Chemical and Biological Inducers of Systemic Resistance to Pathogens Protect Cucumber and Tobacco Plants from Damage Caused by Paraquat and Cupric Chloride

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ABSTRACT

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Oxidative damage has been implicated in the necrotization of plant tissues by incompatible pathogens, including those that induce systemic acquired resistance (SAR). In this study, prooxidant chemicals were employed to evaluate the hypotheses that i) localized oxidative damage that culminates in necrosis can induce SAR and ii) SAR can protect tissues from oxidative damage. Paraquat (PQ), acifluorfen (AF), and sodium chlorate (SC) induced SAR to *Colletotrichum lagenarium* in cucumber. PQ and SC, but not AF, also induced SAR to tobacco mosaic virus (local lesions) and *Peronospora tabacina* in tobacco. Pathogens and chemicals that triggered SAR to pathogens also systemically enhanced resistance to damage by PQ and $CuCl_2$. Local protection from PQ was afforded by salicylate and a synthetic cytokinin known to induce local resistance to pathogens.

Localized infection of plants by necrotizing pathogens can result in a systemic enhancement of disease resistance. This systemic acquired resistance (SAR) is active against a broad spectrum of fungal, bacterial, and viral pathogens and can persist for weeks or months (17,18). Practical application of SAR could lead to reduced dependence on conventional pesticides for plant-disease management (31,34).

The SAR phenomenon has been viewed primarily as an anti-pathogen defense, i.e., as resistance induced by pathogens to pathogens. However, certain chemical treatments (e.g., phosphates and oxalates) cause localized chlorosis and/or necrosis and trigger SAR in cucumber that is comparable to pathogen-induced SAR with respect to elicitation of putative defenses and the spectrum of pathogens against which it protects plants (11,13,14,26). Not all physical or chemical treatments that promote chlorosis and/or necrosis trigger SAR. It may be that the mechanisms by which these types of symptoms are caused determine whether or not a chemical treatment can trigger SAR. Necrotization of plant tissues by incompatible pathogens, which often trigger SAR, appears to involve oxidative damage resulting from enhanced generation of reactive oxygen species by plant tissues in response to the pathogens (9,10,15,16,20). Enhanced generation and/or accumulation of reactive oxygen species has also been implicated in signaling for localized elicitation of plant defensive responses by pathogens, chemicals, and cell wall fragments of plants or pathogens (2,7,20). However, the relationship of SAR to localized oxidative damage leading to necrosis has not been reported. Mercuric chloride, a known inducer of SAR in tobacco (32), was recently shown to promote oxidative damage in animal tissues (22). Thus, we reasoned that promotion of oxidative damage may contribute to induction of SAR by pathogens and hypothesized that chemicals that promote oxidative damage may be efficient inducers of SAR. Because SAR reduces the extent of damage resulting from challenge inoculation with a necrotizing pathogen, we further hypothesized that expression of SAR may involve heightened resistance to oxidative damage. Consistent with these hypotheses, we present evidence that prooxidant chemicals can trigger SAR and that SAR triggered by pathogens or chemicals confers heightened resistance to damage by prooxidant chemicals.

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MATERIALS AND METHODS

Pathogens and hosts. Race 1 of *Colletotrichum lagenarium* (Pass.) Ellis & Halst. and cucumber (*Cucumis sativus* L.) 'Wisconsin SMR 58' and 'Marketer' were grown as previously described (8). Cultivation of burley tobacco (*Nicotiana tabacum* L.) 'Kentucky 14' (which bears the *N* gene for hypersensitive resistance to tobacco mosaic virus [TMV]) and maintenance of the blue mold fungus, *Peronospora tabacina* D. B. Adam (isolate 79) were as described (33). Purified TMV in sterile distilled water was provided by J. Shaw, Department of Plant Pathology, University of Kentucky.

Chemicals. The herbicidal chemicals paraquat (PQ) and acifluorfen (AF) were provided as commercial products by M. Barrett, Department of Agronomy, University of Kentucky. Sodium chlorate (SC) was purchased from Sigma Chemical Co., St. Louis, MO. PQ is a redox-cycling compound, which transfers electrons from photosystem I of chloroplasts to molecular oxygen, forming the superoxide radical, which can in turn give rise to hydrogen peroxide and the hydroxyl radical (3). AF inhibits protoporphyrinogen IX oxidase (and thus chlorophyll and heme biosynthesis), leading to the accumulation of protoporphyrin IX, a potent photosensitizer that generates singlet oxygen in the presence of light and molecular oxygen (12). Chlorate, an analog of nitrate, is converted by nitrate reductase into the strong oxidizing agents chlorite and hypochlorite (19). Sodium salicylate (NaSA), 6-benzylaminopurine (6-BAP), and cupric chloride ($CuC1_2$) were obtained from Sigma, and dibasic potassium

phosphate was from Fisher (Cincinnati, OH). A 25% wettable powder formulation of 2,6-dichloroisonicotinic (INA) was provided by H. Kessman and T. Staub, Ciba-Geigy AG, Basle, Switzerland.

Induction of systemic resistance. Pathogens, chemical solutions, or distilled water were applied to the first one or two true leaves of cucumber (2 to 3 weeks after sowing seed) or to three lower leaves of tobacco plants (about 7 weeks after sowing of seed). A suspension of *C. lagenarium* spores (5×10^4/ml) was infiltrated into 30 sites per leaf (10 µl per site) through the abaxial surfaces of cucumber leaves with a repeating pipettor bearing a plastic syringe. TMV (50 µg/ml) was mechanically inoculated to Carborundum-dusted adaxial surfaces of tobacco leaves. Solutions of dibasic potassium phosphate and suspensions of INA were applied to abaxial surfaces of leaves with a Chromist sprayer (Gelman Sciences, Ann Arbor, MI) to the point of incipient runoff. PQ and AF were diluted in distilled water and applied as droplets (30 and 20 10-µl volumes per leaf for cucumber and tobacco, respectively) to adaxial leaf surfaces, and SC was infiltrated into lamina at 10 and 50 µl per site in cucumber and tobacco, respectively. Doses of the herbicidal chemicals that triggered SAR (see Results) caused localized necrosis but did not cause visible symptoms of systemic phytotoxicity. Negative controls were treated with equivalent volumes of distilled water by infiltration into leaf lamina, spraying, or application of droplets where appropriate.

Induction of local resistance. Solutions of NaSA (0.05%, wt/vol) were applied as single sprays to abaxial leaf surfaces, and solutions of the cytokinin 6-BAP were sprayed three times in a 24-h period, with the last spray applied 4 h before challenge. These chemicals have been previously reported to induce local disease resistance (24,25,33).

Assessment of systemic resistance to pathogens. Challenge inoculation with pathogens was done 7 days (cucumber) or 10 to 14 days (tobacco) after induction. The second true leaves of cucumber received 20 10-µl droplets of a *C. lagenarium* spore suspension (5×10^4/ml), and plants were incubated in a dark, moist chamber as described (8) before they were returned to the greenhouse bench. For tobacco, upper expanded leaves were mechanically inoculated with TMV (50 µg/ml) or entire plants were sprayed with a *P. tabacina* sporangial suspension (2 to 8×10^4/ml). Disease development was evaluated 7 days after inoculation as lesion number and diameter, from which were calculated total necrotic areas (*C. lagenarium*), lesion diameter (TMV; mean of long and short diameters of 20 lesions per leaf), or blue mold rating (*P. tabacina;* 0 = no disease, 1 = 1 to 10%, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% leaf area diseased; depending on plant size, eight to 10 leaves above the last treated leaves were rated, and the average value of all rated leaves designated the disease rating per plant, means of which are presented). Replications per treatment were five for cucumber experiments and five (PQ) or three (SC) for tobacco. Data presented are means ± standard errors.

Assessment of resistance to damage caused by prooxidant chemicals. Challenge applications of PQ were made 7 days (cucumber) or 7 to 10 days (tobacco) after induction treatments for systemic resistance, with the exception of INA-treated plants, which were challenged 4 to 5 days after induction treatment. PQ was diluted in distilled water and applied to the adaxial surfaces of leaves as 10-µl droplets to typically 10 (cucumber) or 20 (tobacco) sites per leaf (droplet challenge), or PQ was diluted in a surfactant solution of 0.02% Silwet L-77 (Union Carbide, Danbury, CT) to promote uniform coverage of leaf surfaces and painted onto leaf surfaces with a camel-hair brush. The rate at which PQ damage developed depended upon plant species and environmental conditions. Challenges were terminated at times at which development of PQ damage in leaves of noninduced plants was judged to be nearly maximal. Thus, the duration of challenge varied with the rate at which damage developed and was typically 3 to 5 days for droplet challenges and 1 to 5 days for brush challenges. Dimensions of necroses resulting from droplet challenge were measured, and total necrotic area (TNA) per leaf was calculated. For brush challenges, the percentage of the challenged leaf area that became necrotic was estimated visually. For statistical analysis, percent data were transformed to the arcsine square root, and back-transformed means and standard errors are presented. In tobacco, a significant ($R^2 = 0.95$) linear correlation was found between visual estimates of necrosis in brush challenges and increases in lipid peroxidation (relative to unchallenged controls) measured by a thiobarbituric acid procedure (27). To assess resistance to copper damage, cucumber leaves were sprayed with $CuCl_2$ solutions, and the percentage of challenged leaf area that became damaged was estimated visually 3 to 6 days later. Data presented are means ± standard errors.

Assessment of lipid peroxidation. Leaf tissue samples were stored at –80°C prior to processing. Tissue extracts were prepared by the method of Cakmak and Marschner (6), and lipid peroxidation was assessed with a thiobarbituric acid procedure (27).

RESULTS

Induction of systemic disease resistance by prooxidant chemicals. *Cucumber*-C. lagenarium *pathosystem.* Application of PQ, AF, and SC to the first true leaves of cucumber as described resulted in the formation of necroses that superficially resembled those caused by *C. lagenarium* in that they expanded for several days and became pale in color from loss of pigments. The herbicidal chemicals also triggered SAR to *C. lagenarium* in a dose-dependent manner. Through several separate tests, it was determined that optimal concentrations for triggering SAR under our experimental conditions were 24 to 72 µM for PQ and 105 to 263 µM for AF. Higher concentrations of PQ and AF resulted in reduction or loss of SAR, and frank systemic phytotoxicity resulted from PQ at ≥1.4 mM and from AF at ≥525 µM. Representative data for protection from anthracnose by optimal doses of PQ and AF (which caused no visible systemic phytotoxicity) are presented in Table 1, experiment A. SAR triggered by these chemicals reduced lesion number and diameter (data not shown) and the calculated TNA per leaf (Table 1). SAR resulting from 263 µM AF approximated that obtained with the biotic inducer, *C. lagenarium,* whereas a lesser degree of protection was obtained by PQ treatment. The optimal dosage for induction of

TABLE 1. Induction of systemic resistance to *Colletotrichum lagenarium* in cucumber by localized application of prooxidant herbicides

Experiment A		Experiment B	
Treatment on leaf 1[a]	Total area of necrotic lesions on leaf 2[b] (mm^2)	Treatment on leaf 1	Total area of necrotic lesions on leaf 2 (mm^2)
Water	229 ± 29	Water	322 ± 73
C. lagenarium	12 ± 3	*C. lagenarium*	3 ± 1
Paraquat		Na chlorate	
24 µM	140 ± 17	1 mM	192 ± 44
36 µM	89 ± 14	2.5 mM	97 ± 32
72 µM	68 ± 9	10 mM	84 ± 22
Acifluorfen			
105 µM	25 ± 7		
263 µM	12 ± 4		

[a] The first true leaf (leaf 1) was infiltrated at 30 sites with 10 µl of water, a conidial suspension or *C. lagenarium* (5×10^4/ml), or an aqueous solution of sodium chlorate; or 30 droplets containing water, paraquat, or acifluorfen were applied to the adaxial leaf surface. Seven days after treatment of leaf 1, plants were challenged on leaf 2 with 20 10-µl drops of a conidial suspension of *C. lagenarium* (5×10^4/ml), and disease was evaluated 7 days after challenge.

[b] Means ± standard errors for five replicate plants per treatment.

SAR by PQ varied between experiments: in one, 72 μM PQ did not trigger SAR (TNA = 151 ± 78 vs. 126 ± 32 for the water control), whereas 36 μM PQ provided SAR comparable to that triggered by *C. lagenarium* (TNA = 17 ± 12 and 9 ± 6, respectively). Application of SC over a concentration range of 1 to 10 mM also triggered SAR to *C. lagenarium;* a greater degree of protection was seen at higher doses of the herbicide (Table 1, experiment B). In another experiment with SC, TNA values were water control, 167 ± 20; *C. lagenarium,* 7 ± 3; SC at 5 mM, 76 ± 12; SC at 10 mM, 20 ± 7; and SC at 20 mM, 11 ± 5, indicating that higher doses of the chemical could provide protection approximating that achieved with the biotic inducer, *C. lagenarium.* Induction of SAR in cucumber was observed in each of three or more experiments conducted with PQ, AF, and SC.

The extent of oxidative damage in cucumber leaf tissues at 7 days after treatment with water or resistance-inducing agents was estimated with a thiobarbituric acid assay for lipid peroxidation. Lipid peroxidation (net A_{532}/mg of protein; ± standard error; n = 3) for tissues treated with water droplets was 0.109 ± 0.005, and for tissues treated with droplets containing 72 μM PQ or 263 μM AF, it was 0.150 ± 0.004 and 0.213 ± 0.008, respectively. Lipid peroxidation was also increased in leaf tissues infiltrated with spores of *C. lagenarium* compared with tissues infiltrated with water (n = 3; 0.144 ± 0.005 and 0.115 ± 0.001, respectively).

TABLE 2. Induction of systemic disease resistance in tobacco by paraquat (PQ) and sodium chlorate (SC)

Treatment [a]	TMV lesion diameter [b] (mm)	Blue mold disease rating [c]
Water	2.2 ± 0.3	4.1 ± 0.3
PQ (144 μM)	1.1 ± 0.1	2.0 ± 0.4
Water	1.6 ± 0.1	5.0 ± 0.0
SC (20 mM)	0.6 ± 0.2	3.7 ± 0.2

[a] Three lower leaves of 7-week-old tobacco plants were treated by application of 10-μl droplets containing PQ to 20 sites per leaf on the adaxial surface or infiltration of lamina with 50-μl volumes of SC. Five replicate plants per treatment were used for the PQ test and three for the SC test.

[b] At 10 (PQ) or 13 (SC) days after treatment, upper expanded leaves were mechanically inoculated with tobacco mosaic virus (TMV) (50 μg/ml), and diameters of 20 local lesions per leaf were measured 7 days later. Data presented are means ± standard errors.

[c] At 10 (PQ) or 13 (SC) days after treatment, entire plants were challenged by spraying them with a spore suspension of *Peronospora tabacina* (2 and 8 × 10^4 sporangia per milliliter for SC and PQ, respectively). Disease development was evaluated 7 days after challenge on a rating scale of 0 to 5, where 0 = no disease, 1 = 1 to 10%, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% of leaf area diseased. Depending on plant size, eight to 10 leaves above the last treated leaf were rated, and the average value of all leaves designated the disease rating per plant. Data presented are means ± standard errors.

Lipid peroxidation was enhanced in tissues with restricted necroses caused by spray application of 100 mM K_2HPO_4 relative to water-sprayed tissues (0.152 ± 0.003 and 0.123 ± 0.016, respectively). K_2HPO_4 was shown previously to trigger SAR in cucumber (13,14,26).

Tobacco pathosystems. Both PQ (144 μM) and SC (10 or 20 mM) triggered SAR in tobacco, which was expressed as a decrease in the size (diameter) of necrotic local lesions incited by TMV and in the extent of chlorotic lesions caused by *P. tabacina* (Table 2). However, induction of disease resistance in tobacco by PQ and SC was somewhat inconsistent: PQ was active against TMV in two of two trials and *P. tabacina* in two of three trials, whereas SC was active against TMV in one of two trials and only moderately active against *P. tabacina* in the single trial conducted. Although AF caused gradually expanding necrotic local lesions when applied to tobacco leaves over a broad range of concentrations (105 μM to 13.1 mM), this agent failed to trigger SAR to TMV or *P. tabacina* in several separate trials.

Systemic and local induced resistance to damage by prooxidant chemicals. *Resistance to damage by PQ.* Diverse biological and chemical treatments known previously, or found in the present work, to induce SAR to pathogens in cucumber and tobacco also enhanced resistance to damage by PQ. Inoculation of lower leaves of cucumber and tobacco plants with necrotizing pathogens (*C. lagenarium* or TMV, respectively) known to induce SAR to pathogens consistently resulted in partial systemic protection from damage (necrosis) caused by brush application of PQ to upper leaves. SAR commonly suppressed the development of necrosis resulting from PQ application by 40 to 90% relative to water controls. Results from several representative experiments with pathogen-induced resistance to PQ damage in cucumber and tobacco are presented in Table 3. Note that SAR induced by *C. lagenarium* protected both cucumber cultivars from PQ damage (Table 3). In addition to *C. lagenarium,* chemical inducers of SAR in cucumber were found to protect this species from necrotic damage caused by challenge of upper leaves with droplets containing PQ. In one representative experiment (n = 5), the TNA (in mm^2) caused by application of 10 droplets containing 28.8 μM PQ to upper leaves were water control, 149 ± 16; *C. lagenarium,* 61 ± 15; 50 mM K_2HPO_4, 46 ± 8; 72 μM PQ, 62 ± 8; 263 μM AF, 64 ± 5; and 10 mM SC, 71 ± 7.

INA, an experimental chemical inducer of disease resistance, promoted resistance to necrotic damage incited by brush application of 144 μM PQ both in lower leaves treated with INA and in upper leaves not directly treated with INA. In cucumber, PQ damage in upper leaves of plants treated with 50 ppm INA was almost completely suppressed (Table 4). This concentration of INA also protected tobacco plants from damage caused by brush application of 288 μM PQ: in lower (treated) and upper

TABLE 3. Induction by pathogens of systemic resistance to necrosis caused by brush-applied paraquat in cucumber and tobacco

Cucumber [a]				Tobacco [c]		
Experiment	Cultivar	Treatment on lower leaf	Challenged area necrotic on upper leaf [b] (%)	Experiment	Treatment on lower leaves	Challenged area necrotic on upper leaf [d] (%)
1	Marketer	Water	52.3 ± 0.8	1	Water	25.0 ± 1.7
		Colletotrichum lagenarium	17.2 ± 1.0		TMV	2.3 ± 0.4
2	Wisconsin SMR 58	Water	85.4 ± 1.5	2	Water	44.8 ± 2.3
		C. lagenarium	29.7 ± 1.2		TMV	1.2 ± 0.1

[a] First true leaves of young plants were infiltrated at 30 sites with 10-μl volumes of water or a *C. lagenarium* spore suspension (5 × 10^4/ml). At 7 days after treatment, third true leaves were challenged with 144 μM paraquat in water containing 0.02% Silwett applied with a camel-hair brush. Necrosis was evaluated 2 days and 1 day after challenge for experiments 1 and 2, respectively.

[b] Mean ± standard error for five (experiment 1) or four (experiment 2) replicate plants per treatment.

[c] Three lower leaves of tobacco plants (cultivar Kentucky 14) were mechanically inoculated with tobacco mosaic virus (TMV) (50 μg/ml) or mock inoculated with water. At 10 days after treatment, the fourth leaf above the last treated leaf was challenged with 288 μM paraquat as described for cucumber. Necrosis was evaluated 4 days after challenge.

[d] Mean ± standard error for four (experiment 1) or three (experiment 2) replicate plants per treatment.

(untreated) leaves, respectively, 44.9 ± 1.0% and 75.1 ± 0.1% of PQ-challenged leaf area became necrotic in water controls and 19.8 ± 0.1% and 9.0 ± 0.7% did so in INA-treated plants ($n = 3$). Protection from PQ damage was consistently greater in upper leaves that developed subsequent to INA application to lower leaves. These leaves exhibited a mild chlorosis prior to PQ challenge. Lower concentrations of INA caused a lesser degree of chlorosis in upper leaves of cucumber and tobacco but afforded less protection from PQ (data not shown).

Other chemicals reported to induce local disease resistance also induced local resistance to PQ damage in tobacco and/or cucumber. Pretreatment by spraying the abaxial surfaces of lower attached leaves of cucumber and tobacco plants with NaSA solutions (0.05%, wt/vol, in water) resulted in partial protection from damage caused by the application 3 days later of droplets containing PQ to adaxial surfaces of the same leaves. In a representative experiment with cucumber ($n = 5$), TNA caused by application of 28.8 μM PQ to 10 sites per leaf on two lower leaves were 493 ± 22 and 635 ± 36 for water controls and 328 ± 12 and 346 ± 36 for NaSA-treated plants. In a representative experiment with tobacco ($n = 3$, 144 μM PQ applied to 20 sites per leaf), TNA (in mm^2) were 1,569 ± 208 and 949 ± 148 for water controls and 634 ± 125 and 399 ± 41 for NaSA-treated plants. Pretreatment of the abaxial surfaces of two lower leaves of cucumber plants ($n = 5$) with the cytokinin 6-BAP afforded local protection from necrosis caused by later application to leaves (adaxial surfaces) of droplets containing 28.8 μM PQ. TNA of 10 sites (in mm^2) were 221 ± 26 and 278 ± 43 for leaves 1 and 2 of water controls and 91 ± 16 and 142 ± 14 for leaves treated with 0.5 mM 6-BAP. A lower degree of protection from PQ damage was afforded by application of 0.1 and 0.25 mM 6-BAP (data not shown).

Resistance to damage caused by $CuCl_2$. Resistance to PQ obtained by other means typically confers cross-resistance to diverse chemical and environmental promoters of oxidative damage (2,4,20,26,27). Thus, we examined the effect of disease resistance-inducing treatments applied to cucumber plants on their sensitivity to spray-applied aqueous solutions (5 mM) of $CuCl_2$, a known promoter of oxidative damage in plants (21). Pretreatment of cucumber plants with *C. lagenarium* or INA (50 ppm) conferred a significant degree of systemic protection against damage (primarily chlorosis) caused by $CuCl_2$. In a representative experiment in which the first true leaves (leaf 1) of young cucumber plants ($n = 4$) were infiltrated with water or with a *C. lagenarium* spore suspension 7 days prior to challenge of upper leaves, the extent of chlorosis, expressed as the percentage of challenged leaf area, caused by $CuCl_2$ on upper leaves 2 and 3 was 63.0 ± 1.8 and 59.7 ± 1.5, respectively, for noninduced water controls and 8.9 ± 0.2 and 37.5 ± 0.0 for *C. lagenarium*-induced plants. Protection by INA was pronounced in untreated leaves that expanded prior to $CuCl_2$ challenge but was also detectable to a lesser extent in leaves directly treated with INA (Table 4, experiment 2), a pattern similar to that seen for protection from PQ damage by INA (Table 4, experiment 1).

DISCUSSION

These results indicate that promotion of localized necrosis by discrete application of prooxidant herbicidal chemicals can trigger SAR to pathogens in cucumber and tobacco. These findings are consistent with the possibility that oxidative damage, which is associated with necrotization of host tissues in incompatible plant-pathogen interactions (9,10,15,16), could contribute to induction of systemic disease resistance. Additionally, the compatible fungal pathogen *C. lagenarium* and dibasic potassium phosphate, which were previously reported to trigger SAR in cucumber, promoted lipid peroxidation in treated leaves. Thus, promotion of oxidative damage is associated with, and may play a causal role in, the triggering of SAR by chemical and biological agents.

However, the promotion of necrosis by prooxidant chemicals was not always sufficient for induction of systemic disease resistance. In cucumber, supraoptimal concentrations of PQ and AF resulted in reduced or no induction of resistance, although these concentrations caused necrosis on treated leaves. Furthermore, necrosis caused by a wide range of AF concentrations did not trigger SAR in tobacco plants. If localized oxidative damage is involved in the triggering of SAR, differential induction of resistance by these chemicals may have resulted from differences in the identity, subcellular locale, and rate of generation of reactive oxygen species during the interaction of plants with these agents. These differences might be particularly important if systemic signal or signals for SAR are oxidation products of specific cellular constituents or if active metabolism of damaged or dying cells is required for signal production. Activity of AF in cucumber but not in tobacco may reflect differences in the identity and production of systemic signal or signals for SAR; e.g., inhibition of heme biosynthesis by AF (12) may prevent production of an inducible enzyme, such as a cytochrome P-450, that is necessary for signal generation in tobacco but not in cucumber. The ineffectiveness of higher doses of PQ and AF in cucumber may have resulted from their promotion of cell death too rapidly for signal to be produced. Other agents that cause rapid necrosis in cucumber (e.g., flame and dry ice) do not trigger SAR.

Treatment of plants with diverse biological and chemical inducers of disease resistance promoted enhanced resistance to damage by the prooxidant chemicals PQ and $CuCl_2$. These results indicate that enhanced resistance to oxidative damage may be a feature of local and systemic induced resistance. We speculate that protection from oxidative damage may contribute to protection of plants from necrotizing (and possibly nonnecrotizing) pathogens by SAR and other types of induced resistance. This speculation is supported by the fact that a PQ-resistant tobacco line, developed by in vitro selection with the herbicide, exhibits cross-resistance to necrotizing pathogens (5).

The mechanism or mechanisms by which induced resistance protects plants from damage by these prooxidant chemicals are unknown. In other reported cases of PQ resistance, activity of one or several antioxidative enzymes was typically elevated prior to PQ challenge (1,5,23,29,30). However, our preliminary investiga-

TABLE 4. Local and systemic protection of cucumber plants by 2,6-dichloroisonicotinic acid (INA) from damage caused by paraquat or cupric chloride

Treatment	Challenge	Challenged leaf area damaged (%)	
		Lower leaf (treated)	Upper leaf (untreated)
Experiment 1[a]			
Water	Paraquat, 144 μM	80.4 ± 0.4	84.7 ± 0.4
INA, 50 ppm	Paraquat, 144 μM	56.9 ± 4.0	0.7 ± 0.1
Experiment 2[b]			
Water	$CuCl_2$, 5 mM	65.5 ± 0.6	63.9 ± 1.5
INA, 50 ppm	$CuCl_2$, 5 mM	37.4 ± 1.1	8.6 ± 1.0

[a] The first true leaves of young plants (cultivar Marketer) were sprayed on the abaxial surfaces with distilled water or a suspension of a 25% wettable powder formulation of INA in water (50 ppm a.i.). Four days after treatment, the first and third true leaves were challenged with brush-applied paraquat (144 μM). The percentage of challenged area that became necrotic was estimated visually at 1 day after paraquat application. Data are means ± standard errors for three replicate plants per treatment.

[b] The third true leaves of slightly older plants were treated with INA or water as described. Four days after treatment, leaves were challenged by spraying 5 mM $CuCl_2$ on the adaxial surface (lower leaf) or abaxial surface (upper leaf). The percentage of leaf area damaged by $CuCl_2$ (primarily chlorosis) was estimated visually 3 days after $CuCl_2$ application. Data are means ± standard errors for three replicate plants per treatment.

tions indicated that SAR did not consistently enhance activities of superoxide dismutase, ascorbate peroxidase, catalase, or glutathione reductase in tobacco leaves before PQ challenge (N. E. Strobel and J. A. Kuć, *unpublished*). It is possible that the increased content of cytokinins associated with SAR (4,28) may protect plants from prooxidant chemical damage as exogenous cytokinin protected cucumber from PQ damage in the present work and was previously shown to protect tobacco leaves from damage by $HgCl_2$ (4), a prooxidant chemical.

The systemic induced cross-resistance among plant pathogens and chemicals that we report here may call for an expanded concept of the SAR phenomenon. This concept must account for observations that include systemic resistance induced i) by pathogens to pathogens, ii) by chemicals to pathogens, iii) by pathogens to chemicals, and iv) by chemicals to chemicals. Although the present work employed prooxidant chemicals as induction and challenge agents, it is not known whether the observed results are related specifically to the prooxidant nature of these chemicals or more generally to the plant cell and tissue damage they promote. Regardless of the precise mechanistic concerns, it was clear that tissues with induced resistance were consistently more resistant to PQ damage than were noninduced tissues. Because PQ resistance derived by other means confers cross-resistance to damage by a broad spectrum of environmental stresses (1,5,23,29,30), it is intriguing to speculate that mechanisms activated in SAR may protect plants against environmental stresses as well as against pathogens. On the other hand, possible overlaps and interactions of SAR with other plant defense and homeostatic mechanisms may render the induction and/or expression of SAR vulnerable to disruption by environmental fluctuations and may contribute to variable performance of SAR in greenhouse and especially field environments.

LITERATURE CITED

1. Aono, M., Kubo, A., Saji, H., Tanaka, K., and Kondo, N. 1993. Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabacum* with high chloroplastic glutathione reductase activity. Plant Cell Physiol. 34:129-135.
2. Apostol, I., Heinstein, P. F., and Low, P. S. 1989. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Plant Physiol. 90:109-116.
3. Babbs, C. F., Pham, J. A., and Coolbaugh, R. C. 1989. Lethal hydroxyl radical production in paraquat-treated plants. Plant Physiol. 90:1267-1270.
4. Balasz, E., Sziraki, I., and Kiraly, Z. 1977. The role of cytokinins in the systemic acquired resistance of tobacco hypersensitive to tobacco mosaic virus. Physiol. Plant Pathol. 11:29-37.
5. Barna, B., Adam, A. L., and Kiraly, Z. 1993. Juvenility and resistance of a superoxide-tolerant plant to diseases and other stresses. Naturwissenschaften 80:420-422.
6. Cakmak, I., and Marschner, H. 1992. Magnesium deficiency and high light enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98:1222-1227.
7. 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.
8. Dean, R. A., and Kuć, J. 1986. Induced systemic protection in cucumber: Time of production and movement of the signal. Phytopathology 76:966-970.
9. Doke, N. 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiol. Plant Pathol. 23:345-357.
10. Doke, N., and Ohashi, Y. 1988. Involvement of an O_2^--generating system in the induction of necrotic lesions on tobacco leaves infected with tobacco mosaic virus. Physiol. Mol. Plant Pathol. 32:163-175.
11. Doubrava, N., Dean, R., and Kuć, J. 1988. Induction of systemic resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalates and extracts from spinach and rhubarb leaves. Physiol. Mol. Plant Pathol. 33:69-79.
12. Duke, S. O., Lydon, J., Becerril, J. M., Sherman, T. D., Lehnen, L. P., Jr., and Matsumoto, H. 1991. Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 39:465-473.
13. Gottstein, H. D, and Kuć, J. 1989. Induction of systemic resistance to anthracnose in cucumber by phosphates. Phytopathology 79:176-179.
14. Irving, H. R., and Kuć, J. 1990. Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by KH_2PO_4. Physiol. Mol. Plant Pathol. 37:355-366.
15. Kato, S., and Misawa, T. 1976. Lipid peroxidation during the appearance of hypersensitive reaction in cowpea leaves infected with Cucumber Mosaic Virus. Ann. Phytopathol. Soc. Jpn. 42:472-480.
16. Keppler, L. D., and Novacky, A. 1987. The initiation of membrane lipid peroxidation during bacteria-induced hypersensitive reaction. Physiol. Mol. Plant Pathol. 30:233-245.
17. Kessman, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. 1994. Induction of systemic acquired resistance in plants by chemicals. Annu. Rev. Phytopathol. 32:439-459.
18. Kuć, J. A., and Strobel, N. E. 1993. Induced resistance using pathogens and nonpathogens. Pages 295-303 in: Biological Control of Plant Diseases: Progress and Challenges for the Future. E. C. Tjamos, G. C. Papavisas, and R. J. Cook, eds. Plenum Press, New York.
19. LaBrie, S. T., Wilkinson, J. Q., and Crawford, N. M. 1991. Effect of chlorate treatment on nitrate reductase and nitrite reductase gene expression in *Arabidopsis thaliana*. Plant Physiol. 97:873-879.
20. Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583-593.
21. Luna, C. M., Gonzalez, C. A., and Trippi, V. S. 1994. Oxidative damage caused by an excess of copper in oat leaves. Plant Cell Physiol. 35:11-15.
22. Lund, B. O., Miller, D. M., and Woods, J. S. 1993. Studies on Hg (II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol. 45:2017-2024.
23. Melhorn, H. 1990. Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. Plant Cell Environ. 13:971-976.
24. Mills, P. R., Gussin, E. J., and Wood, R. K. S. 1986. Induction of resistance in cucumber to *Colletotrichum lagenarium* by 6-benzylaminopurine. J. Phytopathol. 116:11-17.
25. Mills, P. R., and Wood, R. K. S. 1984. The effects of polyacrylic acid, acetylsalicylic acid, and salicylic acid on resistance of cucumber to *Colletotrichum lagenarium*. J. Phytopathol. 111:209-216.
26. Mucharromah, E., and Kuć, J. 1991. Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Prot. 10:265-270.
27. Peever, T. L., and Higgins, V. J. 1989. Electrolyte leakage, lipoxygenase, and lipid peroxidation induced in tomato leaf tissue by specific and nonspecific elicitors from *Cladosporium fulvum*. Plant Physiol. 90:867-875.
28. Sarhan, A. R. T., Kiraly, Z., Sziraki, I., and Smedegaard-Petersen, V. 1991. Increased levels of cytokinins in barley leaves having the systemic acquired resistance to *Bipolaris sorokiniana* (Sacc.) Shoemaker. J. Phytopathol. 131:101-108.
29. Sen Gupta, A., Heinen, J. L., Holaday, A. S., Burke, J. J., and Allen, R. D. 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA 90:1629-1633.
30. Shaaltiel, Y., Glazer, A., Bocion, P. F., and Gressel, J. 1988. Cross tolerance to herbicidal and environmental oxidants of plant types tolerant to paraquat, sulfur dioxide, and ozone. Pestic. Biochem. Physiol. 31:13-23.
31. Strobel, N. E., and Kuć, J. A. Plant resistance to pathogens. Handbook of Pest Management. J. R. Ruberson, ed. Marcel Dekker, New York. (In press.)
32. Van Loon, L. C. 1975. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. "Samsun" and "Samsun NN". IV. Similarity of qualitative changes of specific proteins after infection with different viruses and their relationship to acquired resistance. Virology 67:566-575.
33. Ye, X. S., Pan, S. Q., and Kuć, J. 1989. Pathogenesis-related proteins and systemic resistance to blue mold and tobacco mosaic virus induced by tobacco mosaic virus, *Peronospora tabacina*, and aspirin. Physiol. Mol. Plant Pathol. 35:161-175.
34. Ye, X. S., Strobel, N. E., and Kuć, J. A. 1995. Induced systemic resistance (ISR): Activation of natural defense mechanisms as part of integrated pest management (IPM). Pages 95-113 in: Novel Approaches to Integrated Pest Management. R. Reuveni, ed. Lewis Publishers, Boca Raton, FL.