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Basis for Antagonism by Sodium Bentazon of Tritosulfuron Toxicity to White Bean (*Phaseolus vulgaris* L.)

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White bean (*Phaseolus vulgaris* L.) was used to study the antagonism caused by Na-bentazon on the phytotoxic action of the sulfonylurea (SU) herbicide tritosulfuron. After 168 h, uptake and translocation of [^{14}C]tritosulfuron were reduced by 60 and 89%, respectively, when Na-bentazon was added to the mixture. Addition of $(NH_4)_2SO_4$ or replacement of Na-bentazon with NH_4-bentazon completely eliminated the negative effects on [^{14}C]tritosulfuron uptake but not on its translocation. Scanning electron microscopy revealed that a mixture of Na-bentazon plus tritosulfuron plus DASH HC (0.156%) formed a rough layer of grain-like crystals on the leaf surface, whereas the addition of $(NH_4)_2SO_4$ or replacement of Na-bentazon with NH_4-bentazon resulted in amorphous deposits that may be more easily absorbed. The antagonism of tritosulfuron's phytotoxicity by Na-bentazon involves two separate processes, chemical (uptake effect) and biochemical (translocation effect).

KEYWORDS: Bentazon; tritosulfuron; white bean (*Phaseolus vulgaris* L.); herbicide antagonism; radiolabeled herbicide; uptake; translocation

INTRODUCTION

It has been a common practice to apply mixtures of herbicides with surfactants, safeners, synergists, nutrients, and/or other nonactive ingredients (*1*). Use of herbicide mixtures can achieve broader spectrum and better season-long weed control than one herbicide. Mixtures can also reduce the total amount of herbicide needed and may delay the evolution of herbicide resistance. With these advantages, herbicide mixtures can be a more affordable alternative than the newest herbicide (*1*). Despite the many benefits of herbicide combinations, these mixtures can sometimes result in antagonism of the phytotoxic action of one or more of the constituents within the mixture. Antagonism is defined as "an interaction of two or more chemicals such that the effect, when combined, is less than the predicted effect based on the activity of each chemical applied separately" (*2*). Herbicide antagonism can be attributed to one or more of the following types of mechanisms: (i) chemical, (ii) biochemical (*3*), (iii) physiological, and/or (iv) competitive (*4*).

Many researchers have shown that bentazon (a PSI inhibitor) antagonizes the phytotoxic action of herbicides from chemical groups that have different target sites and modes of action, for example, acetyl-CoA carboxylase (ACCase) inhibitors and acetolactate synthase (ALS) inhibitors as well as paraquat, a PSI electron acceptor. Mixtures of bentazon plus the graminicide sethoxydim (ACCase inhibitors) had less than expected activity on large crabgrass, wire grass, fall panicum, broadleaf signalgrass, giant foxtail, and yellow foxtail (*5*−*8*). It is most likely that bentazon interacts chemically with the other herbicides in the mixture because, for example, the absorption of [^{14}C]sethoxydim was reduced by 30−80%, (*9*, *10*), and the absorption of other graminicides, BAS 625 and haloxyfop, was reduced by 20−50% (*11*, *12*) when mixed with bentazon. The negative effect of bentazon in mixtures on the uptake of other herbicides was not restricted to the graminicides; for example, the uptake of a sulfonylurea (SU) herbicide, halosulfuron-methyl, was reduced by 50−60% when mixed with bentazon compared to its uptake when applied alone (*13*). Conversely, bentazon did not reduce the uptake of prosulfuron when the two herbicides were mixed (*13*).

Bentazon also reduces the translocation of the accompanying herbicide in the mixture. For example, the translocation of halosulfuron-methyl or prosulfuron was reduced by 70−85% (*13*) and the amount of haloxyfop-methyl translocated to the roots of yellow foxtail was reduced by 80% (*12*) when those herbicides were mixed with bentazon. On the other hand, bentazon had no effect on the translocation of BAS 625 (*11*). These results indicate that in addition to chemical interactions, biochemical interactions may also be responsible for bentazon antagonism.

Several studies have shown that the addition of certain ammonium salts to the herbicide mixture improves the efficacy of bentazon mixtures as well as the uptake of the accompanying herbicide. For example, the addition of ammonium sulfate, ammonium phosphate dibasic, or ammonium nitrate completely eliminated the Na-bentazon antagonism of sethoxydim uptake (*14*). The addition of ammonium sulfate or urea with ammonium nitrate improved the efficacy of a bentazon−sethoxydim mixture

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used to control large crabgrass (*15*). Similarly, the addition of ammonium sulfate eliminated the antagonism between Na-bentazon and primisulfuron for the control of shattercane or giant foxtail by improving the uptake of primisulfuron (*16*). In other studies with sethoxydim, Thelen et al. (*15*) suggest that the presence of NH_4^+ simply prohibited the formation of a sodium−sethoxydim complex. Conversely, the addition of ammonium acetate, ammonium hydroxide, or urea failed to overcome the bentazon antagonism of sethoxydim activity (*14*).

The use of appropriate chemicals may also eliminate the antagonism, for example, (a) the addition of the surfactant BCH 815 00, as opposed to crop oil concentrate (COC = Herbimax; *10*, *14*), or (b) the use of bentazon salts other than Na-bentazon (*10*, *14*). Indeed, mixtures of NH_4-bentazon or H-bentazon plus sethoxydim had no antagonistic effect on the uptake of the graminicide (*10*, *14*). Consequently, it has been speculated but not proven that NH_4^+ may counteract the inhibitory effect of bentazon on the activity of plasma membrane H^+-ATPase and hence restores normal symplastic uptake and translocation of weak-acid herbicides (*7*).

In the research presented here, the antagonistic effect conferred by bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] on the activity of a SU herbicide, tritosulfuron [1-[4-methoxy-6-(trifluoromethyl)-1,3,5-triazin-2-yl]-3-[2-(trifluoromethyl) benzenesulfonyl]urea] on white bean (*Phaseolus vulgaris* L.) was studied. White bean was selected as a model plant because it tolerates a postemergence treatment of bentazon (*17*) and is susceptible to tritosulfuron. Thus, the confounding effects of bentazon on white bean were eliminated; as a result, the antagonistic effects of bentazon on the phytotoxicity tritosulfuron could be easily determined.

The hypothesis for this project was that Na-bentazon impaired the uptake and/or the translocation of tritosulfuron when the two herbicides were applied in a mixture. The objectives of these studies were to determine the physiological basis for the antagonistic effect of Na-bentazon and find practical means that could be implemented under agricultural situations to overcome this antagonism. To test the hypothesis and to meet the objectives, various bioassays were conducted to determine the effect of (1) DASH HC (a surfactant) concentration, (2) the timing of bentazon application, (3) the addition of ammonium salts to Na-bentazon−tritosulfuron mixtures, (4) the replacement Na-bentazon with NH_4-bentazon or H-bentazon in mixtures with tritosulfuron, and (5) the addition of sodium salts to NH_4-bentazon−tritosulfuron mixtures on the toxicity of bentazon−tritosulfuron mixtures. Subsequently, studies of the uptake and translocation of [^{14}C]tritosulfuron were compared between treatments that did and did not circumvent the antagonism as determined by bioassay.

MATERIALS AND METHODS

Plant Material and Growth Conditions. White bean (*P. vulgaris* L. cv. Stingray) (W. G. Thompson & Sons Ltd., Blenheim, ON, Canada) seeds were planted in 450-mL pots containing a commercial potting mix, Promix BX (Premier Brands, Brampton, ON, Canada). Plants were grown in a greenhouse at 25 ± 5 °C, with sunlight being supplemented with high-pressure sodium lighting, which provided a constant light intensity between 200 and 500 μEinstein m^{-2} s^{-1}. Light and dark periods were 16 and 8 h, respectively. Water-soluble fertilizer (20% N, 8% P_2O_5, 20% K_2O) was applied twice weekly to promote optimal growth.

Antagonism Studies−Bioassays. All herbicide treatments were applied as foliar sprays 3−4 weeks after planting, when the second trifoliate leaf was approximately equal in area to the first trifoliate leaf. Plants were sprayed using a track sprayer (Mandel Scientific Corp., Guelph, ON, Canada) equipped with a single motorized 8002E flat-jet nozzle, mounted 45 cm above the top of the canopy, which delivered the spray solution at 200 L ha^{-1} at 276 kPa. Plants were harvested 14 days after treatment (DAT). Dry weights (DW) of the foliage were recorded.

Four elementary treatments were included in all of the bioassays: (1) H_2O-sprayed white bean plants, as untreated controls; (2) Na-bentazon formulated as 87% soluble granules (BASF, Limburgerhof, Germany) at 442.5 g of active ingredient (ai) ha^{-1}; (3) tritosulfuron formulated as 71.4% water-dispersible granules (BASF) at 10 g of ai ha^{-1}; and (4) Na-bentazon plus tritosulfuron at 442.5 and 10 g of ai ha^{-1}, respectively. The surfactant DASH HC (BASF) [0.156% (v/v)] was added to all treatments. Surface analysis revealed that this combination of herbicide and surfactant rates conferred the most severe antagonism (data not presented).

DASH HC was added at 0, 0.156, 0.313, or 0.625% (v/v), whereas the concentration of each salt was 0.212 N. Each of these concentrations was applied with the four elementary treatments described previously.

In all treatments bentazon was applied at 442.5 g of ai ha^{-1} and tritosulfuron at 10 g of ai ha^{-1}. The treatment solutions were made with double-distilled water (ddH_2O), and all chemicals were certified ACS grades.

Uptake and Translocation of [^{14}C]Tritosulfuron. The [*phenyl*-U-^{14}C]tritosulfuron (BASF) with a specific activity of 4.22 MBq $\mu$$mol^{-1}$ was dissolved in ddH_2O and kept at −20 °C until required for use. Formulated tritosulfuron and [^{14}C]tritosulfuron were combined to make a 0.112 mM aqueous solution, a concentration equivalent to 10 g of ai ha^{-1}. In addition, Na-bentazon, NH_4-bentazon, and/or DASH HC were added to meet the same herbicidal concentration and composition of the relevant treatment solutions described previously.

The rate of foliar uptake and translocation of [^{14}C]tritosulfuron applied alone or mixed with Na-bentazon was compared with selected treatments that were previously found to eliminate the antagonism in the bioassay experiments. Plants were grown as described previously and were treated at the first trifoliate stage when the first leaf was fully expanded and the second leaf was just starting to expand.

The treatment solution that contained 890 Bq of [^{14}C]tritosulfuron was applied to the adaxial side of the first trifoliate leaf. Nine microliters was applied per plant (3 μL on each of three leaflets as 1-μL droplets) delivered with a 50-μL syringe, equipped with a repeating dispenser (Hamilton Co., Reno, NV). The solutions contained [^{14}C]tritosulfuron and formulated tritosulfuron at concentrations of 0.022 and 0.090 mM, respectively. Two reference treatments were used: (1) [^{14}C]tritosulfuron + DASH (0.156%) and (2) a mixture of [^{14}C]tritosulfuron + DASH (0.156%) + Na-bentazon (9.2 mM, equivalent to 442.5 g of ai ha^{-1} applied at 200 L ha^{-1}). These two treatments represented the best- and worse-case scenarios, respectively, in the bioassays and were compared with three sets of treatments: (i) [^{14}C]tritosulfuron + DASH (0.625%) and [^{14}C]tritosulfuron + DASH (0.625%) + Na-bentazon to evaluate the effect of DASH concentration; (ii) [^{14}C]tritosulfuron + NH_4-bentazon (9.2 mM) + DASH (0.156%) to study the effect of bentazon salts on the uptake and translocation of tritosulfuron; (iii) [^{14}C]tritosulfuron + DASH 0.156% + AMS (0.212 N) and [^{14}C]tritosulfuron +Na-bentazon + DASH + AMS (0.212 N) to determine the effect of AMS.

Two hours after the application, the treated plants were returned to the greenhouse. Plants were harvested 24, 48, and 168 h after treatment (HAT). Treated leaflets were excised, and unabsorbed [^{14}C]tritosulfuron was determined by rinsing the adaxial surface with a 20-mL mixture of acetone/ddH_2O (1:4 v/v) containing 0.5% (v/v) Agral 90 (Norac Concepts Inc., Orleans, ON, Canada). The rinsates from the three leaflets of a plant were pooled. Two aliquots of 1 mL were taken, and each was dissolved in 5 mL of Ecolite(+) (MP Biomedicals Inc., Irvine, CA) and ^{14}C was measured with liquid scintillation spectrometry (LSS; Beckman Instruments Inc., Fullerton, CA). Foliar uptake was determined by subtraction of the total ^{14}C recovered in leaf rinsates from the total [^{14}C]tritosulfuron applied.

The translocation of ^{14}C was estimated by measuring the ^{14}C recovered in the treated leaf (TL) versus the amount of ^{14}C that moved out of the TL to the petiole of the TL, shoot, and root. Plant parts were wrapped in tissue paper and kept in a drying oven at 50−60 °C until

Table 1. Effect of DASH HC Concentrations on Sodium Bentazon Antagonism of Tritosulfuron Toxicity to White Bean Plants[a]

	% of the mean of control plants[b]					
	applied alone		applied as mixture			
DASH HC (%)	Na-bentazon	tritosulfuron	predicted[c]	observed	Pr > |t|[d]	pH[e]
0	94.8 (5.2)	68.1 (3.7)	64.6 (4.4)	83.9 (3.3)	0.0034	7.3
0.156	92.8 (5.8)	54.3 (2.3%)	50.4 (3.7)	79.4 (4.9)	0.0005	3.9
0.312	95.4 (3.0)	53.4 (3.1)	50.9 (4.1)	71.6 (4.7)	0.0015	3.6
0.625	92.7 (2.1)	50.4 (3.4)	46.7 (3.9)	59.6 (3.4)	0.029	3.3
LSD[f] ($\alpha = 0.05$)	12.2%	9.7%	NA[g]	11.8%		

[a] Plants were treated at the second trifoliate stage using H_2O as a control, Na-bentazon at 442.5 g of ai ha^{-1}, tritosulfuron at 10 g of ai ha^{-1}, or a mixture of the two herbicides. Data were taken 14 days after treatment. [b] Data are shoot dry weights (DW) expressed as a percentage of the mean of H_2O-treated control plants. Standard errors of the mean are in parentheses. [c] Colby's (1967) predicted effects of the mixtures, generated with SAS, PROC NLMIXED. [d] Probability of t values = 0; t tests were performed to H_0: observed − predicted = 0. [e] pH of the mixture. [f] Least significant difference; values generated by Duncan's multiple-range test. [g] Not attainable.

combusted. Combustions were carried out with a biological oxidizer (R. J. Harvey Instrument Co., Hillsdale, NJ), set at a 4-min combustion cycle with a flow rate of 350 mL min^{-1} of N_2 and O_2. The resulting $^{14}CO_2$ was trapped in 15 mL of ^{14}C cocktail (R. J. Harvey instrument Co.), and radioactivity was quantified by LSS. Recovery of radioactivity after combustion in the oxidizer was 93.3% (with a standard deviation of 1.4%), as determined by combustion of a known amount of D-manitol-1-^{14}C. The translocation of ^{14}C out of TL was expressed as a percentage of total ^{14}C recovered in planta.

Scanning Electron Microscopy. Plants were treated as in the bioassay section. The cryo-SEM technique allowed fixation without using any reagents or solvent; therefore, the risk of removing or modifying the herbicide deposits was minimal. In each plant the second leaf was harvested 1.5−2 HAT. Leaf sections (approximately 8 × 8 mm) were removed, and the abaxial surface was glued with Tissue Tek (CANEMO Supplies, St Laurent, QC, Canada) and placed against a copper specimen holder. Immediately after gluing, the objects were frozen in slushy liquid nitrogen (∼−210 °C) under vacuum with argon gas bleeding into the freezing chamber to prevent the development of ice through condensation. Immediately after freezing, the samples were transferred to the preparation chamber K1250X (Emitech Ltd., Kent, U.K.), where they sublimated under a 10^{-4} mbar vacuum at −80 °C for 30 min, followed by tandem cycles of sputter coating using a current of 20−25 mA for 1 min at 2×10^{-5} mbar vacuum and −130 °C. Coated samples were kept under vacuum in the preparation chamber at −130 °C for up to 5 h, until they were scanned. A transfer device allowed the specimen to be moved from the freezing chamber to the preparation chamber and eventually to the SEM system under constant vacuum.

Scanning of the samples was conducted under vacuum at −130 °C or lower, with a Hitachi S-570 (Hitachi High-Technologies, Tokyo, Japan) scanning electron microscope equipped with a cold-stage Emscope sp2000A system (Emitech Ltd.). Samples were scanned with a 10-kV electron beam, and images were acquired from two locations within the leaf section using an image capture system Quartz PCI (Quartz Imaging Corp., Vancouver, BC, Canada).

Experimental Design and Statistical Analyses. *Antagonism Studies—Bioassays.* All of the experiments were organized as randomized complete block designs (RCBD), with individual white bean plants as the experimental unit. Treatments were replicated six times ($n = 6$), and the experiments were conducted twice. Data were subjected to analysis of variance (ANOVA) and residual analysis using SAS 8.2 (SAS Institute Inc., Cary, NC) that employed PROC MIXED, PROC UNIVARIATE, and PROC GLM. Type I error was set at 0.05. These analyses revealed whether the distribution of the residuals (the experimental error component of the general linear model) met the criteria for ANOVA, what data could be eliminated as outliers, and the sources of variance (*18*). Quantitative evaluation of the antagonism was conducted using a nonlinear mixed model (PROC NLMIXED; SAS Institute Inc.) to calculate the predicted response of the mixture according to Colby's method (*19*). A specific treatment was considered to be antagonistic when a t test determined that the observed response of the mixture was significantly greater than Colby's predicted response (type I error was set at 0.05).

Uptake and Translocation of [^{14}C]Tritosulfuron Studies. The uptake and translocation experiment was organized as a factorial design with herbicide treatments, time after treatments, and plant parts as the factors. The experimental units were individual plants. Treatments were replicated five times, and the experiment was conducted once.

Total foliar uptake of ^{14}C was expressed as a percentage of [^{14}C]tritosulfuron applied, and the translocation data was expressed as a percentage of the radioactivity recovered in planta. Data were subjected to ANOVA, residuals analyses, and Duncan's multiple-range tests (used to separate treatment means within harvest times) using SAS 8.2 that employed PROC MIXED, PROC GLM, and PROC UNIVARIATE. Type I error was set at 0.05.

RESULTS AND DISCUSSION

Antagonism Studies—Bioassays. Regardless of the DASH HC concentration used, bentazon had no negative effect on white bean 14 DAT. However, compared to tritosulfuron (10 g of ai ha^{-1}) alone, the efficacy of this herbicide improved significantly when combined with DASH HC at 0.156% (**Table 1**). Furthermore, increasing the concentration of DASH HC from 0.156 to 0.625% increased the activity of the herbicide mixture but did not completely eliminate the antagonism (**Table 1**). Because Na-bentazon (442.5 g of ai ha^{-1}) + tritosulfuron (10 g of ai ha^{-1}) + DASH HC (0.156%) caused the most severe antagonism (**Table 1**; **Figure 1A**), this combination was used in all experiments as the worse-case scenario to study the antagonism.

When Na-bentazon was applied at various times up to 24 h after tritosulfuron, the antagonism was eliminated, suggesting that foliar absorption of tritosulfuron occurs rapidly (**Table 2**). Conversely, Na-bentazon antagonized tritosulfuron activity when applied 0−3 h prior to tritosulfuron (**Table 2**). These results indicate that a chemical interaction may occur between Na-bentazon and tritosulfuron on the leaf surface.

The addition of 0.212 N AMS, AMP, or AMN reduced the antagonism between Na-bentazon and tritosulfuron, but the addition of AMA did not (**Figure 1B**; **Table 3**). The ammonium salts specifically affect the toxicity of the mixtures because they did not alter the effects of Na-bentazon or tritosulfuron when each was applied alone (**Table 3**). These results agree with those of Gerwick et al. (*7*), Wanamarta et al. (*14*), Jordan and York (*15*), and Hart et al. (*16*), who reported that the addition of AMN or AMS reduced the antagonism conferred by bentazon on the efficacy of sethoxydim and primisulfuron.

It has been suggested that chemical interactions between sethoxydim and Na^+ from Na-bentazon (and possibly from other sources) caused the formation of the sodium salt of sethoxydim, which has a lower uptake rate compared to the acid form of this herbicide (*10*, *14*, *20*). With this in mind, Na-bentazon was replaced with NH_4-bentazon or H-bentazon; both replacements

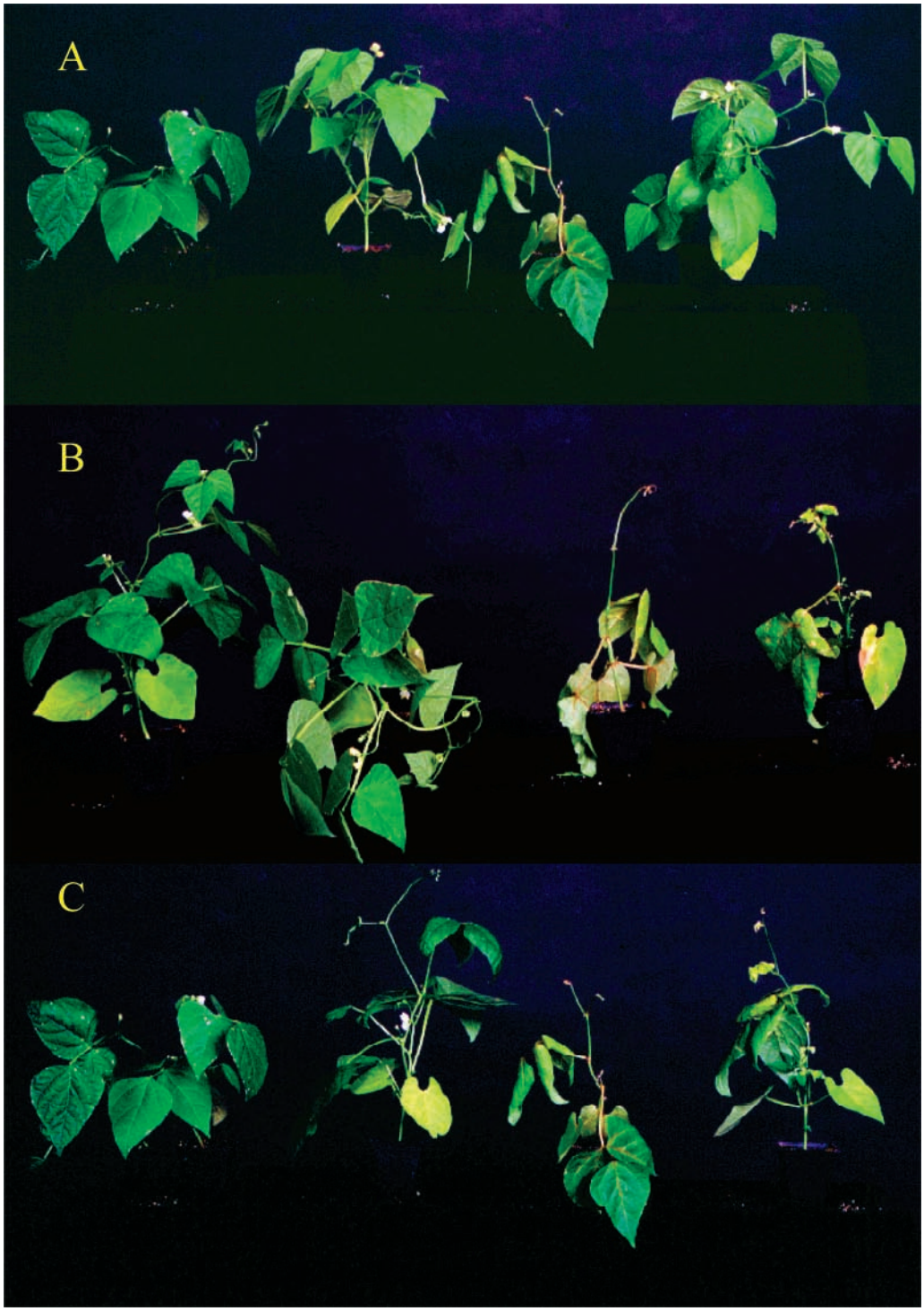

Figure 1. Effect (14 DAT) of tritosulfuron, various chemical formulations of bentazon, and a mixture of these two herbicides on white bean plants treated at the second trifoliate stage: (**A**) plants were treated (left to right) with H_2O, Na-bentazon, tritosulfuron, and a mixture of the two herbicides; (**B**) same treatments as in (**A**) except 0.212 N ammonium sulfate (AMS) was added to all treatments; (**C**) same as in (**A**) except Na-bentazon was replaced with NH_4-bentazon. Herbicide doses were bentazon, 442.5 g of ai ha^{-1}, and tritosulfuron, 10 g of ai ha^{-1}. DASH HC 0.156% (v/v) was added to all treatments.

eliminated the antagonism of tritosulfuron (**Table 4**; **Figure 1C**). Bentazon ammonium and bentazon (at 442.5 g of ai ha^{-1}), applied alone, were not toxic to white bean. Similarly, with mixtures supplemented with inorganic ammonium salts, in terms of dry weight, the antagonism was reduced but not eliminated 14 DAT, because young leaves developed when NH_4-bentazon or H-bentazon was combined with tritosulfuron (**Figure 1C**). These results suggested that Na-bentazon is involved in the antagonistic interaction with tritosulfuron. Furthermore, the fact that H-bentazon eliminated the antagonism implies that NH_4^+ is not an absolute requirement to overcome bentazon/tritosulfuron antagonism.

To clarify the role of ammonium salts and their interplay with Na^+ in the antagonism, 0.212 N SS or SA was added to the mixtures that included NH_4-bentazon. Neither SS nor SA had an effect on the toxicity of NH_4-bentazon or tritosulfuron applied alone (**Table 4**). When 0.2 N Na_2SO_4 or CH_3COONa was added to mixtures containing NH_4-bentazon, tritosulfuron activity was not adversely affected by SS, but was severely antagonized with SA. These results indicated that the Na^+ from Na-bentazon is

Table 2. Effect of Sodium Bentazon Application Time on the Toxicity of Tritosulfuron to White Bean[a]

	% of the mean of control plants[b]															
	effect of Na-bentazon application time (h before tritosulfuron application)								Na-bentazon application time (h after tritosulfuron application)							
	–24	–12	–6	–3	–2	–1	–0.5	–0[c]	0	0.5	1	2	3	6	12	24
	58.3 (2.6)	53.5 (3.3)	52.8 (2.3)	77.7 (7.9)	70.0 (5.9)	58.9 (6.0)	58.8 (4.4)	65.9 (7.4)	56.5 (3.1)	49.5 (3.1)	47.9 (3.0)	51.2 (2.5)	52.2 (3.9)	50.1 (3.1)	47.4 (2.6)	50.8 (1.6)
Pr > |t|[d]	0.0721	0.3033	0.3646	<.0001	0.0008	0.0571	0.0602	0.043	0.069	0.6757	0.9471	0.4396	0.3289	0.5975	0.94741	0.4957

[a] Plants were treated at the second trifoliate stage with H_2O as a control, Na-bentazon at 442.5 g of ai ha^{-1}, tritosulfuron at 10 gof ai ha^{-1}, or a mixture of the two herbicides. Responses of white bean plants treated with Na-bentazon, tritosulfuron or mixture of both were 97.6 (3.4), 48.7 (2.2), and 80.8% (4.8%) of the mean of control plants. Data were taken 14 days after treatment. [b] Data are shoot dry weights (DW) expressed as a percentage of the mean of H_2O-treated control plants. Standard errors of the mean are in parentheses. [c] At time –0, tritosulfuron was applied immediately after Na-bentazon, whereas at time 0 Na-bentazon was applied immediately after tritosulfuron. [d] Probability of t values = 0; t tests were performed to H_o: observed – predicted = 0.

Table 3. Effects of Addition of 0.212 N AMS, AMA, AMN, or Ammonium Phosphate Dibasic (AMP) on the Antagonism of Tritosulfuron's Toxicity to White Bean Plants by Sodium Bentazon[a]

	% of the mean of control plants[b]					
	applied alone		applied as mixture			
ammonium salt	Na-bentazon	tritosulfuron	predicted[c]	observed	Pr > |t|[d]	pH[e]
none	100.7 (5.3)	44.4 (2.9)	44.8 (5.9)	84.7 (7.7)	<0.0001	4.0
AMS	88.7 (6.8)	44.1 (3.4)	36.4 (5.2)	45.2 (4.0)	0.2597	4.0
AMA	96.6 (5.3)	37.2 (3.7)	35.9 (5.6)	73.2 (8.3)	<0.0001	6.6
AMN	94.5 (4.6)	47.7 (4.2)	45. (4.3)	51.7 (3.8)	0.2859	3.9
AMP	97.9 (1.4)	49.6 (4.1)	48.5 (4.1)	52.7 (3.9)	0.4541	7.8

[a] Plants were treated at the second trifoliate stage with H_2O as a control, Na-bentazon at 442.5 g of ai ha^{-1}, tritosulfuron at 10 g of ai ha^{-1}, or a mixture of the two herbicides. DASH HC at 0.156% (v/v) and the corresponding ammonium salt were added to all treatments. Data were taken 14 days after treatment. [b] Data are shoot dry weights (DW) expressed as a percentage of the mean of H_2O-treated control plants. Standard errors of the mean are in parentheses. [c] Colby's (1967) predicted effects of the mixtures generated with SAS, PROC NLMIXED. [d] Probability of t values = 0; t tests were performed to H_o: observed – predicted = 0. [e] pH of the mixture.

Table 4. Effect of Na^+, NH_4^+, or H^+ Derivatives of Bentazon on the Efficacy of Bentazon–Tritosulfuron Mixtures and the Effect of Addition of 0.212 N Sodium Sulfate (SS) or Sodium Acetate (SA) on the Efficacy of NH_4-Bentazon–Tritosulfuron Mixtures[a]

	% of the mean of control plants[b]					
	applied alone		applied as mixture			
bentazon derivative	bentazon	tritosulfuron	predicted[c]	observed	Pr > |t|[d]	pH[e]
Na-bentazon	87.1 (3.9)	42.8 (1.5)	37.3 (2.6)	67.3 (3.1)	<0.0001	4.0
NH_4-bentazon	94.8 (5.6)	44.5 (2.9)	42.2 (4.9)	43.9 (3.2)	0.8045	4.0
H-bentazon	108.7 (2.9)	42.8 (1.5)	46.6 (3.1)	49.2 (1.8)	0.5191	2.6
NH_4-bentazon + SS[f]	90.6 (2.6)	52.2 (2.3)	47.3 (3.6)	57.1 (3.2)	0.0659	4.0
NH_4-bentazon + SA	91.1 (3.2)	51.07 (2.7)	46.5 (3.2)	90.8 (4.3)	<0.0001	7.1

[a] Plants were treated at the second trifoliate stage with H_2O as the control, Na^+, NH_4^+, or H^+ derivatives of bentazon at 442.5 g of ai ha^{-1}, respectively, tritosulfuron at 10 g of ai ha^{-1}, or a mixture of SU and bentazon salts. DASH HC at 0.156% (v/v) was added to all treatments. Data were taken 14 days after treatment. [b] Data are shoot dry weights (DW) expressed as a percentage of the mean of H_2O-treated control plants. Standard errors of the mean are in parentheses. [c] Colby's (1967) predicted effects of the mixtures, generated with SAS, PROC NLMIXED. [d] Probability of t values = 0; t tests were performed to H_o: observed – predicted = 0. [e] pH of the mixture. [f] Sodium salts were added to H_2O, NH_4-bentazon, tritosulfuron, and a mixture of NH_4-bentazon + tritosulfuron.

one of the major causes of antagonism. Nonetheless, the presence of bentazon is required for the antagonism to occur because neither SA nor SS antagonized tritosulfuron applied alone (**Table 4**). Whenever acetic salts were added, either as AMA or as SA, the result was antagonism, whereas the addition of sulfate salts, AMS or SS, resulted in little or no antagonism, suggesting the role of the accompanying cation is as important as the role of the ammonium in overcoming the antagonism. It is likely that the sulfate anion (and other inorganic anions) acts as a sodium "scavenger", thus preventing the interaction of sodium with tritosulfuron while the droplets were drying on the leaf surface. Conversely, when acetate or ammonium was presented in the mixture, they volatilized (data not shown) as ammonia and acetic acid, thus leaving tritosulfuron to interact with Na^+.

Similar to Na-bentazon + tritosulfuron antagonism, sethoxydim antagonim could also be eliminated by replacing Na-bentazon with NH_4-bentazon or H-bentazon or by adding inorganic ammonium salts to Na-bentazon−sethoxydim mixtures (*4*, *14*). The addition of ammonium (as an inorganic salt) to a mixture of Na-bentazon−sethoxydim prevented the sodium from interacting with the graminicide. Furthermore, NH_4^+ associates with sethoxydim in a manner that is dissimilar to that observed with Na^+ and does not impair sethoxydim cuticular permeability (*10*, *14*, *20*).

Uptake and Translocation of [^{14}C]Tritosulfuron. Foliar uptake of [^{14}C]tritosulfuron applied alone (with DASH HC 0.156%) was relatively rapid, with 97% of [^{14}C]herbicide being absorbed 24 HAT (**Table 5**). However, mixing Na-bentazon with tritosulfuron (plus DASH HC 0.156%) reduced the absorption of [^{14}C]tritosulfuron to 23.8 and 42.4% of the total applied [^{14}C]tritosulfuron 24 and 168 HAT, respectively (**Table 5**). Increasing the concentration of DASH HC to 0.625% eliminated the antagonistic effect of Na-bentazon, with >92%

Table 5. Effects of Sodium Bentazon, NH_4-Bentazon, and DASH HC Concentration and the Addition of Ammonium Sulfate on the Foliar Uptake and Translocation of [^{14}C]Tritosulfuron[a]

	uptake, % of applied[b]			translocation out of TL, % recovery in planta		
treatment[c]	24 h	48 h	168 h	24 h	48 h	168 h
tritosulfuron	97.0 (0.4)	96.6 (0.5)	98.1 (0.1)	20.6 (1.4)	25.9 (1.7)	30.8 (2.2)
tritosulfuron + Na-bentazon	23.8 (1.1)	33.9 (0.6)	42.4 (1.2)	4.6 (0.4)	4.9 (1.5)	4.4 (0.6)
tritosulfuron + DASH HC 0.625%	92.7 (0.6)	94.7 (0.4)	98.0 (0.2)	13.5 (1.6)	24.9 (1.4)	27.6 (1.2)
tritosulfuron + Na-bentazon + DASH HC 0.625%	92.6 (0.4)	93.2 (0.3)	96.7 (0.3)	3.5 (0.6)	5.6 (1.1)	6.6 (0.7)
tritosulfuron + NH_4-bentazon	90.0 (1.4)	90.0 (1.4)	96.7 (0.8)	6.1 (1.2)	9.6 (1.6)	7.3 (1.0)
tritosulfuron + AMS 0.212 N	93.9 (0.4)	93.3 (0.6)	95.8 (0.7)	12.6 (0.5)	19.9 (1.4)	25.5 (1.7)
tritosulfuron + Na-bentazon + AMS 0.212 N	94.0 (0.9)	93.5 (0.7)	98.4 (0.4)	4.4 (0.8)	5.9 (1.3)	4.9 (0.6)
LSD ($\alpha = 0.05$)[d]	2.7	2.1	2.1	3.4	4.7	4.2

[a] Data were taken 14 days after treatment. [b] 890 Bq of [^{14}C]tritosulfuron was applied to the first trifoliate leaf. The concentrations of tritosulfuron, bentazon, and DASH HC were 0.112 mM, 9.2 mM, and 0.156%, respectively, unless other concentrations of DASH HC are mentioned in the table. [c] Standard errors of the means are in parentheses. [d] Least significant difference, values were generated by Duncan's multiple range test. The type I error was set at 0.05.

of [^{14}C]tritosulfuron being absorbed 24 and 168 HAT, respectively (**Table 5**). Similarly, AMS (0.212 N) added to the mixture containing 0.156% DASH HC eliminated the negative effect of Na-bentazon, with >92% of the [^{14}C]tritosulfuron being absorbed 24 HAT (**Table 5**). Furthermore, replacing Na-bentazon with NH_4-bentazon also eliminated the antagonism, with 90% of [^{14}C]tritosulfuron being absorbed 24 HAT (**Table 5**).

At 24 and 168 HAT of the trifoliate leaves, white bean exported 20.6 and 30.8%, respectively, of the total ^{14}C recovered in planta, when [^{14}C]tritosulfuron was applied alone (DASH HC 0.156%) (**Table 5**). Similar to the uptake study, addition of Na-bentazon caused severe reduction of translocation, with only 4.4 and 4.6% of the ^{14}C recovered in planta being exported out of TL 24 and 168 HAT, respectively (**Table 5**). Furthermore, unlike in the uptake experiment, increasing DASH HC to 0.625%, adding AMS, or replacing Na-bentazon with NH_4-bentazon did not improve the translocation of [^{14}C]tritosulfuron (**Table 5**). The reduction in translocation of tritosulfuron 24 HAT is probably due to bentazon's negative impact on photosynthesis as was clearly shown by Retzlaff et al. (*21*). However, because bentazon is rapidly detoxified in white bean and has little or no effect on photosynthesis 24 HAT, it is unlikely that the reduction in translocation 168 HAT is due to prolonged, direct effects on photosynthesis. In this case, we speculate that bentazon may have a negative and long-lasting effect on membrane-bound ATPases, thereby reducing symplastic loading of tritosulfuron into the phloem.

These results clearly indicate that Na-bentazon antagonized both the uptake (chemical mechanism) and the translocation of tritosulfuron (biochemical mechanism). Furthermore, because the addition of AMS, increasing DASH HC concentration, and replacement of Na-bentazon with NH_4-bentazon all resulted in a dramatic improvement in the uptake of [^{14}C]tritosulfuron when mixed with bentazon, but had no effect on ^{14}C translocation, it can be concluded that Na^+ from Na-bentazon antagonizes tritosulfuron uptake, but not the translocation. Therefore, although bentazon is involved in both chemical (uptake effect) and biochemical (translocation effect) interactions that reduced the uptake and translocation of tritosulfuron, respectively, the two mechanisms are separate.

Scanning Electron Microscopy. The leaf surface topography of the adaxial side of white bean leaves consisted of raised (periclinical cell walls) and depressed areas (above anticlinical cell walls), with epicuticular wax deposits visible on the surface (**Figure 2A**). The surface also was characterized by trichomes at low density and the presence of stomata. Nevertheless, the addition of DASH HC (0.156%) alone reduced the size of epicuticular wax deposits (**Figure 2I**), whereas some crystalline deposits (probably of DASH HC salts) were evident on the leaf surface. When Na-bentazon was applied with DASH HC, it formed grain-like deposits with a rough surface that covered the periclinical areas, and an amorphous deposit above the anticlinical areas of the leaf (**Figure 2B**). Tritosulfuron (+DASH HC) had a pattern of crystallization similar to that of DASH HC applied alone, with smaller and denser epicuticular wax structures and crystals in the shape of beads in a chain that were scattered on the leaf surface (**Figure 2C**). This pattern of crystal dispersion may be a result of the low concentration of tritosulfuron in the application solution (0.1 mM). The deposits of Na-bentazon plus tritosulfuron (+DASH HC) had a rough surface and grain-like crystals that covered both the raised and the depressed areas of the leaf, which were even rougher with larger grains than Na-bentazon applied without tritosulfuron (**Figure 2B**,**D**). The full coverage with Na-bentazon was likely due to the high concentration of the herbicide in the solution (10.8 mM), which dispersed over the entire surface of the leaf.

The addition of ammonium sulfate (0.212 N) caused the formation of amorphous deposits that were located mainly in the depressed area of the leaf cuticle above the anticlinical cell walls (**Figure 2**). These amorphous structures were evident in the four treatments with the addition of AMS: DASH HC (0.156%) + AMS, Na-bentazon + DASH HC + AMS, tritosulfuron + DASH HC+ AMS, and Na-bentazon + tritosulfuron + DASH HC + AMS (**Figure 2E**−**H**). Amorphous deposits were also detected in leaves treated with NH_4-bentazon alone or in a mixture with tritosulfuron (+DASH HC) (**Figure 2J**,**K**).

It seems that in the case of the Na-bentazon plus tritosulfuron mixture, the presence of Na^+ caused crystalline deposits to form. Conversely, the addition of AMS or the replacement of Na-bentazon with NH_4-bentazon in the mixture resulted in amorphous deposits, which may allow more rapid diffusion of tritosulfuron into the cuticle.

Conclusions. In our previous research on the auxinic herbicides, we showed that ATPase activity can be correlated with light scattering changes (*22*, *23*). Using these methods, we found that bentazon also inhibited ATPase activity, in a dose-dependent manner, in protoplasts of several different plant species (data not shown). In other experiments with white bean plants, we found that when Na-bentazon was replaced with orthovanadate, a known ATPase inhibitor, the inhibitor caused the same pattern of antagonism to the bioactivity of tritosulfuon as did Na-bentazon (data not shown). These results support those of Couderchet and Retzlaff (*24*), who suggested that the inhibitory effect of bentazon on the activity of plasma membrane

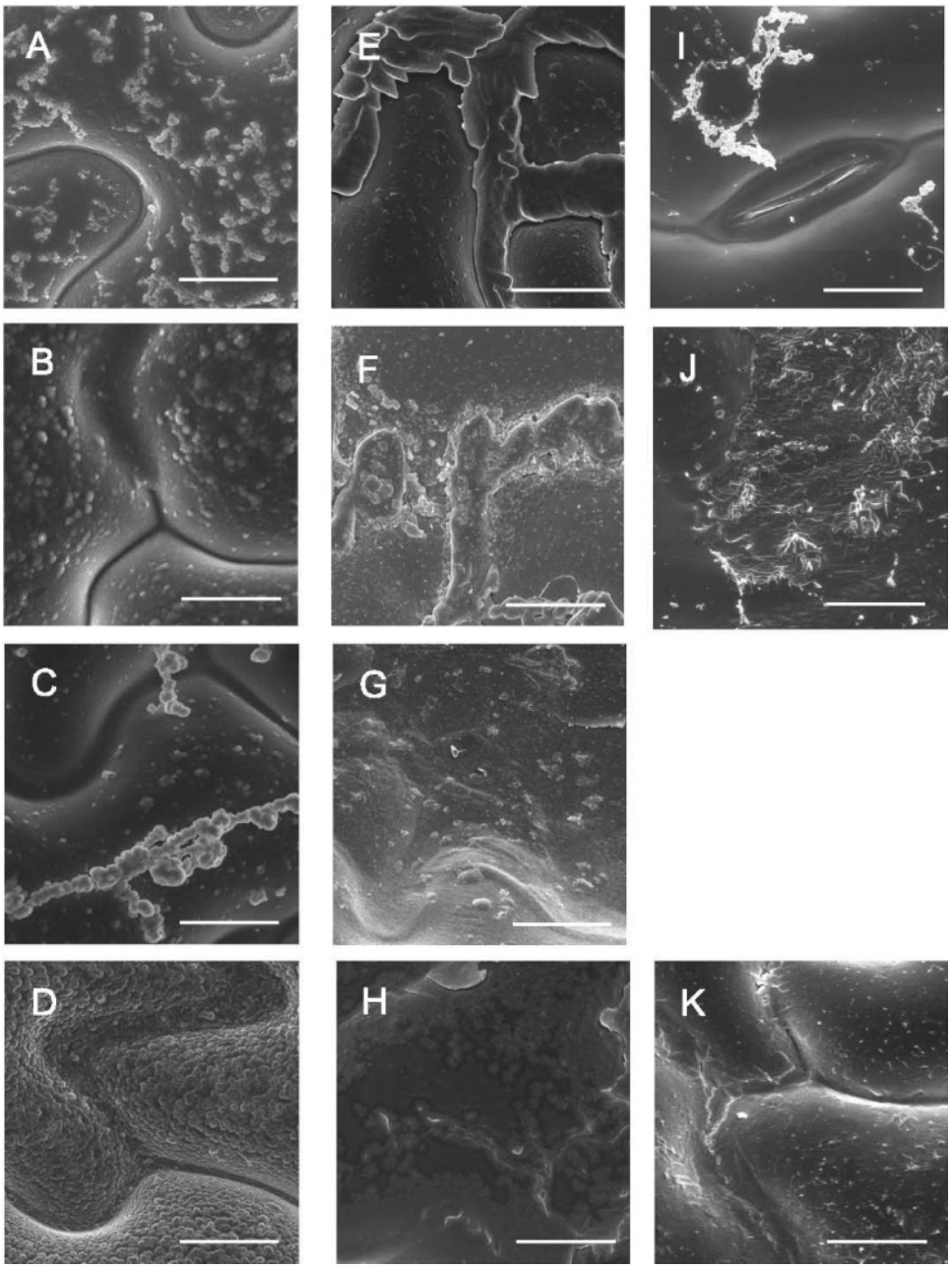

Figure 2. Cryo-SEM images following the deposition of the following treatments: (**A**) nontreated leaf; (**B**) Na-bentazon + DASH HC; (**C**) tritosulfuron + DASH HC; (**D**) Na-bentazon + tritosulfuron + DASH HC; (**E**) ammonium sulfate 0.212 N + DASH HC; (**F**) Na-bentazon + DASH HC + ammonium sulfate 0.212 N; (**G**) tritosulfuron + DASH HC + ammonium sulfate; (**H**) Na-bentazon + tritosulfuron + DASH HC + ammonium sulfate; (**I**) ddH_2O + DASH HC; (**J**) NH_4-bentazon + DASH HC; (**K**) NH_4-bentazon + tritosulfuron + DASH HC. Herbicide doses were bentazon, 442.5 g of ai ha^{-1}, tritosulfuron, 10 g of ai ha^{-1}, and DASH HC, 0.156% (v/v). Bars = 30 μm.

H^+-ATPase explains the role of bentazon antagonism on the translocation of weak acid herbicides (biochemical antagonism). Due to the inhibition of the activity of H^+-ATPase by bentazon, they hypothesize that proton extrusion decreases and the apoplastic pH increases. As a result, the proportion of undissociated weak-acid herbicide molecules in the apoplast is reduced and fewer herbicide molecules accumulate in the symplast (*21*, *25*). Furthermore, because bentazon interferes with photosynthesis and hence production of ATP and NADPH, fewer assimilates are loaded into the phloem; thus, less foliar-applied weak-acid herbicide, when mixed with bentazon, is translocated via the phloem out of a treated leaf. Therefore, it is likely that these two biochemical effects (i.e., plasmalemma ATPase and photosynthesis effects) explain the reduction in translocation of tritosulfuron. Furthermore, these effects cannot be easily ameliorated. Conversely, the addition of DASH HC or some ammonium salts (i.e., AMS, AMP, AMN) or the change of Na-bentazon to NH_4-bentazon can significantly reduce or eliminate the antagonism by Na-bentazon on the absorption of weak-acid herbicides. Consequently, we agree with the conclusions of Thelen et al. (*20*) that the basis for the Na-bentazon antagonism on the absorption of weak acids is chemical; that is, Na^+ from Na-bentazon interacts chemically with weak-acid herbicides, forming a complex that is absorbed poorly. Therefore, although the antagonism by bentazon on the absorption of tritosulfuron can be effectively dealt with in the field, it is unlikely that bentazon's antagonism of tritosulfuons's translocation, or that of any weak-acid herbicide, can be easily and practically overcome.

ABBREVIATIONS USED

AMA, ammonium acetate; AMN, ammonium nitrate; AMP, ammonium phosphate dibasic; AMS, ammonium sulfate; ANOVA, analysis of variance; AR, antagonism ratio; DAT, days after treatment; HAT, hours after treatment; RCBD, randomized complete block design; SA, sodium acetate; SEM, scanning electron microscopy; SS, sodium sulfate; SU, sulfonylurea.

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