretreatment with a salt or sugar solution was done by submerging one of the leaflets for 24 hours in a solution kept in a 100 ml beaker, the other of the pair being similarly submerged in deionized water.

In the experiments with a pretreatment in light or in darkness, one of the leaflets was wrapped in black paper, the outside of which was covered with a thin reflecting layer, in order to prevent the absorption of radiant heat; the other leaflet was exposed to normal daylight. Following pretreatment, the leaflets were washed with deionized water, and after drying were inoculated with conidia of *Botrytis fabae*. The latter were collected from 9-11 day old cultures on hay-agar, that had been grown in light at an average room temperature of 22 °C.

The leaflets were inoculated by putting a drop of a conidial suspension containing about 200,000 conidia per ml on the leaflet, the drop being then spread over the leaflet surface, using a forefinger. The inoculated plants were kept in small moist chambers in the glasshouse for 24 hours at a temperature of 25 °C. All lesions that had become macroscopically visible (the so-called chocolate spots) were then counted and the number of spots on each leaflet finally expressed as the number per 50 cm^2 leaf area.

Leaf areas were estimated by drawing the circumference of the leaflets on thin paper, cutting out the shapes and weighing them. Knowing the weight of 1 cm^2 paper enabled us to calculate the leaf areas.

The germination of the conidia on the leaf was studied by sampling the conidia at different times after inoculation by pressing a piece (1.6 × 5.0 cm) of

"Sellotape" on the leaf surface and removing it carefully. The tape was mounted on a slide upon which a drop of solution of lactophenol-cotton blue had been brought to stain the conidia. In this way the percentage of germinated conidia could easily be estimated.

RESULTS

Effect of pretreatment with a sucrose solution

In the first experiments, one of each pair of leaflets from bifoliate leaves was submerged in a 0.05 M sucrose solution for 24 hours and the other in deionized water. The sucrose pretreatment seemed to have a stimulating effect on infection by *Botrytis fabae*, for in six out of eight tests there were more chocolate spots on the leaflets pretreated with sugar solution than on the water-pretreated ones (Table 1). This might have been due either to an alteration in the permeability of the cell membranes, following which sugars and other substances were leached out from the leaves, or to an increase in sugar content of the pretreated leaves.

TABLE 1. Influence of pretreatment of broad bean leaves with a 0.05 M sucrose solution on infection by *Botrytis fabae*. The number of spots on one leaflet per 50 cm² 24 hours after inoculation.
De invloed van een voorbehandeling van bladeren van tuinbonen met een 0,05 M saccharose-oplossing op de infectie door Botrytis fabae. *Het aantal vlekken op één blaadje per 50 cm² 24 uur na inoculatie.*

Experiment	Pretreatment	
	Sucrose	Water
1	553	448
	435	455
	154	148
2	362	249
	171	88
3	519	561
	779	354
	1024	803
Proef	*Saccharose*	*Water*
	Voorbehandeling	

Effect of pretreatment with different salt solutions

In these experiments one of the leaflets of a bifoliate leaf was pretreated with a 0.0003 M solution of lanthanum chloride or 0.001 M potassium chloride and the other one with water. The effect of pretreatment with the two salt solutions was also compared directly by pretreating one leaflet with $LaCl_3$ and the other with KCl. The leaflets of the control plants were both submerged in water.

TABLE 2. Influence of pretreatment of broad bean leaves with different salt solutions on infection by *Botrytis fabae*. The number of spots on one leaflet per 50 cm² 24 hours after inoculation.
De invloed van een voorbehandeling van bladeren van tuinbonen met verschillende zoutoplossingen op de infectie met Botrytis fabae. *Het aantal vlekken op één blaadje per 50 cm² 24 uur na inoculatie.*

Experiment	Pretreatment							
	LaCl 0.0003³M	H_2O	KCl 0.001 M	H_2O	$LaCl_3$ 0.0003³M	KCl 0.001 M	H_2O	H_2O
1	136	40	94	42	104	24		
	481	262	36	48	454	113	204	157
	39	19	182	88	45	32	45	35
3	500	73	93	26	416	21	119	142
4	1556	323	914	371	1549	916	590	565
Proef	*Voorbehandeling*							

From the results, summarized in Table 2, it is clear that pretreatment with a salt solution had a stimulating effect on infection. Another noteworthy point is that the effect of $LaCl_3$ was more marked than that of KCl. Since the chloride content of the two solutions was the same, it seems unlikely that the chloride ions were responsible for the stimulating effect. In other words, it seems more probable that the effect observed was the result of an action of the cations.

In order to find out which part of the infection process was affected, a study was made of the influence of a pretreatment with $LaCl_3$ solution on the germination of the conidia. The leaflets of a bifoliate leaf were inoculated after pretreatment of one of the leaflets with a 0.0003 M $LaCl_3$ solution and of the other one with water. At different times after inoculation the germination of the spores was examined as already described. On each occasion the percentage of germinated conidia was calculated from the examination of 200–300 conidia. A conidium was recorded as having germinated if a germ tube was clearly visible. At the first check, after three hours in Experiment 1 (Table 3) and after five hours in Experiment 2, there seemed to be a slight effect of pretreatment with the salt solution in the former case, but none in the latter. Three hours later, however, there were, in each case, more germinated conidia on the leaflet pretreated with $LaCl_3$ than on the water-pretreated one. In Experiment 3 the whole plant was kept in the dark after inoculation. Already at the first check, five hours after inoculation, there were more germinated conidia on the leaflet pretreated with the $LaCl_3$ solution. Besides revealing a stimulating effect on spore germination of pretreatment of the leaflets with a salt solution, this experiment also indicated that the germination of conidia of *Botrytis fabae* on broad bean leaves is independent of light.

Influence of pretreatment in darkness and in light

TUKEY, WITTWER & TUKEY (1957) found that light had a stimulating influence on the leaching of carbohydrates from leaves of *Phaseolus vulgaris*. As these substances may be of importance for the infection of broad bean leaves

TABLE 3. Influence of pretreatment of broad bean leaves with a 0.0003 M $LaCl_3$ solution on the germination of *Botrytis fabae* conidia. The percentage of germinated conidia at different times after inoculation.
De invloed van een voorbehandeling van tuinboonbladeren met een 0,0003 M $LaCl_3$-oplossing op de kieming van de conidiën van Botrytis fabae. *Het percentage gekiemde conidiën op verschillende tijdstippen na inoculatie.*

Experiment	Hours after inoculation	Pretreatment	
		$LaCl_3$	H_2O
1	3	7	4
	6	24	4
2	5	12	13
	8	27	11
3	5	31	15
	7	39	18
Proef	*Uren na inoculatie*	*Voorbehandeling*	

by *Botrytis fabae*, we examined the influence of a pretreatment of the leaves in light or in darkness on the infection process.

Table 4 gives the result of two experiments in which one leaflet of a bifoliate

TABLE 4. Influence of pretreatment of broad bean leaves in darkness or light on infection by *Botrytis fabae*. The number of spots on one leaflet per 50 cm^2 24 hours after inoculation.
De invloed van een donker-licht-voorbehandeling van bladeren van tuinbonen door Botrytis fabae. *Het aantal vlekken op één blaadje 24 uur na inoculatie.*

Experiment	Pretreatment	
	Dark	Light
1	413	88
	138	40
	51	19
2	354	221
	266	33
	164	85
	459	44
	Donker	*Licht*
Proef	*Voorbehandeling*	

leaf was kept in the dark for 24 hours before inoculation, while the other was exposed to normal daylight. There were more spots on the leaflet pretreated in the dark than on the one kept in daylight.

It seemed that germination of the conidia is affected by this pretreatment (Table 5). The conidia germinated better on the leaflet pretreated in the dark than on the one kept in light. In the second experiment the whole plant was kept in the dark after inoculation. The germination of the conidia started sooner than in the first experiment, in which the plant had been kept in the light after inoculation. Here again we obtained an indication that light is not needed for the germination of conidia on the broad bean leaf.

TABLE 5. Influence of pretreatment of broad bean leaves in the dark on the germination of *Botrytis fabae* conidia. The percentage of germinated conidia at different times after inoculation.
De invloed van een voorbehandeling van tuinboon-bladeren in het donker op de kieming van de conidiën van Botrytis fabae. *Het percentage gekiemde conidiën op verschillende tijdstippen na inoculatie.*

Experiment	Hours after inoculation	Pretreatment	
		Dark	Light
1	8	9	11
	11	40	16
2	3	15	11
	5	29	18
		Donker	*Licht*
Proef	*Uren na inoculatie*	*Voorbehandeling*	

DISCUSSION

The results of the experiments showed that the infection of broad bean leaves by *Botrytis fabae* was influenced by various pretreatments of the leaves. The number of lesions increased as a result of a 24-hour pretreatment of a leaflet with a sucrose, a potassium chloride or a lanthanum chloride solution (Tables 1 and 2); pretreatment of a leaflet in darkness had a similar effect (Table 4).

It seems that at any rate the first phase of the infection process, the germination of the conidia, is affected. A pretreatment of a leaflet with a lanthanum chloride solution (Table 3) or a treatment in darkness (Table 5) had a stimulating effect on the germination of the conidia. This may have been due to the presence of a greater amount of leached nutrients on the pretreated than on the untreated leaflets.

The influence of various pretreatments on the process of leaching is still being studied. Probably not only the permeability of the cell membranes is altered, but also the composition of the cell contents. These factors may not only influence the total amount of leached substances important for germination, but they may also affect the next phases of the infection process, such as penetration of the germ tubes and further spread of the fungus in the leaves.

SAMENVATTING

De infectie van een blaadje van het jongste tweetallige blad van een tuinboonplant *(Vicia faba)* door *Botrytis fabae* Sard. werd gestimuleerd door een voorbehandeling gedurende 24 uur met een 0,05 M saccharose-, een 0,001 M kaliumchloride- of een 0.0003 M lanthaanchloride-oplossing (tabellen 1 en 2); een voorbehandeling in donker had hetzelfde effect (tabel 4).

Een voorbehandeling van een blaadje met een lanthaanchloride-oplossing (tabel 3) en een voorbehandeling in donker (tabel 5) stimuleerde de ontkieming van de conidiën op het blad. Dit zou het gevolg kunnen zijn van een verhoogde afgifte van kiemingsbevorderende stoffen door het behandelde blaadje in vergelijking met een niet behandeld.

De invloed van een voorbehandeling op de afgifte van stoffen en op de volgende fasen van het infectieproces, het binnendringen van de kiembuizen en de verspreiding van de schimmel in het blad, is nog in onderzoek.

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