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Salicylate Activity. 2. Potentiation of Atrazine

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Atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine] inhibits photosystem II (PSII) and is commonly used to control weeds in maize. It has been found that addition of sodium salicylate (sodium 2-hydroxybenzoate; NaSA) increased the postemergence herbicidal activity of atrazine against dicotyledonous weeds. NaSA also potentiated the activity of bentazon, another PSII-inhibiting herbicide. NaSA increased atrazine activity when applied either as a tank mix or up to 96 h prior to atrazine application. Other salicylates and the plant disease resistance inducers acibenzolar-*S*-methyl [benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester] and 2,6-dichloroisonicotinic acid also increased atrazine activity. Among the compounds tested, 3-chloro-5-fluorosalicylate, 4-chlorosalicylate, or 2,6-dichloroisonicotinic acid combined with atrazine yielded the greatest increase in herbicidal activity. Potentiation of atrazine by NaSA was greater at higher temperatures (35 and 25 > 15 °C). Also, greater potentiation was observed as the light level decreased. In darkness, NaSA alone or in combination with atrazine caused plant death, whereas atrazine alone had little effect. NaSA increased atrazine activity on *npr1-2*, an *Arabidopsis* mutant compromised in SA-induced disease resistance. Atrazine activity was also potentiated by NaSA on the ethylene insensitive mutant *ein2-1*. This indicates that atrazine potentiation is independent of either salicylate-induced disease resistance or ethylene perception.

KEYWORDS: Salicylic acid; bentazon; alternative respiration; salicylhydroxamic acid; SHAM; environmental conditions; nitric oxide

INTRODUCTION

Atrazine is a photosystem II (PSII)-inhibiting herbicide that is commonly used for pre- or postemergent control of broadleaf weeds in maize and sorghum. Unfortunately, atrazine is relatively mobile with water, resulting in leaching and contamination of groundwater or surface waters. Surface water atrazine contamination has been linked to possible amphibian limb deformities (*1*), and groundwater contamination has become a significant public health issue. For these reasons, atrazine-containing herbicides are considered restricted-use pesticides, and lower use rates have been mandated (*2*).

Salicylic acid (SA) is a plant phenolic common throughout the plant kingdom (*3*). Although it was first identified in plants over 150 years ago, the roles of endogenous salicylates became known only in the past two decades. SA was first shown to be the inducer of thermogenesis in the voodoo lily (*4*) and was later shown to be an endogenous signal molecule in systemic acquired resistance (SAR), an induced response to plant pathogens (*5*). SA is also involved in plant stress tolerance, as shown in the protection of mustard plants from heat stress (*6*) and maize from chilling (*7*). SA application also may induce antioxidant defenses (*8*). Moreover, SA levels have been shown to increase in response to the free radical generators ozone and UV illumination (*9*).

Plant growth regulators have previously been shown to modify the activity of PSII-inhibiting herbicides. For example, gibberellic acid potentiated the activity of bentazon on bean and Canada thistle (*10*). It is well-known that synergies and antagonisms of crop protection agents may significantly affect the performance of pesticides in the field. This work is the first reported instance of co-application with a simple plant phenolic enhancing atrazine herbicidal activity. In the present study, we have examined the relationship between salicylates or SAR inducers and atrazine. We have determined that atrazine activity is potentiated by salicylates and SAR inducers, but is independent of SAR.

MATERIALS AND METHODS

Chemicals. Sodium salicylate (sodium 2-hydroxybenzoate; NaSA) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. 3-Fluorosalicylate, 6-methylsalicylate, 3,5-difluorosalicylate, 3-chloro-5-fluorosalicylate, 3-fluoro-5-chlorosalicylate, 3,5-dichloro-6-hydroxysalicylate, 3-methoxysalicylate, and 2,6-dichloroisonicotinic acid were produced by synthesis as described elsewhere (*11*). Aatrex Nine-O with the active ingredient atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and Actigard 50WG with the active ingredient acibenzolar-*S*-methyl [benzo-

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(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester] were obtained from Syngenta Crop Protection (Greensboro, NC). Basagran 4E with the active ingredient bentazon [3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] was obtained from BASF (Research Triangle Park, NC). The crop oil concentrate used for all foliar sprays comprised 83% Orchex 796 (Exxon Co., Houston, TX) and 17% AT Plus 300F (Uniqema, New Castle, DE).

Plant Material. Tobacco plants were grown as previously described (*12*). Briefly, tobacco was sown onto Pro-Mix PGX and grown under cool white fluorescent lamps at 250 μmol·m^{-2}·s^{-1} (16:8 h light/dark cycle; 25 °C). Three weeks after sowing, individual plants were transplanted into pots (7.6 cm diameter) containing Pro-Mix PGX, grown in the greenhouse for 3 weeks, and treated at the 4−5 leaf stage.

Seed for the *Arabidopsis thaliana* (L.) Heyn. mutants *npr1-2* and *ein2-1* was obtained from the Arabidopsis Biological Resource Center (The Ohio State University, Columbus, OH). The corresponding wild type seed was purchased from Lehle Seed (Round Rock, TX). *Arabidopsis* plants were grown in Pro-Mix BX under cool white fluorescent lamps at 150 μmol·m^{-2}·s^{-1} (16:8 h light/dark cycle; 25 °C) and treated at maturity.

Velvetleaf (*Abutilon theophrasti*) seed was purchased from Azelin Seed Service (Leland, MS). Plants were grown in Promix PGX (16:8 h light/dark cycle) in the greenhouse and treated at the 4−6 leaf stage.

Spray Treatments. Herbicides were applied as foliar sprays with a hand sprayer in a volume sufficient to ensure complete coverage. Crop oil concentrate was added at 0.1% (v/v) for *Arabidopsis* and at 0.25% (v/v) for tobacco and velvetleaf. Combinations of herbicide and test compound were tank-mixed and applied as soon as possible after mixing. After spraying, plants were returned to their previous growth conditions, light banks or the greenhouse, and arranged in randomized complete blocks. Herbicidal activity was rated by visual inspection and expressed as percent leaf area damaged. All tobacco herbicide studies were performed in the greenhouse except for studies on the effects of temperature and light level, in which growth chambers were used. Atrazine potentiation was defined as an increase in percent leaf area damaged (herbicidal activity) as compared to treatment with the herbicide alone. The evaluation of percent leaf area damaged was determined between 3 and 6 days after spray application. Potentiation data were normalized for the damage observed on atrazine-treated plants in the same trial.

In studies on temperature effects, tobacco plants were subjected to selected temperatures (15, 25, or 35 °C) after herbicide treatments and held under 30 μmol·m^{-2}·s^{-1} PAR (16 h light/8 h dark cycle) in growth chambers. In studies on light level effects, tobacco plants were subjected to selected light levels (0, 10, or 31 μmol·m^{-2}·s^{-1} PAR) at 25 °C after herbicide treatment.

Statistical Analysis. Data were subjected to analysis of variance, and means were separated by Duncan's new multiple-range test or *t* test using PlotIT software (Scientific Programming Enterprises, Haslett, MI).

RESULTS

Atrazine damage of tobacco was observed within 3 days of foliar spray application and was dose-dependent within the concentration range used (0.5, 1.25, and 2.5 mM; **Figure 1A**,**B**). When atrazine spray was combined with NaSA (5 mM), damage occurred within 24 h and was more severe than with atrazine alone. The damage observed with the combination treatment was similar in appearance to atrazine damage. For example, 7-fold more damage was seen when tobacco was sprayed with 2.5 mM atrazine plus 5 mM NaSA than with atrazine alone at 3 days postapplication.

NaSA potentiated atrazine activity on velvetleaf (**Figure 1C**,**D**). As on tobacco, atrazine damage was dose-dependent within the concentration range used (0.5, 1.25, and 2.5 mM) at 3 days after application (**Figure 1C**). NaSA (5 mM) increased activity for the low rate of atrazine (0.5 mM) at 7 days after application and for the middle rate (1.25 mM) at both 3 and 7 days after application and (**Figure 1D**). As observed on tobacco at 6 days (**Figure 1B**), the combination of NaSA with atrazine on velvetleaf was equivalent to doubling of atrazine rate at 7 days.

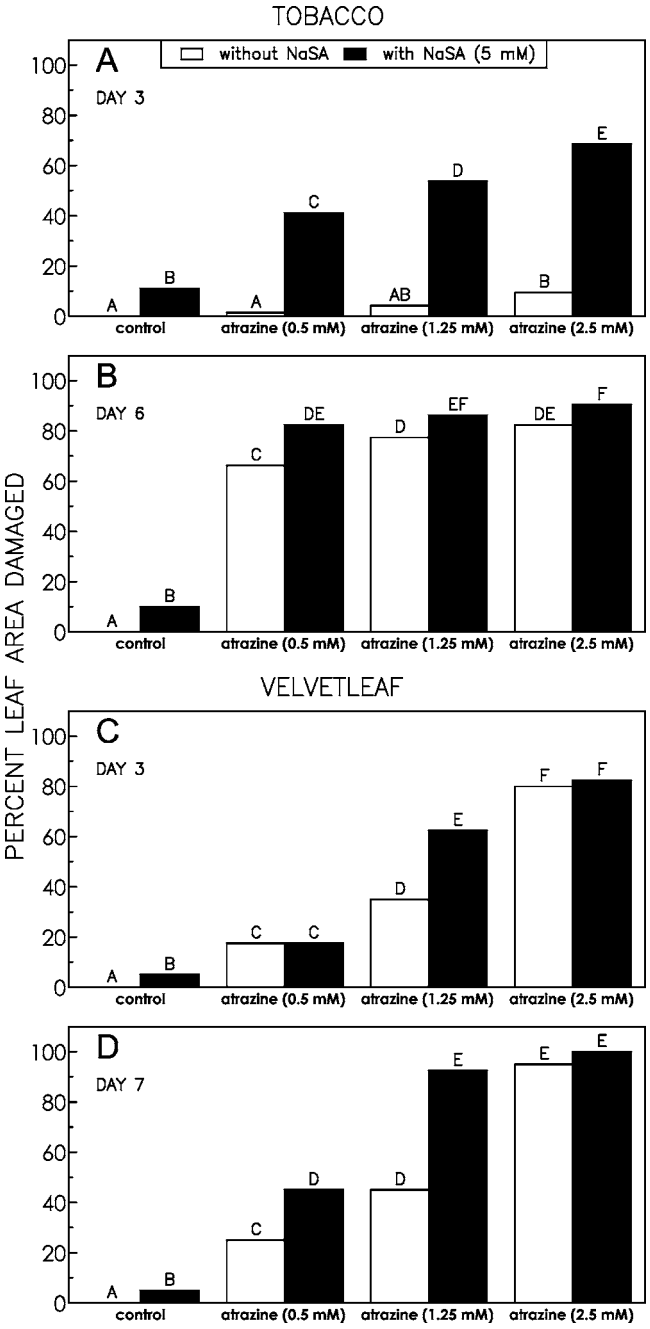

Figure 1. Atrazine concentration response of sodium salicylate (NaSA) potentiation. Simultaneous application of NaSA (5 mM) with atrazine (0.5, 1.25, and 2.5 mM) potentiated herbicide activity in Xanthi-nc tobacco (**A**, **B**) and velvetleaf (**C**, **D**). Crop oil concentrate (0.25% v/v) was used in all treatments. Means were separated by Duncan's new multiple-range test ($p = 0.05$; $n = 6$ plants per treatment) for each day. Means with the same letter are not statistically different.

NaSA also potentiated bentazon herbicidal activity on tobacco. At 4 days after foliar application, leaf area damage was 27% for bentazon (2 mM) alone and 57% for bentazon combined with NaSA (5 mM).

The potentiation of atrazine by other salicylates was structure dependent (**Table 1**). The greatest potentiation was observed among the monochlorinated salicylates. Leaf area damage due

Table 1. Potentiation of Atrazine Activity on Xanthi-nc Tobacco with Selected Salicylates and Systemic Acquired Resistance Inducers

compound[a]	potentiation of atrazine (% change in leaf area damage compared to atrazine alone)[b]	relative activity of atrazine (compared to salicylic acid)[c]
control (atrazine alone)	0	0.78
sodium salicylate (NaSA)	28	1.00
3-chlorosalicylate	249	2.72
3-fluorosalicylate	56	1.22
3-methoxysalicylate	33	1.04
3-methylsalicylate	12	0.87
3-nitrosalicylate	−19	0.63
4-chlorosalicylate	413	4.00
4-fluorosalicylate	282	2.98
4-methoxysalicylate	0	0.78
4-methylsalicylate	1	0.79
5-bromosalicylate	40	1.09
5-chlorosalicylate	253	2.75
5-fluorosalicylate	58	1.23
5-methoxysalicylate	3	0.80
5-methylsalicylate	8	0.84
5-nitrosalicylate	46	1.14
6-fluorosalicylate	44	1.12
6-methoxysalicylate	−36	0.50
6-methylsalicylate	8	0.84
3,5-dichorosalicylate	85	1.44
3,5-difluorosalicylate	85	1.44
3-chloro-5-fluorosalicylate	444	4.24
3-fluoro-5-chlorosalicylate	344	3.46
3,5-dichloro-6-hydroxysalicylate	26	0.99
3,5,6-trichlorosalicylate	24	0.97
benzoic acid	−28	0.56
salicylhydroxamic acid (SHAM)	0	0.78
2,6-dichloroisonicotinic acid (INA)	416	4.03
benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (acibenzolar-*S*-methyl)	148	1.94

[a] Atrazine (0.25 mM) and test compounds (2 mM) were simultaneously foliar-applied to tobacco. Crop oil concentrate (0.25% v/v) was used in all formulations. Acibenzolar-*S*-methyl was applied at (0.5 mM). [b] Potentiation is the percent increase in leaf area damage for compound plus atrazine compared to atrazine alone. Value is the mean activity of at least two trials with $n = 6$ replicate plants per trial. [c] Relative activity is leaf area damage for compound plus atrazine divided by damage for NaSA plus atrazine.

to 0.25 mM atrazine was increased 413, 253, or 249% when 2 mM 4-chlorosalicylate, 5-chlorosalicylate, or 3-chlorosalicylate was combined with atrazine. In comparison, NaSA increased atrazine activity by 28%. Monofluorinated salicylates were also more active than NaSA (282, 58, 56, and 44% increased activity for 4-fluorosalicylate, 5-fluorosalicylate, 3-fluorosalicylate, and 6-fluorosalicylate, respectively). Similar to the monochlorinated salicylates, the 4-position derivative was most active among the monofluorinated salicylates. Monomethylated salicylates were less active than NaSA (12, 8, 8, and 1% increased activity for 3-methylsalicylate, 5-methylsalicylate, 6-methylsalicylate, and 4-methylsalicylate, respectively). Among the methoxylated salicylates, atrazine activity was enhanced by 3-methoxysalicylate by approximately the same amount as with NaSA (33% increased activity), but activity was not affected by 4-methoxysalicylate and 5-methoxysalicylate (0 and 3% increased activity, respectively) and was, in fact, reduced by 6-methoxysalicylate (36% reduced activity). This reduction in atrazine activity was comparable to that produced by benzoic acid and 3-nitrosalicylate (28 and 19% reduced activity, respectively). All four disubstituted salicylates tested increased atrazine activity more than NaSA (444, 344, 85, and 85% increased activity for 3-chloro-5-fluorosalicylate, 3-fluoro-5-chlorosalicylate, 3,5-difluorosalicylate, and 3,5-dichlorosalicylate, respectively). However, both trisubstituted salicylates tested were about as active as NaSA (26 and 24% increased activity for 3,5,6-trichlorosalicylate and 3,5-dichloro-6-hydroxysalicylate, respectively).

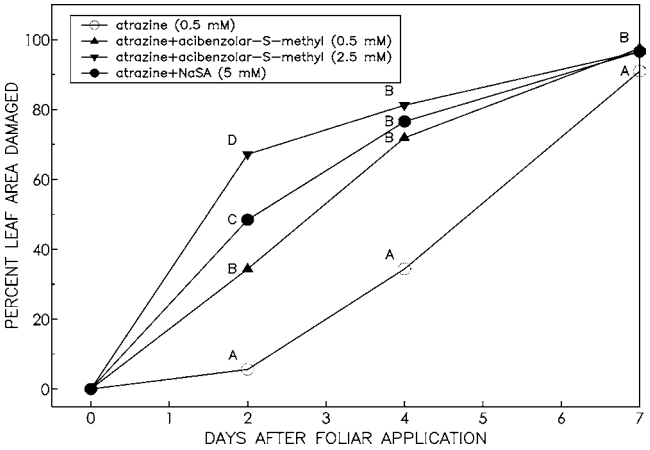

Figure 2. Acibenzolar-*S*-methyl potentiation of atrazine. Application of the systemic acquired resistance inducer acibenzolar-*S*-methyl or sodium salicylate (NaSA) with atrazine (0.5 mM) increased herbicidal activity in Xanthi-nc tobacco. Application of acibenzolar-*S*-methyl alone (0.5 and 2.5 mM) did not damage the leaves, and NaSA alone (5 mM) produced <8% leaf area damage (not shown). Crop oil concentrate (0.25% v/v) was used in all treatments. Means were separated by Duncan's new multiple-range test ($p = 0.05$; $n = 6$ plants per treatment) for each day. Means with the same letter are not statistically different.

Table 2. Effect of Time of Sodium Salicylate (NaSA) Application on Atrazine Herbicidal Damage to Xanthi-nc Tobacco

treatment[b]	leaf area damaged[a] (%) day 3	day 5	day 7
control	0 A	0 A	0 A
NaSA (5 mM)	2 A	3 A	3 A
atrazine (1.25 mM)	0 A	4 A	84 B
NaSA applied with atrazine	23 CD	53 C	96 C
NaSA applied 1 day prior to atrazine	25 D	57 C	97 C
NaSA applied 2 days prior to atrazine	18 C	51 C	97 C
NaSA applied 4 days prior to atrazine	11 B	33 B	95 C

[a] Means were separated by Duncan's new multiple-range test ($p = 0.05$; $n = 6$ plants per treatment). Means followed by the same letter are not significantly different. [b] Crop oil concentrate (0.25% v/v) was used in all treatments.

The SAR inducer 2,6-dichloroisonicotinic acid (INA) (*13*) was one of the most active compounds tested (416% increased activity; **Table 1**). Acibenzolar-*S*-methyl, the active ingredient in disease resistance inducer Actigard 50WG, increased atrazine activity in a dose-dependent manner (**Figure 2**).

To determine the effect of time of application, NaSA was applied at 4, 2, and 1 day prior to or simultaneously with atrazine application (**Table 2**). Leaf area damage 3 days after atrazine application was greatest when NaSA was applied 1 day prior to or simultaneous with atrazine and decreased as the interval between NaSA and atrazine applications increased. However, all of the NaSA pretreatments enhanced atrazine activity equally 7 days after atrazine application. These results do not allow us to determine if NaSA potentiation of atrazine activity is mediated through direct effects on uptake or translocation or through time-dependent processes such as changes in gene expression.

The ability of NaSA to potentiate atrazine activity was evaluated under suboptimal temperature and light conditions.

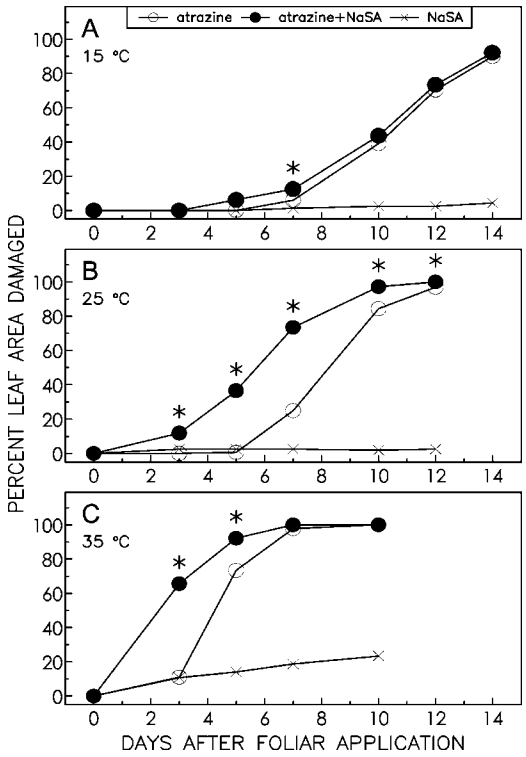

Figure 3. Temperature dependence (**A**, **B**, and **C** for 15, 25, and 35 °C, respectively) of sodium salicylate (NaSA; 5 mM) potentiation of atrazine (1.25 mM) activity on Xanthi-nc tobacco. A light level of 30 μmol·m^{-2}·s^{-1} was used for all temperatures, and crop oil concentrate (COC; 0.25% v/v) was used in all treatments. COC alone produced no leaf area damage under any temperature. Mean separation of atrazine-containing treatments was determined by *t* test ($p = 0.05$; $n = 6$ replicate plants) for each day. Significant differences between atrazine and atrazine plus NaSA are denoted by asterisks.

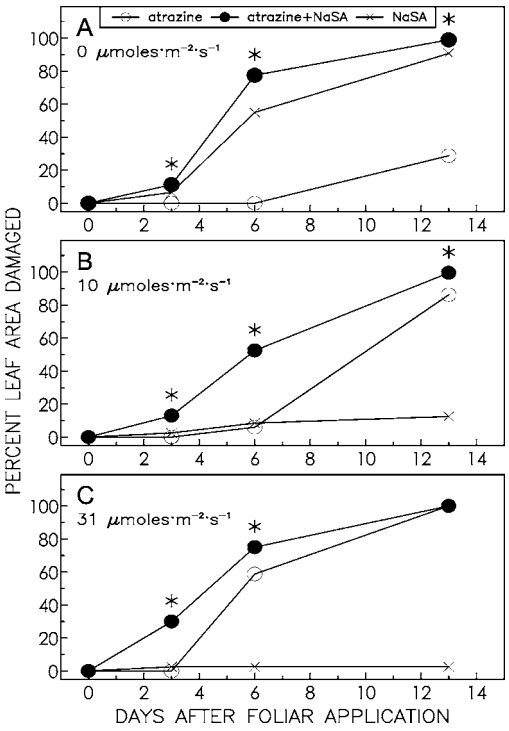

Figure 4. Light dependence of sodium salicylate (NaSA; 5 mM) potentiation of atrazine (1.25 mM) activity on Xanthi-nc tobacco under low light (**A**, **B**, and **C** for 0, 10, and 31 μmol·m^{-2}·s^{-1}, respectively) conditions. A temperature of 25 °C was used for all light levels, and crop oil concentrate (COC; 0.25% v/v) was used in all treatments. COC alone produced no leaf area damage under any light level. Mean separation of atrazine containing treatments was determined by *t* test ($p = 0.05$; $n =$ 6 replicate plants) for each day. Significant differences between atrazine and atrazine plus NaSA are denoted by asterisks.

At room or elevated temperatures (25 and 35 °C, respectively), NaSA decreased time to 50% damage by 2 days (**Figure 3**). However, NaSA was ineffective at potentiating atrazine at 15 °C. Also, 5 mM NaSA alone caused 20% damage at 35 °C, but induced <5% damage at 15 and 25 °C.

NaSA increased atrazine activity under low light conditions on tobacco (**Figure 4**). Under 10 or 31 μmol·m^{-2}·s^{-1} PAR, NaSA with atrazine reduced time to 50% leaf damage by 1 day. An unexpected observation was that NaSA was herbicidal in continuous darkness (**Figure 4A**). Although NaSA alone resulted in plant mortality in continuous darkness, the combination of atrazine and NaSA showed faster herbicidal activity. Atrazine alone was not herbicidal in the dark, as is expected for a light-dependent herbicide.

Nitric oxide (NO), a volatile that has been implicated in plant defense induction, was tested to determine its involvment in salicylate potentiation of atrazine. To test the role of NO, we used the NO generator sodium nitroprusside (SNP; **Table 3**). NO released by SNP has been shown to cause programmed cell death and suppress free radical scavenging in tobacco cell suspension (*14*). At 2 days after spraying, either NaSA or SNP increased atrazine activity. However, the activity of the combination of NaSA and SNP with atrazine was similar to that of atrazine alone, suggesting that although NO and NaSA each independently potentiate atrazine activity, the mechanisms of NO and NaSA enhancement may be antagonistic. By day 3, only NaSA alone significantly potentiated atrazine activity.

The ability of salicylates and disease resistance inducers to potentiate atrazine activity suggested testing the role of SAR in atrazine potentiation with the *Arabidopsis* mutant *npr1-2*. Plants with mutations in *npr1* are more susceptible to pathogen attack and have reduced capacity to express defense genes in response to SA (*15*). NaSA potentiated atrazine activity on *npr1-2* plants to a level similar to that on *NPR1* (wild type) plants (**Figure 5A**). The ethylene insensitive *ein2-1* mutant was used to examine the role of ethylene in atrazine potentiation by NaSA. NaSA potentiated atrazine in the *ein2-1* mutant to the same extent as in wild type *EIN2* (**Figure 5B**). This suggests that neither SAR nor ethylene perception is involved in NaSA activation of atrazine activity.

Table 3. Effect of the NO Generator Sodium Nitroprusside (SNP) on Sodium Salicylate (NaSA) Potentiation of Atrazine Herbicidal Damage to Tobacco

	leaf area damaged[a] (%)	
treatment[b]	day 2	day 3
control	0.0 A	0.0 A
NaSA (5 mM)	1.1 A	1.1 A
SNP (5 mM)	2.0 A	2.5 A
NaSA and SNP	3.5 A	4.5 A
atrazine (1.25 mM)	51.5 B	79.8 B
atrazine applied with NaSA	73.5 C	93.8 D
atrazine applied with SNP	62.5 BC	82.8 BC
atrazine applied with NaSA and SNP	47.0 B	81.3 B

[a] Means were separated by Duncan's new multiple-range test ($p = 0.05$; $n =$ 4 plants per treatment). Means followed by the same letter are not significantly different. [b] Crop oil concentrate (0.25% v/v) was used in all treatments.

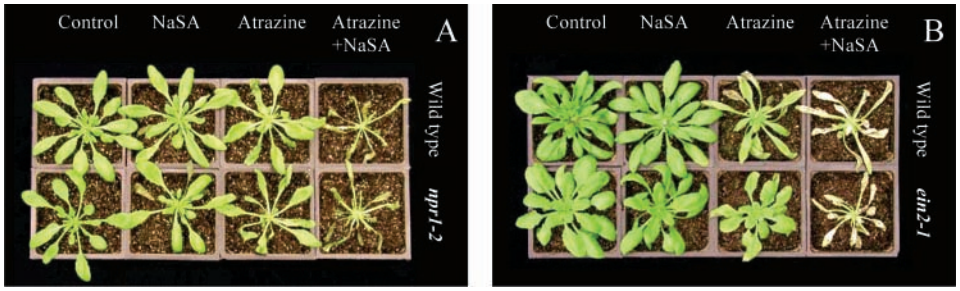

Figure 5. Simultaneous application of sodium salicylate (NaSA; 2.5 mM) potentiates atrazine (0.25 mM) activity on *Arabidopsis*: (**A**) potentiation of atrazine by NaSA of Columbia (wild type) and *npr1-2*, mutant compromised in salicylate-induced acquired resistance; (**B**) potentiation of atrazine by NaSA of Columbia (wild type) and *ein2-1*, mutant insensitive to ethylene. Plants were photographed 4 days after foliar application.

Salicylhydroxamic acid (SHAM), an inhibitor of alternative respiration, did not potentiate atrazine on tobacco (**Table 1**). Moreover, the potentiation observed from the combination of SHAM and NaSA and atrazine was the same as for the combination of NaSA and atrazine. This experiment indicates that alternative respiration is not involved in NaSA potentiation of atrazine.

DISCUSSION

Atrazine is the most commonly used maize herbicide, but it is facing many challenges. Atrazine use has declined in recent years due to several factors, including restrictions of use rates to control leaching, conversion of acreage to glyphosate-resistant maize, concerns about possible endocrine disruption effects (*16*), and the use of alternative herbicides. Even so, atrazine was used on >50 000 000 acres of maize in the United States alone in 2003 (*2*).

Atrazine and other PSII inhibitors function by inhibiting photosynthesis. However, a second mechanism of action may be the induction of free radical-mediated oxidative stress (*17*). Free radical-quenching agents have been shown to limit PSII inhibitor-induced damage (*18*). Another possible mechanism of action of PSII inhibitors is inhibition of nitrite reduction (*19*). The resulting elevated levels of nitrite and NO_x have been postulated to be phytotoxic.

SA is an important regulator of the plant defense pathway. Its ability to induce plant defenses was first shown by White (*20*). Since the discovery of its function as an endogenous regulator of plant defenses (*21*), the role of salicylate has been intensively investigated. The requirement for SA in SAR is demonstrated by the use of the *nahG* transgene, which blocks the accumulation of SA and the subsequent development of SAR (*22*).

In our studies, we examined the ability of NaSA and other SAR inducers to potentiate PSII-inhibiting herbicides. Except in constant darkness (**Figure 4**) or at constant elevated temperatures (**Figure 3**), NaSA alone is not typically herbicidal. However, we have demonstrated that NaSA accelerates atrazine activity (**Figures 1**−**4**) and thus may provide a way to reduce use rates of herbicide in the field. The structure−activity results for the salicylates suggest that more potent synergists for atrazine and PSII inhibition may be available, including the resistance inducers acibenzolar-*S*-methyl (**Figure 2**) and 2,6-dichloroisonicotinic acid (**Table 1**).

The mode of action of NaSA potentiation of atrazine is not known. Klepper (*23*) noted that atrazine and NaSA combinations significantly increased NO_x emissions from intact soybean in the light. Moreover, chlorosubstituted salicylates showed the greatest increases in NO_x emissions (*24*), which is parallel to their ability to potentiate atrazine activity in our results (**Table 1**). The relationship between NO_x emissions and salicylate potentiation of herbicidal activity is unclear. However, one component of the emissions, NO, has been proposed as a signal molecule in the plant defense response. NO reportedly turns on the signal transduction cascade leading to SA accumulation and SAR (*25*). NO has recently been shown to be necessary for the activation of the phenylpropanoid pathway and the production of free radicals in the defense response (*26*). Although NO potentiated atrazine activity by itself (**Table 3**), it antagonized NaSA potentiation. This result may have been influenced by the NO donor employed, SNP, which has recently been shown to suppress free radical scavenging in tobacco (*14*). Thus, we suggest that NO and salicylate have separate and distinct mechanisms for enhancing PSII inhibitor herbicides.

NaSA showed herbicidal effects on its own under higher temperatures (35 °C, **Figure 3C**) and in the absence of light (**Figure 4C**), suggesting that the efficacy of NaSA as a herbicide could be greatly affected by the environment. The herbicidal effect of NaSA in the dark was unexpected, as was the ability of NaSA to increase atrazine activity in the dark (**Figure 4A**). To determine if salicylate toxicity in the dark could be due to induction of the alternate respiratory pathway (*27*), we tested the effect of salicylhydroxamic acid (SHAM), an inhibitor of the alternate oxidase, on atrazine potentiation. SHAM neither potentiated atrazine (**Table 1**) nor modified NaSA potentiation of atrazine. This implies that alternative respiration is not involved in NaSA potentiation of atrazine. Another possible mode of action is that nitrite accumulation is potentiated in the combination treatment, resulting in plant death (*23*).

Modification of herbicide activity by salicylates and SAR inducers is not limited to PSII inhibitors. We have previously shown that NaSA and acibenzolar-*S*-methyl are able to potentiate the activity of protoporphorin oxidase (PPO) inhibitors (*28*) as well as glyphosate (*29*). In contrast, NaSA and acibenzolar-*S*-methyl protect plants from the photosystem I (PSI) inhibitors paraquat and diquat (*30*). It is unclear why plants respond differently to PSI inhibitors with salicylate than they do to other herbicides. One possibility is that NaSA may be inhibiting transport of paraquat to its site of action, because paraquat damage is typically visible within a few hours of application.

The importance of ethylene for atrazine potentiation was tested in *ein2-1*, an ethylene-insensitive mutant. Plants with mutations in *ein2* are insensitive to ethylene (*31*). NaSA increased atrazine activity on the *ein2-1* mutant to the same extent seen in the *EIN* (wild type) plant (**Figure 5B**). Although ethylene levels often increase in response to herbicide application (*32*), ethylene is not implicated in atrazine potentiation.

The independence of atrazine potentiation from the disease resistance pathways was demonstrated by *Arabidopsis* mutants (**Figure 5**). First, NaSA potentiation of atrazine was shown to

be independent of *NPR1*-dependent SAR by the *npr1-2* mutant (**Figure 5A**). Second, the *ein2-1* mutant, in addition to being ethylene-insensitive, is compromised in the induction of the induced systemic resistance (ISR) pathway (*32*). Because NaSA potentiates atrazine on *ein2-1*, a role for ISR-induced disease resistance in atrazine potentiation seems to be unlikely (**Figure 5B**). Atrazine potentiation may be working through an SAR pathway that may be independent of *NPR* (*33*). However, this seems to be unlikely because all commercial SAR inducers to date either mimic SA (e.g., acibenzolar-*S*-methyl) or induce the SA-dependent SAR pathway (*34*). Salicylate protection from paraquat is also independent of the SAR pathways (*30*). Why SA and SAR inducers are able to potentiate the activity of herbicides such as atrazine, while protecting against paraquat, remains an area for further investigation.

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