The Plant Cell, Vol. 6, 959–965, July 1994 © 1994 American Society of Plant Physiologists

Salicylic Acid Is Not the Translocated Signal Responsible for Inducing Systemic Acquired Resistance but Is Required in Signal Transduction

Bernard Vernooij,[a] Leslie Friedrich,[a] Alison Morse,[b] Roland Reist,[c] Rachida Kolditz-Jawhar,[c] Eric Ward,[a] Scott Uknes,[a] Helmut Kessmann,[c] and John Ryals [a,1]

[a] Agricultural Biotechnology, Ciba-Geigy Corporation, P.O. Box 12257, Research Triangle Park, North Carolina 27709
[b] Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695
[c] Plant Protection, Ciba-Geigy Limited, CH-4002 Basel, Switzerland

Infection of plants by necrotizing pathogens can induce broad-spectrum resistance to subsequent pathogen infection. This systemic acquired resistance (SAR) is thought to be triggered by a vascular-mobile signal that moves throughout the plant from the infected leaves. A considerable amount of evidence suggests that salicylic acid (SA) is involved in the induction of SAR. Because SA is found in phloem exudate of infected cucumber and tobacco plants, it has been proposed as a candidate for the translocated signal. To determine if SA is the mobile signal, grafting experiments were performed using transgenic plants that express a bacterial SA-degrading enzyme. We show that transgenic tobacco rootstocks, although unable to accumulate SA, were fully capable of delivering a signal that renders nontransgenic scions resistant to further pathogen infection. This result indicated that the translocating, SAR-inducing signal is not SA. Reciprocal grafts demonstrated that the signal requires the presence of SA in tissues distant from the infection site to induce systemic resistance.

INTRODUCTION

When plants recognize that they are being invaded by a pathogen, a number of responses can be induced surrounding the infection site. These include a programed cell death believed to restrict pathogen spread (the hypersensitive response; Slusarenko et al., 1991), cell wall strengthening as a result of lignification and cross-linking of cell wall proteins (Bowles, 1990), and the production of antimicrobial compounds (Dixon, 1986). In addition, uninfected parts of the plant develop heightened resistance to further infections by viral, bacterial, and fungal pathogens. This nonspecific resistance phenomenon has been termed systemic acquired resistance (SAR) (Ross, 1961). Even though SAR has been known since the beginning of this century (Chester, 1933), little is known about how the initial infection leads to systemic resistance.

Biochemically, the resistant state is characterized by the coordinate expression of a set of genes termed SAR genes (Ward et al., 1991). These include genes encoding the pathogenesis-related (PR) proteins discovered in the 1970s. Evidence is accumulating that the protein products of the SAR genes are causally related to resistance, because several of these proteins have activity against pathogens in vitro and in the plant (Mauch et al., 1988; Alexander et al., 1993; Sela-Buurlage et al., 1993).

[1] To whom correspondence should be addressed.

Very little is known about the signaling pathway that leads to the systemic resistant state following pathogen infection. Salicylic acid (SA, 2-hydroxybenzoic acid) has been implicated as a component of this pathway. This phenolic acid accumulates following infection with a necrotizing pathogen in cucumber, tobacco, and Arabidopsis (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991; Uknes et al., 1993). The accumulation of SA in pathogen-infected leaves is correlated with the induction of both SAR genes and resistance (Malamy et al., 1990; Métraux et al., 1990; Enyedi et al., 1992; Uknes et al., 1993). In addition, treatment of a tobacco leaf with exogenous SA induces the same set of SAR genes as pathogen infection as well as resistance to pathogens (White, 1979; Ward et al., 1991). However, the most compelling evidence that SA has a role in the signal transduction pathway leading to SAR comes from recent experiments with transgenic plants that cannot accumulate SA following pathogen infection (Gaffney et al., 1993). These transgenic tobacco plants, engineered to express a salicylate hydroxylase (*nahG*) gene from *Pseudomonas putida*, are unable to manifest an SAR response. Thus, SA plays a role in the signal transduction pathway leading to SAR (Gaffney et al., 1993). Nonetheless, these experiments did not address whether SA was the long-distance, phloem-transmissible signal that moves from the site of initial pathogen infection throughout the plant.

In this study, we investigated whether SA could be the systemic signal by taking advantage of the observation that the translocating signal can pass through a graft junction (Weintraub and Kemp, 1961; Jenns and Kuc, 1979; Gianinazzi and Ahl, 1983). Despite their inability to accumulate SA, tobacco mosaic virus (TMV)–infected rootstocks of transgenic NahG plants were fully capable of inducing resistance to viral and fungal pathogens in grafted wild-type tissues, indicating that SA is not the systemic signal. In contrast, wild-type and NahG rootstocks were not able to induce resistance in NahG scions, demonstrating that the long-distance signal requires the presence of SA to induce the resistant state in systemic tissues.

RESULTS

Induction of Systemic Resistance to a Viral Pathogen in Chimeric Grafted Tobacco Plants

We previously demonstrated that in tissues expressing salicylate hydroxylase, SA does not accumulate significantly (Gaffney et al., 1993). These NahG plants were used in grafting experiments designed to test whether SA was the systemic signal. If SA is indeed the translocated signal, it would not be transmitted from NahG tissues, or at least transmission and the resulting signal transduction would be dramatically reduced. Using the experimental design shown in Figure 1, grafts were made between transgenic tobacco plants expressing salicylate hydroxylase (lines NahG-8 and NahG-10; Gaffney et al., 1993) and nontransformed (Xanthi-nc) tobacco plants. As controls in the experiment, Xanthi-nc and NahG plants were grafted back onto themselves. We induced rootstocks of grafted plants with TMV and challenged scion leaves 7 days later with one of two pathogens, TMV or *Cercospora nicotianae*. The disease severity in the challenged leaves was compared to that of mock-induced, grafted plants.

In the first set of experiments, TMV was used as the challenge pathogen to assay for SAR. Following TMV treatment of the rootstock of Xanthi/Xanthi (scion/rootstock) grafted plants, increased resistance was clearly induced in the scion as shown in Figure 2. Table 1 shows the lesion sizes in challenged scion leaves of four separate experiments. The average TMV lesion size in the challenged scion leaves decreased to 41% of the lesion size seen in challenged leaves of mock-inoculated control grafted plants (defined as 100%), which is similar to the reduction in lesion size seen in nongrafted plants (Gaffney et al., 1993). This result confirmed that the translocated signal can pass from the rootstock to the scion through the graft junction.

In contrast, NahG/NahG grafted plants were unable to induce a reduction of lesion size in the challenged scion when the rootstock was pretreated with TMV (Figure 2). The average lesion size on the challenged scion leaves of TMV-pretreated, NahG/NahG grafted plants was 99% relative to the mock-inoculated controls (Table 1), which is consistent with our previous results using intact NahG plants (Gaffney et al., 1993).

Similarly, NahG scions grafted onto Xanthi rootstocks (NahG/Xanthi) were unable to mount an SAR response (Figure 2). Lesions on the challenged NahG scion leaves, following TMV pretreatment of the rootstock, were similar (104%) to lesions on mock-pretreated, grafted plants, as shown in Table 1. Thus, even though the Xanthi rootstocks can transmit a systemic signal, it cannot be transduced in the salicylate hydroxylase–expressing scions.

In contrast, a Xanthi scion grafted onto a NahG rootstock (Xanthi/NahG) reproducibly manifested SAR in response to TMV inoculation of the rootstock (Figure 2). TMV pretreatment decreased the average lesion size in challenge leaves to the same extent as observed in control Xanthi/Xanthi grafted plants (40 and 41%, respectively, see Table 1). This result demonstrated that salicylate hydroxylase–expressing plants were fully able to transmit a systemic signal to induce increased resistance to TMV.

The Translocating Signal Induces Nonspecific Resistance

One of the hallmarks of SAR is the induction of broad-spectrum pathogen resistance (Kuc, 1982; Dean and Kuc, 1985). To investigate the specificity of the resistance in the grafting

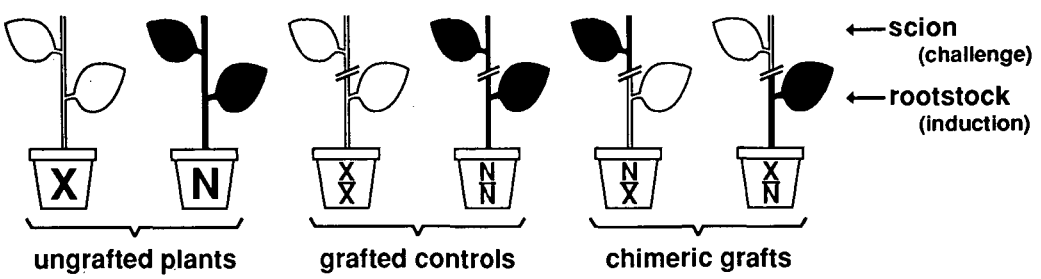

Figure 1. Schematic Diagram of the Grafting Experiments Using Xanthi and NahG Plants.

The rootstocks of the grafted plants were either TMV or mock treated ("induction"), followed by a challenge inoculation of the scion tissue 7 days later. The levels of infection in the challenged leaves were determined 5 or more days after challenge inoculation. X, Xanthi-nc; N, NahG.

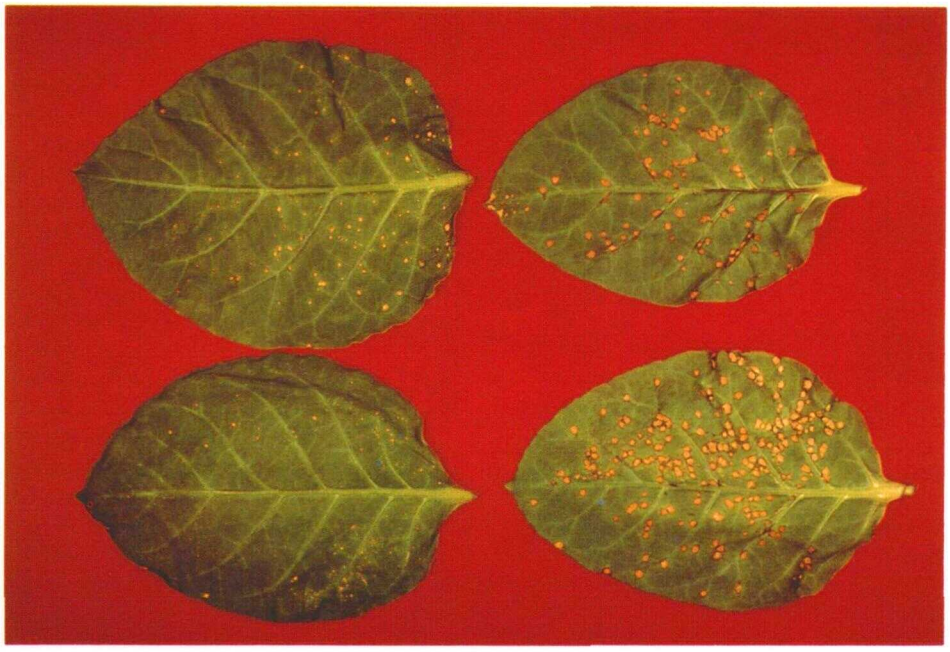

Figure 2. Resistance in Scions of TMV-Pretreated Grafted Plants.

Grafted plants were pretreated with TMV on rootstock leaves and challenged with TMV on the scion leaves 7 days later. The scion leaves, clockwise from top left, are from grafted Xanthi/NahG, NahG/Xanthi, NahG/NahG, and Xanthi/Xanthi plants. Leaves were photographed 5 days after the TMV challenge.

experiment, mock- and TMV-pretreated, grafted plants were challenged on the scion leaves with a fungal pathogen, *C. nicotianae*. Resistance to the fungus was induced by TMV pretreatment of the rootstock in Xanthi/Xanthi grafted plants. The average infected area in the challenged scion leaves of these grafted plants was reduced to 17% of the control as shown in Table 2. Likewise, resistance was inducible in Xanthi/NahG grafted plants in which TMV pretreatment reduced the challenge infected area to 12% of the control. Resistance to *Cercospora* could not be induced in scions of either NahG/NahG or NahG/Xanthi grafted plants by TMV pretreatment of the rootstocks. Thus, NahG rootstocks were capable

Table 1. Induction of Resistance to TMV in Grafted Tobacco Plants by TMV Pretreatment

		Experiment 1	Experiment 2	Experiment 3	Experiment 4	Average
Graft	Inducer	Lesion Size	Lesion Size	Lesion Size	Lesion Size	%
X	Mock	2.70 ± 0.46	0.82 ± 0.13	0.90 ± 0.20	1.56 ± 0.18	
X	TMV	1.40 ± 0.64 (52%)	0.34 ± 0.03 (37%)	0.48 ± 0.40 (53%)	0.31 ± 0.11 (20%)	41%
N	Mock	3.10 ± 0.43	1.94 ± 0.27	1.70 ± 0.40	2.19 ± 0.27	
N	TMV	3.19 ± 0.53 (103%)	1.61 ± 0.22 (83%)	1.76 ± 0.26 (104%)	2.32 ± 0.23 (106%)	99%
N	Mock	2.75 ± 0.40	1.92 ± 0.19	1.20 ± 0.40	2.17 ± 0.16	
X	TMV	3.06 ± 0.54 (111%)	1.63 ± 0.17 (85%)	1.55 ± 0.40 (120%)	2.20 ± 0.22 (101%)	104%
X	Mock	2.08 ± 0.48	1.92 ± 0.07	0.93 ± 0.20	1.38 ± 0.36	
N	TMV	1.19 ± 0.56 (57%)	0.41 ± 0.02 (21%)	0.47 ± 0.18 (51%)	0.44 ± 0.18 (32%)	40%

Grafted plants are indicated in the table (X, Xanthi-nc; N, NahG; scion above, rootstock below the dividing line). Seven days after rootstock-inducing inoculation (mock or TMV), scion leaves were assayed for resistance by a TMV challenge. The lesion size (average diameter ± SD in millimeters) 5 to 10 days postchallenge from four independent experiments are shown. The lesions on the TMV-induced plants are expressed relative to the mock pretreated plants for each experiment and averaged over the four experiments (average %).

Table 2. Induction of Resistance to a Fungal Pathogen by TMV Induction in Grafted Tobacco Plants

Graft	Inducer	Experiment 1 % Infection	Experiment 2 % Infection	Average %
X/X	Mock	18 ± 7	38 ± 9	
X/X	TMV	4 ± 3 (22%)	4 ± 3 (11%)	17%
N/N	Mock	40 ± 15	68 ± 14	
N/N	TMV	41 ± 15 (103%)	67 ± 13 (99%)	101%
N/X	Mock	18 ± 11	70 ± 7	
N/X	TMV	25 ± 18 (139%)	68 ± 6 (97%)	118%
X/N	Mock	12 ± 6	20 ± 12	
X/N	TMV	1 ± 1 (8%)	3 ± 2 (15%)	12%

Grafted plants were challenged with *C. nicotianae* spores following mock or TMV inoculation of the rootstock. The percentage of the infected area on the challenged leaves 12 days postchallenge (% infection) from two independent experiments is shown. The infected area on the TMV-induced plants is expressed relative to the mock induced plants for each experiment and averaged over the two experiments (average %). Grafted plants are indicated in the table as given for Table 1.

of producing a systemic signal that induces resistance to a range of pathogens, whereas NahG scion tissues are unable to respond.

NahG Tissues Do Not Accumulate SA

Because SA and its conjugates accumulate in TMV-infected Xanthi-nc leaves (Malamy et al., 1990, 1992; Enyedi et al., 1992; Enyedi and Raskin, 1993), we measured the levels of both free and total SA in infected and systemic leaves of TMV-induced plants. TMV-inoculated leaves of Xanthi rootstocks accumulated high levels of SA (1.5 to 2.5 μg/g tissue), which represents a 48- to 70-fold increase in SA levels compared to mock-inoculated tissue (30 to 40 ng/g), as shown in Figure 3A. Furthermore, the amounts of free plus glucosylated SA ("total" SA) in Xanthi leaves reached concentrations greater than 8.6 μg/g of tissue (Figure 3B). In contrast, TMV-inoculated NahG leaves showed no significant increase in free (Figure 3A) or total SA levels (Figure 3B), which is consistent with SA levels measured in intact plants (Gaffney et al., 1993). Thus, despite their inability to accumulate SA, NahG rootstock leaves can generate a signal that induced resistance in Xanthi scions.

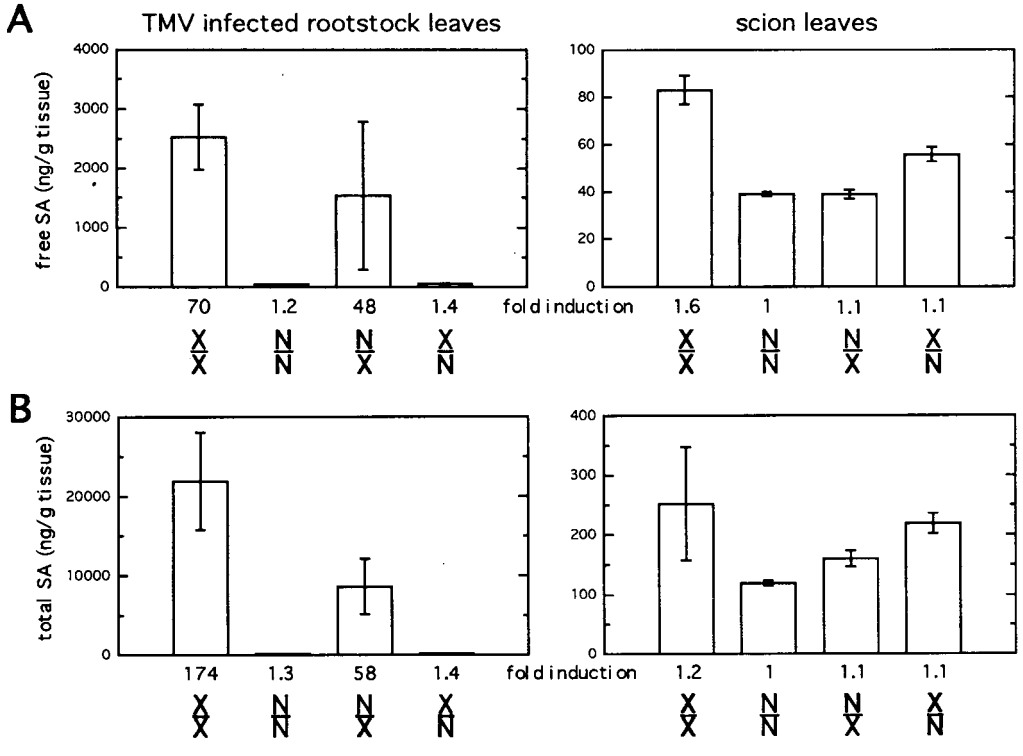

Figure 3. SA Levels in TMV-Pretreated Grafted Plants.

(A) Free SA levels in rootstock and scion leaves.

(B) Free plus glucosylated SA levels ("total") in rootstock and scion tissues.

The levels are shown as the average ± SD of triplicate assays. Grafted plants were inoculated with TMV on rootstock leaves. After 7 days, the rootstock leaves and uninfected scion leaves were harvested and analyzed. Fold induction indicates the ratio of the levels of SA (free or total) in rootstock or scion tissue of TMV-infected plants relative to the levels in mock-inoculated plants (free, 32 to 52; total, 102 to 216 ng/g tissue, respectively). Note the different scales of the graphs. X, Xanthi-nc; N, NahG.

In scion leaves of Xanthi/Xanthi grafted plants, free SA levels increased 1.6-fold after TMV pretreatment of the rootstock (Figure 3A). In the other grafted plants, free SA levels in the scion leaves changed little (1.1-fold or less) as a result of TMV pretreatment (see Figure 3B). Similarly, the total SA levels changed very little in the scion tissues in these grafted plants (1.2-fold or less, see Figure 3B). Thus, SA levels in systemic tissues of the grafted plants increased only slightly when SAR was induced, which is in agreement with our results in intact plants (data not shown).

SAR Gene Expression in Grafted Tissue

Tobacco mosaic virus infection of Xanthi-nc plants induces coordinate expression of a set of genes in inoculated as well as uninoculated leaves. The systemic expression of these SAR genes correlates with the resistant state (Ward et al., 1991). Figure 4 shows the induction of three of the SAR genes (PR-1, PR-2, and PR-3) in the grafted plants. All three genes were strongly expressed in TMV-infected leaves of both types of rootstock, albeit somewhat lower in the NahG tissue (Figure 4). However, in scion tissue of TMV-pretreated, grafted plants, SAR gene expression was only induced in Xanthi tissue, irrespective of rootstock genotype (Xanthi or NahG). Thus, as expected, these molecular markers for SAR were only induced in scion tissue showing resistance.

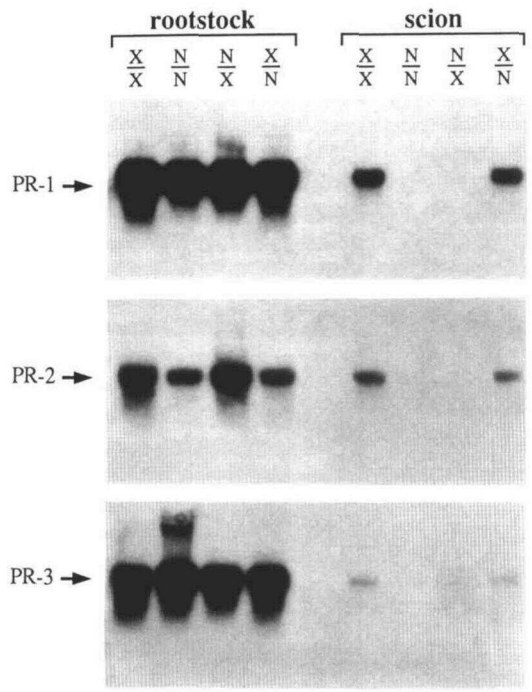

Figure 4. SAR Gene Expression Levels in Rootstock and Scion Leaves of Grafted Plants Following Infection with TMV on Rootstock Leaves.

RNA was isolated from the indicated tissues and analyzed by RNA gel blot hybridization using PR-1, PR-2, and PR-3 as probes. X, Xanthi-nc; N, NahG.

DISCUSSION

In plants, systemic resistance induced in response to a localized infection is characterized by its broad spectrum of activities. In the infected leaf, SA accumulates at the site of infection (Malamy et al., 1990; Uknes et al., 1993) and has been detected in the vascular exudate from infected leaves (Métraux et al., 1990; Rasmussen et al., 1991; Yalpani et al., 1991). When SA is prevented from accumulating in TMV-infected plants (by salicylate hydroxylase in NahG plants), SAR is also inhibited, indicating a clear role for SA in this process. In this study, we clarify the role of SA in SAR by showing that, first, SA is likely not the mobile, systemic resistance–inducing signal, and second, that it is required in systemic tissues for the transduction of the long-distance signal.

Tobacco mosaic virus infection of Xanthi as well as NahG rootstocks generated a signal that was capable of inducing resistance to virus and fungal infection in Xanthi scions (see Tables 1 and 2). NahG rootstocks were as capable as Xanthi rootstocks in inducing resistance: The levels of resistance to viral or fungal infection in Xanthi scions grafted onto either rootstock were similar (TMV lesion sizes were 41 and 40%, and *C. nicotianae*–infected areas were just 12 and 17%, relative to mock-induced controls). These results are consistent with the observations of Rasmussen et al. (1991) in cucumber, which demonstrated that a leaf infected with *P. syringae* could be removed from the plant before a detectable increase in SA without preventing the systemic induction of an acidic peroxidase, which is a biochemical marker for SAR. Taken together, these results strongly suggest that a signal other than SA is transmitted from the infected leaves.

Our experiments further demonstrated that only Xanthi scions were capable of responding to the vascular-mobile signal. NahG tissues appear to be refractory to the translocating, resistance-inducing signal. This implies that SA is required in uninfected parts of the plant for induction of SAR and SAR gene expression. Interestingly, we found only small SA increases in uninfected leaves of TMV-inoculated plants (Figures 3A and 3B), whether or not the plants were grafted. These results are consistent with one report (Enyedi and Raskin, 1993); however, others have reported more substantial increases (Malamy et al., 1990; Enyedi et al., 1992). How a small increase in systemic SA levels can affect SAR induction is not clear. The chloroplast is a likely site for SA synthesis (Löffelhardt and Kindl, 1975; Ranjeva et al., 1977), and conceivably, newly synthesized SA could be stored in that compartment. Upon pathogen infection, SA could be released into the cytosol, making it available to the signal transduction machinery that leads to SAR. Such a mechanism would account for the requirement for SA in distal tissues and the modest increases in accumulation that are detected. This explanation is also consistent with the observation that basal SA levels in

uninfected Xanthi and NahG plants are similar. For example, the compartmentalized SA would not be accessible to the salicylate hydroxylase enzyme, which was engineered for expression in the cytoplasm (Gaffney et al., 1993).

In TMV-infected Xanthi, the SAR genes were expressed at high levels (Figure 4). Surprisingly, in TMV-infected NahG tissues their expression was also highly induced. One possible explanation for this observation is that SAR gene expression in TMV-infected NahG leaves could result from a SA-independent signaling pathway. However, an alternative, more likely explanation is that the large amount of SA produced in the infected cells in NahG tissue overwhelms the salicylate hydroxylase enzyme, and this induces the SAR genes. Consistent with this hypothesis, steady state *nahG* mRNA levels are very low in control tissue and induced when infected by TMV (data not shown). In systemic tissues, the increase in SA levels is much smaller and unlikely to overwhelm the salicylate hydroxylase enzyme. Degradation of SA in these tissues would then prevent SAR gene induction. Further support for this hypothesis comes from our observation that Arabidopsis NahG plants, which express constitutive high levels of the *nahG* message, show more than 10 times lower SAR gene induction in pathogen-infected tissue compared to wild-type tissue (K. Weyman, S. Uknes, E. Ward, and J. Ryals, unpublished data).

Chen and coworkers (Chen et al., 1993) recently demonstrated that high levels of SA inhibit a catalase isozyme in tobacco leaves, leading to increased levels of H_2O_2. Because injected H_2O_2, as well as chemicals that increase H_2O_2 levels, led to PR-1 protein production, the researchers concluded that SA could act via H_2O_2 in inducing SAR gene expression (Chen et al., 1993). In systemic tissues, SA levels apparently do not need to rise substantially for induction of SAR (Figure 3); hence, SA is unlikely to act via H_2O_2 in inducing SAR and SAR gene expression in these tissues.

In summary, our results are consistent with a model for SAR induction in which local pathogen-induced necrosis leads to production of an unidentified vascular-mobile factor, which requires SA in distal tissues for the establishment of the resistant state.

METHODS

Plant Materials and Grafting and Infection Protocols

Nontransgenic Xanthi-nc tobacco plants and plants from transgenic lines NahG-8 and NahG-10, which express salicylate hydroxylase at high levels (Gaffney et al., 1993), were used for grafting. Four- to 6-week-old scions were grafted onto 6-week-old rootstocks. Prospective scion plants were cut from the roots near the soil level or above the second fully developed leaf; the stem was cut in a V shape and placed in a slit, which was made in the stem of a decapitated rootstock plant. The graft junction was stabilized with a twist tie and molten wax or with parafilm and tree wax. Immediately after grafting, plants were grown in high humidity and low light for 5 to 7 days; they were then moved to the greenhouse. Seven days later, three rootstock leaves were inoculated with tobacco mosaic virus (TMV, U1 strain) or mock treated as described previously (Gaffney et al., 1993). After 7 days, one rootstock leaf and two scion leaves were harvested for molecular analysis, and three scion leaves were challenge inoculated with TMV. Five to 10 days later, the diameters of 30 developing lesions on scion leaves were measured on each of at least three grafted plants per graft type.

For challenge with *Cercospora nicotianae*, scion leaves of grafted plants were sprayed with a *C. nicotianae* spore suspension (150,000 spores per mL). The grafted plants were incubated under high humidity for 5 days, and 7 days later the percentage of necrotic leaf area was scored.

Salicylic Acid and RNA Analysis

Three grafted plants of each type were infected with TMV or mock treated on three rootstock leaves. After 7 days, one treated rootstock leaf and two uninfected scion leaves were harvested and pooled according to type of tissue, graft, and treatment. Salicylic acid (SA) levels of triplicate samples were determined as described previously (Gaffney et al., 1993). RNA analysis was performed as previously described (Ward et al., 1991).

ACKNOWLEDGMENTS

We thank Jay Johnson for excellent technical assistance; Sandra Dincher, Patricia Ahl Goy, and George Hengy for sharing grafting techniques; Greg Crawford, Phil Van Bourgondien, Sheila Sips, and Erika Reinhardt for excellent plant care; Gordon Nye for help with the SA analysis; Drs. Danny Alexander, Tim Brears, Mary-Dell Chilton, Christina Hoffman, Kay Lawton, Bruce Lee, Ben Miflin, and Theo Staub for critically reading the manuscript; and Dr. Tom Gaffney for discussion.

Received February 28, 1994; accepted April 27, 1994.

REFERENCES

Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl Goy, P., Luntz, T., Ward, E., and Ryals, J. (1993). Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. Proc. Natl. Acad. Sci. USA **90**, 7327–7331.

Bowles, D.J. (1990). Defense-related proteins in higher plants. Annu. Rev. Biochem. **59**, 873–907.

Chen, Z., Silva, H., and Klessig, D.F. (1993). Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science **262**, 1883–1886.

Chester, K.S. (1933). The problem of acquired physiological immunity in plants. Q. Rev. Biol. **8**, 275–324.

Dean, R.A., and Kuc, J. (1985). Induced systemic protection in plants. Trends Biotechnol. **3**, 125–129.

Dixon, R.A. (1986). The phytoalexin response: Elicitation, signaling, and control of host gene expression. Biol. Rev. Camb. Philos. Soc. **61**, 239–292.

Enyedi, A.J., and Raskin, I. (1993). Induction of UDP-glucose:salicylic acid glucosyltransferase activity in tobacco mosaic virus-inoculated tobacco (*Nicotiana tabacum*) leaves. Plant Physiol. **101,** 1375–1380.

Enyedi, A.J., Yalpani, N., Silverman, P., and Raskin, I. (1992). Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. USA **89,** 2480–2484.

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.

Gianinazzi, S., and Ahl, P. (1983). The genetic and molecular basis of b-proteins in the genus *Nicotiana.* Neth. J. Plant Pathol. **89,** 275–281.

Jenns, A.E., and Kuc, J. (1979). Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus. Phytopathology **7,** 753–756.

Kuc, J. (1982). Induced immunity to plant disease. BioScience **32,** 854–860.

Löffelhardt, W., and Kindl, H. (1975). The conversion of L-phenylalanine into benzoic acid on the thylakoid membrane of higher plants. Hoppe-Seyler's Z. Physiol. Chem. **356,** 487–493.

Malamy, J., Carr, J.P., Klessig, D.F., and Raskin, I. (1990). Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science **250,** 1002–1004.

Malamy, J., Hennig, J., and Klessig, D.F. (1992). Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell **4,** 359–366.

Mauch, F., Mauch-Mani, B., and Boller, T. (1988). Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. Plant Physiol. **88,** 936–942.

Métraux, J.P., 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.

Ranjeva, R., Alibert, A., and Boudet, A.M. (1977). Metabolisme des composes phenoliques chez le petunia. V. Utilisation de la phenylalanine par des chloroplastes isoles. Plant Sci. Lett. **10,** 225–234.

Rasmussen, J.B., Hammerschmidt, R., and Zook, M.N. (1991). Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv *syringae.* Plant Physiol. **97,** 1342–1347.

Ross, A.F. (1961). Systemic acquired resistance induced by localized virus infections in plants. Virology **14,** 340–358.

Sela-Buurlage, M.B., Ponstein, A.S., Bres-Vloemans, S.A., Melchers, L.S., Van den Elzen, P.J.M., and Cornelissen, B.J.C. (1993). Only specific tobacco (*Nicotiana tabacum*) chitinases and β-1,3-glucanases exhibit antifungal activity. Plant Physiol. **101,** 857–863.

Slusarenko, A.J., Croft, K.P., and Voisey, C.R. (1991). Biochemical and molecular events in the hypersensitive response of bean to *Pseudomonas syringae* pv. *phaseolicola.* In Biochemistry and Molecular Biology of Plant-Pathogen Interactions, C.J. Smith, ed (Oxford: Oxford University Press), pp. 126–143.

Uknes, S., Winter, A., Delaney, T., Vernooij, B., Morse, A., Friedrich, L., Nye, G., Potter, S., Ward, E., and Ryals, J. (1993). Biological induction of systemic acquired resistance in *Arabidopsis.* Mol. Plant-Microbe Interact. **6,** 692–698.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.-P., and Ryals, J.A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell **3,** 1085–1094.

Weintraub, M., and Kemp, W.G. (1961). Protection with carnation mosaic virus in *Dianthus barbatus.* Virology **13,** 256–257.

White, R.F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology **99,** 410–412.

Yalpani, N., Silverman, P., Wilson, T.M.A., Kleier, D.A., and Raskin, I. (1991). Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell **3,** 809–818.