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Autor: Engesser, Roland / Gessler, Cesare

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The ability of a cultivar pathogenic race of *Colletotrichum lindemuthianum* to induce resistance to bean anthracnose in etiolated bean hypocotyls

Roland Engesser and Cesare Gessler

Institute of Phytomedicine, Swiss Federal Institute of Technology, ETH. CH-8092 Zürich, Switzerland.

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Abstract

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A model system is presented for the study of induced resistance using the compatible interaction between *Colletrotrichum lindemuthianum* and etiolated hypocotyls of French bean. First results show that similar to systemically induced resistance in whole plants, penetration into the epidermal tissue is reduced. Contrary to induced resistance of leaf tissue, papillae seem to play no role in the activated defense of hypocotyls.

Introduction

Induced resistance in plants is a well-known phenomenon. The mechanisms involved are only partially known and may be specific for each plant-pathogen system, or may even depend on the type of tissue. Pathogens of the genus *Colletotrichum* are often used to study induced defense mechanisms (Elliston et al. 1976 b, Hammerschmidt and Kuć 1982, Stumm and Gessler 1986). Penetration of epidermal cells is easily observed because appressoria are formed on short germination hyphae and the penetration peg forces its way through the cuticular membrane and cell wall, forming a large hypha in the penetrated cell. Host reactions can be observed well in the affected epidermal cells. For observations beyond the epidermal layer, etiolated tissues, for instance of hypocotyls of *Phaseolus vulgaris* and *Colletotrichum* ssp., are suitable.

In the literature the induction of resistance to *C. lindemuthianum,* which causes beananthracnose, by inoculation with cultivar-nonpathogenic races of *C. lindemuthianum* and *C. lagenarium,* nonpathogens of bean (Elliston et al. 1971, 1976 a), is described.

In most cases true systemic resistance can only be induced with a pathogen or at least with organisms that cause slowly developing, restricted necrosis (Kuć 1983). In green plants of French bean this is achieved by superficial application of a pathogenic race of *C. lindemuthianum* or by infiltration of *C. lagenarium* and *Thielaviopsis basicola* (Engesser and Gessler, unpubl.).

This study presents a model system to observe induced mechanisms of resistance using etiolated bean hypocotyls and a pathogenic race of *C. lindemuthianum* without some of the drawbacks known from earlier works.

Materials and methods

Pathogen and host. Colletotrichum lindemuthianum (Sacc. & Magn.) Schribner race Beta (CBS 132.57) was cultivated on Mathur's agar (Mathur et al. 1950). Conidial suspensions were prepared from 10- to 14-day-old cultures. French bean plants (*Phaseolus vulgaris* var. *nana* (L) Aschers, cultivar Top Crop) were grown in 10-cm-diameter plastic pots, containing quartz-sand (corn-size 1 mm) and perlite (1:1 v/v) in a dark phytotron at 22/18 °C (16 h/8 h) and 70% relative air humidity.

Inoculation. Conidial suspension (3.5×10^5 conidia/ml) or water was applied in 10-µl-drops on marked sites on the hypocotyls of 7–8 d old plants (inducer inoculation). Four days later a second (challenge) inoculation was made with a suspension of the same density. To avoid running off of the drops the plants were slightly tilted. Five inoculation sites were marked at 2 cm intervals below the cotyledonary node. For the inducer inoculation, the sites at 2, 6, and 10 cm were used. The drops for the challenge inoculation were applied on the reverse side of the sites at 4 and 8 cm.

Incubation. After the application of the drops the plants were kept for 2 d in a water-saturated atmosphere at a constant temperature (20 °C) in the dark.

Microscopical preparation. Epidermal strips were prepared. For conventional microscopy, the strips were mounted in a solution of lactic acid (10 ml), alcohol (10 ml), glycerine (10 ml), phenol (10 g), and aniline blue (0.02 g) and observed with a differential interference contrast microscope.

For fluorescence microscopy, strips were kept for 1–2 h in ethanol-water with aniline blue (1:1 v/v and 1% w/v) and mounted in water. An Olympus IFC microscope (BHS-TR-NIC) equipped with an exciter filter (20 UB-W) and beam splitting mirror (B4-DMUB) was used.

Evaluation and statistics. Macroscopic symptoms were evaluated 10 d after the challenge inoculation. Each challenge site was classified in one of the following categories: lesion apparent; hypersensitive-like flecking; no visible reaction. Additionally sporulating lesions (presence of acervuli) were classified. Measurements were made under a magnifying binocular. The surface of the lesion was calculated as an ellipse from the length and width measured. Reported values are the mean of 5 experiments with 10 plants each and two sites per treatment. Significance was calculated after the Kolmogoroff-Smirnoff-Test (Sachs 1984) for the median of the evaluated surface of the lesion. For number of lesions and reaction-type significance of difference between hypocotyls with prior fungal infection and controls was calculated by the Student's t-test for all experiments together. Epidermal strips were taken from 10 plants, one site per plant. On each strip 100 appressorial sites were considered and classified into the following three categories: no penetration and no visible papilla; penetration, visible papilla. No case with papilla and penetration at the same site was ever recorded. The experiment was repeated three times. Significance was calculated from the transformed data ($x = \arcsin \sqrt{p}$) in each experiment according to the Student's t-test (Sachs 1984).

Results

Inoculation of healthy hypocotyls with the pathogenic race of *C. lindemuthianum* lead, in almost all cases, to brown lesions. A prior application of water drops (controls) had no effect on development of symptoms. Every lesion which developed ten days later covered an area of 2–4 mm² (Fig. 1 B and Tab. 1). On hypocotyls protected by a prior infection normal symptoms developed only on half of the inoculated sites. At the other sites often no reaction (Fig. 1 A) or hypersensitive flecking could be seen. The

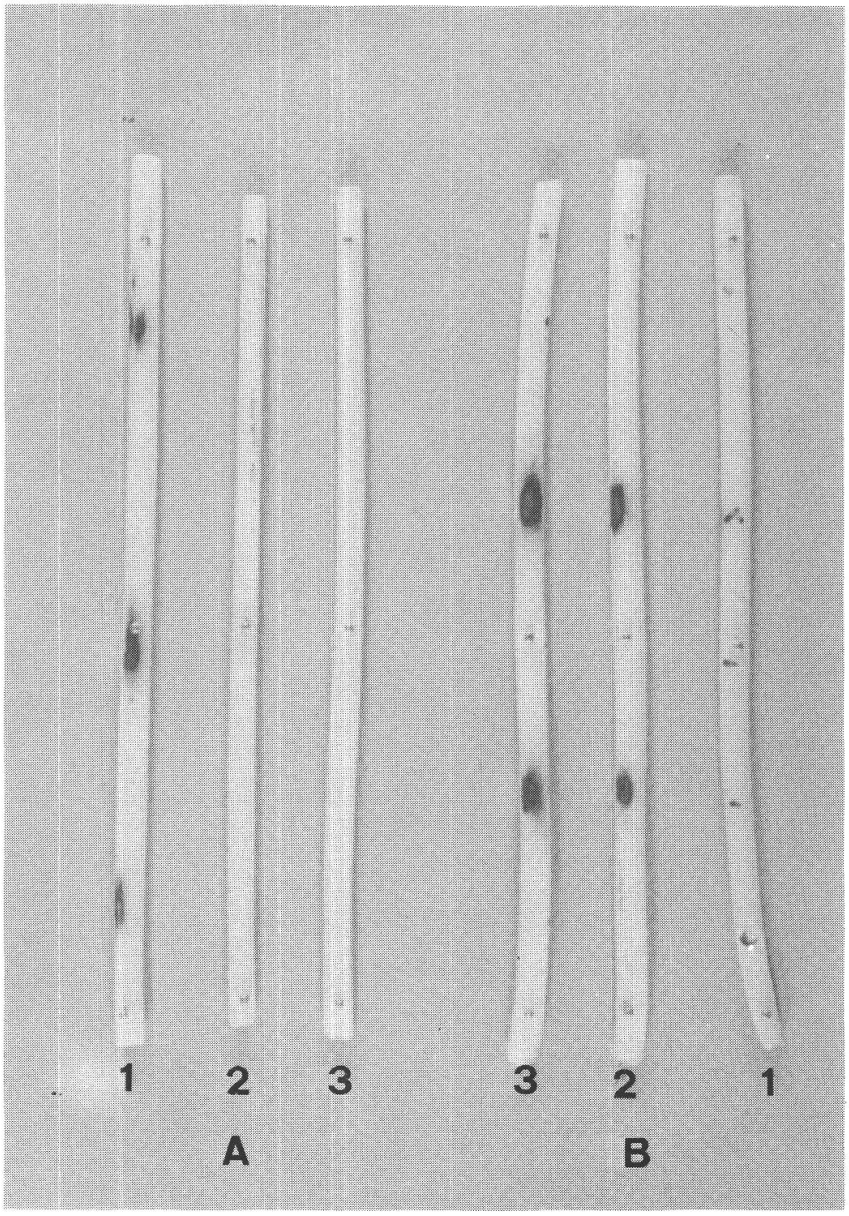
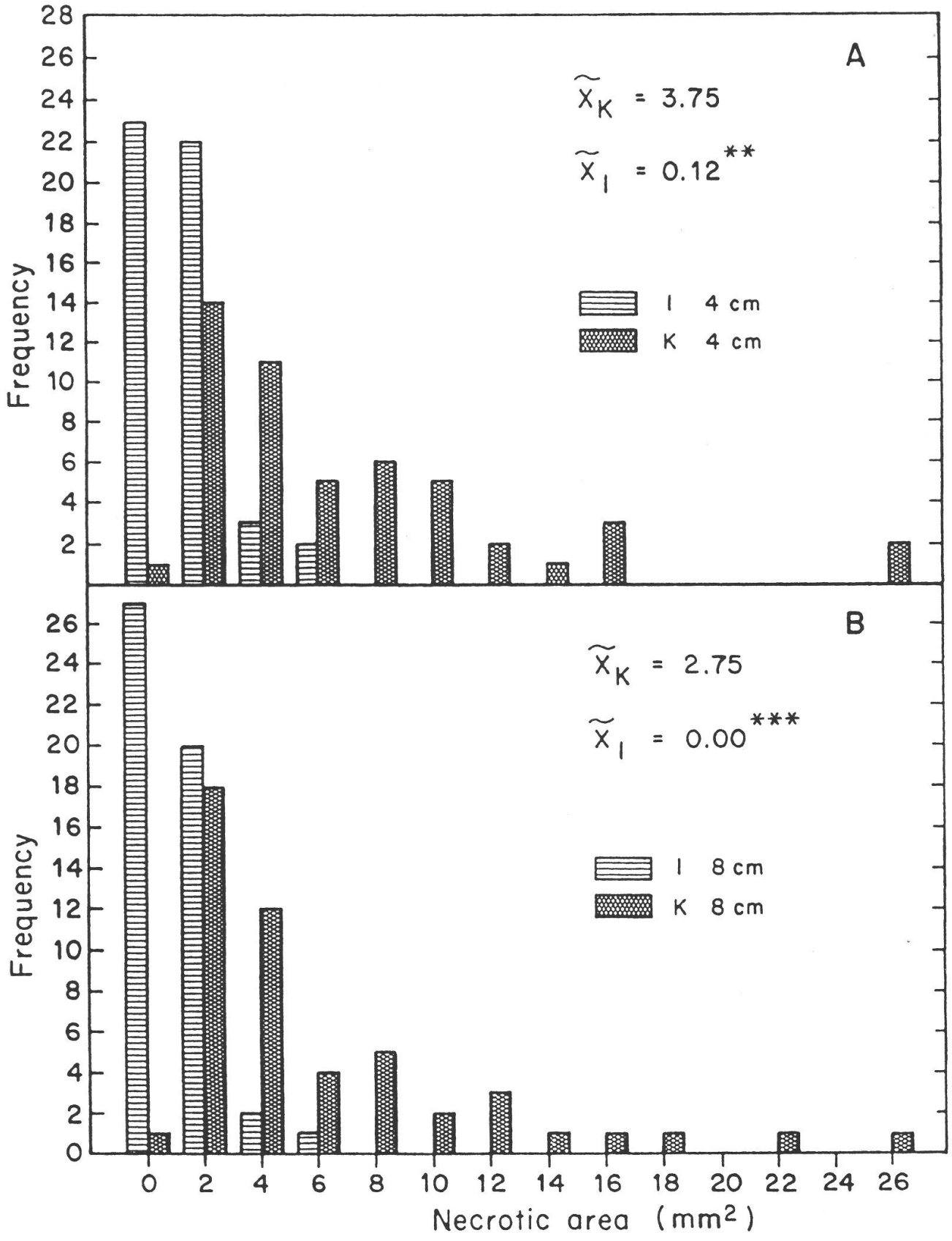


Fig. 1. Symptoms caused by *Colletotrichum lindemuthianum* on etiolated bean hypocotyls. A, induced resistance: 1. Symptoms caused by the inducer inoculation at the sites 2, 6, and 10 cm below the cotyledons; 2 and 3 show no symptoms at the sites 4 and 6 cm 10 d after the challenge inoculation (on 2 slight hypersensitive flecking at site 4 cm is visible). B, controls (unprotected): 1 application of water drops at the sites 2, 6, and 10 cm; 2 and 3 symptoms caused by the challenge inoculation.

number of sporulating lesions was drastically reduced by the induction of resistance (Tab. 1). Variations in reaction type between individual hypocotyls and between experiments were generally great. Induced resistance expressed as reduction of the lesion surface compared to the controls varied from a minimum of 75% to a maximum of 98%, with an average reduction of 87%, depending on the experiment and also on the site along the hypocotyls (4 resp. 8 cm). Overall difference was significant after calculation of the medians and the respective confidence interval, with $p < 0.01$ for the sites at 4 cm and $p < 0.001$ for the sites at 8 cm (Fig. 2).


A
$\tilde{x}_K$ = 3.75
$\tilde{x}_I$ = 0.12**
I 4 cm
K 4 cm
Frequency
B
$\tilde{x}_K$ = 2.75
$\tilde{x}_I$ = 0.00***
I 8 cm
K 8 cm
Frequency
Necrotic area (mm²)

Tab. 1. Type of symptoms developed on etiolated bean hypocotyls 10 d after inoculation with *Colletotrichum lindemuthianum* as a function of a prior inoculation with the same fungus

Treatment	Inoculation site	% sites with symptoms			
		Hyp.	Lesion	0	Sporul.
Induced	4 cm	20* ± 12	54** ± 25	26** ± 15	10*** ± 10
	8 cm	38* ± 24	46** ± 27	16^{ns} ± 19	6*** ± 5
Control	4 cm	2 ± 4	98 ± 4	0	66 ± 21
	8 cm	2 ± 4	98 ± 4	0	56 ± 15

Results are from five experiments, ten plants per treatment. Asterisks represent significant differences from corresponding control values by Student's t-test: *, $P \leqq 0.05$; **, $P \leqq 0.01$; ***, $P \leqq 0.001$.

Tab. 2. Interaction between *Colletotrichum lindemuthianum* and epidermal cells of induced resistant bean hypocotyls

Inoculation site	Replicate	Samples from induced resistant hypocotyls[a]		Samples from control hypocotyls[b]	
		Penetration[c] in %	Papillae[d] in %	Penetration in %	Papillae in %
4 cm	I	9.4 ± 3.4**	1.8 ± 0.8^{ns}	15.4 ± 3.5	2.4 ± 1.0
	II	1.7 ± 1.2**	0.3 ± 0.4*	5.1 ± 2.9	1.8 ± 1.9
	III	1.7 ± 0.9***	0.8 ± 1.0^{ns}	9.3 ± 6.1	0.9 ± 0.9
8 cm	I	7.4 ± 2.7***	1.3 ± 1.2**	19.5 ± 6.8	3.3 ± 1.3
	II	0.6 ± 0.4***	0.2 ± 0.5^{ns}	5.3 ± 3.7	0.8 ± 1.0
	III	1.4 ± 1.2*	0.5 ± 0.6^{ns}	6.6 ± 10.1	0.7 ± 0.5

Mean values and standard deviation were obtained by evaluating the behavior of at least 100 conidia with appressoria 100 h after inoculation on three different occasions. The number of instances where neither penetration nor papillae were found can be obtained by subtracting the sum of penetrations and papillae from 100.

[a] Resistance was induced by previous (4 d) inoculation with *C. lindemuthianum.*

[b] Control hypocotyls were pretreated with water.

[c] Hypha visible in the cells below the appressoria.

[d] Papillae visible below the appressorium, no penetration.

Asterisks represent significant differences from corresponding control values by Student's t-test: *, $P \leqq 0.05$; **, $P \leqq 0.01$; ***, $P \leqq 0.001$.

Fig. 2. Effect of a prior infection with *Colletotrichum lindemuthianum* on the size of lesions 10 d after a drop inoculation with the same fungus. Inoculation sites 4 cm (A) and 8 cm (B) below the cotyledons. Resistance was induced 4 d earlier with a drop inoculation at the sites 2, 6, and 10 cm (I). Control hypocotyls (K) were treated with water only. X = median value of the area developing symptoms. For each treatment 50 application sites were evaluated and classified. **, $P \leqq 0.01$; ***, $P \leqq 0.001$.

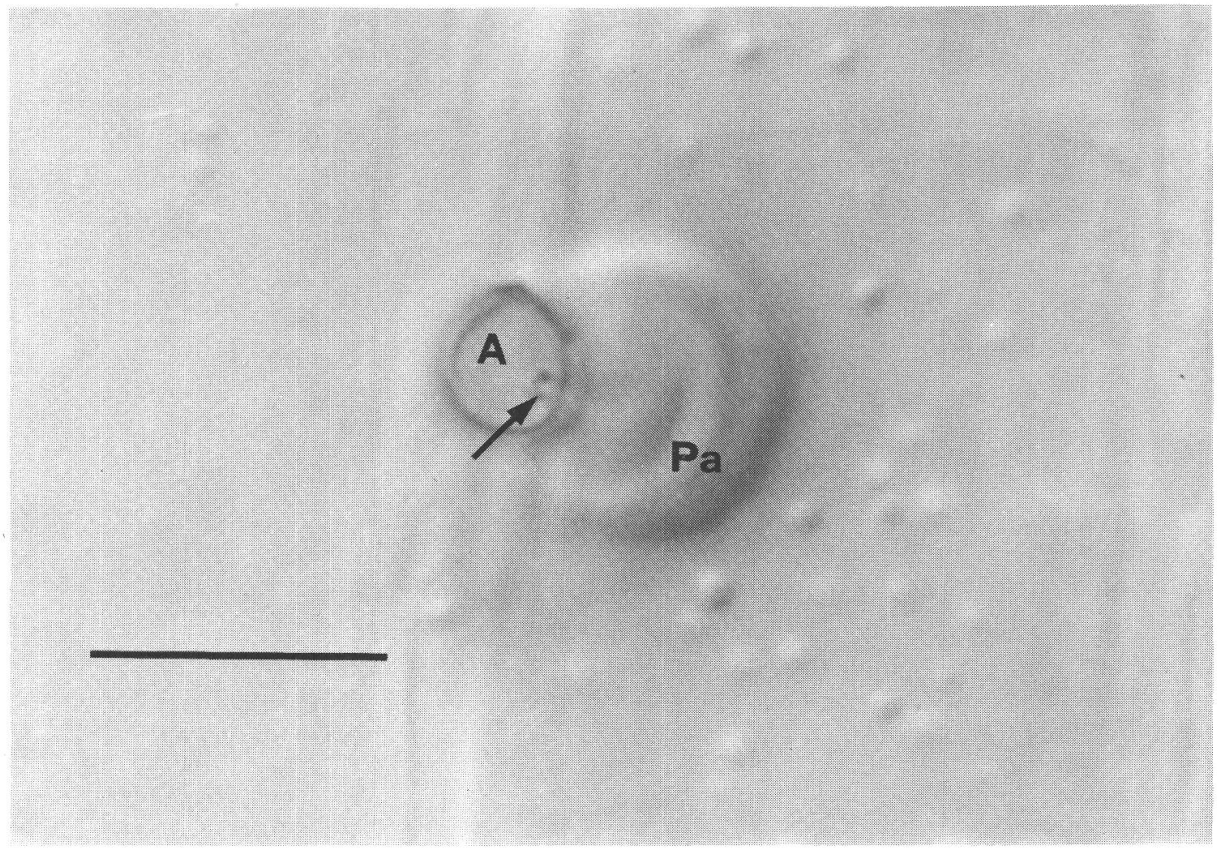


Fig. 3. A papilla (P) in an epidermis cell. Ap. = Appressorium of *Colletotrichum lindemuthianum*. Arrow indicates the penetration peg. Bar represents 20 µm.

Penetration into the epidermal tissue of induced resistant hypocotyls by the fungus with the formation of a visible infection hypha was reduced significantly by 39–89% (Tab. 2) depending on the experiment. The formation of papillae (Fig. 3) was decreased by 20–80%, but only in few cases with a statistical significance (Tab. 2).

Discussion

Induced resistance is expressed in bean hypocotyls as a reduction in number and size of lesions, and a suppression of sporulation. These criteria are commonly used in describing induced resistance (Roberts 1984, Ross 1964, Takahashi et al. 1985). Up to now resistance could be induced in bean hypocotyls only by apathogens of the bean cultivar used (Rahe et al. 1969, Elliston et al. 1976 a, b). Rahe et al. (1969) applied the challenge inoculation at the same site as the inducer inoculation with a cultivar-non-pathogenic race *C. lindemuthianum*. Elliston et al. (1976 a, b) chose a distance of 0.5 cm between the inducer site and the challenge site. Heat attenuated cultivar pathogenic races of the pathogen induced similar resistance effects (Rahe and Kuć 1970, Kuć 1983). In cucumber resistance could be induced by parasites pathogenic to cucumber or by other pathogens (Kuć 1983). In tomato (Heller and Gessler 1986) and tobacco (Kuć 1983) similar results were obtained. Green plants were used in all these cases and

the site of challenge was on plant organs morphologically clearly separated from the induction site.

In all these systems the mechanisms responsible for the induced resistance were investigated. With green tissue only special techniques such as fluorescence microscopy allow a quantitative evaluation of changes due to induced resistance (Stumm and Gessler 1986). Other authors used epidermal strips of petioles (Richmond et al. 1979, Hammerschmidt and Kuć 1982).

Etiolated tissue allows direct observation with a minimum of chemical pretreatments such as clearing. With a novel technique we succeeded in inducing resistance in bean hypocotyls against pathogenic races of *C. lindemuthianum* using the same race as inducing agent. In green plants this resistance could never be induced with nonpathogenic races.

Resistance induced at the same site or at very short distances may not be controlled by the same mechanisms as systemically induced resistance. In bean hypocotyls, Elliston et al. (1971, 1976 b) observed a containment of the penetrating hyphae and not a reduction in penetrations, as was observed in green plants of cucumber. In cucumber the response was dependent on the type of tissue involved. Epidermal tissues from leaves of protected plants reacted against penetration attempts by forming impenetrable papillae in up to 50% of all penetration attempts (Stumm and Gessler 1986). On the other hand, in petiole tissues penetration was blocked by enhanced lignification (Hammerschmidt and Kuć 1982). The model described here allows a comparison between the different systems.

In contrast to induction with an apathogenic race of *C. lindemuthianum* or an apathogen of beans at the same site as the challenge infection, or at a site up to 0.5 cm apart, induction with a pathogenic race at a distance of 2 cm leads to a reduced penetration of *C. lindemuthianum* into the epidermal cells and, if penetration occurs, also to hypha containment. Similar to the results from cucumber petiole tissue, the reduction in penetration was not correlated with an increase in papillae as was observed in cucumber leaf tissue. The ratio of papillae observed had a tendency to be reduced by an almost similar amount as penetration. Common to this model and to systemic induced resistance in cucumber is the reduction in penetration. In cucumber leaf tissue the rapid formation of impenetrable papillae appears to be an important defence mechanism. For cucumber petioles rapid lignification and in bean hypocotyls an unknown mechanism effective prior to the known cytological reactions must be postulated.

It can be concluded that the defence mechanisms effective in induced resistance depend on the type of tissue. Further investigations are necessary to decide whether or not the inducing organism or the distance between the inducer site and challenge site are responsible for the different reactions.

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