

titles of PR1 mRNAs in uninfected leaves as compared to infected leaves (Figs. 1B and 2C). No PR1 gene expression or salicylic acid increase was seen in uninfected leaves of TMV-inoculated Xanthi (nn) plants or in mock-inoculated plants of either genotype (Fig. 2A).

In 1983 Van Loon (15) postulated that salicylic acid acts by mimicking an endogenous phenolic signal that triggers PR gene expression and disease resistance. Our results suggest that the signal is salicylic acid itself. Susceptible Xanthi (nn) plants carry the PR genes, and these genes can be activated by treatment with exogenous salicylic acid (Fig. 1C) but not with TMV. Therefore, it is likely that infection of susceptible plants fails to trigger the signal transduction pathway that leads to salicylic acid production, resistance, and PR gene expression.

Métraux *et al.* (16) present independent evidence suggesting that salicylic acid plays a role in the induction of SAR in cucumber after pathogen attack. In addition, Raskin and co-workers recently demonstrated that salicylic acid is an endogenous regulator of heat and odor production in the inflorescences of some thermogenic lilies (17–19). These three studies suggest that salicylic acid plays a broad and important role in signal transduction in plants.

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20. Using a more sensitive assay, we have since found that basal levels range from <0.005 to 0.02 µg per gram of fresh weight, as indicated in the text.
21. Salicylic acid was extracted from 1 g, fresh weight, of leaf tissue and analyzed by high-performance liquid chromatography and spectrofluorescence as described previously (18). The presence of salicylic acid was confirmed by gas chromatography–mass spectroscopy. Salicylic acid recovery was 55%. The data shown were not corrected for this factor. Total leaf RNA was prepared as previously described [J. O. Berry, B. J. Nikolau, J. P. Carr, D. F. Klessig, *Mol. Cell. Biol.* **5**, 2238 (1985)] and 20 µg of each preparation was analyzed by Northern (RNA) blot and hybridization to ³²P-labeled PR1 cloned cDNA [J. R. Curt, D. C. Dixon, J. P. Carr, D. F. Klessig, *Nucleic Acids Res.* **16**, 9861 (1988)].
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23. Supported in part by the Du Pont Company (Wilmington, DE), where some of the experiments were performed, and in part by the National Science Foundation.

14 May 1990; accepted 10 August 1990

Increase in Salicylic Acid at the Onset of Systemic Acquired Resistance in Cucumber

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In an effort to identify the signal compound that mediates systemic acquired resistance (SAR), changes in the content of phloem sap were monitored in cucumber plants inoculated with either tobacco necrosis virus or the fungal pathogen *Colletotrichum lagenarium*. The concentration of a fluorescent metabolite was observed to increase transiently after inoculation, with a peak reached before SAR was detected. The compound was purified and identified by gas chromatography–mass spectrometry as salicylic acid, a known exogenous inducer of resistance. The data suggest that salicylic acid could function as the endogenous signal in the transmission of SAR in cucumber.

PLANTS INOCULATED WITH NECROTrophic pathogens such as fungi, bacteria, or viruses react by inducing a transient resistance against subsequent fungal, bacterial, or viral infections (1–4). This induced resistance can be restricted to areas of the first inoculation (3) but may spread to other parts of the plant to establish SAR (1). It has been proposed that SAR is mediated by an endogenous signal that is produced in the infected leaf and translocated in the phloem to other plant parts where it activates resistance mechanisms (1, 2, 5).

Cucurbitaceae have the unique property of releasing phloem sap from cut stem or petiole surfaces (6). We used a high-performance liquid chromatograph (HPLC) system, originally devised for the detection of hydroxylated polyamines (7), to analyze phloem sap after inoculation of cucumber leaves (*Cucumis sativus* L. cv. Marketer SMR 580) with either tobacco necrosis virus (TNV) or the fungal pathogen *Colletotrichum lagenarium*. A distinct increase of a fluorescing metabolite was detected in the phloem after inoculation (Fig. 1, A and B). This

increase appeared before necrotization had taken place on the infected leaf and preceded the induction of resistance observed in the upper uninfected leaves, independent of the pathogen used to induce resistance (Fig. 1, C and D).

The timing of the increase was different depending on the pathogen used for the primary infection. Both resistance and the fluorescing peak appeared sooner after infection when TNV was used as an inducer. TNV produces necrosis 3 to 4 days after inoculation, whereas *C. lagenarium* produces necrosis 5 to 6 days after inoculation. The appearance of the fluorescent peak is therefore likely to be dependent on the timing of disease development inherent in the nature of the inoculated pathogen. The metabolite was also observed to increase when both necrosis on the first leaves and SAR were well established (7 to 9 days after inoculation).

Further analysis of the metabolite indicated that it consisted of several ultraviolet-absorbing components (Fig. 2). Only one of these was fluorescent and separated at the position of the initial metabolite. A preparation of this fluorescent fraction was derivatized to convert the polar compounds to their volatile trimethylsilyl derivatives (8), and this was used for analysis by capillary gas chromatography–mass spectrometry (GC-MS). The chemical ionization mass spectra and chromatographic retention data showed that the trimethylsilyl ester of

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2-hydroxybenzoic acid (salicylic acid) was the primary compound and that the trimethylsilyl ester of 4-hydroxybenzoic acid was present at a trace level (less than 1%). Both the HPLC and the GC-MS results were corroborated by coinjection of the respective reference compounds. Thus, salicylic acid is

an endogenous phloem-located metabolite whose concentration increases after infection of a leaf with necrotizing pathogens.

The concentration of salicylic acid in the phloem sap before resistance occurs ranged typically between 0.2 and 7 μM in infected plants, depending on the pathogen, com-

pared to between 0 and 0.7 μM in mock-infected plants (Fig. 1, A and B). Salicylic acid has been reported to induce resistance against *C. lagenarium* when exogenously applied to cucumber cotyledons at a concentration of 14.5 mM (9, 10). Furthermore, it has no direct antifungal activity against *C. lagenarium*, and no fungitoxic metabolites were detected in salicylate-treated cucumber tissue (9). However, the concentration range needed to elicit resistance from exogenous application is substantially higher than the concentration measured in the phloem. To address this disparity, we followed the fate of ^{14}C -labeled salicylic acid infiltrated into a lower leaf and observed that 1.3% of the initial salicylic acid could be found in the treated leaf 1 day after application, whereas in the upper leaf 0.4% was recovered (11). Thus, salicylic acid, like other organic acids, was translocated rapidly to the upper leaf (12), but a large portion of the applied material was either distributed and sequestered or metabolized. This rapid turnover of the compound may explain why high concentrations of salicylate are needed to induce resistance in infiltration experiments.

Among the resistance mechanisms that are correlated with the establishment of SAR is the induction of pathogenesis-related (PR) proteins. In cucumber, the major PR protein is an extracellular endochitinase (13), which may be involved in protection against fungal attack. This protein and its mRNA accumulate in leaves treated with either pathogens or salicylic acid (14). The induction of the chitinase gene by salicylic acid is, therefore, consistent with the proposed role of salicylate as a signal compound that activates SAR mechanisms. In tobacco, exogenously applied salicylic acid also induces PR proteins and resistance in the treated and occasionally in the untreated leaves (15). Moreover, Malamy *et al.* (16) demonstrate that endogenous levels of salicylic acid, as well as expression of certain PR protein genes, increase in both infected and uninfected upper leaves of tobacco after inoculation with tobacco mosaic virus, which supports our observations in cucumber.

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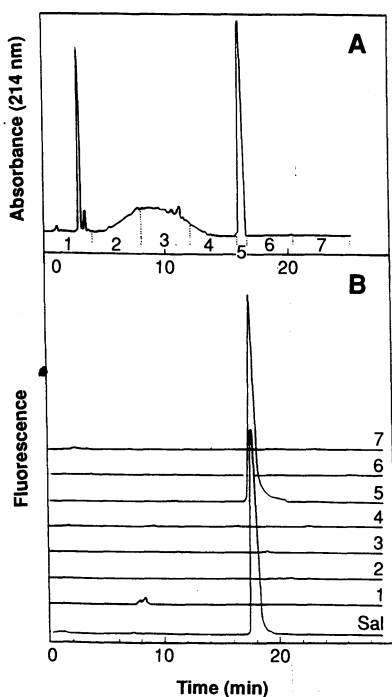
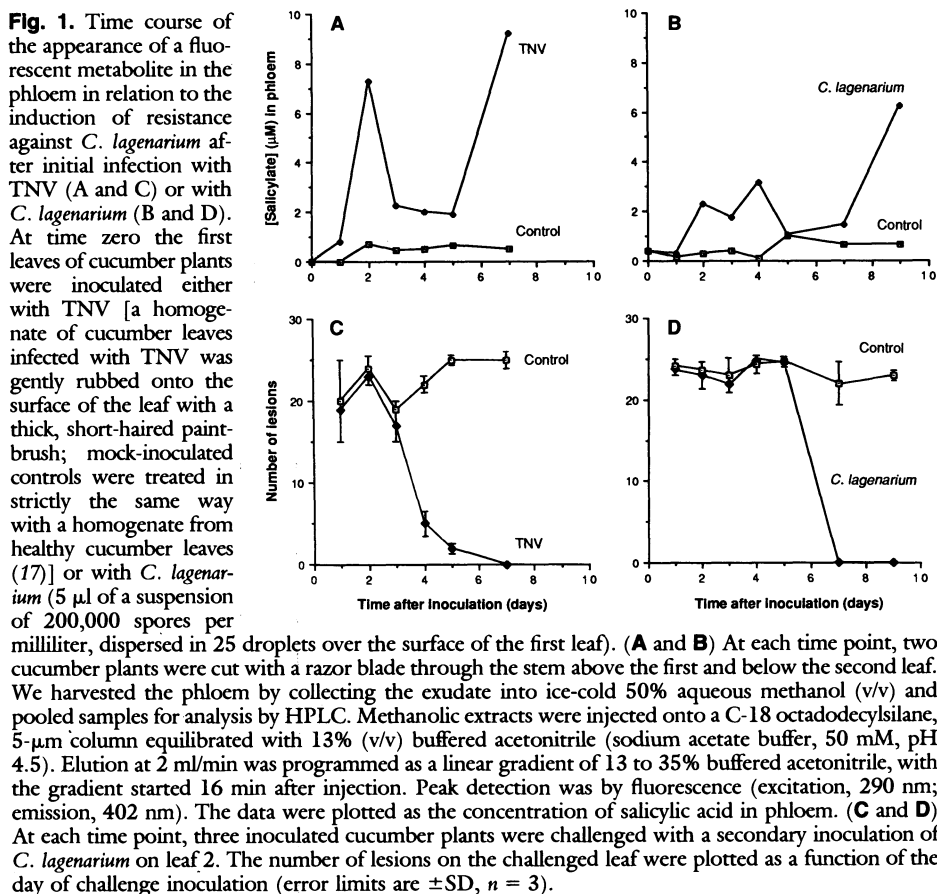


Fig. 2. Characterization of the fluorescent component in phloem sap from plants inoculated on the first leaf with TNV. (A) Chromatogram showing further separation of the crude fluorescent component. The fluorescent peak from the HPLC separation described in the legend to Fig. 1 was collected, lyophilized, and taken up in 50% methanol (v/v). The peak eluate was injected onto a C-18 Vydac 218 TP245 column equilibrated with aqueous trifluoroacetic acid (0.1%, v/v) and eluted at 1.2 ml/min with a continuous gradient of 5 to 95% (v/v) aqueous acetonitrile. Detection was at 214 nm. (B) Elution profiles of the fractions from (A) separated on the system described in the legend to Fig. 1. The eluates were pooled as indicated in (A), lyophilized, and dissolved in 50% methanol (v/v). The elution profile for pure salicylic acid (1 μM) is shown as the bottommost chromatogram (Sal).

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14 May 1990; accepted 6 August 1990



"Sure, genetic fingerprinting can prove someone's innocence –
but not in a case of stock fraud."