

SALICYLATE ACTIVITY. 4 ROLE OF ETHYLENE IN PARAQUAT DAMAGE

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ABSTRACT

Paraquat is a free radical-generating herbicide that inhibits electron transport through photosystem I. We have found that simultaneous application of sodium salicylate (NaSA) with paraquat decrease herbicidal activity. Because NaSA is an inhibitor of ethylene biosynthesis, we wanted to determine if ethylene is causal in salicylate protection from paraquat. Tobacco plants were treated with paraquat amended with inhibitors of ethylene biosynthesis, inhibitors of ethylene action, or ethephon, an ethylene-releasing agent. Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine (AVG) did not significantly affect paraquat activity, while aminooxyacetic acid (AOA), a general inhibitor of pyridoxal phosphate-dependent enzymes, slowed but did not stop paraquat activity. Inhibitors of ethylene perception (1-methylcyclopropane, 1-MCP; silver thiosulphate, STS) had no effect on paraquat activity. Moreover, NaSA protected both Arabidopsis wild type and the ethylene-insensitive mutant *ein2-1* from paraquat. Only NaSA and the ethylene generator ethephon significantly protected tobacco from paraquat. We conclude that although ethylene may modulate paraquat activity, its perception is not essential for paraquat damage or NaSA protection.

INTRODUCTION

Paraquat is a non-selective contact herbicide. Paraquat inhibits photosynthesis by accepting electrons from photosystem I, which in turn generates reactive oxygen species (ROS) in the light. The ROS generated, which include superoxide anion, hydrogen peroxide, and the hydroxyl radical, cause lipid peroxidation and membrane damage. Paraquat has been used experimentally to induce plant stress.

Salicylic acid is a phenolic that is commonly found in plants. It has been shown to be a signal molecule in thermogenesis of certain Arum lilies, and to be a signal in plant defense induction (Raskin, 1992). Applied SA has been shown to reduce the effects of abiotic stresses, including chilling and heat stress (Dat et al., 1998; Janda et al., 1999). Additionally, SA is an inhibitor of the terminal step of ethylene biosynthesis, the oxidation of 1-aminocyclopropane carboxylic acid to ethylene catalyzed by ACC oxidase (Leslie and Romani, 1988). Ethylene is a plant hormone involved in plant growth and senescence. Ethylene is produced in response to herbicide treatment, and it may be one of the causes of herbicide damage. However, we have demonstrated that ethylene production is a consequence of herbicide treatment, but not a necessary component of paraquat action.

The protection of tobacco from paraquat by salicylate provided us an opportunity to test the role of ethylene in paraquat activity. The objectives of these studies were 1) to test to role of ethylene modulators in paraquat activity, and 2) to define any interaction between ethylene and salicylate in protection of plants from paraquat.

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MATERIALS AND METHODS

Plant Material

Xanthi-nc tobacco was grown in an environmentally-controlled growth chamber in a 16 h light/8 h dark photoperiod at 25 °C for 4-5 weeks after sowing. Plants typically had 5 to 7 fully expanded leaves when treated (Silverman et al., 2004).

Cotton (SG-105, Delta Pine Land Company, Scott MS) was sown and grown for 10 d in a growth chamber in a 16 h light/8 h dark photoperiod at 25 EC, at 250 moles m⁻² s⁻¹. At the time of treatment, the cotyledons were fully-expanded and the first true leaves had not expanded. Cotyledons were removed 24 hours after spray applications and sealed in disposable 50 mL polypropylene tubes. Ethylene determinations were made 5-6 hours later as described previously (Greenberg et al., 2000).

Seed for the *Arabidopsis thaliana* (L.) Heyn. *ein2-1* mutant was obtained from the Arabidopsis Biological Resource Center (The Ohio State University, Columbus, OH). The corresponding wild type (Columbia) seed was obtained from Lehle Seed (Round Rock, TX). Arabidopsis plants were grown in Pro-Mix PGX under cool white fluorescent lamps at 150 μmoles m⁻² s⁻¹ under a 16 h light/8 h dark photoperiod at 25 °C and treated at maturity.

Herbicide treatments

Herbicides were foliar-applied with sufficient volume to insure good coverage. Plants were sprayed with either the active ingredient (paraquat, methyl viologen; Aldrich) or the commercial product (Gramoxone Max®; Syngenta) in a solution containing either 0.25% (v/v) crop oil concentrate (COC) (cotton and tobacco), or 0.1% COC (Arabidopsis). Plants were rated for herbicidal damage as percent leaf area affected at selected times following application.

Ethylene modulators

The ethylene biosynthesis inhibitors used were aminoethoxyvinylglycine (AVG; ReTain®; Valent BioSciences Corporation), aminooxyacetic acid (AOA; Sigma-Aldrich), and sodium salicylate (NaSA; Sigma-Aldrich). The ethylene action inhibitors used were 1-methylcyclopropene (1-MCP, EthylBloc®; Floralife® Inc.), silver thiosulfate (STS, Silgard®; Gard Inc.). The ethylene generator used was 2-chloroethylphosphonic acid (Ethepon, Florel®; Southern Agricultural Industries).

With the exception of 1-MCP, all ethylene modulators were dissolved in water for application. STS and AOA were applied by soil drench, while ethepon, AVG and NaSA were applied either alone or in combination sprays with the herbicide. The ethylene action inhibitor 1-MCP was applied by sealing tobacco plants in an airtight 115 liter drum overnight with 200 ppm 1-MCP. Following gas exposure, plants were equilibrated for 2 hours before being spray-treated.

Statistics

Data were subjected to analysis of variance, and means were separated by Duncan's new multiple range test (p=0.05) using PlotIT software (Scientific Programming Enterprises, Haslett, MI).

RESULTS AND DISCUSSION

The conversion of S-adenosylmethionine (SAM) to aminocyclopropane (ACC) is the rate-limiting step in ethylene biosynthesis (McKeon et al., 1995). This step, catalyzed by ACC synthase, is inhibited by either AVG or AOA. The next step, oxidative conversion of ACC to ethylene, is inhibited by salicylate (Leslie and Romani, 1988). NaSA inhibition of the conversion of ACC to ethylene is dose-dependent (Figure 1). The perception of ethylene is blocked by STS or 1-MCP. Finally, the perception of ethylene is blocked in *ein2-1*, which has a lesion in the pathway of ethylene-mediated signal transduction (Alonso et al., 1999).

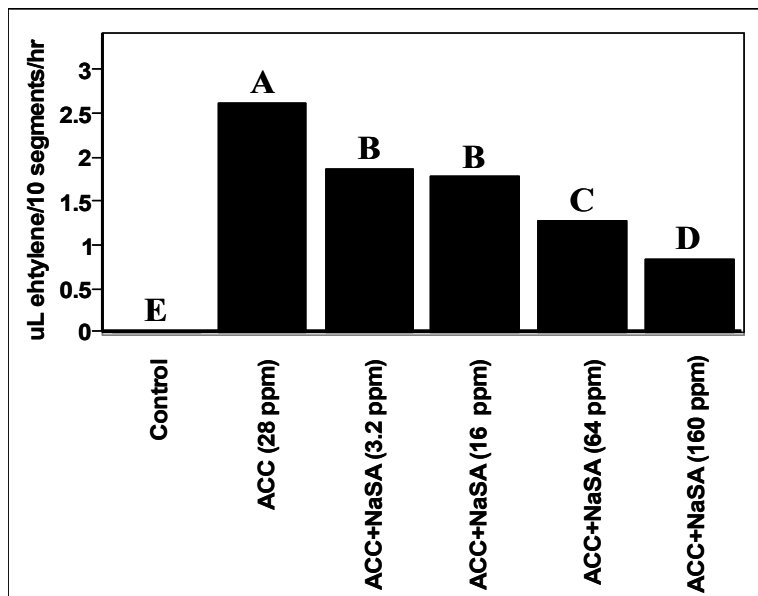


Figure 1. Application of sodium salicylate (NaSA) inhibited conversion of aminocyclopropane to ethylene in mung bean hypocotyls in a dose-dependent manner.

Tobacco plants sprayed with paraquat alone showed signs of leaf damage and desiccation within the first 24 hours. The simultaneous application of sodium salicylate (NaSA) with paraquat inhibited paraquat damage (Figure 2). At 3 days after spray application, a 50% reduction in damage was observed when the combination treatment was used. NaSA alone caused no significant leaf damage.

To better understand the mechanism of action of salicylate-mediated protection from paraquat, the effect of paraquat on ethylene generation of cotton seedlings was determined. Treatment with paraquat alone (500 μ M) increased ethylene evolution of cotton cotyledons from 1.4 pmole/gram/hr to 467.4 pmole/gram/hr. The combination of NaSA (5 mM) with paraquat (0.5 mM) reduced ethylene generation by 9-fold to 51.3 pMoles/gram/hr and resulted in less herbicidal damage.

To better understand the role of ethylene biosynthesis inhibition in protection of tobacco from paraquat, both AVG and AOA were examined (Figure 3). AVG alone (100 ppm) induced a slight amount of chlorosis on the new growth of tobacco. AVG was unable to protect tobacco from paraquat (Figure 3A) and showed no interactions with NaSA protection of paraquat (not shown). In contrast, AOA slowed paraquat damage in

a dose-dependent manner 2 days after application (Figure 3B), but had no effect 4 days after application.

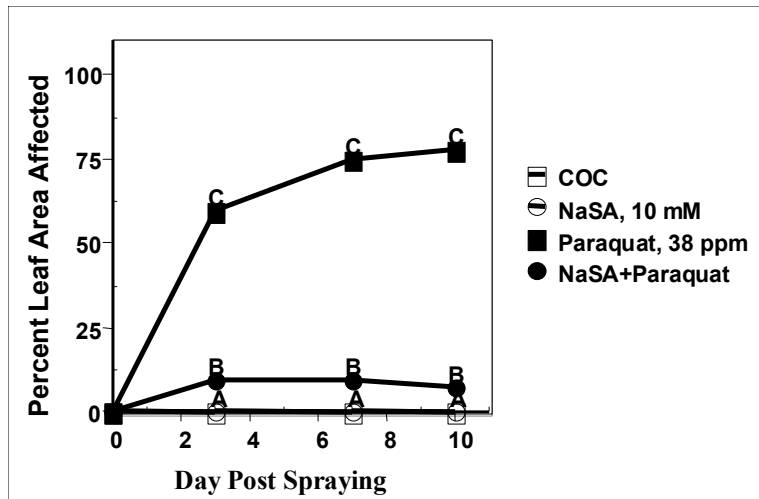


Figure 2. Simultaneous application of sodium salicylate (NaSA) protects tobacco from paraquat damage (COC: 0.25% v/v crop oil concentrate).

The role of ethylene perception was tested with two inhibitors of ethylene action: 1-MCP and silver thiosulfate. Neither inhibitor affected paraquat activity. A further test of the role of ethylene perception was made using the *ein2-1* mutant of Arabidopsis, which is unable to perceive ethylene. Both paraquat damage and salicylate protection were equal in *ein2-1* and in WT Columbia (Table 1).

To determine if ethylene could result in increased protection, the ethylene generator ethephon was used. Simultaneous foliar application of ethephon with paraquat significantly protected tobacco from herbicidal damage (Figure 4). Ethylene is an important endogenous regulator of plant growth and development. In addition to its role in fruit ripening, ethylene is integral to other developmental processes. Ethylene is produced in response to all forms of plant stress and is often considered to be a consequence of stress and not essential for plant response. The salicylate-mediated protection from paraquat provided an opportunity to examine the role of ethylene in the response of plants to herbicide stress.

Salicylic acid is an endogenous growth regulator in plants and has been shown to be involved in thermogenesis and plant defense. We have shown that NaSA protects against paraquat (Silverman et al., 2004). Several workers have previously shown that SA pretreatment can protect plants against paraquat and oxidative stresses (see Strobel and Kuc, 1995). However, we have shown that simultaneous treatment also protects against paraquat.

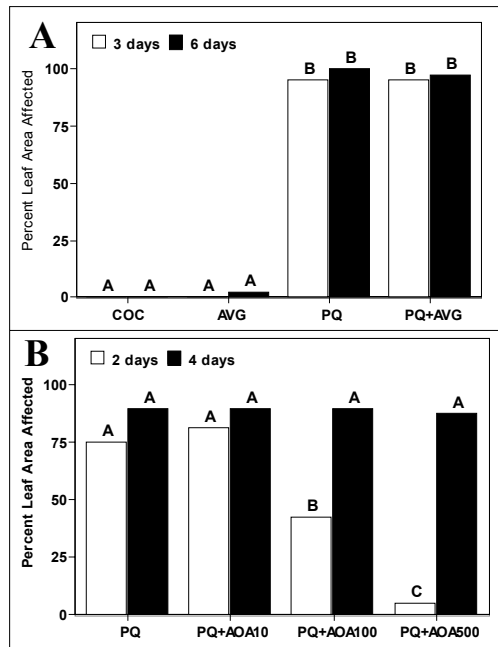


Figure 3. The ethylene biosynthesis inhibitors showed differential affects on paraquat herbicidal activity.

A. Aminoethoxyvinylglycine (AVG) did not significantly affect paraquat (PQ) activity (Treatments: COC, 0.25% v/v crop oil concentrate; AVG, 100 ppm AVG+COC; PQ, 500 ppm PQ+COC; PQ+AVG, PQ+AVG+COC).

B. Aminoxyacetic acid (AOA) delays herbicidal activity of paraquat (Treatments: PQ, 187 ppm PQ + 0.25% v/v COC; PQ+AOA10, PQ+COC+10 ppm AOA pretreatment; PQ+AOA100, PQ+COC+100 ppm AOA pretreatment; PQ+AOA500, PQ+COC+500 ppm AOA pretreatment).

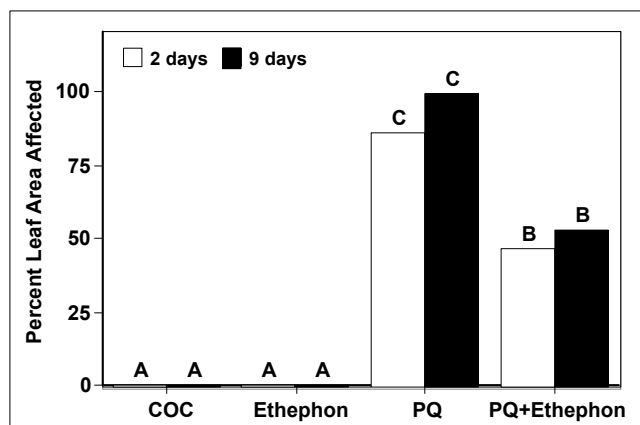


Figure 4. Ethylene protects tobacco from paraquat (PQ) activity (Treatments: COC, 0.25% v/v crop oil concentrate; Ethephon, 975 ppm ethephon+COC; PQ, 375 ppm PQ+COC; PQ+Ethephon, PQ+ethephon+COC).

Of the compounds tested here, only NaSA and ethephon reduced paraquat activity. NaSA is an ethylene biosynthesis inhibitor (Leslie and Romani, 1988). The only other ethylene biosynthesis inhibitor that showed any modulation of paraquat activity is AOA. AOA is a general inhibitor of PLP-dependent enzymes, and it is likely to be acting on other targets. Ethephon is used as a ripening and synchronizing agent in many crops. The mode of action of ethephon protection may be through induction of lignification (reviewed in Enyedi et al., 1992), or through some other mechanism.

Ethylene perception does not appear to be necessary for either paraquat activity or NaSA protection from paraquat. Neither STS nor 1-MCP treatment resulted in paraquat protection. Moreover, NaSA protected both WT (Columbia) and ethylene insensitive *ein-2-1* Arabidopsis from paraquat damage (Table 1).

Table 1. Sodium salicylate safens both Columbia WT and *ein2-1* Arabidopsis from paraquat^a.

Columbia WT		Hours post spray application		
Treatments	48 h	96 h	168 h	
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Sodium salicylate	2.0 ± 0.5	2.5 ± 0.1	2.5 ± 0.1	
Paraquat	83.8 ± 1.5	92.8 ± 2.3	95.3 ± 2.2	
Sodium salicylate + paraquat	13.8 ± 2.3	21.3 ± 1.5	30.0 ± 2.3	
<i>ein2-1</i> (Columbia)		Hours post spray application		
Treatments	48 h	96 h	168 h	
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Sodium salicylate	2.5 ± 0.1	2.5 ± 0.1	2.0 ± 0.5	
Paraquat	76.3 ± 2.3	85.8 ± 3.7	85.0 ± 7.6	
Sodium salicylate + paraquat	8.8 ± 2.5	13.8 ± 3.1	23.8 ± 3.6	

^a All data are expressed as the mean ± the standard error of the mean of the percent leaf area affected.

Taken together, these data suggest that neither ethylene biosynthesis nor perception is necessary for paraquat activity or salicylate protection from paraquat damage. We suggest that sodium salicylate functions in paraquat protection through an ethylene-independent pathway.

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