Mode of Action of Naphthalic Acid as a Safener for Imazethapyr*

Dale L. Shaner

American Cyanamid Co., P.O. Box 400, Princeton, N.J., 08543-0400 U.S.A.

Z. Naturforsch. **46c**, 893–896 (1991); received March 26, 1991

Maize, Imidazolinone, Safeners, Naphthalic Acid, Naphthalic Anhydride, Metabolism

The tolerance of maize to root-applied imazethapyr can be increased by pretreating plants with the potassium salt of naphthalic acid (NAK). This safening effect appears to be the result of NAK stimulating the ability of maize to rapidly metabolize imazethapyr to 5′-hydroxyethyl-imazethapyr, possibly through a mixed function oxidase. The safening effect of NAK can be antagonized by 1-aminobenzotriazole (ABT), a mixed function oxidase inhibitor. The increased rate of hydroxylation of imazethapyr to 5′-hydroxyethyl-imazethapyr immobilizes the herbicide in the root system which decreases the accumulation of herbicide in the meristematic tissue. This decreased accumulation, in turn, lowers the phytotoxicity of imazethapyr on maize.

Introduction

Maize is highly tolerant to imazethapyr. However, this tolerance can be increased if the crop is treated with the safener naphthalic anhydride (NA) [1]. Barrett [1] found that NA treatment of corn seed altered the distribution and rate of metabolism of imazethapyr in maize. The purpose of this research was to further clarify the mode of action of NA as a safener for imazethapyr in maize.

Materials and Methods

Plant material

Maize plants (Pioneer, var. 3475) were grown hydroponically in a modified Hoagland's solution in 120 ml plastic containers in growth chambers (14 h photoperiod; 28/20 °C (day/night); 300 μE/m^2/sec). Plants were treated when the fourth leaf was just emerging from the whorl.

Treatments

Plants were treated with various concentrations of technical materials dissolved in the nutrient solution. Naphthalic acid (NAK) and 1-aminobenzotriazole (ABT) treatments were given 24 h prior to imazethapyr treatment, unless otherwise indicated. Imazethapyr treatments were given for 24 h followed by transfer of plants to untreated nutrient solutions. [^{14}C]carboxy-labelled imazethapyr was used in the radiolabelled experiments.

Growth measurements

Growth of the fourth leaf was measured daily by recording the change in length to the nearest mm from the top of the growing container to the tip of the leaf.

Acetohydroxyacid synthase

Acetohydroxyacid synthase activity was extracted from plant material and measured using the procedures of Shaner *et al.* [2] as modified by Singh *et al.* [3].

Analysis

Plant tissue was extracted in acidified methanol and the radiolabelled material in the extract was analyzed after concentrating the extract. Radiolabelled metabolites of imazethapyr were co-chromatographed with standards on two-dimensional TLC on 60 F-254 silica gel plates. The solvent in the first direction was $CHCl_2$/*n*-propanol/HCOOH (40/50/10, v/v/v) and in the second direction was CH_3CN/*n*-propanol/NH_4OH (40/50/10, v/v/v). The location of the standards of imazethapyr and 5′-hydroxyethyl-imazethapyr were visualized under UV light. Exposing the plates to X-ray film located the radioactive spots.

Chemicals

Technical imazethapyr, 5′-hydroxyethyl-imazethapyr, aminobenzotriazole and the potassium

* Based on a paper presented at the International Conference on Herbicide Safeners, August 12–15, 1990 in Budapest, Hungary.

Reprint requests to Dr. D. L. Shaner.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0900–0893 $ 01.30/0

salt of naphthalic acid were synthesized at American Cyanamid Co., Princeton, N.J.

Results and Discussion

Imazethapyr caused a concentration-dependent inhibition of elongation of the fourth leaf of maize when the herbicide was applied *via* the root system for 24 h (Fig. 1). This inhibition could be largely reversed if the plants were pretreated with 250 μM NAK 24 h prior to imazethapyr treatment (Fig. 2).

The safening effect of NAK reached its maximum effect if it was given to the plant at least 4 h before imazethapyr treatment. Delaying NAK treatment after imazethapyr treatment reduced its safening effect on the elongation of the fourth leaf (Fig. 3). Sweetser [4] found similar effects on the safening of chlorsulfuron by NAK in corn.

NAK-pretreated maize plants metabolized imazethapyr much more rapidly than unsafened plants. The apparent half-life of imazethapyr in the roots of unsafened plants was 20 h, while in NAK-treated plants it was 2 h. Barrett [1] also found that NA treatment increased the rate of metabolism of imazethapyr in maize. Sweetser [4] reported a similar change in the ability of maize to metabolize chlorsulfuron after NAK treatment.

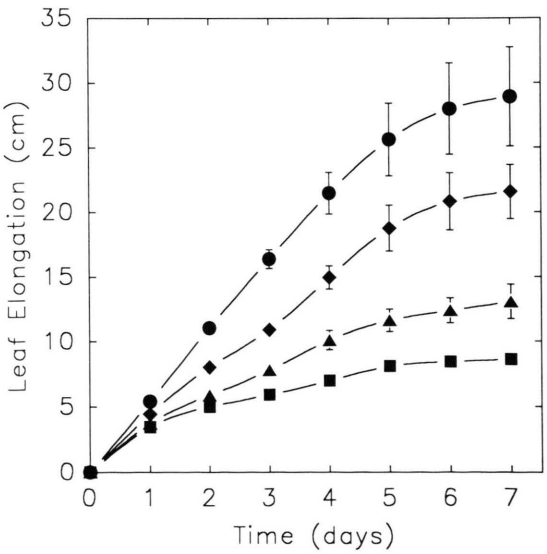


Fig. 1. Inhibition of elongation of the fourth leaf of maize after root application of different concentrations of imazethapyr. Herbicide treatments were given for the first day and then plants were transferred to untreated nutrient solution. (●) Untreated; (◆) 1 μM; (▲) 2.5 μM; (■) 5 μM imazethapyr. Vertical lines designate ±1 standard deviation.

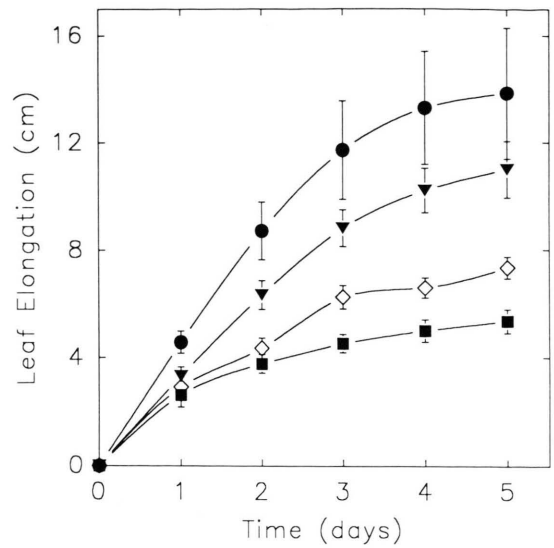


Fig. 2. Interaction of NAK and ABT on the inhibition of the fourth leaf of maize after root application of different concentrations of imazethapyr, NAK, and ABT. NAK and ABT treatments were given 24 h before imazethapyr application. NAK and ABT treatments alone and in combination did not inhibit leaf elongation. Time 0 on the graph denotes when imazethapyr treatment began. NAK and ABT were also included with the imazethapyr treatment. Herbicide treatments were given for the first day and then plants were transferred to untreated nutrient solution. (●) Untreated; (■) 5 μM imazethapyr; (◇) 5 μM imazethapyr + 75 μM ABT + 250 μM NAK; (▼) 5 μM imazethapyr + 250 μM NAK. Vertical lines designate ±1 standard deviation.

Both unsafened and NAK-treated maize metabolized imazethapyr to one metabolite, which was identified by co-chromatography on two-dimensional TLC to be 5′-hydroxyethyl-imazethapyr (Fig. 4). This imidazolinone is almost as active as imazethapyr on acetohydroxyacid synthase, the site of action of the imidazolinones. The I_{50} for AHAS inhibition by imazethapyr is 1 μM and by 5′-hydroxyethyl-imazethapyr is 2.4 μM. However, in whole plant studies, 5′-hydroxyethyl-imazethapyr was more than 100-fold less active than imazethapyr when applied through the root system (Fig. 5).

The reason for the lack of herbicidal activity of 5′-hydroxyethyl-imazethapyr appears to be due to

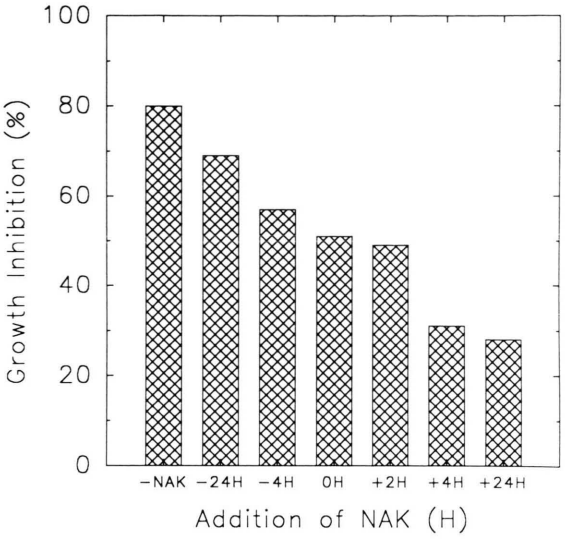


Fig. 3. Effect of timing of application of 250 μM NAK on the safening of imazethapyr inhibition of the fourth leaf of maize after root application of 5 μM imazethapyr. The times indicated on the X-axis are: NAK = no safener; 24H = NAK applied 24 h after beginning herbicide treatment; −4H = NAK applied 4 h after beginning herbicide treatment; 0h = NAK applied with herbicide treatment; +2H = NAK applied 2 h before beginning herbicide treatment; +4H = NAK applied 4 h before beginning herbicide treatment; and +24H = NAK applied 24 h before beginning herbicide treatment. Herbicide treatments were given for the first day and then plants were transferred to untreated nutrient solution.

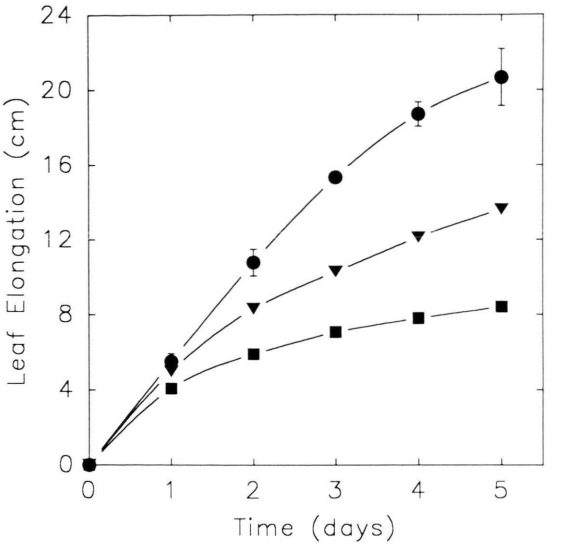
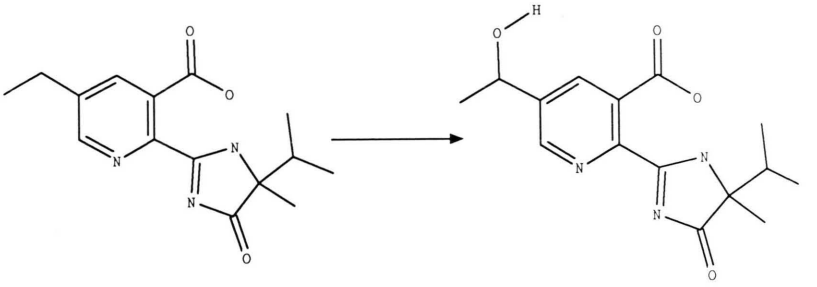


Fig. 5. Inhibition of elongation of the fourth leaf of maize after root application of 2.5 μM imazethapyr or 250 μM 5′-hydroxyethyl-imazethapyr. Herbicide treatments were given for the first day and then plants were transferred to untreated nutrient solution. (●) Untreated; (▼) 250 μM 5′-hydroxyethyl-imazethapyr; (■) 2.5 μM imazethapyr. Vertical lines designate ±1 standard deviation.



Imazethapyr

5'-hydroxyethyl-imazethapyr

Fig. 4. Metabolism of imazethapyr in maize.

the immobility of this compound in maize. Examination of the distribution of 5′-hydroxyethyl-imazethapyr in NAK-treated and unsafened plants showed that NAK treatment resulted in an accumulation of this metabolite in the roots whereas in unsafened plants there is an accumulation in the leaves and apical meristematic tissue (Fig. 6). Barrett [1] had previously found that NA treatment affected the distribution of imazethapyr in maize by causing an increase in the amount of radioactivity remaining in the roots after herbicide treatment.

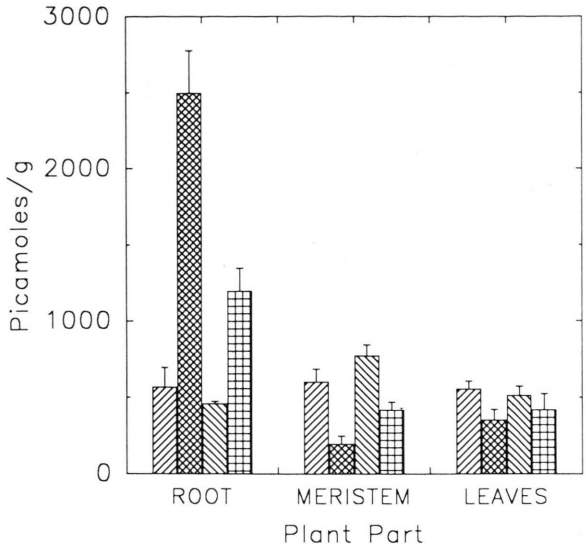


Fig. 6. Distribution of 5′-hydroxyethyl-imidazolinone 26 h after application of 5 μM [^{14}C]imazethapyr in combination plus or minus 250 μM NAK and/or 75 μM ABT to the roots of maize. NAK and ABT were given to the plants 24 h prior to introduction of the radiolabelled herbicide. NAK and ABT alone and in combination had no effect on leaf elongation. (▨) Imazethapyr; (▩) imazethapyr + NAK; (▧) imazethapyr + ABT; (⊞) imazethapyr + NAK + ABT. Vertical lines designate ±1 standard deviation. g refers to fresh wt.

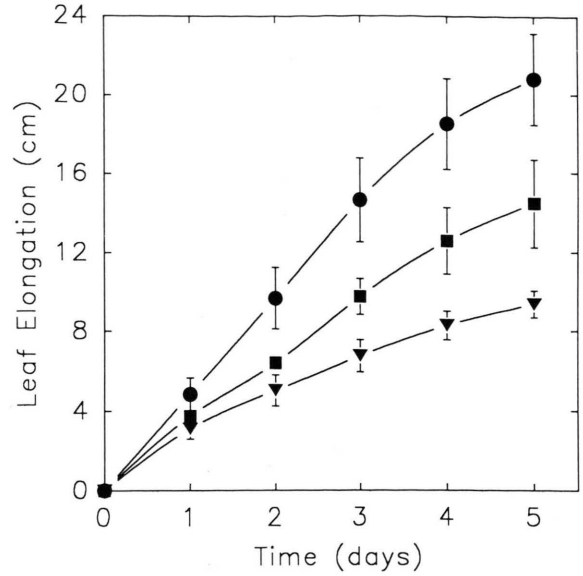


Fig. 7. Effect of ABT on inhibition of elongation of the fourth leaf of maize after root application of imazethapyr. ABT was applied 24 h prior to introduction of the herbicide and during herbicide treatment. ABT alone had no effect on leaf elongation. Herbicide treatments were given for the first day and then plants were transferred to untreated nutrient solution. (●) Untreated; (■) 1 μM imazethapyr; (▼) 1 μM imazethapyr + 75 μM ABT. Vertical lines designate ±1 standard deviation.

Further support that it is the rate of hydroxylation of imazethapyr that determines its phytotoxicity on maize is shown by the interactions between imazethapyr and the mixed function oxidase inhibitor, ABT. ABT increased the inhibitory activity of imazethapyr on maize (Fig. 7) and partially reversed the safening effect of NAK (Fig. 2). ABT treatment increased the half-life of imazethapyr in unsafened from 22 h to 30 h and in NAK-treated plants from 2 h to 5 h. This inhibitor also increased the amount of herbicide accumulating in the apical meristem (Fig. 6). ABT treatment has been shown to have a similar effect on the phytotoxicity of chlortoluron, another herbicide that is detoxified *via* a mixed function oxidase system [5].

These results support the hypothesis that the mode of action of NAK in safening imazethapyr in maize is due to a stimulation of the rate of hydroxylation of imazethapyr in the plant at the site of uptake. This hydroxylation essentially immobilizes the herbicide and prevents it from accumulating in the meristematic tissue. This hydroxylation occurs *via* a mixed function oxidase which can be inhibited by ABT, which, in turn, can partially prevent the safening effect of NAK.

[1] M. Barrett, Weed Sci. **37,** 296–301 (1989).
[2] D. L. Shaner, P. C. Anderson, and M. A. Stidham, Plant Physiol. **76,** 545–546 (1984).
[3] B. K. Singh, M. A. Stidham, and D. L. Shaner, J. Chromatogr. **444,** 251–261 (1988).
[4] P. B. Sweetser, Proc. Brit. Crop Prot. Conf. – Weeds **3,** 1147–1154 (1985).
[5] M. Gonneau, B. Pasquette, F. Cabanne, and R. Scalla, Weed Res. **28,** 19–25 (1988).