

# The Effect of BAS 145138 Safener on Chlorimuron Ethyl Metabolism and Toxicity in Corn\*, \*\*

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BAS 145138 was moderately effective in protecting corn from injury due to chlorimuron ethyl. BAS 145138 increased the  $I_{50}$  of chlorimuron ethyl to corn, determined by root-growth in a sandy loam soil, from 6 g chlorimuron ethyl/ha to 25 g/ha. BAS 145138 caused an increase in the rate of chlorimuron ethyl metabolism, but it did not effect the rate of chlorimuron ethyl uptake or the nature of the metabolites produced. Major metabolites included chlorimuron ethyl hydroxylated in the pyrimidine ring (hydroxychlorimuron ethyl), the glucoside of hydroxychlorimuron ethyl, the glutathione conjugate formed by displacement of chlorine, a metabolite characterized as the glutathione conjugate sulfoxide or a hydroxylated glutathione conjugate, four unidentified polar metabolites, and 2-sulfonylamino benzoic acid ethyl ester. Metabolites were characterized by mass spectrometry and hydrolysis. It was concluded that BAS 145138 partially protects corn from chlorimuron ethyl by causing an increase in the rate of herbicide metabolism by hydroxylation, glucoside conjugation and by glutathione conjugation.

## Introduction

BAS 145138 (1-dichloroacetylhexahydro-3,3,8 $\alpha$ -trimethylpyrrolo-[1,2- $\alpha$ ]-pyrimidin-6-(2*H*)-one) caused a 10-fold increase in the tolerance of corn to the herbicide metazachlor. Increased tolerance appeared to be related to a 2-fold increase in the rate of glutathione (GSH) conjugation of metazachlor and a 7-fold reduction in the concentration of metazachlor in the developing leaves [1, 2]. The reduction in the concentration of metazachlor in the developing leaves was attributed to the increased rate of metazachlor metabolism. The mode of action of metazachlor has not been established; therefore, it is difficult to prove that BAS 145138 protects corn from metazachlor injury by causing an acceleration in the rate of metazachlor metabolism by GSH conjugation. Chlorimuron ethyl (2-[[[4-chloro-6-methoxy-2-pyrimidinyl)-

amino]carbonyl]amino]sulfonyl]benzoic acid ethyl ester) is a sulfonyleurea herbicide. The toxicity of chlorimuron ethyl is due to inhibition of acetolactate synthase, and selectivity between tolerant and susceptible species is due to a more rapid rate of chlorimuron ethyl metabolism in the tolerant species [3]. The major metabolites of chlorimuron ethyl in tolerant soybean, a homoglutathione (hGSH) conjugate and the free acid of chlorimuron, are not inhibitors of acetolactate synthase [3]. Since safeners can protect corn from sulfonyleurea injury [4, 5], and since chlorimuron ethyl tolerance in soybean is primarily due to rapid detoxification by conjugation with hGSH [3], chlorimuron ethyl and BAS 145138 appeared to be an excellent herbicide/safener combination to study the relationship between the effect of a safener on herbicide metabolism, presumably by GSH conjugation, and protection from herbicide injury.

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## Materials and Methods

[<sup>14</sup>C-phenyl]Chlorimuron ethyl (3.48  $\mu$ Ci/ $\mu$ mol, 99% radiochemical purity) and analytical grade chlorimuron ethyl were from E. I. DuPont de Nemours and Company and BAS 145138 (98.4% purity) was from BASF Corporation. Hybrid Northrup King PX 9144 corn (*Zea mays* L.) was used throughout this study. Growth response



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studies were conducted in the presence or absence of 2.7 kg/ha BAS 145138 and variable concentrations of chlorimuron ethyl incorporated into a previously described sandy loam soil [6]. Growth response studies were also conducted with corn germinated and grown between rolls of absorbent paper partially immersed in an aqueous solution containing various concentrations of chlorimuron ethyl  $\pm$  68  $\mu$ M BAS 145138. Plants were germinated and grown in a growth chamber at a constant 21 °C, 50% relative humidity, and a 14 h photoperiod [145  $\mu$ E/(m<sup>2</sup> × sec)].

For metabolism studies, plants were germinated between rolls of moistened paper  $\pm$  68  $\mu$ M BAS 145138 for 4 days before they were treated by application of 1.27 to 12.3 nmol [<sup>14</sup>C]chlorimuron ethyl in 2.5  $\mu$ l of acetone to the roots of the individual seedlings (60 mg fresh weight per root). These seedlings were incubated in the dark at 21 °C on screens held 1 cm above trays of water. The treated root tissue was excised and frozen immediately following incubation. The frozen tissue was extracted with cold aqueous 70% acetonitrile, the extracts were concentrated under vacuum, and the concentrated extracts were applied to open columns of C<sub>18</sub> and eluted with aqueous 67% acetonitrile. The resulting radioactive eluates were concentrated and chromatographed on a 3.9 mm × 30 cm C<sub>18</sub>  $\mu$ Bondapak column (Millipore, Corp.) eluted at 1.5 ml/min with a gradient of aqueous acetonitrile in 1% acetic acid. Radioactive products were detected, quantified, and characterized as previously described in other metabolism studies [6].

## Results

BAS 145138, incorporated into a sandy loam soil at 2.7 kg/ha, partially protected corn seedlings from injury due to chlorimuron ethyl. The I<sub>50</sub> for root growth was increased from 6 g chlorimuron ethyl/ha to 25 g/ha; however, the response curve for protection was not as favorable as had been previously observed when 0.5 to 5 ppm BAS 145138 was used to protect corn from metazachlor injury [2]. Corn germinated between layers of moist absorbent paper was also protected from chlorimuron ethyl injury by BAS 145138, but protection was most effective at low levels of chlorimuron ethyl (Fig. 1).

BAS 145138 did not effect the uptake of chlorimuron ethyl by the roots of corn seedlings (data

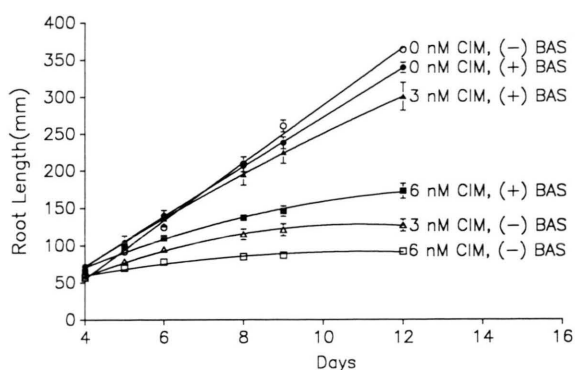


Fig. 1. Protection of corn from chlorimuron ethyl by BAS 145138. Corn was germinated and grown between rolls of paper partially immersed in 0, 3, or 6 nM chlorimuron ethyl (CIM)  $\pm$  68  $\mu$ M BAS 145138 (BAS). The experiment was replicated three times.

not shown); however, the rate of chlorimuron ethyl metabolism was increased significantly (Fig. 2). BAS 145138 at concentrations to 5 ppm was most effective at increasing chlorimuron ethyl metabolism, but additional stimulation of chlorimuron ethyl metabolism was observed with concentrations of BAS 145138 through 25 ppm.

BAS 145138 did not appear to cause any qualitative changes in chlorimuron ethyl metabolism in the roots of intact corn seedlings. An HPLC chromatogram of an extract from corn roots grown in the presence of BAS 145138 and treated with [<sup>14</sup>C]chlorimuron ethyl is shown (Fig. 3). Qualitatively similar results were observed with extracts from corn that had not been exposed to BAS

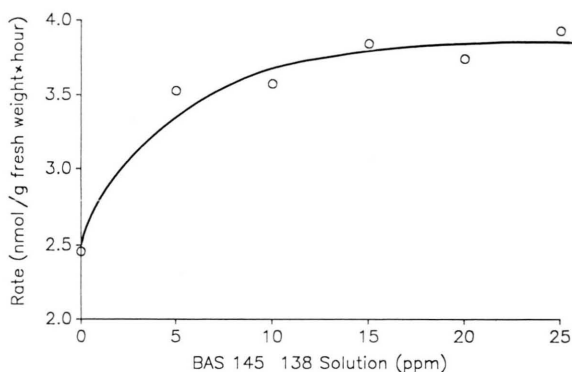


Fig. 2. The effect of BAS 145138 on chlorimuron ethyl metabolism. At four days the roots of intact seedlings were treated with [<sup>14</sup>C]chlorimuron ethyl and the rate of metabolism was determined. Each data point represents a true rate determined in a three-data-point time course.



The quantitative effects of BAS 145138 on chlorimuron ethyl metabolism were determined in intact corn seedlings. The roots of these seedlings were treated with [ $^{14}$ C]chlorimuron ethyl and analyzed after 7 h (Table I). BAS 145138 stimulated the production of hydroxychlorimuron ethyl, the corresponding O-glucoside, the GSH conjugates, three unidentified metabolites, and the bound residue. Hydroxychlorimuron ethyl is probably a precursor for other metabolites, including the O-glucoside; therefore, the results in Table I may underestimate the stimulation of chlorimuron ethyl hydroxylation by BAS 145138. A dramatic 6-fold increase in the concentration of the O-glucoside was observed in response to BAS 145138. This increase could have been due to changes in hydroxychlorimuron ethyl formation and/or to changes in the glucosyltransferase system.

The effect of BAS 145138 on the *in vivo* glucosyltransferase system from corn root was evaluated using hydroxychlorimuron ethyl as the substrate. [ $^{14}$ C]Hydroxychlorimuron ethyl was synthesized *in vivo* by corn and isolated by HPLC. The purity and structure of the isolated substrate was verified by HPLC and FAB/MS. Corn seedlings germinated in the presence or absence of BAS 145138 were treated by application of [ $^{14}$ C]hydroxychlorimuron ethyl to the roots. After incubation of the intact seedlings, the roots were rinsed,

extracted, and the extracts were analyzed by HPLC. As evidenced by Fig. 5, the rate of glucosylation was approximately doubled as a result of treatment with BAS 145138. This increase in the rate of glucosylation, and perhaps an increase in the rate of hydroxylation, resulted in the 6-fold increase in the concentration of the O-glucoside observed after 7 h (Table I). This suggests that the glucosyltransferase system is directly effected by BAS 145138.

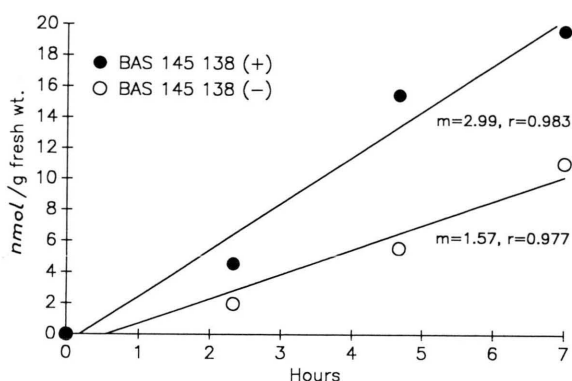


Fig. 5. The effect of BAS 145138 on the glucosylation of [ $^{14}$ C]hydroxychlorimuron ethyl in the roots of intact corn seedlings. Corn was grown in the presence of  $\pm 68 \mu\text{M}$  BAS 145138 and treated with 168 nmol hydroxychlorimuron ethyl/g fresh weight of roots.

Table I. The effect of BAS 145138 on the levels of chlorimuron ethyl and its metabolites in corn roots 7 h post-treatment.

Compound	nmol/gram fresh weight		Ratio $\pm$ BAS
	(-) BAS	(+) BAS	
Chlorimuron E.	76.5 $\pm$ 7.7	34.5 $\pm$ 3.9	0.45
HO-Chlorimuron E.	28.0 $\pm$ 6.0	40.4 $\pm$ 0.9	1.44
O-Glucoside conjugate	2.3 $\pm$ 0.2	13.5 $\pm$ 2.1	5.93
GSH conjugate	5.1 $\pm$ 0.4	8.9 $\pm$ 0.4	1.74
HO- or SO-GSH conjugate	5.1 $\pm$ 0.2	8.6 $\pm$ 0.4	1.67
Sulfonamide	6.2 $\pm$ 1.2	5.8 $\pm$ 1.5	0.93
Unknown 1	8.8 $\pm$ 1.8	12.3 $\pm$ 0.3	1.40
Unknown 2	4.7 $\pm$ 0.9	3.3 $\pm$ 0.3	0.71
Unknown 3	4.4 $\pm$ 0.8	8.3 $\pm$ 0.4	1.90
Unknown 4	2.4 $\pm$ 0.4	3.3 $\pm$ 0.4	1.38
Bound residue	2.1 $\pm$ 0.2	3.3 $\pm$ 0.3	1.57

Corn (80/replicate, 3 replicates) was germinated between rolls of paper  $\pm 68 \mu\text{M}$  BAS 145138, treated with [ $^{14}$ C]chlorimuron ethyl (12.2 nmol/seedling applied to the roots), incubated for 7 h, extracted, and analyzed by HPLC. Deviations are standard error.

## Conclusions

It appears that BAS 145138 protects corn from chlorimuron ethyl injury by causing an elevation in the rate of chlorimuron ethyl metabolism. Although the metabolism of both chlorimuron ethyl and metazachlor were stimulated to approximately the same extent, BAS 145138 was not as effective in protecting corn from chlorimuron ethyl injury (approximately 4-fold) as from metazachlor (approximately 10-fold). Because safeners are known to effect several enzyme systems and processes, including glutathione S-transferases [7], glutathione

reductases [8], sulfur assimilation [9], and monooxygenases [10], herbicide safeners probably protect plants from herbicide injury by more than one mechanism. This appears to be one of the first reports to suggest that the glucosyltransferase pathway may be stimulated in response to a herbicide safener. However, the relative roles of hydroxylation and glucoside conjugation in chlorimuron ethyl detoxification remains to be determined, as does the effect of BAS 145138 on the levels of acetylactate synthase, the target enzyme.

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