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# Effect of Ammonium Sulfate on the Phytotoxicity, Foliar Uptake, and Translocation of Imazamethabenz in Wild Oat

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**Abstract.** Experiments were conducted in greenhouse, growth chamber, and laboratory conditions to determine the effect of ammonium sulfate [$(NH_4)_2SO_4$] on the phytotoxicity, foliar uptake, and translocation of imazamethabenz on wild oat. Rates of $(NH_4)_2SO_4$ up to 5% (w/v) applied with a greenhouse sprayer did not affect the phytotoxicity of the herbicide when the mix was applied at the one- to two-leaf stage. However, inclusion of 1 and 2% $(NH_4)_2SO_4$ increased the phytotoxicity of the herbicide when the mix was sprayed at the two- to three-leaf, or the three- to four-leaf stage. At 10%, $(NH_4)_2SO_4$ decreased the phytotoxicity of the sublethal dosage of the herbicide. When the herbicide was applied as individual drops to the growth chamber-grown plants, inclusion of $(NH_4)_2SO_4$ at 1% did not affect phytotoxicity as measured by shoot growth. The presence of $(NH_4)_2SO_4$ did not affect the amount of imazamethabenz retained by wild oat foliage, but it decreased [$^{14}$C]imazamethabenz absorption, slightly antagonized acropetal translocation, and increased the basipetal translocation of [$^{14}$C]imazamethabenz. It was concluded that application methods greatly modify the effect of $(NH_4)_2SO_4$ on imazamethabenz phytotoxicity. Herbicide absorption and translocation as determined by one method do not necessarily represent the absorption and translocation patterns when different application methods are used. Absorption and translocation were not the factors that were responsible for the observed effect of $(NH_4)_2SO_4$ on the herbicide phytotoxicity.

**Key Words.** Herbicide adjuvant—Additive—Application method—Growth stage—Weed control

**Abbreviations:** SC; suspension concentrate
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The methyl ester of imazamethabenz, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2) (hereafter referred to as imazamethabenz) selectively controls wild oat (*Avena fatua*) in spring wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum*), and barley (*Hordeum vulgare*) (Kneeshaw et al. 1983, Pillmoor and Caseley 1987). Tank mixing with MCPA ester, (4-chloro-2-methyl-phenoxy)acetic acid, extends the spectrum of weed control without adversely affecting imazamethabenz phytotoxicity to wild oat, but the addition of MCPA amine or fenoxaprop, (±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid, antagonizes wild oat control (Liu et al. 1995a). The addition of adjuvants also affects the phytotoxicity of imazamethabenz. The nature of the effect of sodium bisulfate on imazamethabenz phytotoxicity to wild oat has already been reported (Liu et al. 1995b).

Ammonium sulfate can increase the phytotoxicity of many herbicides (Hatzios and Penner 1985). Ammonium sulfate consistently mediated increases in cyclohexanedione herbicide phytotoxicity (Harker 1995). Control of yellow nutsedge (*Cyperus rotundus*) with glyphosate, *N*-phosphonomethyl)glycine, at 0.2 kg a.i. ha$^{-1}$ plus $(NH_4)_2SO_4$ was as effective as glyphosate alone at 0.8 kg a.i. ha$^{-1}$ (Suwunnamek and Parker 1975). Ammonium sulfate is reported to increase phytotoxicity of fenoxaprop-ethyl (Blackshaw 1989), and sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one (Jordan and York 1989, Smith and Vanden Born 1992). However, $(NH_4)_2SO_4$ did not consistently affect control of grassy weeds by quizalofop, (±)-2-[4(6-chloro-2-quinoxalinyl)phenoxy]propanoic acid (Beckett et al. 1992).

We have reported that $(NH_4)_2SO_4$ increases wild oat control with imazamethabenz (Liu et al. 1992). The mechanism of the effect, the dose relationship of

$(NH_4)_2SO_4$ to the herbicide, and the growth stage response were not studied. Ammonium sulfate is reported to increase herbicide uptake but to have little effect on translocation (Costa and Appleby 1986, Jordan et al. 1989, Kent et al. 1991, Suwunnamek and Parker 1975, Turner and Loader 1980, 1984). Ammonium sulfate increased sethoxydim translocation in wild oat and barley (Smith and Vanden Born 1992) but did not affect the absorption of quizalofop in volunteer corn (*Zea mays*) (Beckett et al. 1992).

This paper reports the effects of $(NH_4)_2SO_4$ on the phytotoxicity, foliar uptake, and translocation of imazamethabenz in wild oat.

## Materials and Methods

Wild oat seeds were obtained from the Agriculture and Agri-Food Canada Regina Station in Regina, Canada. The suspension concentrate (SC) formulation of imazamethabenz, containing 300 g a.i. liter$^{-1}$, was provided by American Cyanamid Inc. (Princeton, NJ). Ammonium sulfate was a 49% (w/v) solution supplied as a companion pack for sethoxydim (BASF, Calgary, Alberta, Canada).

### *Phytotoxicity Experiments*

Afterripened wild oat seeds were germinated and planted at 1.5-cm depth in a loamy soil contained in 1-liter waxed-paper pots. Pots were placed in a greenhouse and were watered daily with tap water. Supplementary illumination supplied a 16-h photoperiod with a photosynthetic photon flux density at the canopy of not less than 400 $\mu$E m$^{-2}$ s$^{-1}$. Seedlings were thinned from six to three/pot 1 week after emergence. At the one-leaf stage, 50 mL of a water-soluble N:P:K (20:20:20) fertilizer at a concentration of 3 g of fertilizer/liter of tap water was supplied to each pot. Greenhouse temperature varied from 18 to 30°C during the day and 14 to 22°C at night.

Plants were sprayed with a moving-nozzle sprayer, calibrated to deliver 100 liters ha$^{-1}$ at 210 kPa and equipped with a flat fan nozzle tip (730039). Before herbicide treatment, the soil surface was covered with a layer of coarse vermiculite to prevent soil interception of the herbicide and subsequent root absorption. The vermiculite was removed after spraying, and the pots were arranged in the greenhouse in a randomized design. Three weeks after herbicide application, shoot fresh weight/pot was determined. The treatments were replicated seven or eight times.

### *Experiments on Uptake and Translocation*

Three germinated wild oat seeds were planted 2 cm deep in individual silica sand-filled styrofoam cups (6.7-cm diameter; 8 cm deep with drainage holes). On the initial day and every 2nd day the sand was saturated with 30 mL of one-half strength Hoagland's solution, with distilled water added on alternate days. Growth chamber incubation was at a 20/15°C day/night temperature cycle, 16-h day length, 50% relative humidity, and a light intensity of 450 $\mu$E m$^{-2}$ s$^{-1}$. Three days after emergence, plants were thinned to two uniform plants/cup. When they reached the one-leaf stage, they were thinned to one/cup. Treatments were applied at the three-leaf stage.

[$^{14}$C]Imazamethabenz (labeled in the 4 position carbon of the imidazolin ring, specific activity of 1.47 × 10$^3$ kBq mg$^{-1}$) was obtained from American Cyanamid Inc. It had radiochemical purity >98% (89.95% methyl ester of imazamethabenz and 8.18% imazamethabenz acid) as determined by a two-dimensional thin layer chromatography [solvent system 1 of $CHCl_3$:MeOH:HAc (20:1:1, v/v) and system 2 of benzene:acetone:HAc (75:25:5, v/v)]. Solid [$^{14}$C]imazamethabenz was dissolved in 10 mL of methanol, and the radioactivity in this stock solution was determined. An aliquot containing 105 kBq was evaporated to dryness and redissolved in 0.5 mL of commercially formulated imazamethabenz, diluted with distilled water. Ammonium sulfate at 1% (w/v) was added where appropriate. Quantification by liquid scintillation spectrometry (Tri-Carb 1900 TR, Canberra Packard Canada, Mississauga, Ontario, Canada) confirmed the activity of solutions. The final imazamethabenz concentration was 10.5 mM.

Two 2-$\mu$L droplets containing 850 Bq of radioactivity were applied to the midpoint of the upper leaf surface of the second true leaf on either side of the midvein. The amount of [$^{14}$C]imazamethabenz applied per plant was checked periodically during treatment by depositing two droplets into vials for quantification. After treatment of plants, the sand was kept moist but not leached.

Plants were harvested 24, 48, and 96 h after treatment by dividing each into the following four components: the tip of the treated leaf, the treated region of the treated leaf, the remainder of the shoot, and roots. Unabsorbed [$^{14}$C]imazamethabenz was washed from the freshly harvested treated leaves by rinsing the treated region three times under a light stream of 10 mL of Tween 20 solution at 0.1% (v/v). Ten mL of Scinti-Verse I (Fisher Scientific Company, Fairlawn, NJ) scintillation liquid was added to each 10-mL wash solution, and radioactivity was quantified. The sum of the radioactivity from these three washes was considered to constitute unabsorbed herbicide. Plant sections were cut into strips and oxidized (Biological Oxidizer model OX 500, R. J. Harvey Instrument Corp., Hillsdale, NJ). Roots were washed under a stream of water and then air-dried and oxidized. The trapped $^{14}CO_2$ was measured. Recoveries of $^{14}$C as $^{14}CO_2$ from plant tissues fortified with $^{14}$C standards were greater than 98%. In addition, microscopic examination of herbicide residue on the leaf surface was carried out 96 h after application of the various treatments. Each treatment had six replications.

Data were presented by the following parameters.

*Absorption.* The absorbed radioactivity was total recovered activity less the activity in the leaf washes expressed as a percentage of the applied activity.

*Acropetal Translocation.* The radioactivity recovered in the tip of treated leaf represents acropetal translocation, expressed as a percentage of absorbed activity.

*Basipetal Translocation.* Basipetal translocation was the sum of the counts in the remaining shoot and root components, expressed as a percentage of absorbed activity.

### *Statistical Analysis*

A completely randomized design was used. The phytotoxicity experiments were repeated once, and the radioactive uptake and translocation experiments were repeated twice. The repeated experiments were subjected to the homogeneity tests, and data were pooled where permis-

**Table 1.** Parameters calculated from the logistic model and relative potencies of effect of ammonium sulfate on imazamethabenz efficacy in wild oats. Greenhouse-grown wild oat plants at the two- to three- leaf stage were sprayed. Values show means and 95% confidence intervals. The terms D, C, b, and $ED_{50}$ are defined in the Materials and Methods section.

| D | C | b | $ED_{50}$ |
|---|---|---|---|
| (g $pot^{-1}$) | (g $pot^{-1}$) | | (g $ha^{-1}$) |
| 13.17 | 0.29 | 2.58 | 159.21 |
| (11.78 to 14.56) | (−0.46 to 1.05) | (1.73 to 3.42) | (133.36 to 185.07) |

| Treatment | Relative potency |
|---|---|
| Imazamethabenz alone | 1.00 |
| Imazamethabenz plus ammonium sulfate | 1.46 (1.23 to 1.68) |
| Test for lack of fit: $F_{,19,63} = 0.86$ | |
| Test for assumption of parallel dose-response curves: $F_{,1,82} = 2.42$ | |

sible. If the repeated experiments were not homogeneous, then each run for an experiment was reported individually. Data from the herbicide dose-response experiments were analyzed with a logistic regression model of wild oat fresh weight (g $pot^{-1}$) ($Y$) on dose ($z$) of imazamethabenz,

$$Y_{ij} = C + \frac{D-C}{1+\exp[b_i(\log(z_j) - \log(ED_{50i})]} \qquad (1)$$

was fitted simultaneously to the dose-response data with imazamethabenz alone ($i$ = 1) and those with imazamethabenz plus $(NH_4)_2SO_4$ ($i$ = 2), using nonlinear regression procedures of the Statistical Analysis System (SAS Institute Inc., Cary NC). The term $z_j$ denotes the $j^{th}$ dose of imazamethabenz. The upper limit, $D$, at zero dose and the lower limit, $C$, at large doses were assumed to be similar for the response curves within an experiment because $(NH_4)_2SO_4$ alone had no effect upon the shoot fresh weight. The term $ED_{50i}$ is the dose required to reduce fresh weight 50% between the upper and the lower limit; $b_i$ denotes the slope around the $ED_{50i}$. Because of the rather large ratio $Y_{max}/Y_{min}$, a transform both sides method was used to stabilize the variance (Rudemo et al. 1989).

The goodness of the sigmoid dose-response curve was tested by comparing the residual sum of squares of an analysis of variance and a nonlinear regression assuming nonparallel dose-response curves. If the model was not rejected by this goodness test, the assumption of parallel dose-response curves was tested by comparing the residual sum of squares of nonlinear regressions assuming nonparallel and parallel dose-response curves. If the assumption was not rejected, then we re-ran the analysis by assuming the curves were similar apart from a horizontal displacement determined by the relative potency $r$ parameter,

$$Y_{ij} = C + \frac{D-C}{1+\exp[b(\log(r_1 z_j) - \log(ED_{50i})]} \qquad (2)$$

If the relative potency $r$ was significantly different from 1.00, the addition of $(NH_4)_2SO_4$ had no effect on the imazamethabenz responses. If $r$ was smaller or bigger than 1.00, the mixture with imazamethabenz and $(NH_4)_2SO_4$ was less or more potent than the imazamethabenz applied alone. Models 1 and 2 have been used on several occasions to describe herbicide dose-response curves (Kudsk et al. 1987, Liu et al. 1994).

The data from the radioactivity studies were subjected to analysis of variance. Fisher's protected LSD at the 5% level of probability was used for comparison of treatment means.

## Results and Discussion

### *Phytotoxicity Experiments*

The summary of the regression analysis showed that the relative potency value ($r$ = 1.46) was significantly different from 1, indicating a significant increase in imazamethabenz efficacy when $(NH_4)_2SO_4$ was added to spray solution (Table 1 and Fig. 1). The efficacy of 1 kg $ha^{-1}$ imazamethabenz plus $(NH_4)_2SO_4$ equals the efficacy of 1.46 kg $ha^{-1}$ imazamethabenz alone. This supported our previous work (Liu et al. 1992), which showed that the addition of 1% $(NH_4)_2SO_4$ increased the phytotoxicity of the imazamethabenz in wild oat control.

In experiments using standard spraying techniques, the effect of $(NH_4)_2SO_4$ addition on imazamethabenz phytotoxicity varied with the plant growth stage and with the concentrations of the herbicide and $(NH_4)_2SO_4$ used. At low herbicide levels of 0.05 and 0.1 kg $ha^{-1}$, the addition to the mix of $(NH_4)_2SO_4$ at any of the concentrations used did not alter imazamethabenz phytotoxicity at any leaf stages (Figs. 2–4). In contrast, wild oat control by imazamethabenz at 0.2 kg $ha^{-1}$ was influenced by both growth stage and $(NH_4)_2SO_4$ concentration. The addition of 1–2% $(NH_4)_2SO_4$ had no effect on imazamethabenz phytotoxicity when the mix was applied to plants at the one- to two-leaf stage (Fig. 2); but at both the two- to three-leaf stage (Fig. 3) and the three- to four-leaf stage (Fig. 4), a similar addition of $(NH_4)_2SO_4$ increased the herbicide phytotoxicity. Increasing the $(NH_4)_2SO_4$ concentration to 10% antagonized the efficacy of 0.2 kg $ha^{-1}$ imazamethabenz (Fig. 3).

The finding that low rates of $(NH_4)_2SO_4$ tend to enhance and high rates of $(NH_4)_2SO_4$ decrease the activity of imazamethabenz may apply to other herbicides. Wilson and Nishimoto (1975) found that picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) activity tended to increase with an increasing $(NH_4)_2SO_4$ concentration up to 10% (w/v), but by the end of each experiment, $(NH_4)_2SO_4$ concentrations above 0.5% were generally equally effective. Ammonium sulfate concentrations from about 1 to 10% had approximately similar

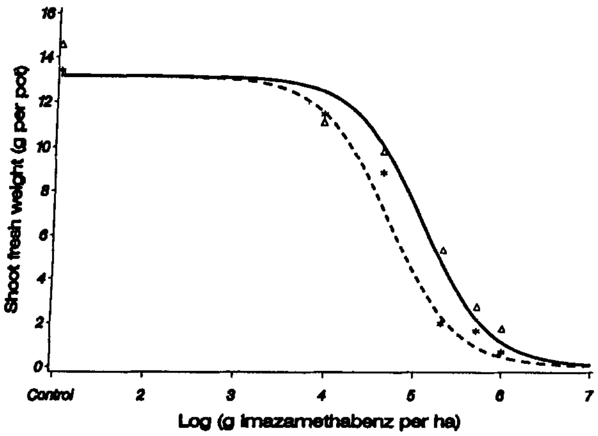


**Fig. 1.** Dose-response curve of wild oat shoot fresh weight on imazamethabenz alone (——, △) and imazamethabenz plus ammonium sulfate (– – –, *). Greenhouse-grown wild oat plants at the two- to three-leaf stage were sprayed. Data from two experiments were homogeneous.

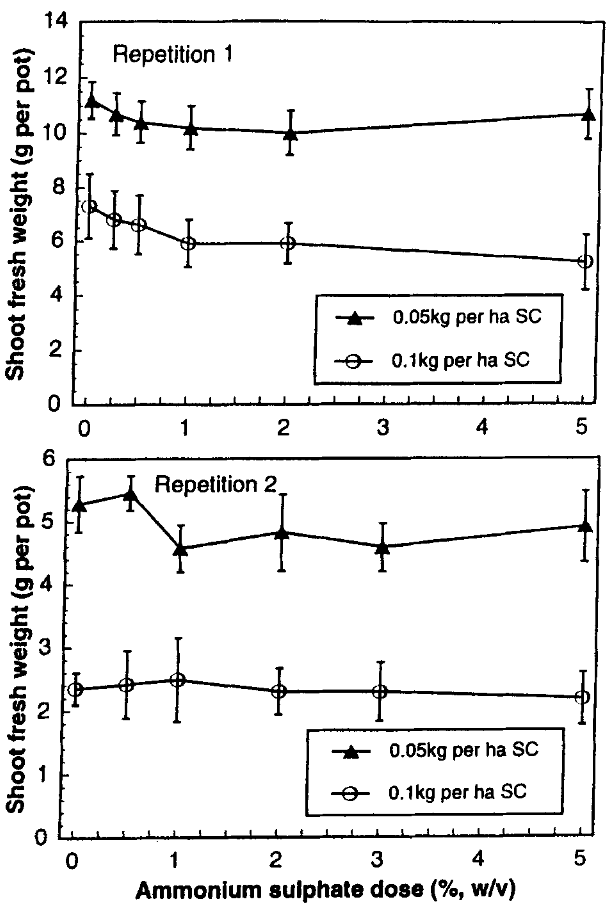


**Fig. 2.** Effect of different concentrations of ammonium sulfate on the phytotoxicity of imazamethabenz (SC) on shoot fresh weight of wild oat treated at the one- to two-leaf stage. *Vertical bars* represent standard errors. Data from two repeated experiments were not homogeneous.

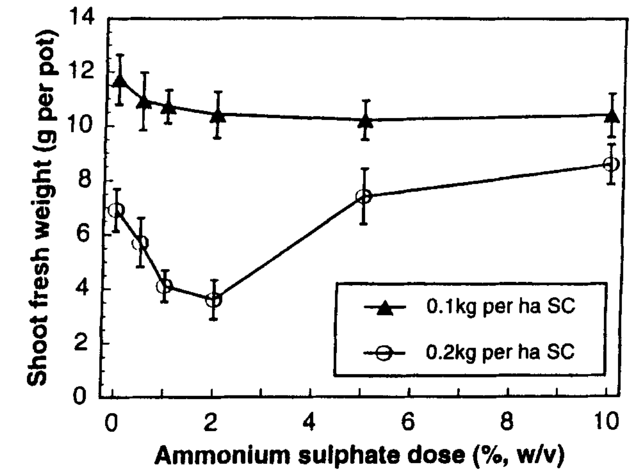


**Fig. 3.** Effect of different concentrations of ammonium sulfate on the phytotoxicity of imazamethabenz (SC) on shoot fresh weight of wild oat treated at the two- to three-leaf stage. *Vertical bars* represent standard errors. Data from two repeated experiments were pooled.

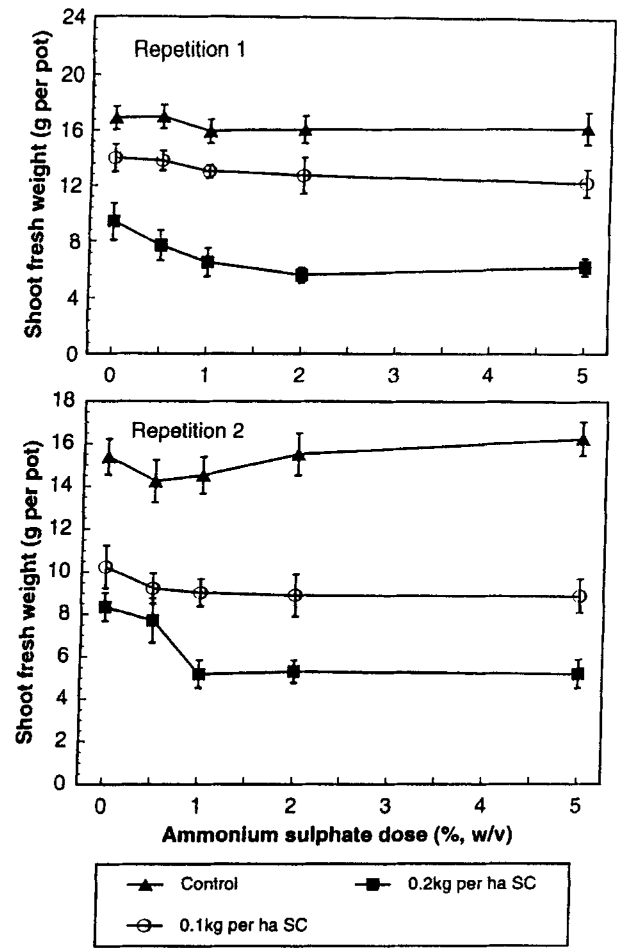


**Fig. 4.** Effect of different concentrations of ammonium sulfate on the phytotoxicity of imazamethabenz (SC) on shoot fresh weight of wild oat treeated at the three- to four-leaf stage with one to two tillers. *Vertical bars* represent standard errors. Data from two repeated experiments were not homogeneous.

enhancing effects on the phytotoxicity of the low doses of glyphosate plus surfactant Agral 90, 90% nonylphenoxyethanol. Concentrations of 20% $(NH_4)_2SO_4$ and above were antagonistic (Turner and Loader 1980).

The reduced sensitivity to imazamethabenz of wild oat plants at later growth stages is well established (Harker and O'Sullivan 1991, Hedlund and Anderson 1987). Applied at a later growth stage, the addition of a higher rate of $(NH_4)_2SO_4$ appeared to increase herbicide phytotoxicity. Thus, $(NH_4)_2SO_4$ at 5% had no effect on the phytotoxicity of imazamethabenz at the two- to three-leaf stage (Fig. 3) but improved the efficacy of the 0.2 kg a.i. $ha^{-1}$ imazamethabenz when applied at the three- to four-leaf stage (Fig. 4). This suggests a strategy for weed control at a wide variety of leaf stages.

### *Experiments on Uptake and Translocation*

The addition of $(NH_4)_2SO_4$ significantly decreased the absorption of [$^{14}$C]imazamethabenz by wild oat at the three-leaf stage at all time intervals tested (Fig. 5*A*). It slightly decreased acropetal translocation of [$^{14}$C]imazamethabenz at 96 h after application (Fig. 5*B*). However, $(NH_4)_2SO_4$ increased the basipetal translocation of [$^{14}$C]imazamethabenz into the remaining shoot and root portions (Fig. 5*C*).

For several herbicides $(NH_4)_2SO_4$ addition has tended to increase uptake although some inconsistencies occur (Beckett et al. 1992, Jordan et al. 1989, Kent et al. 1991, Smith and Vanden Born 1992, Wilson and Nishimoto 1975). None of these studies suggested that in some cases $(NH_4)_2SO_4$ decreased uptake of herbicides, as indicated in this report. Our use of a suspension concentrate formulation of imazamethabenz, which is not readily water soluble, may have contributed to our results. The addition of $(NH_4)_2SO_4$ did not clarify the suspension, in contrast to the effect of sodium bisulfate addition (Liu et al. 1992). Much more crystalline deposit, which is characteristic of imazamethabenz presence, was microscopically visible on leaf surfaces treated with imazamethabenz plus $(NH_4)_2SO_4$ than on leaves treated with imazamethabenz alone. This suggested either a decreased imazamethabenz solubility or a shorter drying time of applied drops on the leaf surface. Either or both of these factors could account for decreased absorption of the herbicide.

There are some apparent discrepancies between greenhouse and field results. Differences in drying time of droplets and consequent absorption differences could partially account for such differences. Harker (1995) suggested that ultraviolet degradation of some herbicides and differences in the permeability to herbicides of plant cuticles may also play a role in such differences. Our efficacy experiments were conducted using standard spraying procedures, whereas the uptake and transloca-

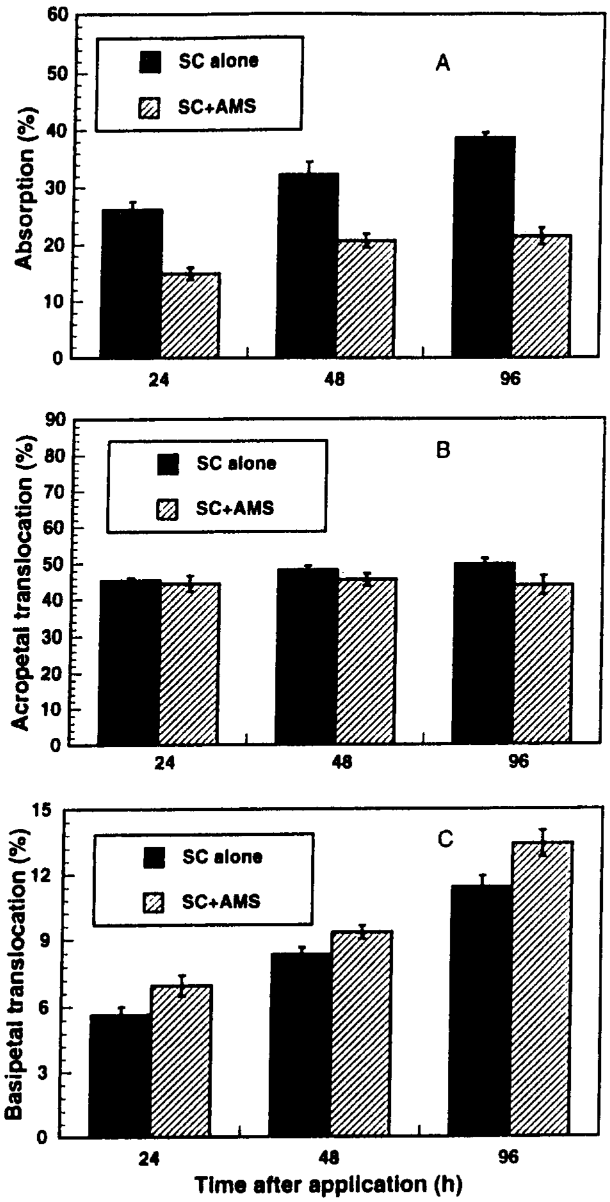


**Fig. 5.** Effect of ammonium sulfate (*AMS*) applied at the three-leaf stage on *A*, foliar uptake (percent of applied); *B*, acropetal translocation (percent of absorbed); and *C* basipetal translocation (percent of absorbed) of [$^{14}$C]imazamethabenz (SC) in wild oats. *Vertical bars* represent standard errors. Data from three repeated experiments were pooled.

tion studies were carried out by applying two relatively large (2 μL) drops of test chemicals to parts of the plant. The latter method appears to be the most commonly used procedure for applying radioactive labeled herbicides. We found only one report in which a spray cabinet was used for such a purpose (Wink et al. 1984). The mode of application, together with the nature of the herbicide used, the dosage employed, leaf morphology, cuticle development, and environmental conditions all potentially play a role affecting the efficacy of a given herbicide in association with $(NH_4)_2SO_4$ (Whitehouse et al. 1982, Xie et al. 1994, 1995). It is also possible that a small positive effect on crop plant development may be due to foliar absorption of ammonium nitrogen. Our results, using the large droplet method, do not indicate an increase in absorption of herbicide when $(NH_4)_2SO_4$ is added (Fig. 5*A*). Where changes in herbicide efficacy are reported they almost invariably describe increased phytotoxicity. Whether the augmentation in phytotoxicity be large or small, the point remains that $(NH_4)_2SO_4$ appears to maintain more stable herbicide performance, which is why it remains a part of agricultural practice. Our results suggest that changes in absorption or translocation are not the cause of the observed changes of enhancement of imazamethabenz phytotoxicity.

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