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Induction of systemic resistance in potato (*Solanum tuberosum L.*) plants to late blight by local treatment with *Phytophthora infestans* (Mont.) de Bary, *Phytophthora cryptogea* Pethyb. & Laff., or dipotassium phosphate

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Summary

Induced systemic resistance to late blight was found in the potato cultivar Bintje which has no major or minor resistance genes to late blight. The prerequisite was a local inoculation with 5×10^3 zoospores/ml of *Phytophthora infestans* race 1, 3, 4, 7, 8, a local inoculation with 2×10^3 zoospores/ml of *Phytophthora cryptogea* isolated from wheat or local treatment with 50–100 mM solution of K_2HPO_4. Potato plants used in the experiments were propagated in vitro from nodal cuttings. Protection caused by the different inductions was 30 to 70 % (assessed as a reduced number of lesions/plant and reduced necrotic tissue/leaf or plant) and tended to positively influence yield and dry matter of the haulm. The induced protection in the potato plant was better in young developing leaves than in fully expanded leaves.

Introduction

Induced systemic resistance by prior inoculation with pathogenic organisms or viruses has been described in many pathogen-host relationships. In *Solanaceae* it has been well studied in tobacco, in which induced resistance to blue mold (*Peronospora tabacina* Adam) with *P. tabacina* is persistent and gives protection during the growing season and yield increases comparable to treatment with metalaxyl (Tuzun et al., 1986). Doke et al. (1986) found induced systemic resistance in potatoes, cultivar Irish cobbler, with no major resistance genes to late blight, when upper or lower leaves were treated with hyphal wall components of *Phytophthora infestans* (Mont.) de Bary. Avdyushko et al. (1987) and Chalova et al. (1989) reported that potato tubers (*Solanum tuberosum* L.) treated with a lipoglycoprotein (LGP)-complex from *P. infestans*, in which the active principle was found to be arachadonic and eicosapentaenoic acid, were less susceptible to the fungus compared with untreated tubers. The increase of resistance also spread to plants obtained from the treated tubers. Gottstein & Kuć (1989) reported induction of systemic resistance to anthracnose (*Colletotrichum lagenarium* (Pass.) Ell. & Holst) on cucumber plants by phosphates. The response was associated with the gradual appearance of chlorotic and necrotic stippling.

There is little knowledge about induced systemic resistance in potato plants. The aim of this investigation was to study whether potato, cultivar Bintje, which has no

major or minor resistance genes to late blight, had the potential to induce systemic resistance to late blight and to compare the induction by a pathogen, a non-pathogen, and a salt. In this study we have used potato cuttings grown in vitro in order to obtain a rapid and uniform propagation, and we determined the level of induced resistance by estimates of the number of lesions per plant, necrotic tissue per leaf or per plant, dry weight of the haulm, and tuber yield.

Materials and methods

Plant material. The potato cultivar Bintje was propagated in vitro from nodal cuttings (Roca et al., 1978). Medium MS (Murashige & Skoog, 1962), without IAA and kinetin, was supplemented with 10 % sucrose and 1 g l^{-1} edamin (casein hydrolysate). After two weeks growth in liquid MS the plants were transplanted to pots (one plant per pot 9 cm diam.) containing sterile standardized soil (Enhetsjord P) mixed with sand 80:10 and a fertilizer (Osmocote Plus mikro N-P-K-Mg 5-5-11-1.2) at the rate of 1.5 kg m^{-3}. Three weeks after transplanting, when the plants were about 40 cm tall and had 10–12 leaves, they were used in the experiments.

Growth conditions. The experiments were performed in a growth chamber maintained at 19 °C day and 14 °C night and 30–40 % humidity. The day length was 12 h and the light intensity 7000–10000 Lux at the plant-top level (irradiance 30–35 W m^{-2}, light source Osram HPIT/HQI400W).

Fungi. Race 1, 3, 4, 7, 8 of *P. infestans* was isolated from cultivar Bintje and maintained on surface sterilized tuber slices. Zoospore suspensions prepared according to Berggren et al. (1988). *Phytophthora cryptogea* Pethyb. & Laff. was isolated from wheat and maintained on V8-juice agar and zoospore suspensions prepared according to Larsson & Gerhardson (1990).

An inducer inoculum was applied to the first 3–5 leaves of the plant. On each of the leaves was placed a filter paper disc (6 mm diam.) that had been dipped in a zoospore suspension of 5×10^3 per ml of *P. infestans* or of 2×10^3 per ml of *P. cryptogea*. For induction with phosphate, 1–2 ml of a 50–100 mM (pH 8.5–9.2) solution of K_2HPO_4 was sprayed on each inducer leaf. The plants were placed in a moist chamber, sited within the growth chamber, and incubated for 24 h at 15–20 °C and 100 % humidity. Control plants were treated as the induced plants but without any induction.

Plants were challenge inoculated one week after induction by spraying the plants to run off with a zoospore suspension of about 10^4 per ml from *P. infestans* or by placing 4–8 filter paper discs (6 mm diam.) dipped in a zoospore suspension on the leaves to be challenged. Leaf 3 or 4 from the top, about two-third expanded at the time of induction, is referred to as a young developing leaf and leaf 5 from the bottom as a fully expanded leaf.

The estimation of late blight was done on each plant at different times after challenge inoculation. The number of lesions per plant were recorded after 4 days. The diameter of the lesions was measured after 6–8 days. The overall amount of necrotic tissue per leaf or plant was assessed on a 1–9 scale of increasing resistance according to Malcolmson (1976) or Cruickshank et al. (1982) after 6 days. Sporulation is one indicator of resistance but to avoid secondary infections in the experiments, the sporulation was deliberately kept low by keeping the humidity low in the growth chamber.

One or two weeks after challenge the dry weight of the haulm and fresh weight of the tubers from each pot were recorded. The haulm was harvested and dried at 120 °C for 24 h and tubers were washed and dried over-night at room temperature.

The experiments were done in a completely randomized design with 5–8 replications and, except for the experiment described in Fig. 2 which was done once, were repeated at least three times. Statistical analyses were done with the computer program Statworks (Statistics with Graphics for the Macintosh). Analysis of variance (ANOVA) and means were calculated, means were compared by the Student's t-test at $P<0.05$.

Results

Induction with Phytophthora infestans. Induction by *P. infestans* caused water soaked lesions, that turned grey to brown in their centre within a few days, to develop on the induced leaves. The lesions continued to expand and after about 10 days the inducer leaves fell off. Protection after challenge inoculation was evidenced by a reduced number of lesions per plant and reduced necrotic tissue per leaf or plant (Table 1). There were no statistically significant differences between induced plants and control plants for dry weight of their haulm and fresh weight of their tubers. The mean from four experiments shows, however, a tendency in induced plants for increased dry weight of the haulm and fresh weight of the tubers (Table 1). The protection tended to increase with the number of leaves induced but not with the inoculum concentration (Figs 1

Table 1. Influence on the development of foliage blight and yield of potato, of an inducer inoculation with *P. infestans, P. cryptogea* or dipotassium phosphate to a challenge inoculation with *P. infestans*.

Treatment	Lesions (number)	Necrotic tissue (scale 1 – 9)	Fresh weight of the tubers (g)
Experiment-series 1			
P. infestans	26.0 b	7.5 b	4.1
P. cryptogea	13.0 b	8.2 b	4.4
Control	45.0 a	6.0 a	3.1
ANOVA, P =	0,001	0,0001	*

Treatment	Lesions (number)	Necrotic tissue (scale 1 – 9)	Dry weight of the haulm (g)	Fresh weight of the tubers (g)
Experiment-series 2				
P. infestans	135.0 b	6.5 b	0.98 ± 0.38	8.20 ± 5.46
K_2HPO_4	141.0 b	5.4 a	1.01 ± 0.39	7.64 ± 4.19
Control	203.0 a	4.7 a	0.95 ± 0.36	7.49 ± 4.21
ANOVA, P =	0.039	0.0001	ns	ns

Means followed by the same letter are not significantly different (Student's t-test) at $P<0.05$.
Disease severity is expressed on an increasing 1 – 9 scale.
* Not applicable.

Table 2. Induced resistance to late blight in a potato plant, comparing a young developing leaf with a fully expanded leaf.

Treatment	Lesions (number)	Lesions day 6 (diam. in cm)	Lesions day 8 (diam. in cm)
young leaf on induced plant	0.5 a	3.4 a	4.3 a
young leaf on non-induced plant	1.6 b	3.5 a	5.3 b
fully exp. leaf on induced plant	2.8 c	3.8 a	*
fully exp. leaf on non-induced plant	4.5 c	4.3 a	*
ANOVA, P =	0.0001	0.355	0.049

Means within a column followed by the same letter are not significantly different (Student's t-test) at $P<0.05$.
* The lesions covered more than the leaves.

and 2). Young developing leaves were more responsive to induced resistance than fully expanded leaves (Table 2).

Induction with Phytophthora cryptogea. Induction caused dark brown lesions about 1 cm in diameter. The lesions did not expand further on the induced leaves and the leaf area surrounding the lesions was green. The inducer leaves did not fall off. Protection after challenge inoculation was evidenced by a reduced number of lesions per plant, a reduced necrotic tissue per leaf and a tendency, in one experiment, for increased fresh weight of the tubers (Table 1). The protection induced by *P. cryptogea* tended to be better than that with *P. infestans.*

Induction with dipotassium phosphate. Induction caused chlorotic, sometimes necrotic, stippling about 3 mm in diameter. When the stronger concentration was used the leaves sometimes fell off after 10 days. Protection after challenge inoculation was evidenced by a reduced number of lesions per plant, and a reduced amount of necrotic tissue per plant (Table 1). The protection tended to be less than for *P. infestans.* The mean from four experiments shows a tendency in induced plants for increased dry weight of the haulm and fresh weight of the tubers (Table 1).

Discussion

Increased resistance to late blight from 30 to 70 % was found in potato cultivar Bintje which has no major or minor genes for resistance to *P. infestans.* The prerequisite was prior inoculation with either a complex race of the potato-pathogen *P. infestans*, a wheat isolate of the non-potato pathogen *P. cryptogea* or with dipotassium phosphate (Table 1). The results support the hypothesis that the genetic potential for induced resistance is present in all plants (Kuć, 1982).

The common feature of the inducer's action was a slight injury to the induced leaves. This is in agreement with the requirements for induction suggested by Dean & Kuć (1985), i.e. a living leaf with a small lesion that will signal a continuous stress message. The induction given by *P. cryptogea* tended to be better than that given by *P. infestans.* Furthermore, the induction given by the fungi was better than that given by the salt

(Table 1), perhaps because a living microorganism gives a more continuous stress message (injury) than does a salt.

Kalra et al. (1989) found that induction by *P. infestans* against potato virus Y on potatoes was at an optimum at about 5×10^3 zoospores per ml and decreased with increasing inoculum concentration. We found with *P. infestans* as inducer that the protection increased with the number of induced leaves and that it decreased with increasing inoculum concentration (Figs 1 and 2). In contradiction, Kuć & Richmond (1977) found that increasing inoculum concentration and number of lesions on a leaf resulted in increased resistance to anthracnose on cucumber when the inducer was *C. lagenarium*. One difference between the two pathogens is that *P. infestans* causes lesions on a susceptible potato leaf that expand and finally kill it, whereas *C. lagenarium* causes

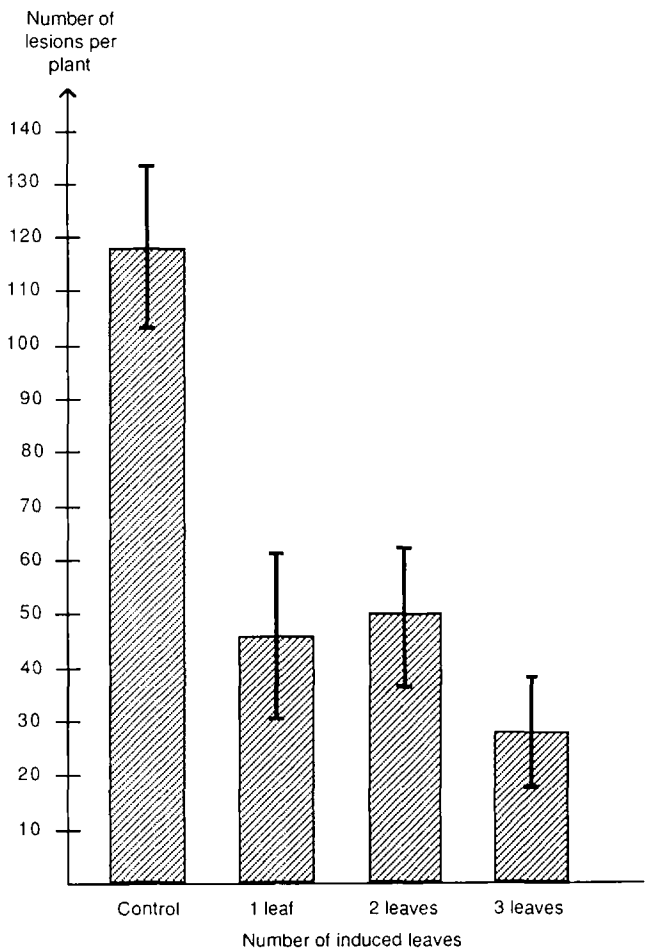

Fig. 1. Systemic induced resistance with *P. infestans* to late blight as a function of the number of leaves induced. ANOVA $P < 0.001$. The vertical bars show S.D.

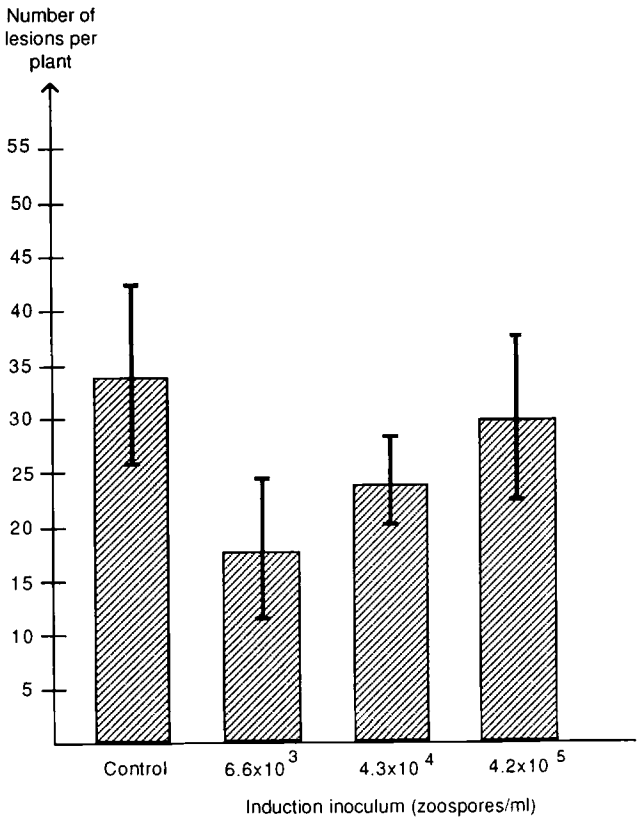

Fig. 2. Systemic induced resistance with *P. infestans* to late blight as a function of the inoculum concentration on the inducer leaves. ANOVA $P<0.224$. The vertical bars show S.D.

lesions on a cucumber leaf that are restricted in size. This may mean that an increased amount of an inducer giving restricted lesions on a living potato leaf, as did *P. cryptogea* in our experiments (Table 1), could increase the protection to late blight.

Dry weight of the haulm and fresh weight of the tubers tended to increase in induced plants (Table 1). In longer lasting experiments these tendencies would be likely to increase as the green assimilating leaf area is larger on induced plants.

Young developing leaves of a potato plant are more likely to be induced than fully expanded leaves (Table 2). This is in agreement with results from tobacco where leaves developing in the time interval between induction and challenge with *Peronospora tabacina* exhibited a high degree of resistance to blue mold (Mandryk, 1961). This suggests that if young potato plants in the field are induced, the attack of late blight may be hampered later on in the season when new induced leaves have developed. Riviera-Peña observed in Mexico that if cultivar Criolla (*Solanum tuberosum L. subsp. andigena* (Juz. et Buk.), Hawkes) was attacked in the field by late blight as a young plant, it was resistant when attacked as a developed plant (personal communication, 1989). One reason for the absence of similar observations in Sweden may be that late blight occurs late in the season when the plants already are well developed.

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