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Uptake and Phytotoxicity of the Herbicide Metsulfuron Methyl in Corn Root Tissue in the Presence of the Safener 1,8-Naphthalic Anhydride

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ABSTRACT

Growth of *Zea mays* L. cv Potro roots was inhibited by the herbicide metsulfuron methyl (MSM) at the lowest concentration tested: 5 nanomoles per liter. Pretreatment of corn seeds with commercial 1,8-naphthalic anhydride (NA) at 1% (w/w) partially reversed MSM-induced root growth inhibition. MSM at a concentration of 52 nanomoles per liter was taken up rapidly by roots and accumulated in the corn tissue to concentrations three times those in the external medium; the safener NA increased MSM uptake up to 48 hours. The protective effect of NA was related to the ability of the safener to increase the metabolism of MSM; tenfold increases in the metabolic rates of MSM were observed in NA-pretreated corn seedlings grown for 48 hours on 52 nanomolar [^{14}C]MSM solution. DNA synthesis determined by measurement of [^{3}H]thymidine incorporation into DNA was inhibited by root MSM applications; after a 6-hour application period, 13 nanomolar MSM solution reduced DNA synthesis by 64%, and the same reduction was also observed with NA-pretreated seedlings. Pretreatment of corn seeds with safener NA did not increase the acetolactate synthase activity in the roots and did not change, up to 13 micromoles per liter, the *in vitro* sensitivity of roots to MSM.

MSM[1] is the active ingredient in Du Pont "Ally"® weed killer. This new sulfonylurea herbicide is active on most vascular plants and is used for weed control in small grains. An important feature of this compound is its high herbicidal activity at extremely low application rates; recommended amounts for weed control in wheat are between 6 and 8 g ai/ha.

[1] Abbreviations: MSM, metsulfuron methyl (methyl 2-[({[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl} amino) sulfonyl] benzoate); ai, active ingredient; ALS, acetolactate synthase, used in the more general sense of acetohydroxyacid synthase; RDP, ribonucleoside diphosphate; NA, 1,8-naphthalic anhydride; EPTC, S-ethyl *N*,*N*-dipropylthiolcarbamate; chlorsulfuron, 2-chloro-*N*-[{(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino} carbonyl] benzene sulfonamide; acetoin, 3-hydroxy-2-butanone; LSC, liquid scintillation counting; TPP, thiamine pyrophosphate chloride; FAD, flavin adenine dinucleotide; IC_{50}, concentration of substance producing 50% inhibition; dichlormid, 2,2-dichloro-*N*,*N*-di-2-propenylacetamide; glyphosate, *N*-(phosphono-methyl)-glycine; PAL, phenylalanine ammonia-lyase; GST, glutathione S-transferase; imazapyr, 2-(4,5-dihydro-4-methyl-4-[1-methylethyl]-5-oxo-1*H*-imidazol-2-yl)-3-pyridinecarboxylic acid.

The mode of action of sulfonylurea herbicides is through the inhibition of ALS (EC 4.1.3.18) which directly catalyzes the first common step in the biosynthesis of the branched chain amino acids, leucine, isoleucine, and valine (7, 9, 16), and indirectly disrupts DNA synthesis (28), possibly via feedback inhibition of RDP reductase (EC 1.17.4.1) (27). The apparent K_i for ALS extracted from various species is 10 to 100 nM (4).

There has been considerable interest in the use of herbicide safeners to enhance crop tolerance to a number of herbicides. The most successful protection reported is on corn and sorghum against thiocarbamate and acetanilide herbicide injury (11). Pretreating corn seeds with the herbicide safener NA significantly reduces injury caused by EPTC (6) and from pre-emergence application of chlorsulfuron (22, 23); more recently, a NA-mediated protection against chlorsulfuron inhibition was shown in sorghum (12) and corn (10, 21). In addition to work with chlorsulfuron, Mersie and Foy (18) have reported the safening of corn against MSM injury by seed dressing with NA.

In spite of the general interest in herbicide safeners, little information exists on the mode of safener action. According to Hatzios (14), safeners may act either as 'bioregulators' influencing the amount of a herbicide that reaches its target site in an active form or as 'antagonists' of herbicidal effects at a common site of action.

Research has been directed at understanding the protective effect of the safener NA. However, while there is acceptance of the phenomenon of chemical protection of corn from chlorsulfuron injury, there is less agreement on the protective mechanisms. Naphthalic acid treatment decreased the chlorsulfuron half-life more than 50% in the experiments of Sweetser (29) but did not increase the rate of chlorsulfuron metabolism in the subsequent experiments of Frear *et al.* (10).

The objectives of the research described in this paper were to examine the degree of protection by NA-pretreatments against MSM injury to corn roots when MSM was applied at different times and to explain the observed difference between unsafened and pretreated seedlings by two ways: the effectiveness of NA relative to the fate of MSM in corn seedlings (absorption, translocation, and metabolism) and the effects of the safener relative to the ALS activity and DNA synthesis. Corn was used as the test plant because it is relatively sensitive to MSM and sulfonylurea herbicide injury to corn is marginally alleviated by NA (13, 18).

MATERIALS AND METHODS

Chemicals

Technical grade MSM (99.2% purity) was a gift from Du Pont Company. It was dissolved in buffer (50 mM phosphate [pH 7.0]) (500 mg/L), and further diluted in 20 mM citrate-phosphate buffer (pH 5.0) to the desired concentration. Acetoin was from Fluka; NA and thymidine were from Aldrich; all other organic chemicals were from Sigma.

Plant Material

Corn (*Zea mays* L. cv Potro) seeds were supplied by Maïsadour (Mont de Marsan, France). They were surface sterilized by a 5-min soaking with gentle stirring in 20% (v/v) commercial bleach (2.5% [w/v] NaOCl), then soaked for 30 s in 95% (v/v) ethanol, and rinsed with distilled water. After surface sterilization, the seeds were sown on moist filter paper in square dishes and grown in darkness at 28° C.

Root Elongation Study

Solution culture experiments were carried out with corn seedlings (48-h stage) in darkness at 28° C. Five seedlings were laid on three 9-cm diameter filter discs placed in a Petri dish containing 8.0 mL of MSM solution in buffer (20 mM citrate-phosphate [pH 5.0]). The MSM concentrations were: 0, 5, 13, 26, and 52 nM. After 1 and 2 d, root lengths were determined, and the results were expressed as the net growth over the initial lengths. The results are expressed as the mean of 18 replicates, half of which were run on separate days.

Absorption and Metabolism of MSM in Roots

MSM ([2-^{14}C]triazine; specific activity, 137 Bq/nmol) was synthesized by Bastide *et al.* (3) and prepared in buffer (50 mM phosphate [pH 7.0]) so that the final herbicide concentrations, when 15 or 75 μL of this solution was added to 480 mL buffer, were 52 or 262 nM.

Metabolism studies with the [^{14}C]MSM were carried out in darkness at 28° C with seedlings which were harvested 66 h after planting. The roots were subsequently blotted dry and five seedlings were laid on one 9-cm diameter filter disc placed in a Petri dish containing 8.0 mL of 52 or 262 nM MSM solution in buffer (20 mM citrate-phosphate [pH 5.0]). The seedlings were removed from the Petri dish at specified time intervals, and the roots were subsequently washed in three batches of 10 mL distilled water, blotted dry, and excised.

The ^{14}C content of the bathing solutions was measured by LSC of 1-mL aliquots removed at $t = 0$ and immediately after the seedlings were harvested.

Approximately 2.6 g (fresh weight) of roots (always 30 individual roots) were weighed into a glass mortar containing 150 mg Fontainebleau's sand. These roots were ground and extracted with cold 80% (v/v) acetone. The extract was centrifuged at 6000*g* for 6 min. The supernatant was concentrated with an evaporator at ambient temperature and a sample (1 mL) was taken for the ^{14}C content determination as described above. MSM absorption was expressed as pmol MSM absorbed/g fresh root tissue.

The solution was acidified with acetic acid and extracted three times with 1.5 mL chloroform. The chloroform layers were combined and dried over sodium sulfate. The remaining solution was filtered and the excess chloroform was removed with an evaporator at ambient temperature. The ^{14}C content was measured in a sample (1 mL) of the organic and aqueous fractions. The concentrations of MSM and MSM metabolites were determined on the concentrated extract which was applied on TLC plastic sheets (0.2-mm silica gel plastic sheets [60 F_{254}], Merck) and developed in dichloromethane:acetonitrile:formic acid (50:50:1, v/v/v). Radioactive spots were measured with a Berthold TLC linear analyzer.

pH Dependence

MSM absorption was measured over a 24-h period in roots placed in 6.1 μM [^{14}C]MSM solutions that ranged from pH 5.0 to 8.0, using a series of citrate-phosphate buffers (20 mM). Total MSM absorption was calculated on the basis of the ^{14}C taken up by the tissue. Measurements of the pH at the end of the experiments indicated that the solution pH did not change by more than 0.7 units during the 24-h uptake period.

DNA Synthesis

The seedlings were harvested 66 h after planting. The roots of ten intact seedlings were blotted dry, immersed in 13 nM MSM for up to 6 h in darkness at 22° C, and placed into vials containing 1.0 mL buffer (20 mM citrate-phosphate [pH 5.0]) with [6-^{3}H]thymidine (specific activity, 0.92 TBq/mmol, 0.4 MBq/mL) to measure DNA synthesis. After 1 h in the isotope solution, the roots were rinsed in tap water for 30 s and soaked in 0.86 mM thymidine for 30 s. The roots were then blotted dry and the apical 1-cm portion of the roots including the tip was excised. Approximately 70 mg (fresh weight) of roots (always 10 individual root tips) were weighed into a glass mortar containing Fontainebleau's sand and cold 80% (v/v) ethanol. Tips were macerated in the glass tissue grinder and poured onto Whatman 3MM filter discs on a vacuum filter apparatus. The filter was washed six times with cold 80% (v/v) ethanol and air dried. The radioactivity remaining on the filter and the radioactivity in the filtrate were measured by LSC.

ALS Extraction and Determination

The method used was similar to described procedures (7, 25) with modification in the preparation of plant extracts. Plant material was homogenized in three volumes (fresh weight basis) of cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM sodium pyruvate, 0.5 mM magnesium chloride, 0.5 mM TPP, and 10 μM FAD, using a cold mortar and pestle. The homogenate was centrifuged at 27,000*g* for 20 min at 4° C. The supernatant fraction was brought to 50% saturation with ammonium sulfate and allowed to stand 2 h at 0 to 4° C. Then the mixture was centrifugated at 17,000*g* for 30 min and the supernatant was discarded. The precipitate was dissolved in cold 50 mM potassium phosphate buffer (pH 7.0) containing 20 mM sodium pyruvate, and 0.5 mM magne-

sium chloride. The dissolved protein ('enzyme solution') was placed on ice and used immediately for the enzyme assay.

The reaction mixture in 1-mL total volume contained 20 mM sodium pyruvate, 0.5 mM magnesium chloride, 0.5 mM TPP, 10 μM FAD, various concentrations of MSM and/or NA, and 0.4 mL enzyme solution in 50 mM potassium phosphate buffer (pH 7.0). MSM was dissolved in buffer (50 mM phosphate [pH 7.0]) (500 mg/L) and diluted as appropriate with 20 mM potassium phosphate buffer (pH 7.0). Incubations were carried out for 60 min in darkness at 30° C and the enzyme reactions terminated by addition of 6 N sulfuric acid (0.1 mL). The acidified reaction mixtures were assayed for acetolactate by decarboxylation at 60° C for 15 min and subsequent measurement of the acetoin formed (17). Total protein was determined by the Bradford method (5). Under these assay conditions, acetolactate formation was directly proportional to the protein concentration. ALS activity is expressed as nmol acetoin/mg protein/h or as percent of the untreated control.

In Vitro Effect of MSM and NA on ALS Activity

One-hundred-fifty corn seeds were germinated on moist filter paper in darkness at 28° C. Sixty-six h after sowing, 140 seedlings were harvested and the roots were separated for assays immediately. This experiment was repeated three times, each with two replicates.

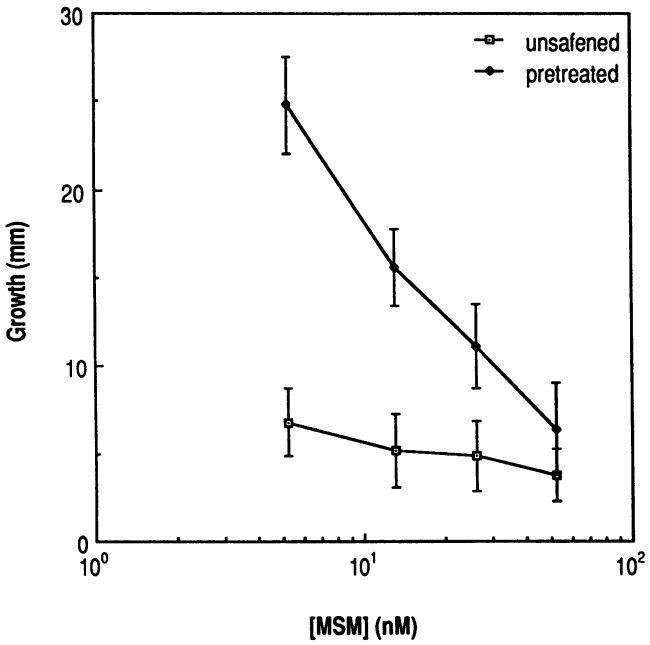

Figure 1. Net growth of roots of corn seedlings grown for 48 h in the presence of MSM in solution culture. The safener NA at 1% (w/w) was applied separately as a seed dressing and the herbicide as a filter paper drench. The zero-MSM concentration values are 38 ± 5 mm for unsafened seedlings and 35 ± 4 mm for pretreated seedlings. Each point is the mean of 18 replicates. The error bars are the standard errors of the means.

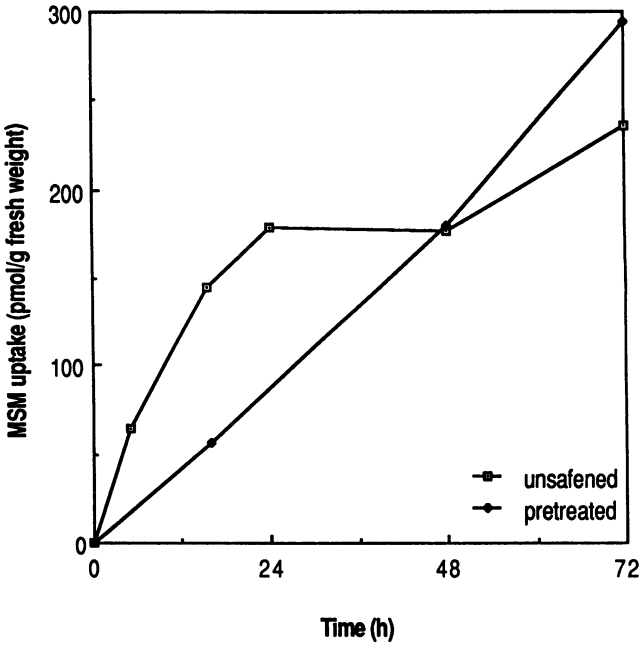

Figure 2. Time course of [^{14}C]MSM uptake in corn root tissue. The bathing solution was 20 mM citrate-phosphate buffer (pH 5.0). Initial MSM concentration was 52 nM.

In Vivo Effect of MSM on ALS Activity

Corn seeds were germinated as above but the moist filter paper was treated with MSM. The MSM concentrations were: 0, 5, 13, 26, 52, 262, and 524 nM. Sixty-six h after sowing, 40 seedlings were harvested, and the roots were separated for fresh weight determination and ALS activity measurement immediately. This experiment was repeated two times, each with three replicates.

Safener Experiments

Each of the above types of experiment was repeated with NA-pretreated seeds. Pretreated seeds received the herbicide safener NA applied as a dust (10 g/kg seed weight). The seeds were shaken with a known amount of NA powder in a glass container for 60 min. The weight of the container before and after removal of the seeds was determined to ensure as nearly as possible that each batch of seeds received the same amount of NA (1%, w/w). This amount was the maximum amount that could be applied with adequate adherence to the seeds.

RESULTS

Effects of MSM and NA on Corn Root Elongation

One of the most noticeable plant responses of MSM is inhibition of plant growth. The inhibition of corn root elongation by MSM was decreased when the seeds were pretreated with NA. Similar results were found at this early seedling stage with chlorsulfuron by O'Leary and Prendeville (21). The effect of MSM on the net root growth of corn seedlings in the

Table I. pH Dependence of MSM Uptake during 24 h in Corn Root Tissue

Bathing solutions were 20 mM citrate-phosphate buffer (pH 5.0, 6.0, 7.0, and 8.0). Initial MSM concentration was 6.1 μM. C_i/C_o = ratio of MSM concentration in the tissue to that in the bathing solution.

pH	C_i/C_o
5.0	1.33
6.0	0.57
7.0	0.45
8.0	0.42

presence of several MSM concentrations (0, 5, 13, 26, and 52 nM) is shown in Figure 1. The net growth values were determined 2 d after adding MSM. As little as 5 nM MSM inhibited root growth in unsafened and pretreated seedlings.

These studies revealed that pretreatment of corn seeds with NA at 1% (w/w) 2 d prior to application of MSM significantly protects seedlings from MSM injury and alleviates MSM action at 5 nM but not at 52 nM (Fig. 1). NA provides a 'safening factor' (defined as the ratio of the IC_{50} value for the pretreated seedlings versus the unsafened seedlings) of approximately four; calculated IC_{50} concentration values are 3 nM for unsafened seedlings and 11 nM for pretreated seedlings. The safener allows greater elongation of root and causes only marginal inhibition of lateral root growth.

Absorption of MSM in Corn Roots

The MSM concentration in the seedlings was affected in a manner consistent with a protective mechanism; in the unsafened seedlings, [^{14}C]MSM was absorbed more rapidly than when corn seeds were dressed with NA (Fig. 2). Uptake into the unsafened seedlings was rapid initially but leveled off after approximately 20 h and increased slowly for the duration of the experiment. Uptake into the NA-pretreated seedlings proceeded linearly for the duration of the experiment.

After 48 h of incubation, unsafened and pretreated seedlings absorbed approximately 16% and 11%, respectively, of the applied radiolabeled MSM. The bathing solutions contained 86% of the applied [^{14}C]MSM. The average total recovery of [^{14}C]MSM and its radiolabeled metabolites was nearly 100% of the applied [^{14}C]MSM.

MSM reached a C_i/C_o (ratio of MSM concentration in the tissue to that in the bathing solution) of 1.0 after approximately 5 h in the unsafened seedlings, and a value of approximately 2.9 was reached after 24 h. After approximately 16 and 48 h, respectively, concentrations of MSM in the pretreated seedlings were identical and slightly higher than the external concentrations (1.0 and 2.7, respectively).

pH Dependence

Uptake of MSM was influenced markedly by the pH of the bathing solution, with greatest uptake at low pH (Table I). This relationship was especially clear in the pH range 5.0 to 7.0.

Metabolism of MSM in Corn Roots

Metabolism has been shown to be the basis for crop tolerance to chlorsulfuron (30), but Frear *et al.* (10) found no difference between the rates of chlorsulfuron metabolism in unsafened and NA-protected corn seedlings. The following investigation was to establish whether the protective effect of NA was due to its action on MSM metabolism.

The fate of MSM in corn roots was examined. The majority (62–91%) of the absorbed radioactivity was found in a polar fraction (Fig. 3). MSM was rapidly metabolized in the roots, yielding polar metabolites and 2-amino-4-methoxy-6-methyl-1,3,5-triazine which was identified by TLC with an authentic

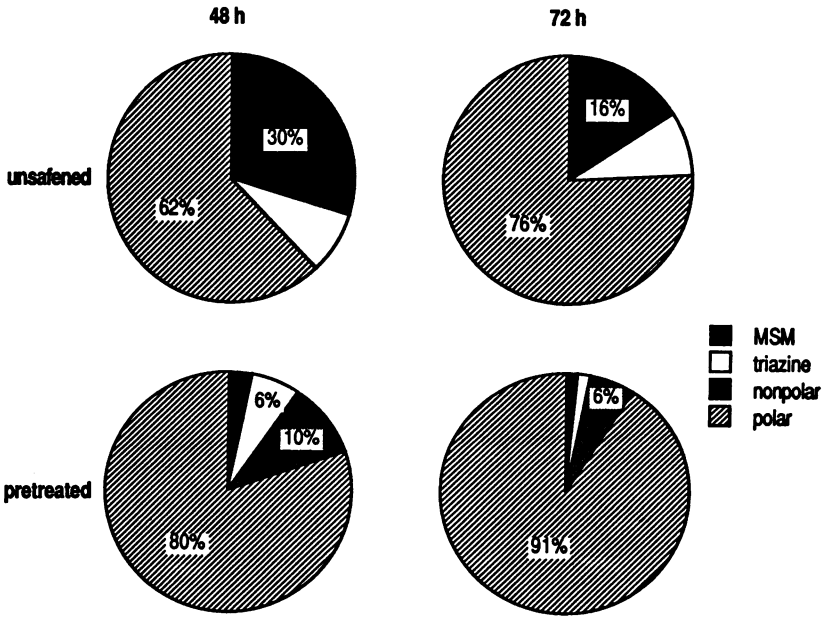

Figure 3. Metabolism of [^{14}C]MSM in corn roots showing MSM and radiolabeled metabolites in the chloroform- and water-fraction for the unsafened and the pretreated seedlings. The data are for applications of MSM at 52 nM but were essentially the same for MSM at 262 nM.

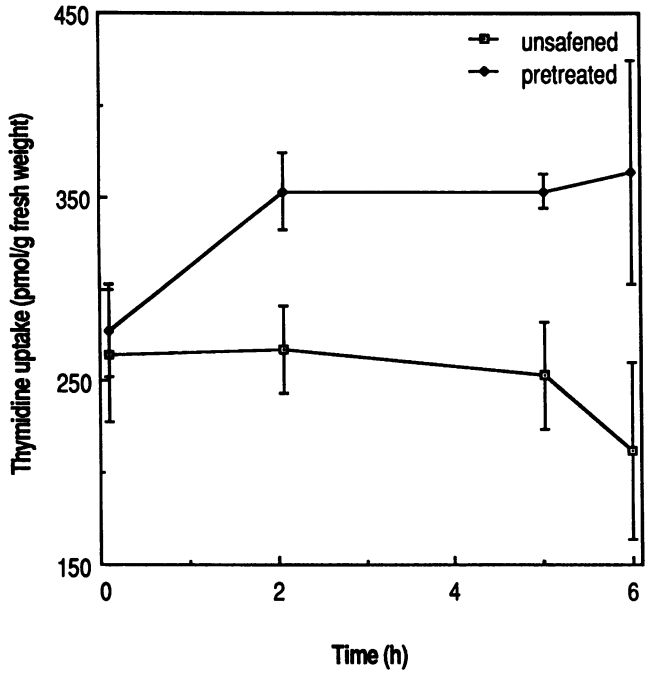

Figure 4. Time course for the effects of 13 nM MSM on the uptake of [^{3}H]thymidine as measured in roots of corn seedlings. Seedlings were incubated in the herbicide up to 6 h followed by a 1-h incubation in [^{3}H]thymidine. The error bars are the standard errors of the means.

standard. In the pretreated seedlings, MSM was metabolized to an additional nonpolar metabolite. Pretreated seedlings were much more efficient in converting absorbed MSM to metabolites; the final MSM concentration in the roots after 3 d was about 16% of the ^{14}C uptake in the unsafened seedlings and only 1% of the ^{14}C uptake in the pretreated seedlings (Fig. 3).

In the unsafened seedlings, root-associated MSM decreased immediately after application to about 16% within 3 d, while polar metabolites increased to 76%. Free 2-amino-4-methoxy-6-methyl-1,3,5-triazine was a minor constituent of radioactive metabolites (at the most, 8% after 3 d in the unsafened seedlings). The polar metabolite fraction consisted of unidentified products; additional work is required to establish the identity and potential phytotoxicity of these MSM metabolism products.

Effect of MSM on DNA Synthesis

Plant growth can be considered to have two components, cell expansion and cell division. To better understand how MSM inhibits plant growth it was necessary to determine how MSM affects these components.

One method for analyzing cell division in plant tissue is to measure [^{3}H]thymidine incorporation into DNA. The effects of MSM on plant cell division as measured by [^{3}H]thymidine incorporation into DNA of roots of corn seedlings is shown in Figures 4 and 5. The roots of the intact seedlings were treated for up to 6 h in 13 nM MSM followed by a 1-h incubation with [^{3}H]thymidine.

In unsafened seedlings, analysis of the amount of radioactivity in the DNA of the roots indicated that 2 h of treatment was sufficient to inhibit subsequent thymidine incorporation; by 6 h the amount of [^{3}H]thymidine incorporated was only 35% of the initial value (Fig. 6). Measurement of total uptake of [^{3}H]thymidine by the root tips showed little effect of MSM during the first 5 h of treatment (Fig. 4) when thymidine incorporation was inhibited by 48% (Fig. 5), indicating that the inhibition of thymidine incorporation into DNA by MSM is not due to an inhibition of thymidine uptake.

Uptake of [^{3}H]thymidine by the root tips was greater in pretreated seedlings than unsafened seedlings also exposed to MSM (Fig. 4), but thymidine incorporation into DNA was not affected by NA-pretreatment (Fig. 6).

In Vitro Effects of MSM and NA on ALS Activity

In previous research, Frear *et al.* (10) separated extractable ALS activity from corn into two fractions and showed that NA seed treatments did not appear to affect *in vitro* ALS I and II activities in seedling shoot tissues.

Figure 7 shows the average activity of ALS in extracts from corn roots over a wide range of MSM concentrations. The seed pretreatment with 1% (w/w) NA did not significantly change the ALS activity in response to MSM. With another safener, an increased ALS activity was induced by dichlormid pretreatment (26).

In vitro experiments with ALS extracted from seedlings not

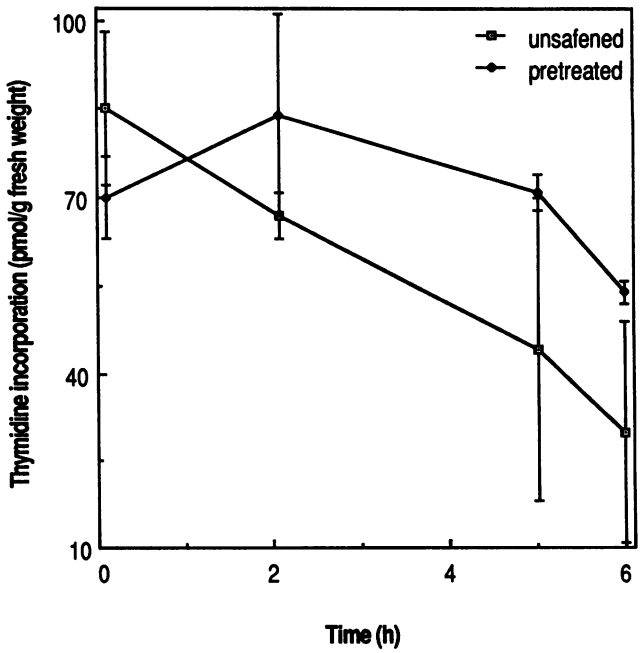

Figure 5. Time course for the effects of 13 nM MSM on incorporation of [^{3}H]thymidine into DNA by roots of corn seedlings. Seedlings were incubated in the herbicide up to 6 h followed by a 1-h incubation in [^{3}H]thymidine. The error bars are the standard errors of the means.

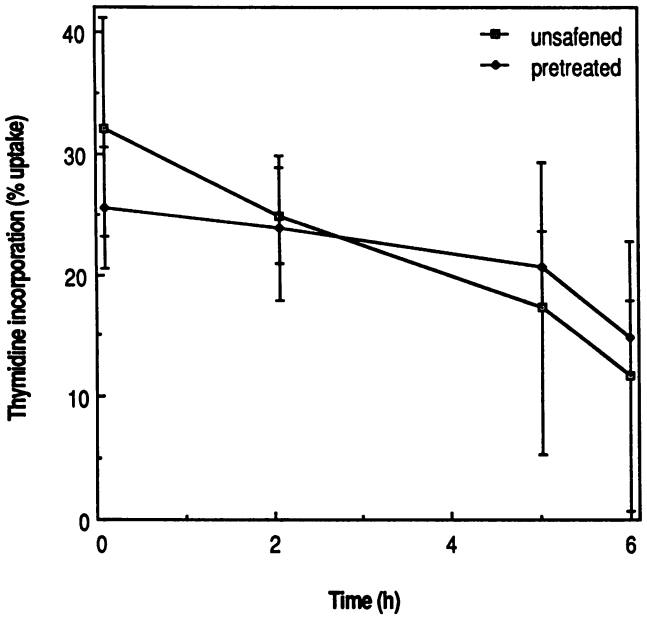

Figure 6. Time course for the effects of 13 nM MSM on incorporation percentage of [^{3}H]thymidine into DNA by roots of corn seedlings. Seedlings were incubated in the herbicide up to 6 h followed by a 1-h incubation in [^{3}H]thymidine. The error bars are the standard errors of the means.

pretreated with NA showed no effect of including the safener in the ALS assay (Fig. 8). In short, NA does not act *in vitro* to change the root enzyme response to MSM; it is an ineffective safener for extract-applied MSM. Previously, with another sulfonylurea herbicide, Frear *et al.* (10) also showed that NA did not protect against chlorsulfuron inhibition *in vitro.*

In Vivo Effects of MSM on ALS Activity

ALS activity rates for unsafened seedlings of the *in vivo* study were half as much as those of the *in vitro* study. This significant difference in the rate of ALS activity was an expected result because the unsafened seedlings in these experiments differ from one another in treatment solution; seeds were germinated in pH 5.0 buffer for the *in vivo* study and in distilled water for the *in vitro* study.

Frear *et al.* (10) showed that chlorsulfuron treatment reduced the specific activity of ALS II by approximately 50% but did not affect ALS I. However, in our study, MSM increased the total extractable ALS activity, with the greatest response in the pretreated seedlings (200% of the zero-MSM concentration value for a 0.5 μM MSM application) and the least in the unsafened seedlings (153% of the zero-MSM concentration value for a 26 nM MSM application) (Fig. 9). *In vivo* inhibition of an enzyme often leads to overproduction of that enzyme; for example, Duke and Hoagland (8) have shown that the herbicide glyphosate induced PAL activity in the roots of dark-grown corn seedlings. MSM-applications induced an increase of protein contents of seedlings roots (Fig. 10).

Pretreatment of corn seeds with 1% (w/w) NA enhances the ALS activity in the roots, and the pretreated seedlings contain higher ALS levels *in vivo* than the unsafened seedlings at high MSM concentrations (>0.1 μM).

DISCUSSION

The possible effects of MSM described in this paper were measured after relatively short treatment times. It can be assumed that with longer treatment times secondary effects would become apparent. Concentrations of MSM used in this study were relatively low, 1 nM to 13 μM. These low concentrations were used not only because adequate responses could be obtained, but because high concentrations could cause secondary effects which are not related to the primary mode of action.

Laboratory experiments described here showed that MSM is active as a plant growth inhibitor at 5 nM in a sensitive plant such as corn and NA partially alleviates MSM injury. Similar results have been obtained with chlorsulfuron by O'Leary and Prendeville (21).

Herbicides can accumulate in plant tissue by several mechanisms, including ion trapping of weak acids (20, 24). MSM is weak acid (pK_a value of 3.3), and its accumulation in corn root tissue is consistent with weak acid accumulation. The ratio of undissociated to dissociated molecules in the external solution was proportional to the solution pH, and the greater uptake at low pH (Table I) suggests that MSM crosses the plasmalemma as the undissociated acid. A pH-dependent

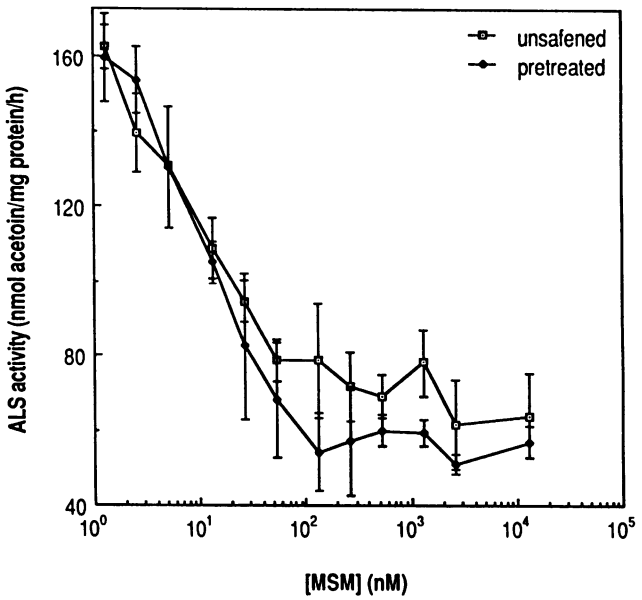

Figure 7. *In vitro* ALS activity in extracts from unsafened and pretreated seedlings at various MSM concentrations. Uninhibited control rates (expressed as nmol acetoin/mg protein/h) are 162 ± 1 for unsafened seedlings and 175 ± 12 for pretreated seedlings. Each point is the average of data from duplicate assays in three separate experiments. The error bars are the standard errors of the means.

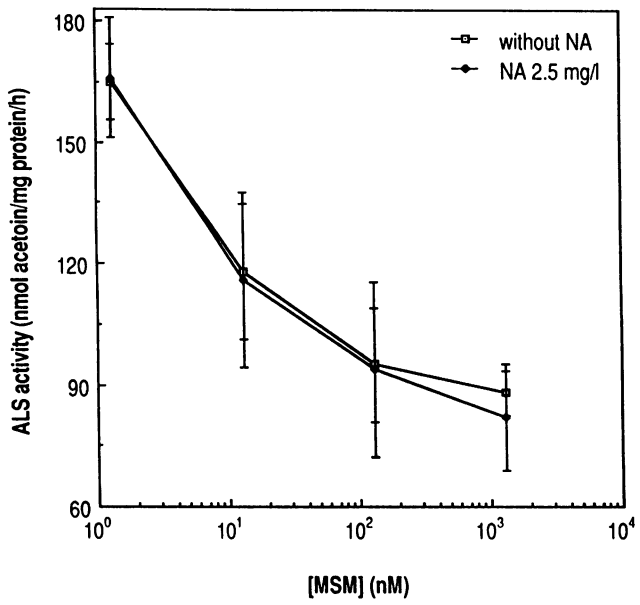

Figure 8. *In vitro* effects of MSM and NA on the ALS activity in extracts from roots of 66-h-old corn seedlings. Uninhibited control rates (expressed as nmol acetoin/mg protein/h) are 163 ± 3 without NA and 181 ± 29 with NA at 2.5 mg/L. Mean values based on two separate experiments. The error bars are the standard errors of the means.

uptake of several other classes of weakly acidic herbicides has been reported (24).

The different investigations on the fate of MSM have shown that accumulation of a nonpolar metabolite is a major difference between metabolism of MSM in unsafened and pretreated seedlings. MSM metabolism in corn roots was significantly enhanced by seed pretreatment with NA at 1% (w/w); after 48 h on 52 or 262 nM [^{14}C]MSM solutions, distributions of the absorbed radioactivity was altered and only 3% of the ^{14}C absorbed was recovered as MSM in the roots of pretreated seedlings.

Activation of metabolism of sulfonylurea compounds by safeners was previously reported by Sweetser (29); in his treatment conditions the half-life of MSM was four times less, but Frear *et al.* (10), with chlorsulfuron, found no effect of NA-pretreatment on the metabolic rate in corn. The observation that auxins afford protection to corn from chlorsulfuron damage (10) is interesting, because NA is converted into naphthalic acid in corn (2), and the potential safening activity of this auxin analog could be effective in protecting corn against MSM injury.

The metabolism of MSM in higher plants generally involves three different pathways; a hydroxylation reaction on the phenyl ring followed by glucosylation; a hydroxylation on the methyl group of the triazine ring; and a sulfonylurea bridge cleavage (1, 4). The mechanism by which NA elevates MSM metabolism is of prime interest. NA does not change the levels of Cyt P-450 but enhances herbicide metabolism which is correlated in corn with an induced increase in the GST (EC 2.5.1.18) level (10, 15). This enzyme does not seem to be directly involved in the MSM metabolism and, thus, another enzyme must be activated by NA-pretreatment or new forms of detoxifying enzymes must be produced.

The *in vitro* sensitivity of crude ALS to MSM inhibition was approximately the same for ALS extracted from unsafened and NA-pretreated seedlings. (Fig. 7). Similarly, in seedling shoot tissues, Frear *et al.* (10) separated extractable ALS activity from corn into two fractions and showed that NA seed treatments did not affect *in vitro* ALS I and II activities. In contrast to dichlormid (26), NA does not significantly increase ALS activity in the pretreated corn seedlings.

In the same way, the safener NA does not act *in vitro* to change the ALS response to MSM (Fig. 8). Similar results were found with dichlormid (26) which does not modify the ALS sensitivity to chlorsulfuron.

On the other hand, the levels of extractable ALS were increased by application of MSM to the seeds prior to the enzyme extraction (Fig. 9). Similar results were found with chlorsulfuron by Frear *et al.* (10) but with imazapyr (an ALS inhibitor of another chemical group), a completely different result has been obtained by Muhitch *et al.* (19). The level of extractable ALS was drastically reduced by the pretreatment of seedlings with imazapyr prior to enzyme extraction.

The extractable ALS activity was practically the same in the unsafened and pretreated seedlings when the MSM application rate was low (<50 nM); if this concentration was high (>0.2 μM), the ALS activity was higher in the pretreated seedlings than in the unsafened seedlings. These results may

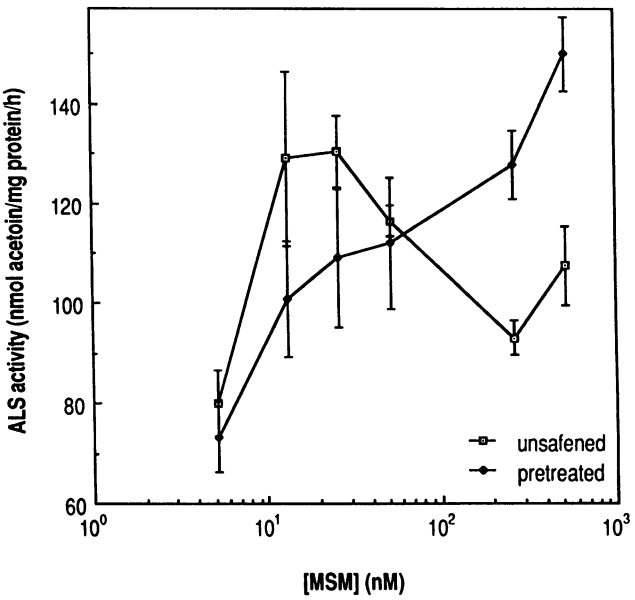

Figure 9. *In vivo* effect of various MSM concentrations on the ALS activity in extracts from unsafened and pretreated seedlings at 1% (w/w) NA concentration. Uninhibited control rates (expressed as nmol acetoin/mg protein/h) are 85 ± 10 for unsafened seedlings and 75 ± 8 for pretreated seedlings. Each point is the average of data from triplicate assays in two separate experiments. The error bars are the standard errors of the means.

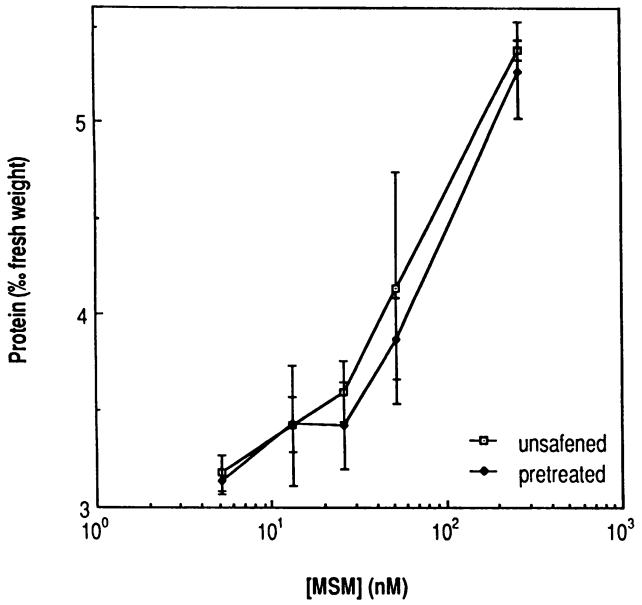

Figure 10. Protein contents of corn seedlings roots grown in solution culture for 66 h in presence of MSM. The zero-MSM concentration values (expressed as mg protein/g fresh weight) are 3.3 ± 0.3 for unsafened seedlings and 2.9 ± 0.1 for pretreated seedlings. Each point is the average of data from triplicate assays in two separate experiments. The error bars are the standard errors of the means.

be explained by a decreased MSM phytotoxicity in the pretreated seedlings.

DNA synthesis is not the prime site of sulfonylurea herbicides, but it was investigated because it is a rapid secondary site upon which the safener might act. The NA-pretreatment modifies [^{3}H]thymidine uptake but not DNA synthesis corresponding to the [^{3}H]thymidine incorporation into DNA. Since the uptake does not represent a normal physiological activity in plants, the NA-pretreatment does not indicate an effect on corn DNA synthesis.

According to Barrett (2), extractable ALS levels were not increased sufficiently to account for the safening effect; only the enhanced metabolism of MSM was considered a potential cause of the safening. In conclusion, corn protection against MSM is based on increased detoxification of the parent herbicide by the safener NA, which seems to act as a "bioregulator" influencing the amount of MSM that reaches its target site in an active form.

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