

## Effects of pretreating broad bean leaflets with decenylsuccinic acid on subsequent attack by *Botrytis fabae*

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### Abstract

Pretreating with decenylsuccinic acid (DSA) increased numbers of lesions developing when *Vicia faba* leaflets were inoculated with conidia of *Botrytis fabae*. The increase is partly attributed to an effect on conidial germination. More conidia germinated on DSA than on water-pretreated leaflets, an effect associated with greater concentrations of sugars and amino acids in leachates from the former.

### Introduction

Sol (1966) found that the susceptibility of *Vicia faba* to attack by *Botrytis fabae* was enhanced by pretreating leaves with substances that change the permeability of the host's plasma membranes. Because alkenyl succinic acids increase cell permeability to water when incorporated into the lipid layer of cytoplasmic membranes (Kuiper, 1964), experiments were done with *Vicia faba* to test the influence of decenylsuccinic acid ( $\text{CH}_3\text{-(CH}_2\text{)}_8\text{-CH=CH-CH}_2\text{-CH(COOH)-CH}_2\text{-COOH}$ ) on (a) the composition of leaf leachates, and (b) susceptibility to attack by *Botrytis fabae*.

### Material and methods

Broad beans *Vicia faba* var. 'Vroege brede witkiem', were grown in a glasshouse, where all experiments were done at ca. 23°C. After five or six bifoliate leaves, ageing seedlings usually developed tri- and quadrifoliate leaves. The youngest bifoliate leaf was used experimentally when the oldest trifoliate leaf was fully expanded. One of the pair of leaflets was submerged for 2 h in  $3 \times 10^{-5}$  M decenylsuccinic acid (DSA); the other was similarly submerged but in distilled water. After drying, leaflets were either used for the collection of leachates or inoculated with *B. fabae* conidia.

Leachates were collected from dried leaflets which were first covered with atomized droplets of distilled water. The sprayed plants were put in small, moist and dark chambers and, after 6 h incubation, leaflets were individually washed with 50 ml distilled water, the liquid being collected and subsequently dried at 30°C and reduced pressures. The residu was later dissolved in 5 ml distilled water, aliquots being analysed

for sugars and amino acids, the former being estimated colorimetrically using the anthron method described by Hewitt (1958), and amino acids with ninhydrin (Rosen, 1957). Glucose and leucine were the standards for sugar and amino acid determinations, respectively

The techniques used for sampling *B. fabae* conidia, inoculating leaves, studying in vivo germination and estimating leaf areas, have already been described (Sol, 1966).

## Results

Leachates were collected from leaves of five replicate plants in each of two experiments. In most instances larger concentrations of sugars and amino acids occurred in the washings from DSA-pretreated leaflets, than in those from leaflets pretreated with water (Table 1), suggesting that DSA increased the permeability of leaf cells to these substances. Although leaf cells were not injured during 2 h pretreatment with  $3 \times 10^{-5}$  M DSA, some cells were water-soaked when higher concentrations were tested. Pretreating leaflets with DSA increased the mean number of *B. fabae* lesions from 397 on the water controls to 531. In eleven of fifteen comparisons DSA appreciably increased numbers and in the other four there was no significant divergence from the controls (Table 2). The increased numbers of lesions may be partly attributed to leachate effects on conidial germination (Sol, 1966, 1967), because more germinated on DSA, than on water-pretreated leaflets (Table 3). The percentage of 200 conidia germinating on leaflets was assessed, in situ, 3 and 6h after inoculation, germination being considered to have occurred when germ tubes were clearly visible.

Table 1. Concentrations of sugars and amino acids in leachates from broad bean leaflets after pretreatment with either  $3 \times 10^{-5}$  M decenylsuccinic acid (DSA) solution or distilled water ( $H_2O$ ). Amounts of sugars and amino acids per 50 cm<sup>2</sup> leaf area expressed as  $\mu$ g glucose and  $\mu$ Mol leucine, respectively.

Experiment	$\mu$ g glucose		$\mu$ Mol leucine	
	Leaflets pretreated with:		Leaflets pretreated with:	
	DSA	$H_2O$	DSA	$H_2O$
1	204	135	1.49	0.36
	190	219	0.78	0.41
	146	111	0.46	0.24
	351	237	0.41	0.22
	440	322	0.22	0.28
2	165	128	1.40	0.61
	188	217	0.77	0.68
	157	96	0.40	0.26
	143	79	0.84	0.35
	89	64	0.83	0.41

Tabel 1. Hoeveelheden suikers en aminozuren afgespoeld van blaadjes van tuinboonplanten na een voorbehandeling met  $3 \times 10^{-5}$  M decenylbarnsteenzuuroplossing (DSA) of gedistilleerd water ( $H_2O$ ). De hoeveelheden suikers en aminozuren van 50 cm<sup>2</sup> bladoppervlak gespoeld, respectievelijk berekend als  $\mu$ g glucose en  $\mu$ Mol leucine.

## Discussion

More *B. fabae* lesions (= chocolate spots) developed on leaflets pretreated with decenylsuccinic acid than on water-pretreated leaflets (Table 2), part of the increase being attributed to an external effect on germination. The increased proportion of conidia germinating on DSA-pretreated leaflets (Table 3) was associated with greater

Table 2. Effects of pretreating broad bean leaflets with  $3 \times 10^{-5}M$  decenylsuccinic acid (DSA) solution on lesion numbers per leaflet, counted 24 h after inoculating with *Botrytis fabae*

Experiment	Leaflets pretreated with:	
	DSA	H <sub>2</sub> O
1	994	690
	413	298
	506	295
	684	342
	326	334
2	535	340
	423	264
	718	725
	261	237
3	824	512
	462	336
	392	405
	631	527
	514	443
	287	209
Mean	531	397

Tabel 2. Effect van een voorbehandeling van tuinboonblaadjes met  $3 \times 10^{-5}M$  decenylbarnsteenzuuroplossing (DSA) op het aantal vlekjes per blaadje, geteld 24 uur na inoculatie met *Botrytis fabae*

Table 3. Effects of pretreating broad bean leaflets with  $3 \times 10^{-5}M$  decenylsuccinic acid (DSA) solution on *Botrytis fabae* conidia germinating in the phylloplane (% germination of at least 200 conidia counted 3 and 6 h after inoculation)

Experiment	Hours after inoculation	Leaflets pretreated with:	
		DSA (%)	H <sub>2</sub> O (%)
1	3	35	18
	6	60	41
2	6	86	69

Tabel 3. Effect van een voorbehandeling van tuinboonblaadjes met  $3 \times 10^{-5}M$  decenylbarnsteenzuuroplossing (DSA) op *Botrytis fabae* conidia, kiemend in de "phylloplane" (% gekiemde conidia van de 200 getelde, 3 en 6 uur na inoculatie)

amounts of nutrient leached from these leaflets than from water-pretreated leaflets (Table 1). Although substances other than sugars and amino acids may have been responsible for the effect on germination, there was a close association with changing concentrations of sugars (Sol, 1967).

## Samenvatting

*Voorbehandeling van bladeren van tuinbonen met decenylbarnsteenzuur en de vatbaarheid voor Botrytis fabae*

Eén van de blaadjes van het jongste tweetallige blad van een tuinbouwplant werd voor de inoculatie met conidiën van *Botrytis fabae* gedurende 2 uur ondergedompeld in een  $3 \times 10^{-5}M$  decenylbarnsteenzuuroplossing (DSA), het andere blaadje van het bladpaar in gedistilleerd water. Op de met DSA behandelde blaadjes waren 24 uur na de inoculatie meer vlekjes aanwezig dan op de met water behandelde (Tabel 2). Deze verhoging van de vatbaarheid moet voor een deel worden toegeschreven aan een effect op de ontkieming van de conidiën op het blad. Hogere percentages ontkiemde conidiën werden gevonden op de met DSA behandelde blaadjes (Tabel 3). Dit zou het gevolg kunnen zijn van een grotere afgifte van kiemingsbevorderende stoffen door het met DSA behandelde blaadje in vergelijking met een met water behandeld (Tabel 1).

## References

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