

Possible involvement of salicylic acid in systemic acquired resistance of *Cucumis sativus* against *Sphaerotheca fuliginea*

Gian Guido Conti¹, Alberto Pianezzola¹, Anna Arnoldi², Guido Violini¹ and Dario Maffi¹

¹Centro CNR di Studio per il Miglioramento Sanitario delle Colture Agrarie c/o Istituto di Patologia Vegetale, Università di Milano, via Celoria 2, 20133 Milano, Italy (Fax: 2 70631287); ²Dipartimento di Scienze Molecolari Agroalimentari (DISMA), Università di Milano, via Celoria 2, 20133 Milano, Italy

Accepted 24 January 1996

Key words: cucumber, powdery mildew, salicylic acid, SAR, tobacco necrosis virus

Abstract

The possible involvement of salicylic acid in systemic acquired resistance of *Cucumis sativus* against *Sphaerotheca fuliginea* was studied. Cucumber plants were inoculated with tobacco necrosis virus on the cotyledons and the level of endogenous salicylic acid in the first true leaf was determined by gas chromatography. Salicylic acid increased continuously from the second day after virus inoculation to the fifth day, when the same leaf was inoculated with *Sphaerotheca fuliginea*. In healthy plants, the efficiency of exogenous salicylic acid in inducing resistance was assayed by applying aqueous solutions at different times before *Sphaerotheca fuliginea* inoculation. To evaluate the level of induced resistance, the following parameters were examined by light microscopy: percentage of conidial germination, length of the hyphae derived from single conidia, number of haustoria, percentage of epidermal cells with lignified walls and of necrotic cells underlying fungal hyphae. In treated plants conidial germination was reduced, the total length of the hyphae was shorter, the number of haustoria was lower and the haustorium-containing epidermal cells had more frequently lignified walls. Moreover, an evident increase in callose deposition was observed leading to the formation of oversized papillae around the penetration pegs. These results indicate that the application of salicylic acid before inoculation with *Sphaerotheca fuliginea* reduces the intensity of the infectious process and that salicylic acid is involved in the expression of systemic resistance in cucumber challenged by the biotrophic pathogen *Sphaerotheca fuliginea*.

Introduction

Pre-inoculation with infectious agents or treatment with chemicals can make plants resistant to different pathogens. This active resistance results from a series of processes which are induced in the host cells by structures or products of a parasite (inducing agent). By this way the plant is protected against a subsequent attack by a pathogen. This induced resistance can operate in the areas of the first inoculation, but can also spread to uninfected parts of the plant in which defence mechanisms are activated (Ross, 1966; Sequeira, 1979; Heitefuss, 1982). This protection is known as systemic acquired resistance (SAR). In cucumber, a localized infection by *Colletotrichum lagenarium* or tobacco necrosis virus (TNV) insures systemic protec-

tion against a broad range of foliar and root pathogens (Kuc, 1982). Further studies have demonstrated that pre-inoculation of cucumber with TNV induces systemic resistance to *Sphaerotheca fuliginea* (Bashan and Cohen, 1983; Conti et al., 1990) and that this resistance is probably due to mechanisms which are triggered by a complex system involving one or more 'signal' molecules (Conti et al., 1994). To be qualified as a signal in SAR, a molecule should be synthesized by the plant, increase systemically following attack by a pathogen or pest, move throughout the plant, induce defence-related proteins and phytochemicals, and enhance resistance to pathogens or pests (Enyedi et al., 1992a).

Salicylic acid (SA) could be one of the endogenous signals which activate SAR against pathogens

causing necrosis of the host tissue (Cutt and Klessig, 1992; Malamy and Klessig, 1992; Metraux and Raskin, 1993). The endogenous levels of SA increase both locally and systemically as a consequence of the inducing inoculation (Malamy et al., 1990; Metraux et al., 1990; Rasmussen et al., 1991) and seem to be correlated with the accumulation of PR proteins (Yalpani et al., 1991; Enyedi et al., 1992b; Malamy et al., 1992; Yalpani et al., 1993) and disease resistance (Malamy and Klessig, 1992; Klessig et al., 1993; Lawton et al., 1993). Exogenous SA, administered in different ways, induces defence responses in plants and enters the signalling pathway somewhere between recognition of the pathogen and induction of defence responses. This effect has been studied mainly in plants bearing viral local necrotic lesions: the treatment reduces the number and the size of lesions formed, induces accumulation of PR-proteins, modulates the expression of the same genes induced by pathogens which cause resistance and protects the leaves against a subsequent attack. The induction of resistance by exogenous SA is effective also against various necrotizing systemic viruses, fungi and bacteria (for a review, see Malamy and Klessig, 1992). About the signal transduction pathway leading to acquired resistance, Chen and Klessig (1991) isolated a soluble protein with an high affinity for SA suggesting that this protein plays an important role in perceiving and transducing the signal. This protein exhibits a catalase activity which is inhibited by the binding of SA, with an induced increase in H_2O_2 concentration thereby inducing the expression of defence-related genes associated with SAR (Chen et al., 1993).

In this work, we have tried to verify if SA is involved in systemic resistance against the biotrophic agent *S. fuliginea* in *Cucumis sativus*. Therefore, we have measured the changes of endogenous SA level induced in the first true leaf by the inoculation of the cotyledons with TNV. At the same time, the defence responses evoked in this plant-pathogen interaction by exogenous SA were characterized and discussed in comparison with those induced by virus inoculation.

Materials and methods

Plant growth conditions and inoculation procedures

Seeds of cucumber (*Cucumis sativus* L. cv Marketer) were sown in 9 cm-pots and grown in an environmentally controlled growth chamber at 22 ± 1 °C, 70% RH, 14-h photoperiod and $190 \mu\text{Em}^{-2}\text{s}^{-1}$ light intensity.

The cotyledons of plants with two fully expanded leaves (about 3 weeks after sowing) were mechanically inoculated with a purified suspension ($4 \mu\text{g ml}^{-1}$) of TNV in phosphate buffer in order to obtain at least 12 local necrotic lesions per cotyledon, a number sufficient to induce systemic resistance (Conti et al., 1990). These plants will be referred to as 'protected plants'. Control plants were sham-inoculated with carborundum and buffer.

Extraction and quantitation of salicylic acid

At various intervals after TNV inoculation (1, 2, 3, 4 and 5 days), 5 protected and 5 control plants were harvested. The first true leaves were sliced, mingled, divided into two groups of 0.5 g each and stored at -25 °C until use. Each sampling was repeated twice.

For gas chromatographic analysis the method described by Niemann et al. (1991) was followed, with some modifications. Leaf samples were ground in acetone (4 ml), the mixture was filtered and the residue washed with acetone. The combined filtrates were evaporated to dryness, the residue was taken up in 100% ethanol (10 ml) and partitioned with a mixture of hexane (10 ml) and distilled water (4 ml). The organic phase was dried with anhydrous Na_2SO_4 and evaporated. Samples were dissolved in 100 μl of dichloromethane and derivatized as trimethylsilyl ethers by adding 100 μl of 1:1 pyridine: *N,O*-bis-(trimethylsilyl)trifluoroacetamide before injecting. Gas chromatographies were performed on a DANI 3800 gas chromatograph equipped with a FID detector and a PTV injector. A capillary column SPB5 Supelco, 30 m \times 0.25 mm i.d., 0.25 mm film thickness, was used. Analysis conditions were: carrier gas helium; flow, 0.75 ml min^{-1} ; oven temperature, from 140 °C to 270 °C at 12 °C min^{-1} ; injector from 30 °C to 290 °C in 30 s; splitless injection; after 30 s split 30 ml min^{-1} . Data were recorded by a Shimatzu CR2A integrator. 2,4-dichlorobenzoic acid was used as internal standard.

Gas chromatography/mass spectrometry analysis was performed on a Finnigan TSQ70 instrument equipped with the same column. Analysis conditions were: oven, the same program; injector, 280 °C; transfer line, 280 °C; electron energy, 70 eV; electromultiplier, 900 V. The mass spectrum of bis(trimethylsilyl)salicylic acid was compared with that of an authentic sample and with the NIST data bank.

Recovery of SA, estimated by extracting tissue to which a known amount of SA had been added, aver-

aged 89% and therefore results for recovery were not corrected.

Application of exogenous SA

Aqueous SA solutions adjusted to pH 6 with 0.1 M Na₂CO₃ were applied to 3 weeks-old plants in 3 different ways: i) 10 droplets (0.7 µl each; 3 mM SA) were deposited on each cotyledon; ii) the first true leaf was sprayed with 1 ml of 2 mM SA; iii) the petiole of the first true leaf was injected with 1 ml of 2 mM SA.

Fungal inoculation

At different times (from 12 h to 2 days) after SA application, as specified below, the upper surface of the first true leaf was sprayed with an aqueous suspension containing 100,000 conidia ml⁻¹ of *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. as previously described (Conti et al., 1990).

Light microscopy observations

Thirty-six hours after *S. fuliginea* inoculation, 12 leaf discs (15 mm diameter) were cut out of the inoculated leaves from 6 SA-treated and 6 untreated plants. The discs were cleared in 70% hot ethanol, stained with toluidine blue (O'Brien et al., 1965) and observed with a Leitz Dialux photomicroscope: 50 conidia were examined on each disc and the percentage of germination was calculated on a total of 600 conidia. The experiment was repeated three times.

At 72 hours, other sets of leaf discs were cleared and stained as above. Eight conidia were randomly selected in each disc set. The images were recorded by camera lucida. The total length of the hyphae derived from each conidium was measured and the number of haustoria was counted. The experiment was repeated three times.

Two and 10 days after fungal inoculation, some leaf discs were collected from the infected leaves and subjected to the aniline blue fluorescent test for callose (Eschrich and Currier, 1964). The discs were discoloured in hot ethanol, rehydrated in water and placed in aniline blue for 10–15 min. They were observed with a photomicroscope equipped with a fluorescent attachment (excitation filter 340–380 nm). Samples of the same leaves were fixed in phosphate-buffered 3% glutaraldehyde, dehydrated in ethanol and embedded in Technovit 7100 (Gerrits and Smid, 1983). Thin sections were stained and observed as above.

Two, 5 and 10 days after fungal inoculation, other sets of leaf discs were cleared and stained with toluidine blue: for each set of discs, 500 epidermal cells

Table 1. Levels of endogenous salicylic acid (µg g⁻¹ fresh weight) in the first true leaf of protected cucumber plants, at different times after inoculation of the cotyledons with TNV

Days after TNV inoculation	Salicylic acid (µg g ⁻¹ fresh weight) ^a
1	<5
2	48.6 ± 7.0
3	64.2 ± 2.2
4	76.7 ± 3.3
5	91.7 ± 3.7

^a Values are averages ± SD (N = 2). In control plants, SA was less than 5 µg g⁻¹ f.w. 5 µg is the limit of detection of SA with the used method.

underlying fungal hyphae were examined. The numbers of haustorium-containing cells, cells with lignified walls and necrotic cells were recorded separately.

All the obtained data were subjected to variance analysis followed by Duncan's multiple range test. In each figure, significant differences are indicated by different letters.

Results

Endogenous SA levels in the first true leaf after TNV inoculation of the cotyledons

Gas chromatographic analysis showed that the level of endogenous SA in the first true leaf of protected plants increased continuously from the second day after TNV inoculation until the fifth day, when the first true leaf was inoculated with *S. fuliginea*. In control plants, SA level remained undetectable in each analysis (Table 1).

Effects of exogenously applied SA on fungal development

Compared with control plants, the percentage of conidial germination calculated 36 h after inoculation with *S. fuliginea* was significantly reduced when droplets of 3 mM SA were applied on the cotyledons 2 days before fungal inoculation. A reduction, though not statistically significant, was also observed when SA was applied 1 day before inoculation. A significant decrease of the percentage of conidial germination was also measured when 2 mM SA was applied by spraying the surface of the first true leaf two times (24 h and 12 h before fungal inoculation) or one time (12 h before fungal inoculation) (Figure 1).

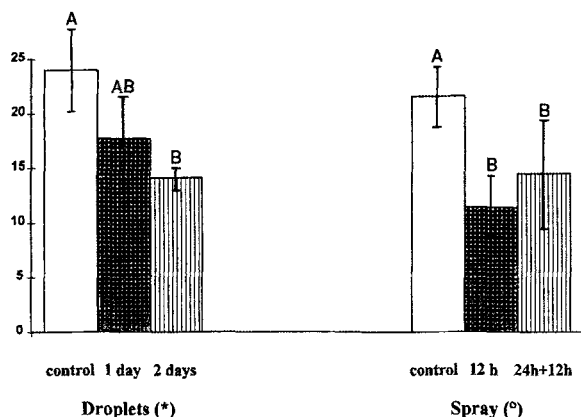


Figure 1. Percentage of germinated conidia 36 hours after inoculation with *S. fuliginea*. Means of 12 replications (N = 600) repeated 3 times. Bars represent standard deviation. Differences significant at $P = 0.01$.

(*): 10 droplets (0.7 μ l each) of 3 mM SA were applied on each cotyledon 1 or 2 days before fungal inoculation.

(°): 1 ml of 2 mM SA was sprayed on the first true leaf, once (12h) or twice (24 h and 12 h) before fungal inoculation.

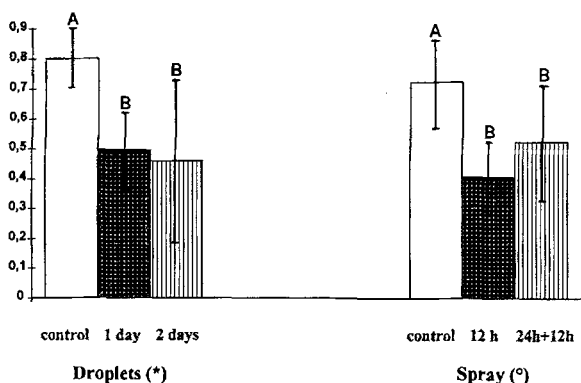


Figure 2. Length of hyphae (mm) developed from single conidia of *S. fuliginea* 3 days after fungal inoculation. Means of 8 measurements repeated 3 times. Bars represent standard deviation. Differences significant at $P = 0.01$.

(*): 10 droplets (0.7 μ l each) of 3 mM SA were applied on each cotyledon 1 or 2 days before fungal inoculation.

(°): 1 ml of 2 mM SA was sprayed on the first true leaf, once (12 h) or twice (24 h and 12 h) before fungal inoculation.

Three days after inoculation, the total length of the hyphae derived from single conidia was significantly shorter when droplets of 3 mM SA were applied on the cotyledons either 2 days or 1 day before fungal inoculation. Similar results were obtained when the first true leaf was sprayed with 2 mM SA both two times (24 h and 12 h before inoculation) and one time (12 h before inoculation) (Figure 2).

For each treatment, the total number of haustoria visible under the measured hyphae was reduced 3 days

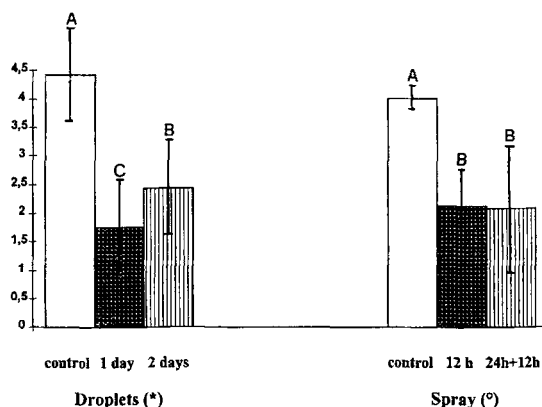


Figure 3. Number of haustoria per germinated conidium. Means of 8 counts repeated 3 times. Bars represent standard deviation. Differences significant at $P = 0.01$.

(*): 10 droplets (0.7 μ l each) of 3 mM SA were applied on each cotyledon 1 or 2 days before fungal inoculation.

(°): 1 ml of 2 mM SA was sprayed on the first true leaf, once (12 h) or twice (24 h and 12 h) before fungal inoculation.

after fungal inoculation. Droplets of 3 mM SA on the cotyledons gave the maximum reduction when supplied 2 days before inoculation. The spraying of 2 mM SA significantly reduced the number of haustoria, without any difference due to the time and the number of treatments (Figure 3).

Effects of exogenous SA on epidermal cells underlying fungal hyphae

Compared with untreated plants (Figure 6), exogenous 2 mM SA applied by injection through the petiole 3 days before inoculation of *S. fuliginea* induced an evident increase in callose deposition leading to the formation of oversized papillae around the penetration pegs (Figures 4, 5, 7). Many epidermal cells underlying the hyphae had lignified walls both in SA-treated plants and in control plants (Figure 8). In SA-treated plants, the number of haustorium-containing cells with lignified walls was significantly higher than in untreated plants 2 and 5 days after fungal inoculation, while at 10 days the difference was not statistically significant (Figure 9).

The number of necrotic epidermal cells was low both in plants treated with SA and in controls; no statistical differences were observed (data not shown).

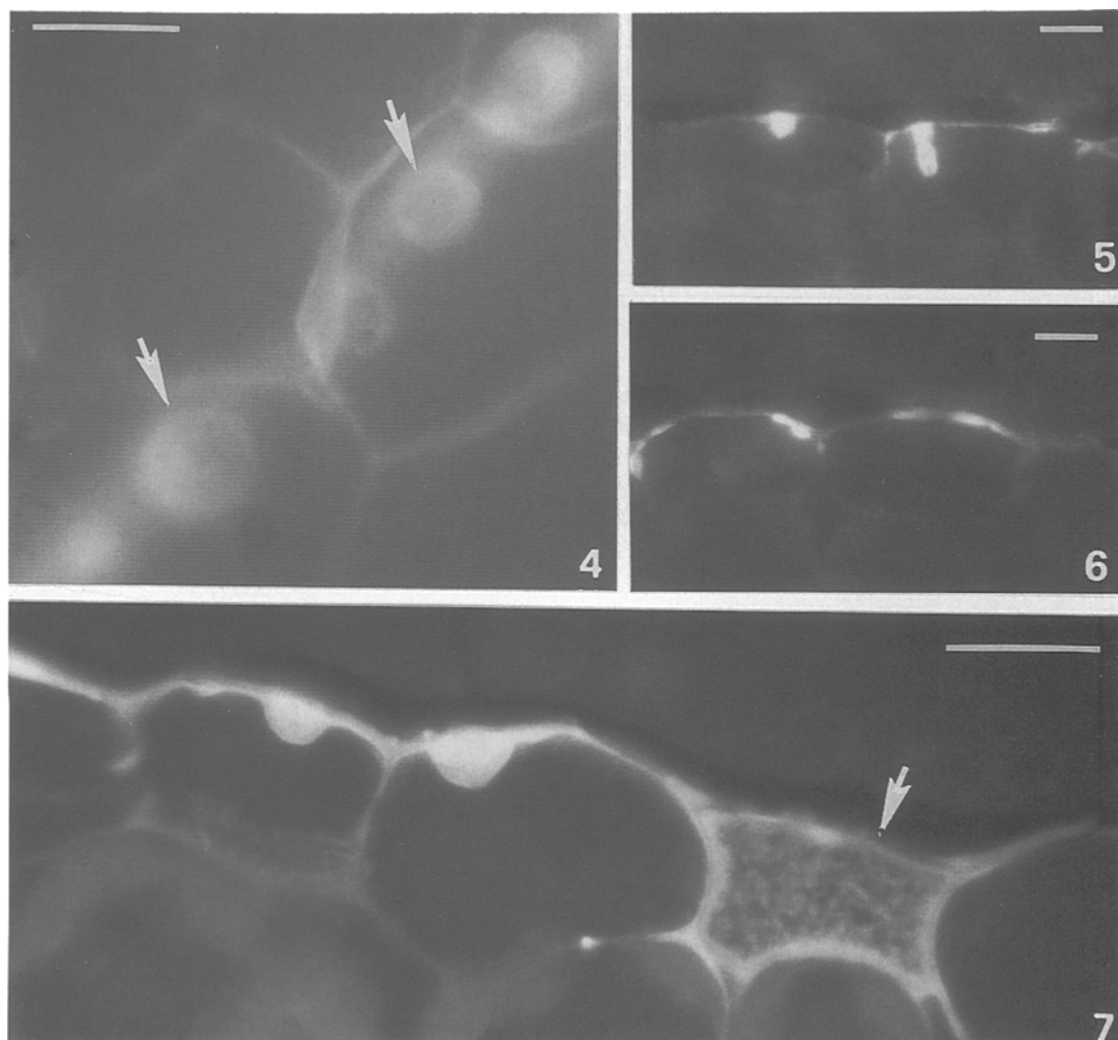


Figure 4–7. Light micrographs of cucumber primary leaves from plants treated with salicylic acid (injection of 2 mM SA) and from control plants, 10 days after challenge inoculation with *S. fuliginea*. All bars = 10 μ m.

Figure 4. In SA-treated plants, the walls of epidermal cells underlying fungal hyphae and the haustoria (arrows) show an intense fluorescence after aniline blue staining for callose.

Figure 5 and 7. In SA-treated plants, strong callose depositions form oversized papillae around the penetration pegs in transverse leaf sections. Note in **Figure 7** a necrotic epidermal cell with thickened walls (arrow).

Figure 6. In control plants, only minor callose depositions take place and no oversized papillae are formed.

Discussion

Systemic acquired resistance (SAR) requires a lag period between induction and challenge and is probably mediated by an endogenous signal produced in the leaf which had previously received the inducing inoculation, and then translocated to uninoculated parts of the plant (Jenns and Kuc, 1979 and 1980; Dean and Kuc, 1986a and 1986b; Conti et al., 1990). Several evidences indicate that endogenous salicylic acid (SA) is a signal molecule in SAR and that exogenous appli-

cation of this compound also stimulates resistance to a variety of pathogens (for reviews, see Cutt and Klessig, 1992; Enyedi et al., 1992; Malamy and Klessig, 1992; Raskin, 1992a and 1992b; Hammerschmidt, 1993; Kessmann et al., 1994).

The results presented here indicate that endogenous SA accumulates in the first true healthy leaf of *Cucumis sativus* soon after pre-inoculation of the cotyledons with TNV. SA was detectable 2 days after the inducing inoculation, when the necrotic lesions were not yet visible, and its concentration increased in the follow-

ing days, at least until the moment of the challenge inoculation with *Sphaerotheca fuliginea*. These results are in agreement with other evidences indicating the SA involvement in SAR in cucumber, since at the onset of SAR a strong increase in SA was found in the phloem sap after inoculation of the first leaf with TNV or *C. lagenarium* (Metraux et al., 1990). Similar results were obtained following inoculation of cucumber leaves with *Pseudomonas syringae* pv. *syringae* (Rasmussen et al., 1991). An increase of endogenous SA, often related to accumulation of pathogenesis-related proteins and resistance, were also found in Xanthi-nc tobacco, in *Arabidopsis thaliana* and in the hybrid *Nicotiana glutinosa* × *Nicotiana debney* inoculated with TMV or *P. syringae* (Malamy et al., 1990; Yalpani et al., 1991; Enyedi et al., 1992; Yalpani et al., 1993; Summermatter and Metraux, 1994).

Our results show that the application of exogenous SA before inoculation with *S. fuliginea* reduces the intensity of the infectious process. A strong inhibitory effect on the conidial germination was observed 36 h after fungal inoculation both at a local (spraying of the first true leaf) and at a systemic level (droplets on the cotyledons), with slight changes of this effect depending on the concentrations tested and on the time between treatment and inoculation. A similar effect was observed in plants showing systemic resistance after the inoculation of the cotyledons with TNV (Conti et al., 1994) and could be due to the formation of surface chemical barriers (Blakeman and Atkinson, 1981; Cohen et al., 1983; Goodman et al., 1986). A toxic effect of locally applied SA on the conidia cannot be excluded, though some evidences indicate that the compound has not a direct antifungal activity (Mills and Wood, 1984; Okuno et al., 1991).

A major resistance mechanism observed to act at the penetration stage after treatment with SA, was the frequent formation of papillae, which inhibited both fungal penetration and haustoria development as observed by other authors (Sherwood and Vance, 1976; Koga et al., 1980; Stumm and Gessler, 1986; Xuei et al., 1988). These papillae were frequently over-sized and gave a strong positive reaction for callose, as already found in plants protected by TNV (Conti et al., 1990).

Moreover, in SA-treated plants the total length of the hyphae derived from single conidia was reduced and the number of haustoria visible in the epidermal cells underlying the hyphae was lower. A great number of these cells (50% as maximum) had lignified walls. This result seems to indicate that injection of

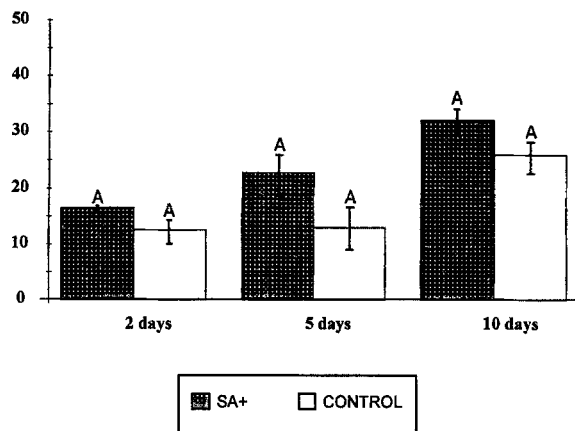


Figure 8. Percentage of epidermal cells with lignified walls 2, 5 and 10 days after *Sphaerotheca fuliginea* inoculation. 500 cells were examined both in SA-treated and in control plants. Means of 10 replications (N = 500). Bars represent standard deviation. No statistical differences were found at P = 0.01.

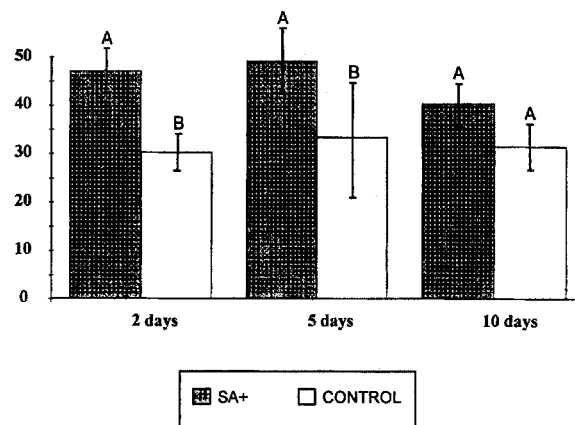


Figure 9. Percentage of cells having lignified walls on the total of haustorium-containing cells 2, 5 and 10 days after *Sphaerotheca fuliginea* inoculation. 500 cells were examined both in SA-treated and in control plants. Means of 10 replications (N = 500). Bars represent standard deviation. Differences significant at P = 0.01.

SA through the petiole is efficient to induce this effect though it was not compared with other ways of administration assayed. This increased lignification is probably related to an activation of cell wall peroxidases (Hammerschmidt and Kuc, 1980; Hammerschmidt et al., 1982). Parallel effects on the infectious process were observed also in plants protected by TNV (Bashan and Cohen, 1983; Conti et al., 1990 and 1994). Lignification may reduce or block the diffusion of nutrients from the neighbouring host cells to the haustorium, inhibit fungal enzymes following the accumulation of toxic lignin precursors or act as a physical barrier that

impedes haustorial growth (Kuc and Preisig, 1984; Conti et al., 1994), thus affecting the severity of infection.

In conclusion, the onset of SA-induced resistance is a quite early event starting in the pre-penetration stage and leading to a partial inhibition of conidial germination. Resistance seems to become more effective in the post-penetration stage, when the defence mechanism based on increased lignin biosynthesis and deposition becomes fully active. As a general consequence of the observed effects (reduction in conidial germination, reduced hyphal length and reduced number of haustoria), fungal invasion was strongly inhibited, although not completely stopped, by the application of SA. Our results clearly show that infection was impeded during the first steps of its development. However the short duration of the experiments (10 days after fungal inoculation) did not allow to determine the final level of plant protection reached.

The results presented here strongly suggest that salicylic acid plays an important role in the induction of systemic resistance to *S. fuliginea* in cucumber. Experiments with transgenic plants clearly demonstrate that SA is required for the establishment of SAR, at least in tobacco and *Arabidopsis thaliana* (Gaffney et al., 1993; Lawton et al., 1993). However, it is not yet fully ascertained whether salicylic acid is the primary mobile signal or is simply involved in a cascade of events which culminates in the expression of SAR, as already indicated by Rasmussen et al. (1991). On the basis of grafting experiments using transgenic plants, Vernooij et al. (1994) affirm that SA is not the translocating SAR-inducing signal in tobacco, though the presence of SA is required to induce systemic resistance in tissues distant from the infection site. In our experiments, the effects of SA treatment did not depend on the site of application of the compound. The local effects are consistent with the conclusion of Vernooij et al. (1994). The systemic effects of SA application to the cotyledons could be interpreted in two ways: either SA itself, being translocated throughout the plant and accumulating in distant tissues, triggers resistance mechanisms or, following SA application, an unknown long distance signal is liberated which acts in distant tissues *via* the local production of SA.

Acknowledgements

The skilful technical assistance of Mr. Renato Bosoni is gratefully acknowledged. Research supported by the

National Research Council of Italy, Special Project RAISA, Sub-project N. 2, Paper N. 2520.

References

- Bashan B and Cohen Y (1983) Tobacco necrosis virus induces systemic resistance in cucumbers against *Sphaerotheca fuliginea*. *Physiol Plant Pathol* 23: 137–144
- Blakeman JP and Atkinson P (1981) Antimicrobial substances associated with the aerial surface of plants. In: Blakeman JP (ed) *Microbial Ecology of the Phylloplane* (pp. 245–263) Academic Press, London
- Chen Z and Klessig DF (1991) Identification of a soluble salicylic acid-binding protein that may function in signal transduction in the plant disease resistance response. *Proc Natl Acad Science USA* 88: 8179–8183
- Chen Z, Silva H and Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* 262: 1883–1886
- Cohen Y, Goldschmidt Z and Sklarz B (1983) A preformed chemical inhibitor of tobacco powdery mildew on leaves of *Nicotiana glutinosa*. *Physiol Plant Pathol* 22: 143–150
- Conti GG, Bassi M, Carminucci D, Gatti L and Bocci AM (1990) Preinoculation with tobacco necrosis virus enhances peroxidase activity and lignification in cucumber, as a resistance response to *Sphaerotheca fuliginea*. *J Phytopathology* 128: 191–202
- Conti GG, Bassi M, Maffi D, Violini G, Magnani L and Gatti L (1994) Induced systemic resistance against *Sphaerotheca fuliginea* in cucumber: efficiency of Tobacco Necrosis Virus (TNV) and copper sulphate (CuSO₄) in eliciting defence reactions. *J Phytopathology* 140: 123–132
- Cutt JR and Klessig DF (1992) Salicylic acid in plants: a changing perspective. *Pharmaceutical Technol* 16: 26–34
- Dean RA and Kuc J (1986a) Induced systemic protection in cucumbers: the source of the 'signal'. *Physiol Mol Plant Pathol* 28: 227–233
- Dean RA and Kuc J (1986b) Induced systemic protection in cucumber: time of production and movement of the signal. *Phytopathology* 76: 966–970
- Enyedi A, Yalpani N, Silverman P and Raskin I (1992a) Signal molecules in systemic plant resistance to pathogens and pests. *Cell* 70: 879–886
- Enyedi A, Yalpani N, Silverman P and Raskin I (1992b) Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to Tobacco Mosaic Virus. *Proc Natl Acad Science USA* 89: 2480–2484
- Eschrich W and Currier HB (1964) Identification of callose by its diachrome and fluorochrome reactions. *Stain Technology* 39: 303–307
- Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H and Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261: 754–756
- Gerrits PO and Smid L (1983) A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *J Microscopy* 132: 81–85
- Goodman RN, Kiraly Z and Wood KR (1986) *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, Columbia
- Hammerschmidt R (1993) The nature and generation of systemic signals induced by pathogens, arthropod herbivores, and

- wounds. In: Andrews JH and Tommerup IC (eds), *Advances in Plant Pathology* (pp. 307–337) Academic Press Ltd, London
- Hammerschmidt R and Kuc J (1980) Enhanced peroxidase activity and lignification in the induced systemic protection of cucumber. *Phytopathology* 70: 689
- Hammerschmidt R, Nuckles E and Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol Plant Pathol* 20: 73–82
- Heitefuss R (1982) General review of active defence mechanisms in plants against pathogens. In: Wood RKS (ed) *Active Defense Mechanisms in Plants* (pp. 1–18) Plenum Press, New York
- Jenns A and Kuc J (1979) Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and Tobacco Necrosis Virus. *Phytopathology* 69: 753–756
- Jenns A and Kuc J (1980) Characteristics of anthracnose resistance induced by localized infection of cucumber with Tobacco Necrosis Virus. *Physiol Plant Pathol* 17: 81–91
- Kessmann H, Staub T, Ligon J, Oostendorp M and Ryals J (1994) Activation of systemic acquired disease resistance in plants. *European Journal of Plant Pathology* 100: 359–369
- Klessig DF, Malamy J, Hennig J, Chen Z, Sanchez-Casas P, Indulski J and Gryniewicz G (1993) Induction, modification and reception of the salicylic acid signal in plant defense. In: Fritig B and Legrand M (eds) *Mechanisms of Plant Defense Responses* (pp. 185–195) Kluwer Academic Publishers, Dordrecht
- Koga H, Mayama S and Shishiyama J (1980) Correlation between the deposition of fluorescent compounds in papillae and resistance in barley against *Erysiphe graminis hordei*. *Can J Bot* 58: 536–541
- Kuc J (1982) Plant immunization mechanisms and practical implication. In: Wood RKS (ed) *Active Defence Mechanisms in Plants* (pp. 157–178) Plenum Press, New York
- Kuc J and Preisig C (1984) Fungal regulation of disease resistance mechanisms in plants. *Mycologia* 76: 767–784
- Lawton K, Uknes S, Friedrich L, Gaffney T, Alexander D, Goodman R, Mettraux JP, Kessmann H, Ahl Goy P, Gut Rella M, Ward E and Ryals J (1993) The molecular biology of systemic acquired resistance. In: Fritig B and Legrand M (eds) *Mechanisms of Plant Defense Responses* (pp. 422–432) Kluwer Academic Publishers, Dordrecht
- Malamy J and Klessig DF (1992) Salicylic acid and plant disease resistance. *The Plant Journal* 2: 643–654
- Malamy J, Hennig J and Klessig DF (1992) Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to Tobacco Mosaic Virus infection. *The Plant Cell* 4: 359–366
- Malamy J, Carr JP, Klessig DF and Raskin I (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250: 1002–1004
- Mettraux JP and Raskin I (1993) Role of phenolics in plant disease resistance. In: Chet I (ed) *Biotechnology in Plant Disease Control* (pp 191–209) Wiley-Liss
- Mettraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W and Inverardi B (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250: 1004–1006
- Mills PR and Wood RKS (1984) The effects of polyacrylic acid, acetylsalicylic acid and salicylic acid on resistance of cucumber to *Colletotrichum lagenarium*. *Phytopathol Z* 111: 209–216
- Niemann GJ, Van Der Kerk A, Niessen WMA and Versluis K (1991) Free and cell wall-bound phenolics and other constituents from healthy and fungus-infected carnation (*Dianthus caryophyllus* L.) stems. *Physiol and Mol Plant Pathol* 38: 417–432
- O'Brien TP, Feder N and McCully ME (1965) Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59: 367–373
- Okuno T, Nakayama M, Okajima N and Furusawa I (1991) Systemic resistance to downy mildew and appearance of acid soluble proteins in cucumber leaves treated with biotic and abiotic inducers. *Ann Phytopath Soc Japan* 57: 203–211
- Raskin I (1992a) Role of salicylic acid in plants. *Annu Rev Plant Physiol Plant Mol Biol* 43: 439–463
- Raskin I (1992b) Salicylate, a new plant hormone. *Plant Physiol* 99: 799–803
- Rasmussen JB, Hammerschmidt R and Zook MN (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*. *Plant Physiol* 97: 1342–1347
- Ross AF (1966) Systemic effects of local lesion formation. In: Beemster ABR and Dijkstra J (eds) *Viruses of Plants* (pp. 127–150) North Holland Publishing Company, Amsterdam
- Sequeira L (1979) The acquisition of systemic resistance by prior inoculation. In: Daly JM and Uritani I (eds) *Recognition and Specificity in Plant Host-Parasite Interactions* (pp. 231–251) University Park Press, Baltimore
- Sherwood RT and Vance CP (1976) Histochemistry of papillae formed in reed canarygrass leaves in response to noninfecting pathogenic fungi. *Phytopathology* 66: 503–510
- Stumm D and Gessler C (1986) Role of papillae in the induced systemic resistance of cucumbers against *Colletotrichum lagenarium*. *Physiol Mol Plant Pathol* 29: 405–410
- Summermatter K and Mettraux JP (1994) The role and the mode of action of salicylic acid in systemic acquired resistance in *Arabidopsis thaliana*. *Intl. Congress on Molecular Plant-Microbe Interactions*, Edinburgh, UK (Abstract 374)
- Vernooij B, Friedrich L, Morse A, Reist R, Kolditz-Jawhar R, Ward ER, Uknes SJ, Kessmann H and Ryals JA (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *The Plant Cell* 6: 959–965
- Xuei XL, Jarlors U and Kuc J (1988) Ultrastructural changes associated with induced systemic resistance of cucumber to disease: host response and development of *Colletotrichum lagenarium* in systemically protected leaves. *Can J Bot* 66: 1028–1038
- Yalpani N, Shulaev V and Raskin I (1993) Endogenous salicylic acid levels correlate with accumulation of pathogenesis-related proteins and virus resistance in tobacco. *Phytopathology* 83: 702–708
- Yalpani N, Silverman P, Wilson TMA, Kleier DA and Raskin I (1991) Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. *The Plant Cell* 3: 809–818