SESSION 10B

MODE OF ACTION AND METABOLISM OF HERBICIDES: III (continued)

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RESEARCH REPORTS   10B-1 to 10B-6

10B—1

BEHAVIOUR OF GLUFOSINATE-AMMONIUM IN WEEDS

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ABSTRACT

Glufosinate-ammonium, under the commercial name Basta^R, is a non-selective herbicide for post-emergence weed control. Translocation and metabolic pathways were examined in a number of plant species using ^{14}C-labelled active ingredient.

Three days after application, the radioactive substance exported from the treated leaves and detected in the untreated parts of the plants amounted to 2.0 % in Calopogonium mucunoides, 2.2 % in Lathyrus tuberosus, 2.5 % in Convolvulus arvensis, 3.3 % in Trifolium subterraneum, and 4.3 % in Sorghum halepense.

In the three investigated plant species, S. halepense, T. subterraneum and C. arvensis, the ^{14}C-labelled active ingredient was metabolised by formation of non-phytotoxic 3-methyl-phosphinico-propionic acid, and of three other substances, which occurred only in very small quantities. Unchanged active ingredient translocated to the roots amounted to 6.6 % in S. halepense, 3.3 % in T. subterraneum, and only 1.2 % in C. arvensis.

The differences in specific sensitivity could be accounted for by the varying concentrations of unchanged active ingredient transported into the roots. The intensity of basipetal transport also played an important part.

The sensitivity of the investigated plant species to glufosinate-ammonium increased in the following order: C. mucunoides, L. tuberosus, C. arvensis, T. subterraneum and S. halepense.

INTRODUCTION

Glufosinate-ammonium (HOE 039866) is an organophosphorus compound. The free amino acid on which it is based is referred to in the literature as phosphinothricin and is a metabolic product of Streptomycetes viridochromogenes (Gugel 1971, Bayer et al. 1972).

Glufosinate-ammonium is a contact herbicide with a particular systemic mode of action. It has a non-selective action on a wide spectrum of annual and perennial weeds and grasses when applied post-emergence at a rate of 0.3 - 1.5 kg active ingredient/ha. The substance has a rapid effect, causing the plants to wither and die after as little as 2 - 5 days, depending on environmental factors (Hoechst AG 1982).

The substance is degraded within the plant to 3-methyl-phosphinico-propionic acid, which has no further phytotoxic effect (Götz et al. 1983).

Studies with ^{14}C-labelled glufosinate-ammonium after foliar treatment show marked translocation in the leaf, extending from base to tip. Transport from the treated leaf to the other parts of the plant takes place only to a very limited extent (Hoechst AG 1982).

MATERIALS AND METHODS

Plant material

The studies were conducted with the species Sorghum halepense (L.), Lathyrus tuberosus (L.), Convolvulus arvensis (L.), Trifolium subterraneum (L.) and Calopogonium mucunoides (L.). Except for C. mucunoides, all of the test plants were cultivated in greenhouses (17 - 22 °C, 50 - 70 % relative humidity, 15 hours daylight, with additional lighting when required). C. mucunoides was cultivated in the greenhouse of the Institute under tropical conditions (20 - 25 °C, 12 hours additional lighting, 60 - 70 % humidity). As soon as the cotyledons had formed, the seedlings were transferred into water jars containing nutrient solution at half ionic concentration; 3 days later they were further cultivated in a nutrient solution at full ionic concentration.
(Composition of nutrient solution per 1000 ml: 1 330 mg $Ca(No_3)_2$ x 4 H_2O; 200 mg KNO_3; 330 mg KH_2PO_4; 170 mg $MgSO_4$ x 7 H_2O; 20.00 mg $MnCl_2$ x 4 H_2O; 29.00 mg H_3BO_3; 50.00 mg Fe-EDTA (SEQUESTREN 138 Fe); 0.22 mg $ZnSO_4$ x 7 H_2O;; 0.08 mg $CuSO_4$ x 5 H_2O; 0.03 mg Na_2MoO_4 x 2 H_2O.)

Radioactive substance

The trials were conducted with ^{14}C-glufosinate-ammonium (Hoe 039866 - ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate-(1,2-^{14}C)) manufactured by Hoechst AG, Frankfurt am Main (spec. activity 24.36/uCi/mg, 4.87 mCi/mM, 901 mBq/mg)

$$\left[CH_3 - \overset{\overset{O}{\|}}{\underset{\underset{O^{\ominus}}{|}}{P}} - CH_2^* - CH_2^* - \underset{\underset{NH_2}{|}}{CH} - COOH \right] NH_4^{\oplus}$$

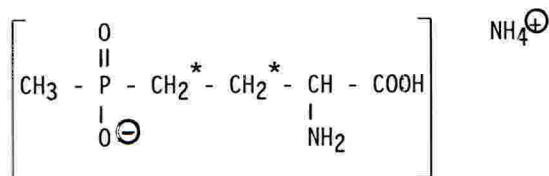Fig. 1. Structural formula of glufosinate-ammonium

Application

In the translocation studies, foliar treatment of each plant was conducted with 0.5 /uCi in 10 /ul aqueous solution. With reference to active substance content, this amounted to a 0.2 % solution of glufosinate-ammonium. In the metabolism studies with S. halepense, C. arvensis and T. subterraneum, the application rate per plant was 1 /uCi in 20 /ul aqueous solution. The weeds were treated as follows: S. halepense (4 - 5 leaf stage), lower third of second leaf; T. subterraneum (2 - 3 leaf-triplet stage) and C. arvensis (5 -6 leaf stage), first foliage leaf; L. tuberosus (4 -5 leaf stage), second pair of foliage leaves; and C. muconoides (1 - 2 leaf-triplet stage), middle leaf of first leaf-triplet.

Exposure period

The exposure time was 3 days.

Harvesting and processing of test material

For the translocation studies, the plants were placed after harvesting in a climatic cabinet with circulating air at 95 °C and allowed to dry for 2 hours. The treated leaf in each case had been separated from the leaf-stem. After the dry weight had been determined, the plant samples were

combusted in a Packard TRI-CARB 306; the radioactivity was measured with a Packard TRI-CARB 3380 liquid scintillation spectrometer (Packard, Downers Grove, Ill., U.S.A.). For the metabolism studies, the plants were separated into shoots and roots, and the green weights of each were determined. The plant material was then briefly rinsed with methanol and distilled water.

Extraction
    The homogenised plant material was first extracted three times with ethyl acetate, after which the same process was repeated with methanol, methanol/water (1 : 1), and distilled water. The centrifugates obtained by each extraction medium were evaporated to dryness and finally dissolved in the same medium.

Thin layer chromatography (TLC)
    The extracts were separated in a mixture of 1-pentanol, formic acid and water (48.8/48.8/2.4; v/v/v), on TLC plates manufactured by Merck, Darmstadt, FRG (silica gel 60 F_{254}, thickness of layer 0.25 mm). The TLC plates were evaluated with an LB 2821 TLC LINEAR ANALYSER (manufactured by Berthold, Wildbad, FRG).
Reference substances for identification
    Glufosinate-ammonium (Hoe 039866) and 3-methyl-phosphinico propionic acid (Hoe 061517) were identified with ^{14}C-labelled reference substances provided by Hoechst AG.

RESULTS AND DISCUSSION

Translocation
    S. halepense, T. subterraneum, C. arvensis, L. tuberosus and C. mucunoides were investigated in order to establish how much radioactive substance is translocated from the treated leaf to the other parts of the shoots and to the root system. Three days after application, the radioactive substance exported from the treated leaves amounted to 2.0 % in Calopogonium mucunoides, 2.2 % in Lathyrus tuberosus, 2.5 % in Convolvulus arvensis, 3.3 % in Trifolium subterraneum, and 4.3 % in Sorghum halepense (Table 1).

Taking the total radioactive substance exported from the leaf as 100 %, the major portion of this, i.e. 64.1 % to 88.7 %, was in the remaining parts of the shoot, except for S. halepense, where it was only 48.1 %. Thus ^{14}C-glufosinate-ammonium applied via the leaf is easily taken up by all 5 plant species. However, marginal exportation from the treated leaf and basipetal translocation down to the root system also took place. Transportation was via the phloem. Thus the translocation of glufosinate-ammonium in plants is partly symplasmatic and partly apoplasmatic. Many other herbicides, e.g. amitrol, are also transported in this way (Crafts and Yamaguchi 1964; Müller 1976). However, transportation via the phloem represents only a minor part of total translocation, a fact which accounts for the limited transportation to the subterranean parts of the plants. In the case of the easily translocatable glyphosate, as much as 33 % of the radioactivity applied e.g. to S. halepense had been transported to other parts of the plant after 6 days (Lolas and Coble 1980). But 3 days after application of glyphosate, only 3.5 % of the applied radioactive substance had been translocated to the untreated parts of C. arvensis (Sandberg et al. 1980).

Taking into account the different plant sizes, S. halepense and T. subterraneum showed the highest concentrations (1 200 and 1 160 dpm/mg dry weight respectively). C. arvensis and L. tuberosus, with 590 and 500 dpm/mg respectively, occupied an intermediate position (Table 1). Based on the

different concentrations of radioactive substance in the roots, it is reasonable to assume that the sensitivity of the studied plant species increased in the following order: C. mucunoides, L. tuberosus, C. arvense, T. subterraneum and S. halepense.

TABLE 1

Translocation of radioactive substance in S. halepense, T. subterraneum, C. arvensis, L. tuberosus and C. mucunoides after foliar treatment with ^{14}C-glufosinate-ammonium. Applied radioactive substance: 0.5 /uCi/plant. Exposure time 3 days. Mean values from 6 replicates. Readings: dpm and %.

Plant species	^{14}C-substance exported from treated leaf [dpm] (%)	^{14}C-substance in shoots (dpm)	roots (dpm)	^{14}C-substance in shoots (dpm/mg dry wt.)	roots (dpm/mg dry wt.)
S. halepense	38 160 4.3 100 %	18 360 48.1 %	19 800 51.9 %	690	1 200
T. subterran.	36 220 3.3 100 %	24 580 67.9 %	11 640 32.1 %	530	1 160
C. arvensis	26 650 2.5 100 %	17 090 64.1 %	9 560 35.9 %	310	590
L. tuberosus	23 190 2.2 100 %	14 880 64.2 %	8 310 35.8 %	680	500
C. mucunoides	16 760 2.0 100 %	14 870 88.7 %	1 890 11.3 %	380	280

Metabolism

The metabolism of ^{14}C-glufosinate-ammonium was studied in the three plant species S. halepense, T. subterraneum and C. arvensis. The principal interest here was to determine how much unchanged substance reached the roots by basipetal transportation. For this purpose, the plant material after foliar treatment and 3 days exposure time was divided into roots and shoots and extracted with various solvents, ranging from hydrophobic to highly hydrophilic. The results are set out in Table 2.

Table 2 clearly shows a correlation between the total radioactivity measured in dpm/g fresh weight in the shoots and the sensitivity of the three plant species to glufosinate-ammonium. Thus the sensitive species S. halepense, with 4 732 550 dpm/g fresh weight, showed the highest concentration, compared with only 4 162 660 dpm/g fresh weight in the relatively tolerant C. arvensis. T. subterraneum occupied an intermediate position with 4 554 700 dpm/g fresh weight. As in the shoots, the total concentration in the roots also showed a correlation with plant susceptibility to the substance. Thus the highest concentration was 409 830 dpm/g fresh weight in S. halepense, and the lowest 114 840 dpm/g fresh weight in C. arvensis. T. subterraneum was in between, with 192 690 dpm/g fresh weight.

Most of the radioactive substance was extractable from the various parts of the plant with ethyl acetate, methanol, methanol/water, and distilled water. The insoluble portion of the total radioactivity was only 1.3 - 2.7 % in the shoots, and 0.6 - 3.5 % in the roots.

TABLE 2

Extractability of radioactive substances from shoots and roots of S. halepense, T. subterraneum and C. arvensis after foliar treatment with ^{14}C-glufosinate-ammonium (extraction with ethyl acetate, methanol, methanol/water, and distilled water. Exposure time: 3 days. Applied radioactive substance: 30 /uCi. Readings: dpm/g fresh weight and %.

Plant	Total radio-activity (dpm)	^{14}C-labelled substances in extracts from shoots				
		Ethyl acetate (dpm)	Methanol (dpm)	Methanol/ water (dpm)	distilled water (dpm)	non-extractable residues (dpm)
S. halepense (4-5 leaf stage)	4 732 550 100 %	47 330 1.0 %	4 273 490 90.3 %	298 150 6.3 %	52 060 1.1 %	61 520 1.3 %
T. subterraneum (2-3 leaf stage)	4 554 700 100 %	22 770 0.5 %	3 803 180 83.5 %	573 890 12.6 %	31 880 0.7 %	122 980 2.7 %
C. arvensis (5-6 leaf stage)	4 162 660 100 %	37 460 0.9 %	3 700 610 88.9 %	283 060 6.8 %	41 630 1.0 %	99 900 2.4 %

Plant	Total radio-activity (dpm)	^{14}C-labelled substances in extracts from roots				
		Ethyl acetate (dpm)	Methanol (dpm)	Methanol/ water (dpm)	distilled water (dpm)	non-extractable residues (dpm)
S. halepense (4-5 leaf stage)	409 830 100 %	20 900 5.1 %	316 390 77.2 %	55 740 13.6 %	14 340 3.5 %	2 460 0.6 %
T. subterraneum (2-3 leaf stage)	192 690 100 %	12 900 6.7 %	150 520 78.1 %	18 290 9.5 %	4 240 2.2 %	6 740 3.5 %
C. arvensis (5-6 leaf stage)	114 840 100 %	7 810 6.8 %	91 070 79.3 %	9 760 8.5 %	2 640 2.3 %	3 560 3.1 %

Table 3 shows the quantities of radioactivity in the separate extracts and the quantities of radioactive substances in the TLC extracts obtained with methanol and methanol/water from shoots and roots.

The metabolisation of glufosinate-ammonium in the shoots proceeded fairly slowly in all three species. The quantities of glufosinate-ammonium in the shoot material after 3 days exposure were 84.1 % in S. halepense, 82.4 % in T. subterraneum, and 81.8 % in C. arvensis. The portions of unchanged active ingredient were rather lower in the roots. A possible reason for this was that metabolites are more readily transported from the shoot to the roots. As compared with this, approximately the same amount of the systemic herbicide active ingredient glyphosate (82 %) was present after 3 days in weeds such as Convolvulus arvensis, Convolvulus sepium, Cirsium arvensis and Polygonum amphibium (Sandberg et al. 1980).

In the parts of the various plant species investigated in this study, glufosinate-ammonium is metabolised to 3-methyl-phosphinico-propionic acid (Rf value 0.34) and to a very minor extent to other compounds, ranging from hydrophilic to fairly hydrophobic, with Rf values of 0.03, 0.55 and 0.77 (Table 3). According to Götz et al., glufosinate-ammonium is degraded in the plant to 3-methyl-phosphinico-propionic acid, which has no phytotoxic effect.

It becomes possible to form an exact picture of the distribution of radioactive substance in the treated plants only if the various compounds extracted from shoots and roots separately are considered together with the degree of their extractability in the different solvents, based on the data given in Table 3. It emerges that the greatest portion of unchanged active ingredient reaching the roots by basipetal translocation was 6.6 % in the most susceptible species, S. halepense. The portion was 3.3 % glufosinate-ammonium in the less susceptible T. subterraneum, and only 1.2 % in the moderately susceptible C. arvensis.

It is probable that the different sensitivity of the three plant species is due not only to the varying intensity of basipetal transportation, but also to these active ingredient levels.

Additional HPLC separations of the plant extracts confirmed the TLC findings concerning the sensitivity of the three weed species.

TABLE 3

Quantities of radioactive substances after application of ^{14}C-glufosinate-ammonium to shoots of S. halepense, T. subterraneum and C. arvensis; TLC-separation of shoot and root extracts after a 3-day exposure time.
Readings: dpm/g fresh weight and %. Solvent system: 1-pentanol/formic acid/water (48.8/48.8/2.4; v/v/v/)

Substance	S. halepense shoots (dpm)	roots	T. subterraneum shoots (dpm)	roots	C. arvensis shoots (dpm)	roots
Ethyl acetate extract *	47 330 1.0 %	20 900 5.1 %	22 770 0.5 %	12 900 6.7 %	37 460 0.9 %	7 810 6.8 %
Methanol and methanol/water extracts:						
Radioactivity at origin (Rf value 0.03)	17 590 0.4 %	-	-	-	7 080 0.2 %	-
Glufosinate-ammonium (Rf value 0.19)	3 981 500 84.1 %	338 270 82.5 %	3 751 310 82.4 %	155 470 80.7 %	3 404 970 81.8 %	91 100 79.3 %
3-methyl-phos-phinico-pro-pionic acid (Rf value 0.34)	559 730 11.8 %	31 900 7.8 %	625 770 13.7 %	13 340 6.9 %	565 120 13.6 %	8 240 7.2 %
Substance 4 (Rf value 0.55)	-	-	-	-	6 510 0.2 %	950 0.8 %
Substance 5 (Rf value 0.77)	12 820 0.3 %	1 950 0.5 %	-	-	-	540 0.5 %
Water extract *	52 060 1.1 %	14 340 3.5 %	31 880 0.7 %	4 240 2.2 %	41 630 1.0 %	2 640 2.3 %
Non-extractable residue	61 520 1.3 %	2 460 0.6 %	122 980 2.7 %	6 740 3.5 %	99 900 2.4 %	3 560 3.1 %
Total radio-activity	4 732 550 100 %	409 820 100 %	4 554 710 100 %	192 690 100 %	4 162 670 100 %	114 840 100 %

* could not be detected, due to the lower amount of radioactivity

REFERENCES

Bayer, E.; Gugel, K.H.; Hägele, K.; Hagenmeier, H.; Jessipow, S.; König, W.A.; Zähner, H. (1972) 25 Stoffwechselprodukte von Mikroorganismen. 98. Mitteilung (1), Phosphinothricin und Phosphinothricyl-Alanyl-Alanin. Helv. Chim. Acta 55, 224 - 239.

Crafts, A.S.; Yamaguchi, S. (1964) The autoradiography of plant material. Calif. Agr. Expt. Sta. Extension Serv. Manual 35.

Götz, W.; Dorn, E.; Ebert, E.; Leist, K.-H.; Köcher, H. (1983) Hoe 039866, a new non-selective herbicide: Chemical and toxicological properties - mode of action and metabolism. Proc. Asia Pacific Weed Sci. Soc. 9th Conf., Manila, 401 - 404.

Gugel, K.-H. (1971) Isolierung und Wirkungsweise von Phosphinothricyl-Alanyl-Alanin, ein Antibiotikum aus Streptomyces viridochromogenes. Diss. Tübingen.

Haas, P. (1986) Verhalten von Glufosinate-ammonium in Unkräutern. Diss. Hohenheim.

Hoechst AG (1982) Basta (Glufosinate) - Produktinformation. Hoechst AG, Frankfurt/M., FRG.

Lolas, P.C.; Coble, H.D. (1980) Translocation of ^{14}C-glyphosate in Johnson grass (Sorghum halepense L. Pers.) as affected by growth stage and rhizome length. Weed Res. 20, 267 - 270.

Müller, F. (1976) Translokation von ^{14}C-markiertem MCPA in verschiedenen Entwicklungsstadien mehrjähriger Unkräuter. Acta Phytomedica, Heft 4, Verlag Paul Parey, Berlin, Hamburg, 160 S.

Sandberg, C.L.; Meggitt, W.F.; Penner, D. (1980) Absorption, translocation and metabolism of ^{14}C-glyphosate in several weed species. Weed Res. 20, 195 - 200.

MODE OF CROP TOLERANCE TO PYRIDATE IN CORN AND PEANUTS

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ABSTRACT

Pyridate,   O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-car-
bonothioate,   (formerly coded CL 11344), is a new herbicide
from the chemical group of the phenylpyridazines developed
by Chemie Linz /Austria for post-emergence control of
weeds.
Pyridate undergoes rapid hydrolysis after penetration into
the plant yielding 6-chloro-3-phenyl-pyridazine-4-ol (coded
CL 9673) which has been established as the herbicidal
active principle by inhibiting photosynthetic electron
transport of photosystem II. Crop tolerance in corn and
peanuts is based on metabolic inactivation of CL 9673.
Metabolic conversion into conjugates of N- and O-glucosidic
types are found to be the first detoxification steps.
Formation of N-conjugates was discussed to be a key-reac-
tion for subsequent substitution of chlorine by compounds
with sulfhydryl groups. No interference of CL 9673-con-
jugates of corn and peanuts with the Hill reaction using
isolated broken spinach chloroplasts was found indicating
that inactivation by conjugation is the basis for selecti-
vity.

INTRODUCTION

Pyridate,   O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-car-
bonothioate,   (formerly coded CL 11344), is a new herbicide from
the chemical group of the phenylpyridazines developed by Chemie
Linz, Austria, for post-emergence control of weeds in corn,
peanuts, wheat, alfalfa and cole crops and is marketed under the
registered trade names 'LENTAGRAN' and 'TOUGH' (Figure 1).

Figure 1. Structural formula
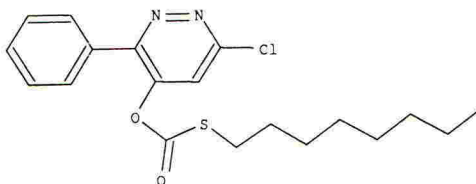
Herbicidal properties of pyridate were first described by Diskus _et al._, 1976. Pyridate penetrates easily into the plant tissue due to the lipophilic property of the ester component. After penetration pyridate undergoes rapid hydrolysis yielding 6-chloro-3-phenyl-pyridazine-4-ol (coded CL 9673) which has been established as the herbicidally active principle. Pyridate was shown to be extremely safe for corn and peanuts when used in postemergence weed control. This report outlines the mode of action of pyridate and the mechanism of metabolic detoxification of CL 9673 in corn and peanuts.

MATERIALS AND METHODS

Photosynthesis measurements

The time course of photosynthetic rates of pyridate treated leaves of corn and peanuts (tolerant plants) and of cleavers (_Galium aparine_, sensitive plant) was measured over a period of four days. Fully developed leaves were treated at intervals of 4, 2, and 1 days and 8, 6, 4, 2, 1 and 0,5 hours by brushing 0,25 % a.i. aqueous pyridate suspension (formulated as wettable powder) on the upper leaf surface. Treated plants were kept under the normal day-night rhythm in a green house.

The photosynthetic rates were evaluated on excised entire leaves of peanuts and cleavers or leaf discs (corn, area approximately 5 cm^2) measuring the uptake of gaseous ^{14}C-carbon dioxide for 30 minutes in a plexiglass box (volume 1 L) under light from fluorescent tubes (distance 20 cm). ^{14}C-radiolabelled carbon dioxide was liberated from 10 μCi sodium-(^{14}C)-bicarbonate solution (specific activity 0,1 mCi/mM) with dilute sulphuric acid. Fixation of ^{14}C-carbon dioxide was terminated by aeration and by shock heating of the leaf discs at 80 °C. Leaves were dried and oxidized in a sample oxidizer (mod. TRI CARB 306, Packard Instr., U.S.A.). Radioactivity was trapped in CARBOSORB (Packard Instr., U.S.A.) and measured in a PERMAFLUOR V cocktail (Packard Instr., U.S.A.) in a liquid scintillation counter (mod. TRI CARB 300 C, Packard Instr., U.S.A.).

Metabolic conversion of ^{14}C-pyridate in leaves of corn and peanuts.

^{14}C-pyridate (^{14}C-labelled in the 4,5-position of the pyridazine ring) was synthesized by A. Zohner, Chemie Linz with a spec. activity of 5,07 mCi/mM and a radiochemical purity $\geq$ 98 % determined by thin layer chromatography.

Corn (5-leaf stage) and peanuts plants (12 cm diameter) were treated with ^{14}C-pyridate by spraying an aqueous suspension (0,25 % a.i.) of a wettable powder formulation (application rate corresponding to approximately 1,8 kg a.i. /ha). Leaves samples were taken dayly and extracted with cold acetone and acetone/water. Extracts were chromatographed without further

cleaning by thin layer chromatography (tlc) on silicagel 60 plates with a fluorescence indicator in the solvents acetone/water (9:1). The radioactive spots were located and evaluated with a linear analyzer (mod. LB 2842, Ludwig Berthold, FRG). Radioactive spots on the tlc-plates were identified by co-chromatography of standards.

Measurements of photosynthetic electron flow

Metabolites of ^{14}C-pyridate were tested for their ability to inhibit the Hill reaction of isolated broken spinach chloroplasts (thylakoids). The test followed a procedure described by Kovac et al. 1976 and Zohner et al.,1977, using a spray technique with a Hill reagent on freshly developed thin layer chromatograms.

Preparation of the spraying solution:

Chloroplasts were isolated from spinach leaves according a normal procedure (Jacobi et al., 1974). Broken chloroplasts were resuspended in a 10 % glycerol solution at a chlorophyll concentration of about 30 mg/ml (Solution 1). This suspension was prepared and stored at a temperature between 0 - 4 °C.

The Hill reagent solution (Solution 2) was prepared by dissolving 40 mg of 2,6-dichlorophenol indophenol (DPIP, Sigma, FRG) in 100 ml of a 66 mM buffer solution of sodium potassium phosphate of pH 6,5.

Procedure for the Hill reaction test:

Acetone extracts of ^{14}C-pyridate treated leaves were chromatographed on silicagel 60 thin layer plates 20 x 20 cm in the solvents chloroform/methanol (6:4) and dried at room temperature. To avoid unspecific interferences with the Hill reagent, the plates were pre-developed in the same solvents and dried before use. The plates were then sprayed with a mixture of chloroplast homogenate (1 volume of Solution 1) and DPIP solution (2 volumes of Solution 2), covered with a glass plate and exposed to the light of fluorescent tubes at a distance of 20 cm until the blue background color turns to yellow-green due to the photoreduction of DPIP by the chloroplasts (= Hill reaction). Compounds which inhibit the photosynthetic electron flow were visualized by the blue colour remaining on the sprayed plate after exposure to light.

Pyridate and CL 9673-conjugates could be also visualized by the Hill reaction test if the developed plates were heated at 150 °C for 30 minutes. By this procedure, pyridate and conjugates of CL 9673 were decomposed partly to free CL 9673 which inhibited the Hill reaction.

RESULTS AND DISCUSSION

The photosynthetic rate of pyridate treated leaves of peanuts dropped to approx. 80 % within 3 hours and recovered fully within 8 hours. In treated leaves of corn the rate dropped below 5 %, recovered slowly and reached approx. 80 % after 4 days indicating a less powerfull detoxification capacity than that found in peanuts. The photosynthesis of treated leaves of cleavers was inhibited totally (more than 98%) and irreversbly (Figure 2).
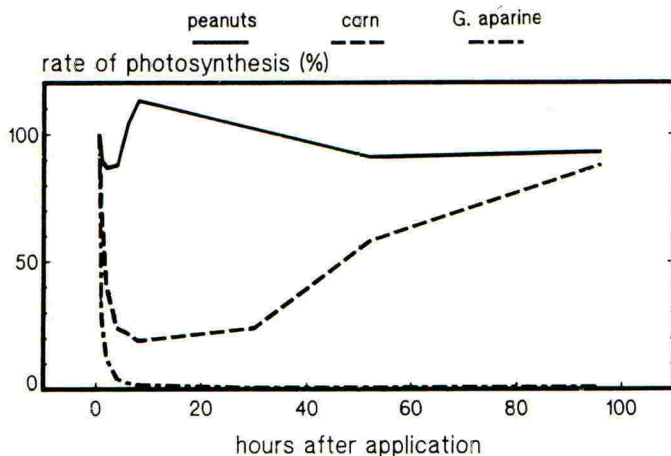

Figure 2. Time course of the photosynthetic rates of leaves of peanuts, corn and cleavers (<u>Galium</u> <u>aparine</u>) after treatment with pyridate.

<u>Time</u> <u>course</u> <u>of</u> <u>metabolic</u> <u>conversion</u> <u>of</u> ^{14}C-pyridate <u>in</u> <u>leaves</u> <u>of</u> <u>peanuts</u> <u>and</u> <u>corn</u>

Fast hydrolization of ^{14}C-pyridate to ^{14}C-CL 9673 was the first metabolic step. Low intermediate concentrations of free ^{14}C-CL 9673 were found in both, peanuts and corn. From the photosynthetic measurements discussed above, it could be derived that concentrations of free CL 9673 in the leaf tissue were too low to inhibit photosynthesis. In leaves of peanuts C-CL 9673 was detoxified mainly to ^{14}C-CL 9673-N-glycoside (Figure 3). In leaves of corn only small amounts of C-CL 9673-N-glycoside and ^{14}C-CL 9673-O-glycoside but higher amounts of more polar ^{14}C-metabolites were detectable at lower Rf-values (Figure 4). Hydrolization of these more polar metabolites did not yield free ^{14}C-CL 9673.
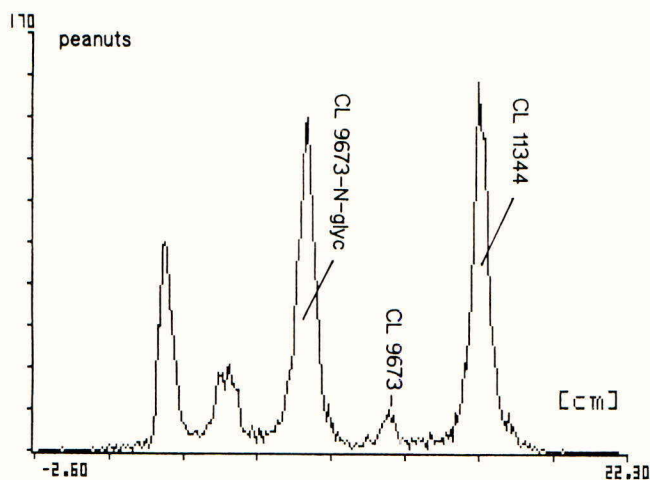
Figure 3. Metabolization of ^{14}C-pyridate in peanuts (3 days after application; tlc-scanning, silicagel; solvents: chloroform/methanol, 10 : 4)
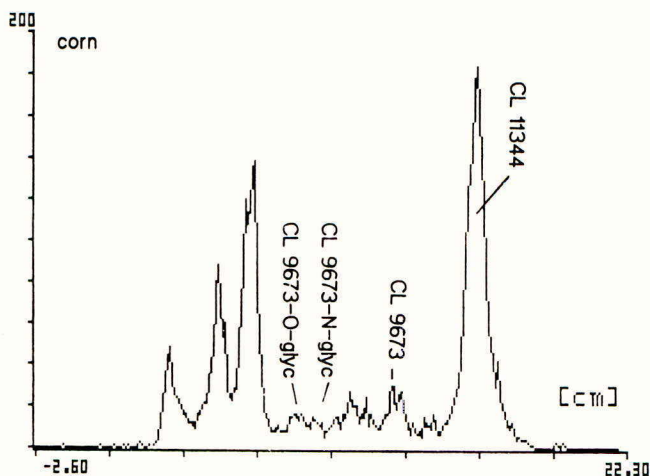

Figure 4. Metabolization of ^{14}C-pyridate in corn (6 days after application; tlc-scanning, silicagel; solvents: chloroform/methanol, 10 : 4)

Investigations of the detoxification mechanism of atrazine in corn leaves have shown that the key step for detoxification

is a conjugation reaction with glutathione by substitution of the chlorine (Lamoureux, G.L. et al.; 1972). It was found by chemical synthesis that CL 9673-N-glucoside reacted readily with SH-compounds like glutathione or cysteine by substitution of the chlorine whereas free CL 9673 and CL 9673-O-glucoside showed no reaction (unpublished). N-glycosylation of CL 9673 appears to be a necessary metabolic step which activates the pyridazine ring for conjugation reactions by substitution of chlorine. Assuming a same reactivity in vivo, the following metabolic pathway for detoxification of pyridate in corn and peanuts is proposed (Figure 5):
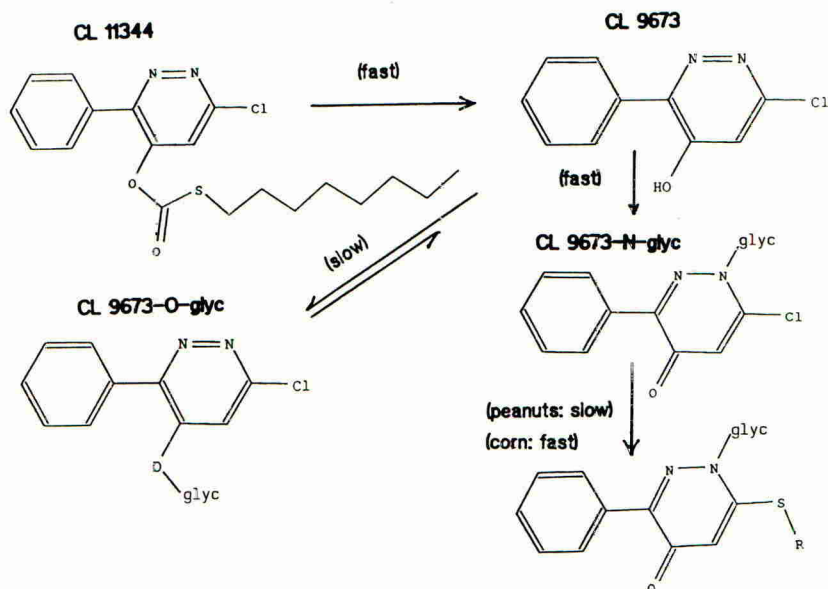
Hydrolysis and formation of CL 9673-N-glucoside are the common first metabolic steps. Formation of CL 9673-O-glycoside is not relevant for detoxification in corn and peanuts. CL 9673-N-glycoside is metabolized then to highly polar conjugates, but with low rates for peanuts and high rates for corn. The low intermediate concentrations of CL 9673-N-glycoside in corn could be explained by a fast conjugation with glutathione or other SH-compounds by substitution of the chlorine.

Interference of pyridate metabolites with the Hill reaction.
     The phytotoxicity of the main metabolites of pyridate was
investigated on broken spinach chloroplasts by studying the Hill
reaction on thin layer chromatograms of leaf extracts of peanuts
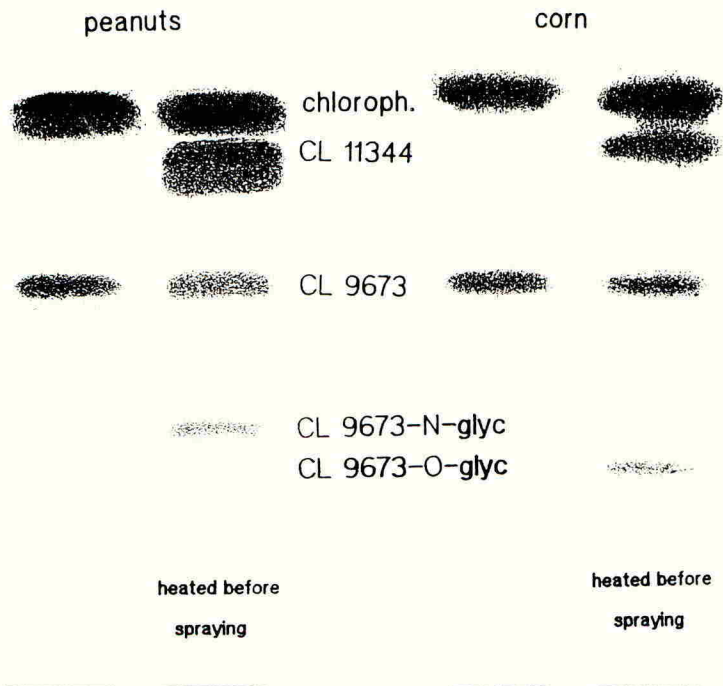and corn (Figure 6).

peanuts   corn

chloroph.

CL 11344

CL 9673

CL 9673-N-glyc
CL 9673-O-glyc

heated before   heated before

spraying   spraying

Figure   6.   Interference of pyridate metabolites of peanuts (3
    days after treatment) and corn (6 days after treatment)
    with the Hill reaction on spinach chloroplasts
    (visible spots indicate inhibition of the Hill reaction;
    tlc: silicagel; solvent: chloroform/methanol, 10:4)

     CL 9673, was the only compound which inhibited the photo-
syntetic electron flow of the chloroplasts (the spot with the
highest Rf-value belongs to extracted chlorophyll). Pyridate by
itself and the metabolites of CL 9673 showed no inhibition of
the Hill reaction. The nature of the CL 9673-metabolites were
investigated by studying their interference with the Hill reac-
tion after cleavage on heated chromatograms. Additionally to

CL 9673 also pyridate and CL 9673-N-glucoside became visible in peanuts. Pyridate and even the small amounts of CL 9673-O-glycoside became visible in corn by this method (CL 9673-N-glycoside decomposed not as compleately as CL 9673-O-glycoside and was not detectable by the Hill reaction at low concentrations after heating). The polar metabolites with Rf-values below that of CL-9673-N-glucoside showed no inhibition of the Hill reaction after heating, indicating that these metabolites could not be decomposed to CL 9673.

CONCLUSION

Due to the lipophilic property of the ester component, treated leaves absorb pyridate readily. In the plant pyridate hydrolyzes quickly to a phenolic compound (CL 9673) which has been established as the herbicidally active metabolite. The mode of action of CL 9673 is based on the inhibition of the photosynthetic electron transport. Pyridate tolerant plants such as peanuts and corn detoxify CL 9673 into conjugates. In peanuts a one-step detoxification forming CL 9673-N-glycoside was found. In corn a two-step mechanism of further conjugation of CL 9673-N-glycoside with glutathione is proposed. Formation of CL 9673-N-glycoside was found to be an essential metabolic step for substitution of chlorine by endogenous SH-compounds.

REFERENCES

Diskus, A.; Schoenbeck, R.; Auer, E.; Kloimstein, E. (1976) CL 11.344 - a new selective herbicide for use in cereals and maize. Proceedings 1976 British Crop Protection Conference - Weeds 2, 717 - 722.

Jacobi, G.; et al; Biochemische Cytologie der Pflanzenzelle, ein Practicum, (1974), 72 - 108.

Kovác, J,; Henselová, M.(1976) A rapid method for detection of Hill rection inhibitors. Photosynthetica 10, 343 - 344.

Lamoureux, G.L.; Stafford, L.E.; Shimabukuro, R.H. (1972) Conjugation of 2-chloro-4,6- bis (alkylanine) - s-triazines in higher plants. Journal of Agricultural and Food Chemistry 20, 1004 - 1010.

Zohner, A.; Bayzer, H. (1977) Interference of the herbicides buthidazole and methazole with the Hill reaction of isolated chloroplasts. Proceedings 11th Int. Velsicol Symp. (1977).

THE MODE OF ACTION OF THE HERBICIDAL QUINOLINECARBOXYLIC ACID, QUINMERAC[1] (BAS 518 H)

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ABSTRACT

Quinmerac (BAS 518 H, 7-chloro-3-methyl-quinoline-8-carboxylic acid) is a new experimental herbicide for the selective control of cleavers (Galium aparine) in wheat and other crops. Several test systems for the detection of auxin activity have demonstrated the hormone-type character of the quinoline-carboxylic acids. Studies with ^{14}C labelled BAS 518 H revealed that quinmerac is taken up by leaves and roots. It is translocated acro- and basipetally. Further studies demonstrated significant differences between the root growth of wheat and cleavers when these were exposed to quinmerac. These results led to the conclusion that quinmerac controls cleavers by root inhibition and hormone-type effects.

INDRODUCTION

BAS 518 H (quinmerac) (Fig. 1) is a new post-emergence herbicide currently being development by BASF Aktiengesellschaft to control cleavers (Galium aparine), Veronica spp. and Lamium spp. in cereals, rapeseed and sugarbeet. Chemical properties and field trial results are

Fig. 1. Chemical structure of BAS 518 H (quinmerac).

published elsewhere (Wuerzer et al. 1985, Nuyken et al. 1985, Haden & Menck 1986). In this paper results covering uptake, translocation and the mode of action are presented.

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[1]proposed common name

MATERIALS AND METHODS

Auxin tests

The cucumber test was performed as described by Sloan and Camper, 1986. Ethylene biosynthesis was determined using soybean (Glycine max. cv. SRF 450 P) leaf discs treated with herbicide solutions for 24 h in sealed test tubes. Ethylene production was then measured by gas chromatography (Packard, model 419).

Uptake and translocation

Plants were grown in controlled growth chambers (22 °C, 400 $\mu E \cdot m^{-2} s^{-1}$. For uptake studies, ^{14}C BAS 518 H (spec. radioact. 549,5 MBq/mM) was applied with an adjuvant to the second leaf of wheat (Triticum aestivum cv. Caribo) or the first whorl of cleavers (Galium aparine).

Treated leaf samples taken at different time intervals were washed with water/nekanil (0,05 %) to remove residues that had not penetrated. Samples were then combusted in an oxidizer (Zinser, Oxymat, OX 300) and the evolved $^{14}CO_2$ was absorbed in a LS cocktail and radioassayed in a scintillation counter (Packard, Tricarb, 460 CD). Root uptake was investigated in a hydroponic culture. Samples were combusted and treated as described above.

Root growth

Cleavers and wheat seeds were surface sterilized in a 1 % sodium hypochlorite solution for 30 min and then stored at 4 °C until the primary root emerged. Germinated seeds were transfered to Petri dishes lined with filter paper that had been treated with different herbicide solutions and incubated for 4 days at 25 °C. The root length was then measured and compared with that of seeds incubated in distilled water.

Chemicals

All experiments were performed with analytical grade chemicals. 2,4-D, picloram and dicamba were purchased from Riedel de Haen, Seelze, FR-Germany.

RESULTS

Auxin activity

Cleavers plants treated with BAS 518 H were stunted, and their leaves were reduced in size, twisted and dark green. Phytotoxic symptoms resembled morphological changes that occur after the application of hormone-type herbicides such as benzoic acids or some pyridine compounds.

Experiments with cucumber seedlings (Fig. 2 A) revealed that BAS 518 H inhibited root growth to the same extent as 2,4-D, picloram or dicamba. Visual symptoms were also similar. At high herbicide concentrations, the roots were stunted and resembled a plant cell callus.
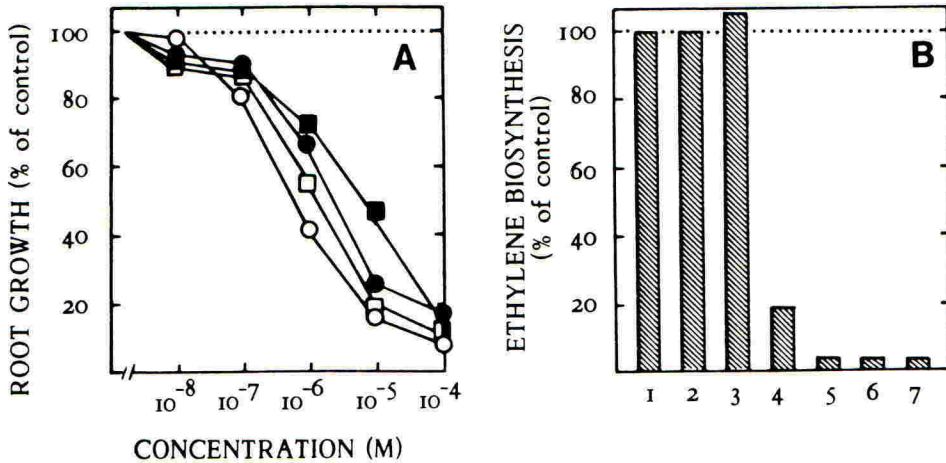

Fig. 2. A: Influence of BAS 518 H (-●-), 2,4-D (-○-), dicamba (-□-) and picloram (-■-) on root growth of cucumber seedlings.
B: Influence of BAS 518 H (4), 2,4-D (1 = control), dicamba (2), picloram (3), sethoxydim (5), bentazon (6) and water (7) on ethylene biosynthesis.

BAS 518 H induced ethylene biosynthesis in soybean leaf discs. Although the activity was lower than that of 2,4-D, dicamba or picloram it nevertheless revealed a distinct auxin activity that was significantly different to that of leaf discs treated with water or herbicides that have a completely different mode of action (Fig. 2 B).

Uptake and translocation

BAS 518 H was rapidly absorbed after a leaf application, particularly under conditions of high humidity and in combination with an adjuvant. Within 24 h, 90 - 100 % of the herbicide had penetrated into the leaf.

In cleavers, the major portion of BAS 518 H was translocated to the green parts of the shoot below the treated leaf during the first 24 h. Similar amounts were transported to the upper parts of the plant and to the root (Fig. 3 A). In wheat, the major part of quinmerac remained in the treated 2nd leaf, and 40 % of the applied substance was transported during the following days mainly to the 3rd and 4th leaf. Only small amounts were found in the roots and the nutrient solution (Fig. 3 B).
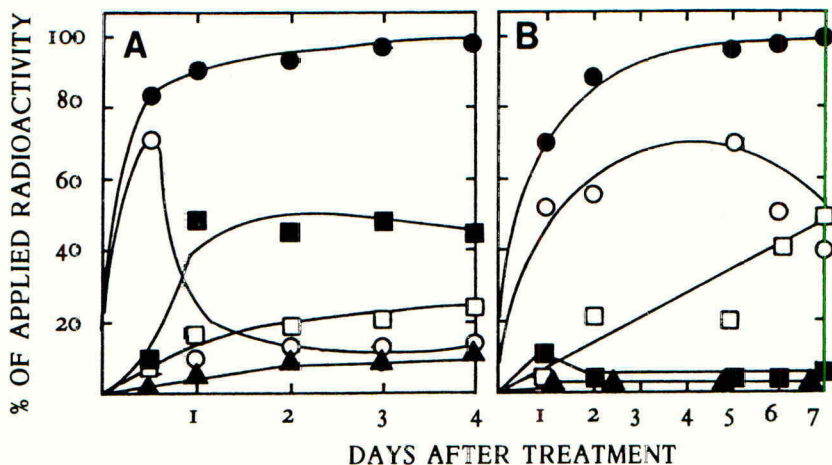
Fig. 3. Uptake and translocation of quinmerac by leaves of cleavers (A) and wheat (B).
- -●- total uptake
- -○- treated leaf
- -■- stem below treated leaf or primary leaf (wheat)
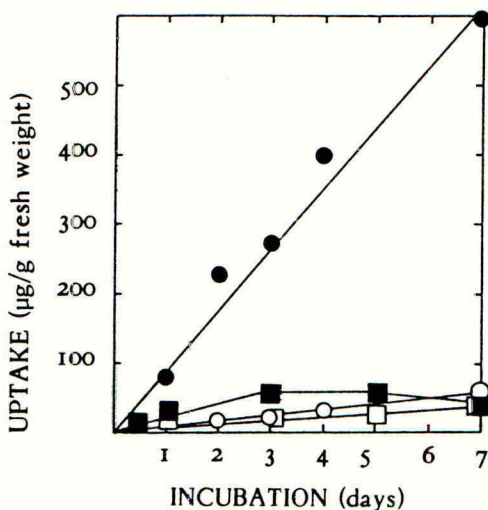- -□- stem above treated leaf or 3rd and 4th leaf (wheat)
- -▲- root and nutrient solution

Fig. 4. Uptake and translocation of BAS 518 H by roots of cleavers and wheat.
- -●- wheat root
- -■- cleavers root
- -○- wheat leaves
- -□- cleavers shoot

BAS 518 H was initially well absorbed by roots of cleavers and wheat (Fig. 4). Within a few hours, a 10 fold concentration of the active compound could be detected in the roots compared with that in the nutrient solution. After 24 hours, the uptake of quinmerac by cleavers roots had stopped. In contrast, wheat accumulated BAS 518 H during the entire incubation period, which resulted in a 200 fold root concentration compared with that of the nutrient solution. On the other hand, similar radioactivity was measured in the green parts of cleavers and wheat. The differences in root uptake could be considered as an expression of varying sensitivity of cleavers and wheat roots to quinmerac (Fig. 5).
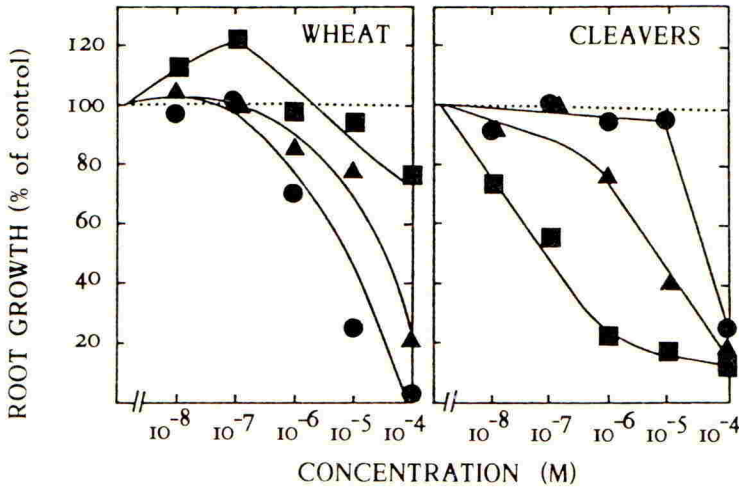
Fig. 5. Root growth of wheat and cleavers seedlings after application of BAS 518 H (-■-), 2,4-D (-●-) and picloram (-▲-).

Whereas wheat roots were almost insensitive to BAS 518 H, the compound was perceptible to cleavers roots at concentrations as low as 10^{-6}M. In contrast, root growth of germinating wheat seedlings was inhibited by 2,4-D and to a lesser extent by picloram. However, both herbicides were only weak inhibitors of cleavers root growth.

DISCUSSION

The visible morphological alterations which followed an application of BAS 518 H, together with the results of auxin test systems clearly demonstrated a quinmerac auxin activity. Consequently, in addition to the pyridine derivatives, benzoic acids and phenoxy compounds, quinoline-carboxylic acids must be regarded as a new form of hormone-type herbicides. In addition, quinolinecarboxylic acids with total different substitution patterns have been known as plant hormones for a long time (SAHASHI 1925, MATSUSHIMA et al. 1973).

The mode of leaf uptake of quinmerac resembled that of fluroxypyr (Sanders & Pallett 1987). Like other weak acids (Lichtner 1984), BAS 518 H was translocated acro- and basipetally. The lower translocation rate of quinmerac in wheat in comparison to cleavers may be due to morphological differences between mono- and dicotyledonous plants (Vanden Born 1966). Hormone-type herbicides are known to lead to an imbalance in the auxin level of the plants, however the exact mechanism for this type of herbicide has not been fully elucidated.

Quinmerac gives excellent control of cleavers and since our test systems have revealed only a rather weak auxin activity, the results indicate an activity at two levels. The high cleavers sensitivity was expressed in a strong inhibition of root growth which in turn disturbed the nutrient and water balance, and caused a cessation of growth or stunting of the plant. Furthermore the auxin activity caused morphological changes by influencing the hormone balance.

Quinmerac is selective in wheat because wheat roots are absolutely insensitive to BAS 518 H and auxin injury is usually weak in monocotyledonous plants. Whether additional metabolic differences between cleavers and wheat may favour the selectivity will be investigated in the future.

REFERENCES

Haden, E.; Menck, B.H. (1986) BAS 518 .. H - ein Spezialherbizid zur Bekämpfung von Galium aparine in Raps, Getreide und Zuckerrüben. Med. Fac. Landbouww. Rijksuniv. Gent, 51/2a, 399 - 408.

Lichtner, F.T. (1984) Phloem transport of xenobiotic chemicals. What's New Plant Physiology, 15, 29 - 32.

Matsushima, G.; Fukumi, H.; Arima, K. (1973) Isolation of Zeanicacid, a natural plant growth-regulator from corn steep liquor and its chemical structure. Agricultural and Biological Chemistry 37, 1865 - 1875.

Nuyken, W.; Haden, E.; Menck, B.H.; Klingenschmitt, D. (1985) BAS 518 H - A new herbicide for weed control in cereals, rapeseed and sugarbeets. Proceedings 1985 British Crop Protection Conference - Weeds. Volume 1, 71 - 76.

Sahashi, Y. (1925) Über das Vorkommen von Di-hydroxy-chinolin-carbonsäure (ß-Säure von U. Suzuke) in der Reiskleie. Biochemische Zeitschrift, 159, 221 - 234.

Sanders, G.E.; Pallett, K.E. (1987) Comparison of the uptake, movement and metabolism of fluroxypyr in Stellaria media and Viola arvensis. Weed Research, 27, 159 - 166.

Sloan, M.E.; Camper N.D. (1986) Effects of alachlor and metolachlor on cucumber seedlings. Environmental and Experimental Botany 26, 1 - 7.

Vanden Born, W.H. (1966) Translocation and persistence of dicamba in canada thistle, tartrary buckwheat, quackgrass and kentucky bluegrass. WSSA Abstracts p. 62.

Wuerzer, B.; Berghaus, R.; Hagen, H.; Kohler, R.-D.; Markert, J. (1985) Characteristics of the new herbicide BAS 518 H. Proceedings 1985 British Crop Protection Conference - Weeds. Vol. 1, 63 - 70.

THE SELECTIVITY OF CLOPYRALID IN SUGAR BEET;   STUDIES ON ETHYLENE EVOLUTION.

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ABSTRACT

Clopyralid induces rapid, concentration-dependent evolution of ethylene from excised scentless mayweed leaves, which precedes the onset of visible epinastic symptoms, and is comparable to that induced by exogenous auxin. However, incubations using inhibitors (AVG and Co^{++}) and a precursor of ethylene biosynthesis (ACC) have shown symptom development to be independent of ethylene production in this experimental system. Incubation of excised sugar beet leaves with clopyralid does not result in increased ethylene production or the development of visual epinastic symptoms after 24 h. However, an intact ethylene biosynthetic pathway does exist in sugar beet since sensitivity to IAA and 2,4-D was demonstrated. These observations indicate that the selectivity of clopyralid is not solely related to differential ethylene evolution, but is more likely to result from differential binding affinity to an "auxin site".

INTRODUCTION

The herbicide clopyralid, a discovery of The Dow Chemical Company, is recommended for selective weed control in Brassica crops and sugar beet. It is particularly active against weeds of the families Compositae and Leguminosae (Dow Technical Information). Recent studies in this laboratory have shown scentless mayweed (Matricaria perforata Merat.) to be highly susceptible to clopyralid whilst sugar beet (Beta vulgaris cv. Salohill) is tolerant. The basis of this selectivity remains obscure, being independent of differential herbicide uptake, translocation or metabolism (Thompson & Cobb, 1986).

Clopyralid is a synthetic pyridine compound which, like certain benzoic acid and phenoxyalkanoic acid derivatives, is described as an "auxin-type" herbicide. This classification arises from the fact that many effects of such herbicides on plant growth and anatomy are similar to those caused by exogenously applied auxin (Haagsma, 1975; Hall et al, 1985). One common symptom is the rapid induction of young stem and leaf epinasty which has been linked to the production of ethylene gas by treated tissues (Baur & Morgan, 1969; Hall et al, 1985). This association is supported by the findings that auxin increases the rate of ethylene production in vegetative tissue (Imaseki, 1981), and that exposure of whole plants to ethylene at very low concentrations can lead to epinastic symptom development (Crocker, 1948). It is now established that auxin-type herbicides stimulate ethylene production in a number of species, and a recent report suggests that enhanced ethylene production following the application of clopyralid to sunflower (Helianthus annus) is a factor involved in resulting symptom development (Hall et al, 1985). These authors also cite differential ethylene production in clopyralid-tolerant and susceptible species, although no inference is made of the significance of this difference in terms of a possible contribution to clopyralid selectivity.

This paper describes the measurement of ethylene production in response to clopyralid application in excised leaves from tolerant sugar beet and

susceptible mayweed. Its relationship to symptom development and selectivity has also been assessed with reference to our current understanding of ethylene biosynthesis. Ethylene is produced from methionine via intermediates S-adenosylmethionine (SAM) and 1-aminocyclopropane carboxylic acid (ACC) (Yang & Hoffman, 1984). The conversion of SAM to ACC is catalysed by the enzyme ACC synthase and forms the rate-limiting step in the pathway. The production of ACC synthase is auxin sensitive and the enzyme is specifically inhibited by aminoethoxyvinylglycine (AVG) (Imaseki, 1981). The conversion of ACC to ethylene is catalysed by an enzyme complex referred to as the ethylene forming enzyme (EFE) and is not rate-limiting. This final reaction step may be inhibited by cobalt ions (Yang, 1981).

MATERIALS AND METHODS

Plant Material

Plants were propagated in J. Arthur Bowers seed and potting compost in 5 cm deep trays. Sugar beet seeds were sparsely sown at a depth of 5 mm, whilst mayweed seeds were surface sown. Trays were maintained under glasshouse conditions with a 14h day (200-400 μmole photons/m^2/s, photosynthetic photon flux density, PPFD) and 20-25°C. In all experiments plants were used at the three to four leaf stage.

Chemicals

All test solutions were first dissolved in methanol (final concentration (<0.2%), and diluted with 10^{-3} M MES (2-[N-Morpholino]-ethane sulfonic acid) buffer adjusted to pH6 with NaOH. AVG, ACC, 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-acetic acid (IAA) were obtained from the Sigma Chemical Company. Cobaltous chloride was obtained from BDH chemicals. Technical grade clopyralid was a gift from The Dow Chemical company.

Incubation system

For consistency with previous work (Thompson & Cobb, 1986), leaf 3 of both species was used. Following excision at the stem, leaves were exposed to test chemicals in gas tight vials as illustrated in figure 1.
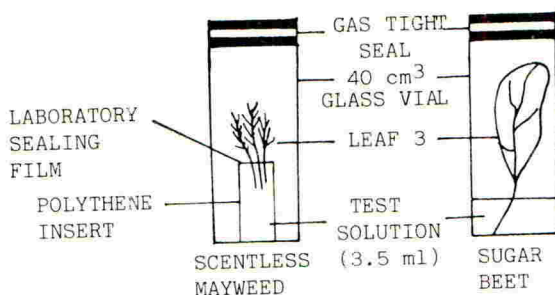

Fig. 1. Experimental system for measurement of ethylene production by excised leaves.

The vials were incubated in a controlled temperature water bath at 25°C and illuminated at 50 μmoles photons/m^2/s (PPFD) using natural white fluorescent tubes (Phillips). One cm^3 gas samples were withdrawn at regular intervals

using a gas-tight syringe. Ethylene concentration was then determined in a gas chromatograph (Sigma 3B-Perkin Elmer) equipped with a Poropack Q. 80-100 mesh column (Perkin Elmer) and a flame ionisation detector. Oven temperature was maintained at $100°C$. A laboratory computing integrator (Perkin Elmer LC1-100) was used to calculate the amount of ethylene in each sample with reference to appropriate standards. After each withdrawal, gas volumes in each vial were amended with ambient air and results adjusted to account for dilution during sampling. Data are expressed on a fresh weight basis and are corrected, where necessary, for any ethylene present in blank tubes. Epinastic symptom development was both visually assessed and recorded photographically. Each experiment was fully replicated on 3 or 4 separate occasions and mean values plus or minus standard errors are presented.

RESULTS

Initial studies clearly demonstrated a differential ethylene response in clopyralid - susceptible and tolerant species (Figure 2).
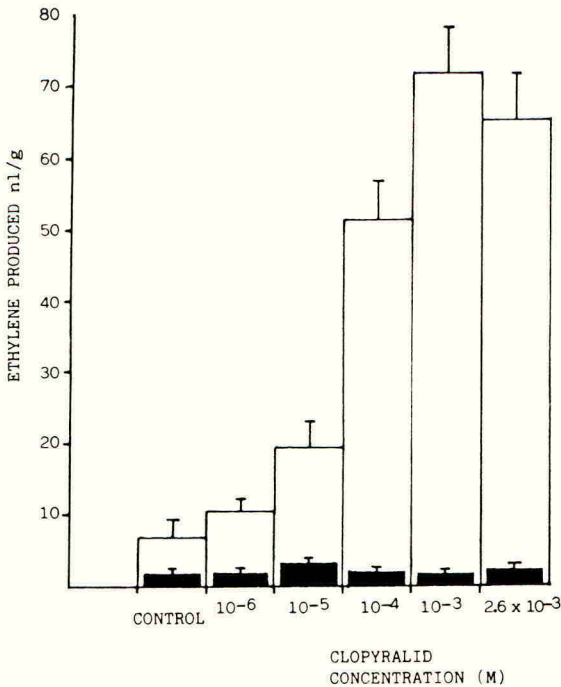
Fig. 2. The effect of clopyralid upon ethylene evolution in mayweed (☐) and sugar beet (■) following incubation of excised leaves for 24h. Each point is the mean from 8 separate plants. Bars represent S.E.'s.

After 24h incubation of excised leaves with a range of clopyralid concentrations, tolerant sugar beet showed no increased ethylene production with respect to controls, whilst the identical treatment of scentless mayweed resulted in a concentration-dependent increase in ethylene production of up to 10 fold. This increase was highly significant ($P<0.001$) at 2.6×10^{-3} M and 1×10^{-3} M clopyralid and significant ($P<0.01$) at 10^{-4} M. Sugar beet leaves appeared unaffected by the herbicide after the 24h period, whereas mayweed exhibited classic epinastic symptoms which increased in severity with increasing herbicide dose. Short-term studies revealed that the clopyralid-induced increase in ethylene production in mayweed was an extremely rapid and concentration-dependent response (Figure 3), being measurable after only 1h incubation and sustained for the following 5h.
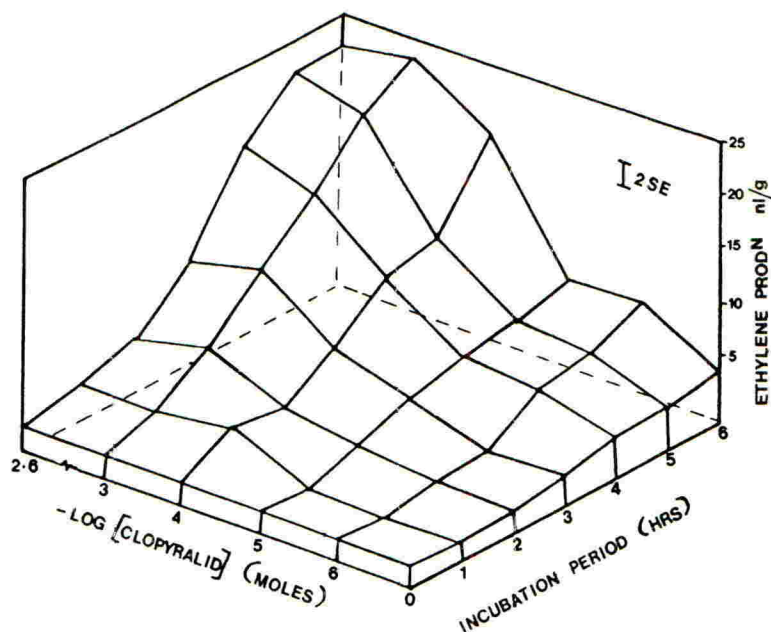
Fig. 3. Effect of clopyralid upon ethylene evolution in scentless mayweed, dose-response and time-course over 6h. Each point is the mean from 8-12 separate plants. Average standard error is indicated.

Increases were statistically significant ($P<0.01$) after only 2h at the two highest clopyralid concentrations and after 3h at 10^{-4} M. Clopyralid concentrations of 10^{-5} M and 10^{-6} M also gave consistently increased ethylene production although values were not statistically different from controls. As found in the 24h experiment, incubation of excised mayweed leaves with 10^{-3} M clopyralid gave the greatest ethylene production with 2.6×10^{-3} M yielding no further increase. Symptom development commenced after 4h at the highest clopyralid doses, and by 6h leaf epinasty was noted at all concentrations. The response of mayweed to 10^{-3} M clopyralid was equivalent to that induced by 10^{-3} M IAA over both 6 and 24h, as illustrated in figure 4.
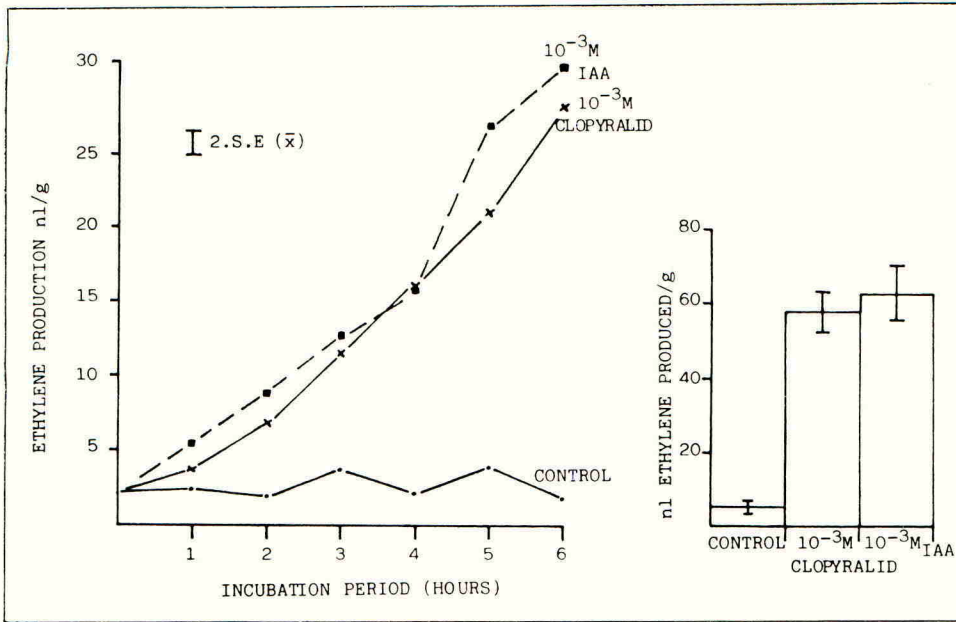
Fig. 4. Effect of clopyralid and IAA on ethylene evolution in scentless mayweed over 6 and 24h. Each point is the mean from 8 separate plants. Bars represent S.E.'s.

The onset and development of visual epinastic symptoms was similar with both clopyralid and IAA. The ethylene biosynthesis inhibitors AVG and Co^{++} reduced mayweed ethylene production in response to 10^{-3} M clopyralid by approximately 50% over 6 and 24h incubations (Figure 5). However, although ethylene production was reduced in the presence of these inhibitors, symptom development was not diminished.

Although sugar beet showed no symptom development or increased ethylene production in response to 10^{-3} M clopyralid, the same concentration of IAA or 2,4-D increased ethylene production by 5 and 12 fold respectively (Figure 6).

These marked increases were accompanied by typical symptom development. However, although sugar beet was insensitive to clopyralid, both species were capable of converting exogenous ACC to ethylene over a 24h period. (Table 1). No symptom development was noted with either species after 24 hours.
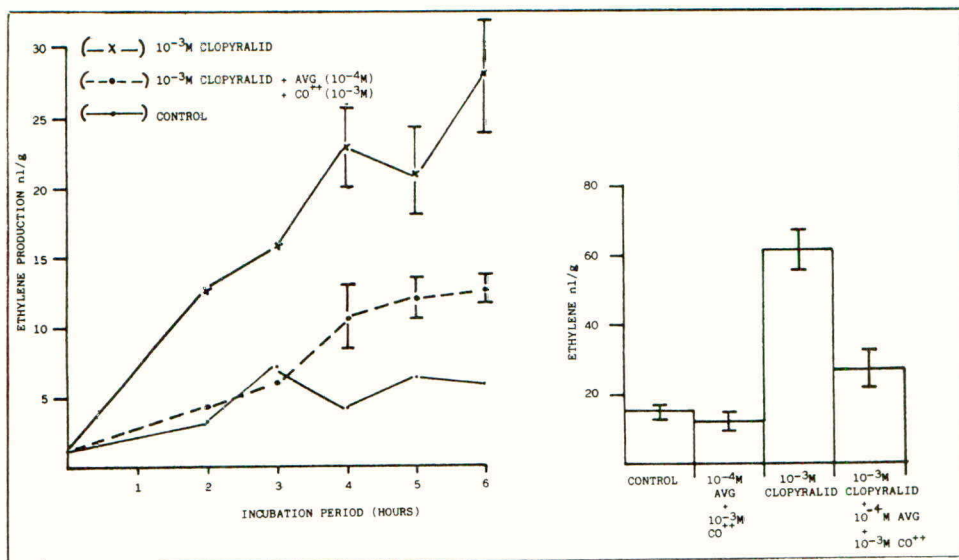
Fig. 5. Effect of AVG and Co^{++} on clopyralid-induced ethylene production in mayweed over 6 and 24h. Data are means of 6-8 replicates. Bars represent S.E.'s.
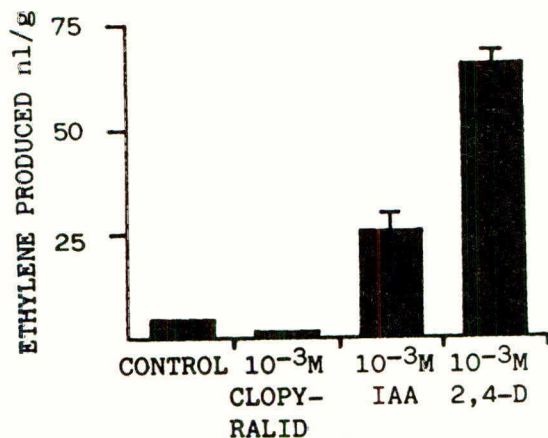

Fig. 6. Effect of clopyralid, IAA and 2,4-D on ethylene evolution in sugar beet after 24h incubation. Each point is the mean from 8-12 separate plants. Bars represent S.E.'s.

Table 1. Production of ethylene from 10^{-3}M ACC in mayweed and sugar beet. Data are means of six replicates plus or minus standard errors.

INCUBATION PERIOD (H)	ETHYLENE PRODUCTION, nl/g	
	Mayweed	Sugar beet
2	20.03 ± 3.92	11.69 ± 1.17
4	38.99 ± 4.52	21.12 ± 1.19
24	163.62 ± 10.90	245.91 ± 32.41

DISCUSSION

Using a relatively simple assay system of incubating excised leaves in herbicide solutions, this study has demonstrated that clopyralid induces a rapid and concentration-dependent production of ethylene from a susceptible species, scentless mayweed, that precedes the onset of epinastic symptom development. Furthermore, no epinastic symptom development was observed in sugar beet as a result of clopyralid application in this system. This observation reveals an apparent inconsistency between our previously reported whole plant data (Thompson & Cobb, 1986) and the current study, in that symptoms were observed in the previous but not the present study. However, it should be borne in mind that the former study used whole plants with foliar application of formulated clopyralid, whilst the current experiments employed the direct, vascular uptake of unformulated clopyralid-acid into leaf explants.

These findings suggested that the observed leaf epinasty is directly caused by ethylene production in mayweed. However, the apparent link is disproven by the further observations that (1) although ethylene evolution was induced by incubation with the precursor ACC alone, leaf epinasty was not observed, (2) exposure to ethylene gas (using prepared standard gases, data not shown) also failed to produce epinastic symptoms in our system, and (3) whilst AVG and Co^{++} inhibited clopyralid-induced ethylene evolution by 50%, symptom development was not affected. Hence, we conclude that ethylene evolution alone is not the sole cause of epinastic symptoms in response to clopyralid incubation in our experimental system.

Neither ethylene production nor epinastic symptom development were observed in excised sugar beet leaves in response to clopyralid incubation. This species is, however, capable of producing ethylene when supplied with the precursor ACC or with IAA and 2,4-D, but symptom development was only observed following incubation with IAA and 2,4-D. Thus, this species has the physiological capacity to produce leaf epinasty in response to herbicides in this experimental system, but is insensitive to clopyralid over 24h. From our present results we conclude that differential ethylene production is not the sole basis of clopyralid selectivity in sugar beet and scentless mayweed.

It is our opinion that ethylene production is a symptom of auxin-type herbicide activity in susceptible species, and we consider it to be one of a cascade of events initiated by binding at an auxin receptor site. There are many published reports of responses to auxin which occur on a time scale from a few minutes to several hours (Evans, 1974). These effects range from almost immediate changes in cell membrane permeability (Fitzsimons et al,

1987), to stimulations in protein synthesis and carbohydrate deposition after 1 to 2 hours, which later appear as measurable growth responses (Evans, 1974). The production of ethylene and visible symptoms therefore appear to be separate measurable responses to the common stimulus of herbicide binding. In this sense, we speculate that clopyralid does not induce epinasty in sugar beet, in this system, due to a relatively poor binding affinity for the auxin binding site in this species.

Finally, the measurement of ethylene production may be a valuable tool in determining potential sensitivity to auxin-type herbicides, and assessing selectivity and structure/activity relationships.

ACKNOWLEDGEMENTS

REFERENCES

Baur, J.R.; Morgan, P.W. (1969) Effects of picloram and ethylene on leaf movement in Huisache and Mesquite seedlings. Plant Physiology 44, 831-838.

Crocker, W. (1948) Physiological effects of ethylene and other unsaturated carbon-containing gases. Growth of Plants, Reinhold Publishing Corporation, 139-171.

Evans, M.L. (1974) Rapid responses to plant hormones. Annual Review of Plant Physiology 25, 195-223.

Fitzsimons, P.J.; Miller, P.R.; Cobb, A.H. (1987) Auxin-induced H^+-efflux; herbicide activity and antagonism. Volume 3, these proceedings.

Haagsma, T. (1975) Dowco 290 - a coming new selective herbicide. Down to Earth 30 (4), 1-2.

Hall, C.J.; Bassi, P.K.; Spencer, M.S.; Vanden Born, W.H. (1985) An evaluation of the role of ethylene in herbicidal injury induced by picloram or clopyralid in Rapeseed and Sunflower plants. Plant Physiology 79, 18-23.

Imaseki, H. (1981) Regulation of ethylene biosynthesis in auxin-treated plant tissues. The New Frontiers in Plant Biochemistry, Eds. Akazawa, Asaki and Imaseki, Japan Scientific Society Press, Tokyo. 153-166.

Thompson, L.M.L.; Cobb, A.H. (1986) Experimental studies into the selectivity of clopyralid in sugar beet. Aspects of Applied Biology 13, 17-24.

Yang, S.F. (1981) Mechanism and regulation of ethylene biosynthesis. The New Frontiers in Plant Biochemistry, Eds. Akazawa, Asaki and Imaseki, Japan Scientific Society Press, Tokyo. 133-151.

Yang, S.F.; Hoffman, N.E. (1984) Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35, 155-189.

SYNERGISM AND ANTAGONISM OF HERBICIDES WITH MONOOXYGENASE INHIBITORS

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ABSTRACT

Triazole compounds, known to be inhibitors of monooxygenase enzymes were found to be antagonistic with EPTC in corn but synergistic with EPTC in sorghum. Interactions were also observed with other herbicides (e. g. metazachlor, SC-0774), but, the type of interactions varied with different rates of herbicide application. One of the monooxygenase inhibitors, BAS-110, had no apparent influence on rates of EPTC metabolism to water soluble metabolites. However, BAS-110 did significantly reduce EPTC uptake in both corn and sorghum.

INTRODUCTION

Various thiocarbamate and chloroacatamide herbicides can be antagonized and made more selective for use in crops such as corn and sorghum through the use of chemical antidotes such as dichlormid and other chloroacetamide compounds (Pallos & Casida, 1978). The most studied mechanism for these safeners involves the elevation of glutathione levels and their enhancement of herbicide detoxication through conjugation with glutathione (Lay & Casida, 1976, Leavitt & Penner 1979, Lay & Niland, 1985). However, in our own studies it has been difficult to correlate the rates of these processes with the degree of antidote action in different crop species (Ekler and Stephenson, 1987). Thus, we have begun to speculate that the glutathione conjugation system is not the only mechanism involved in the safening of these herbicides. One of the primary herbicides involved in these interactions is EPTC, and its metabolism in plants first involves an oxidation step to a sulfoxide then a subsequent conjugation of the sulfoxide with glutathione (Lamoureux & Rusness, 1987).

Because the metabolic pathway for EPTC in plants may involve an oxidation by monooxygenase enzymes, some investigators have speculated that monooxygenase inhibiting compounds (MOI) could have a role in antidote action (Lay & Casida, 1976, Fedtke and Trebst, 1987). It is difficult to compare the phytotoxicity of EPTC and its sulfoxide metabolite because the sulfoxide is likely to be unstable. Kőmíves and Dutka (1980) observed that a monooxygenase inhibitor (MOI), piperonyl butoxide, was synergistically phytotoxic with EPTC in corn plants. However, they did not examine the effects of piperonyl butoxide on EPTC metabolism, thus the mechanism for that synergism is uncertain. Furthermore, to avoid uptake problems, they had to inject piperonyl butoxide into the plant stems. A family of new triazole plant growth regulators has recently been developed

(Rademacher & Jung, 1986) which retard plant shoot growth without causing obvious malformations. Their mechanism is an inhibition of giberrelic acid synthesis through an inhibition of membrane bound monooxygenases.

The purpose of this study was to examine the possible role of monooxygenase enzymes in regulating the phytotoxicity of herbicides such as EPTC and metazachlor. The triazole compounds appear to have an advantage over other monooxygenase inhibitors like piperonyl butoxide, because they are taken up more easily.

MATERIALS AND METHODS

Common and chemical names and the function of the active ingredients used in the experiments are listed in Table 1. The experimental data were statistically analyzed by descriptive statistics followed by a Duncan's Multiply Range Test (DMRT) with $P < 0.05$.

Interaction of herbicides and monooxygenase inhibitors on plant growth

Seeds of corn (PAG SX-111) and sorghum (King Grain P 508 GB) were soaked overnight in tap water and then planted into premoistened vermiculite in foam cups (350 mL). The chemicals were applied immediately after planting as a treatment solution in 100 mL of water. Two corn or four sorghum plants per cup were grown in a growth room at 26 °C in light for 16 h and at 21 °C in dark for 8 h. The relative humidity in the growth room was 75 %. The plants were watered three times per week with 100 mL of water or half-strength Hoagland's nutrient solution (every third watering was nutrient). The experiments were set up in a completely randomized design. All treatments were applied to a total of four replicates. Corn and sorghum shoot heights were measured and the plants were harvested after three weeks.

Metabolism experiments

Corn and sorghum plants (planted and grown in a growth room as written above) were pretreated with a solution of BAS-110 (80 μg/L) or dichlormid (6.7 μM) 2 and 4 days after planting, respectively. Six days after pretreatment, the shoots were excised with a razor blade and placed into glass scintillation vials, containing propyl-labeled [^{14}C]EPTC (sp. act. 1.32 GBq/mmol) in 1 mL of water. EPTC concentration in the vials for corn was 40 μM: 5 μM (6.3 kBq) [^{14}C] and 35 μM unlabeled compound. The vials for sorghum contained only [^{14}C] labeled EPTC in a concentration of 2 μM (2.6 kBq). Three corn or five sorghum shoots were placed into each vial. One hour later, some of the shoots were weighed, wrapped in a small sheet of plastic film, immersed into liquid nitrogen and stored in a freezer until sample extraction. The remaining shoots were transferred to plastic scintillation vials containing 2 mL of water for 1, 3, 5 and 7 hours and then frozen as described above. To measure the [^{14}C] content of the tissue and the rate of metabolism of the herbicides, the frozen samples (fresh weights were 0.8-1.2 g and 1.5-2.2 g for sorghum and corn, respectively) were cut into small parts and extracted in 4 mL of a mixture of acetone and water (7:3) using a Brinkman homogenizer. After centrifugation (7,500 g, 15 min), the supernatants were extracted with 1 mL of dichloromethane to separate water soluble conjugates from the less polar parent herbicides.

The samples were extracted in conical centrifuge tubes (15 mL) with a vortex shaker. To get a proper separation of the liquid phases, the samples were centrifuged at 2,000 g for 5 min. The efficiency of the extraction method was 94 ± 3 % for EPTC and 97 ± 4 % for metazachlor. The radioactivity in both the aqueous and the organic phases was determined by liquid scintillation counting (LSC).

TABLE 1

Active ingredients used in the experiments.

Function	Common name	Chemical name
Herbicides	Chlorsulfuron	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)-aminocarbonyl]-benzenesulfonamide
	EPTC	S-ethyl dipropylthiocarbamate
	Metazachlor	N-(2,6-dimethyl-phenyl)-N-(1-pyrazolyl-methyl)-chloroacetamide
	SC-0774	not released (Stauffer)
safener	Dichlormid	N,N-diallyl-2,2-dichloroacetamide
mono-oxygenase enzyme inhibitors (MOI)	BAS-110	2-(2,4-dichlorophenyl)-2-hydroxy-3-methoxy-3-(1H-1,2,4-triazole-1-yl)-propane
	BAS-111	1-phenoxy-3-(1H-1,2,4-triazole-1-yl)-4-hydroxy-5,5-dimethylhexane
	Tetcyclacis	5-(4-chlorophenyl)-3,4,.5,9,10-pentaaza-tetracyclo-5,4,1,0^{2,6},0^{8,11}-dodeca-3,9-diene

RESULTS

 Numerous increases (synergism) or decreases (antagonism) in the phytotoxicity of the various herbicides to corn or sorghum can be elucidated by careful enumeration of the significant differences in Fig. 1 and 2. However, many of these interactions were not consistently repeated

in the various bioassays conducted. The major interactions that were consistently observed are as follows.

Herbicide - MOI interactions in corn

The triazole MOI-s, BAS-110 and BAS-111, were significantly antagonistic with EPTC in corn at all rates of EPTC application (Fig. 1b). BAS-110 and BAS-111 were antagonistic with the new herbicide SC-0774 in corn (Fig. 1d) at the lowest rate, but, BAS-111 had no effect and BAS-110 became synergistic at the higher herbicide rates applied. BAS-110 was antagonistic with metazachlor (Fig 1c) at the middle herbicide rate, but, BAS-111 and tetcyclacis were synergistic at the highest herbicide rate. None of the three MOI compounds had any type of interaction with chlorsulfuron (Fig 1a).
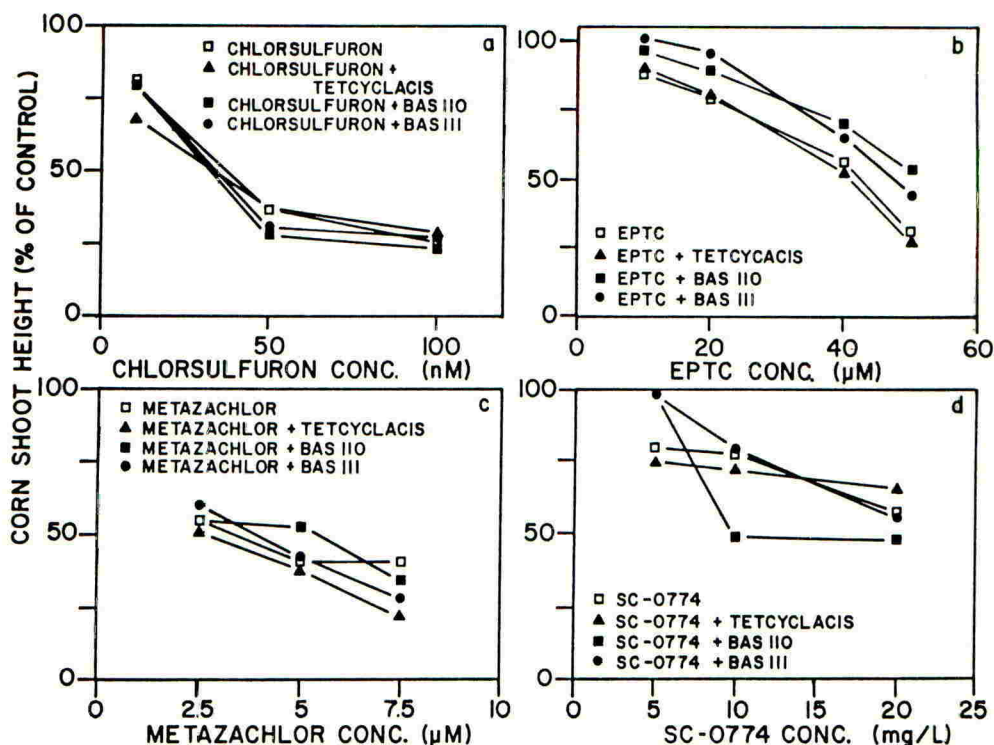
Fig. 1. Herbicide - monooxygenase inhibitor (MOI) interactions in corn. Rates for BAS-110, BAS-111, and tetcylacis were 80, 80, and 15 µg/L, respectively, in combination with the various herbicides at the rates shown.

Herbicide - MOI interactions in sorghum

   In contrast to results with corn where antagonistic interactions were observed, all of the MOI-s, particularly BAS-110, were synergistically phytotoxic with EPTC in sorghum (Fig. 2b). Synergistic interaction was also found between BAS-111 and metazachlor (Fig. 2c) at the lowest herbicide rate, but, BAS-110 was antagonistic at the two highest metazachlor rates. Slight synergism was observed between the MOI-s and low rates of SC-0774 (Fig. 2d), but, no significant interactions were observed between the MOI-s and chlorsulfuron (Fig. 2a) in sorghum.
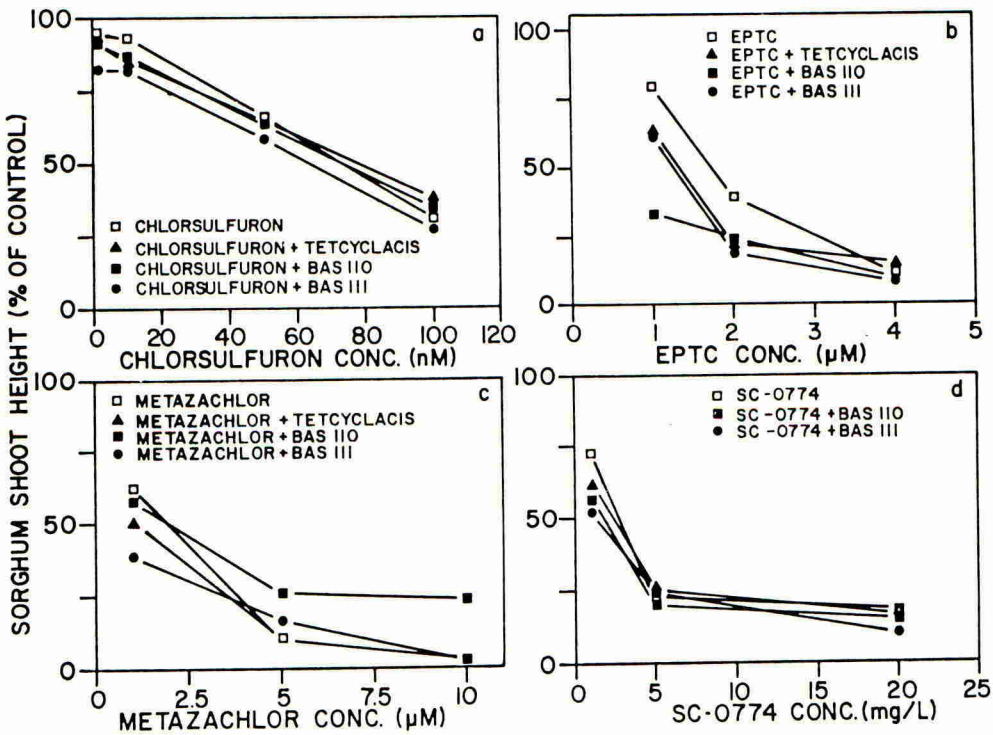
Fig. 2. Herbicide - monooxygenase inhibitor (MOI) interactions in sorghum. Rates for BAS 110, BAS 111 and tetcyclasis were 80, 80 and 15 µg/L, respectively, in combination with the various herbicides at the rates shown.

The effect of BAS-110 and dichlormid on EPTC uptake and metabolism

Of the many different herbicide – MOI interactions observed, those involving BAS-110 or BAS-111 with EPTC were the most interesting. These MOI compounds were antagonistic with EPTC in corn, but, synergistic with EPTC in sorghum. In an attempt to explain the conflicting behaviour in the two species, herbicide metabolism experiments were performed. [14C]EPTC uptake and the rate of the metabolism of the herbicide were compared in plants after pretreating them with BAS-110 for six days, in comparison with EPTC alone. Dichlormid, the most widely used thiocarbamate safener was also included as a pretreatment.

Because of the different sensitivity to the herbicide, corn shoots were treated with 20 times more EPTC than sorghum shoots. Herbicide uptake by corn was 7-9 times higher than by sorghum (Table 2). Sorghum metabolized EPTC much quicker than corn (Fig. 3). There were no significant differences found in the rate of metabolism to water soluble metabolites when the plants were pretreated with either dichlormid or with BAS-110, compared with the unpretreated plants. Nevertheless, both corn and sorghum absorbed significantly less EPTC when the plants were pretreated with BAS-110 (Table 2). Dichlormid pretreatment did not significantly change the uptake of EPTC either by corn or by sorghum.
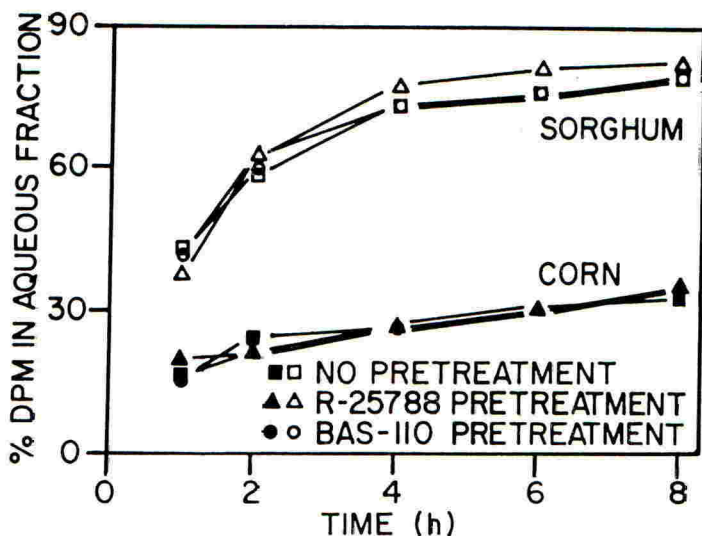
Fig. 3. Influence of 6-day pretreatment with dichlormid (6.7 µM) or BAS 110 (80 µg/L) on the kinetics of [14C]EPTC conversion to water soluble metabolites in corn and sorghum.

TABLE 2

Effect of safener (dichlormid) and monooxygenase inhibitor (BAS-110) pretreatment on EPTC uptake by corn and sorghum shoots[1].

| Pretreatment | EPTC uptake after 1 h (DPM/g fresh weight) | |
for 6 days	Corn	Sorghum
None	298 ± 13 (100 %)	42.4 ± 3.7 (100 %)
Dichlormid	312 ± 17 (105 %)	39.5 ± 4.2 (93 %)
BAS-110	223 ± 11 (75 %)	24.0 ± 3.4 (57 %)

DISCUSSION

The first step in the metabolic pathway for EPTC is oxidation (Lay & Casida, 1976) to a sulfoxide moiety which in turn may be further oxidized to a sulfone (Horváth and Pulay, 1980) and/or conjugated with glutathione (Lay and Casida, 1976). In studies with acetanilide herbicides, Leavitt and Penner (1979) established that oxidation was not a prerequisite to their conjugation with glutathione. This is likely to be true for metazachlor (a chloroacetamide) as well. The importance of oxidation step(s) in the metabolism of EPTC can have a role in the significant interaction of EPTC with compounds known to inhibit monooxygenase enzymes in plants. Lay and Casida (1974) have indicated that EPTC-sulfoxide is more toxic than EPTC to most plants, but, less toxic than EPTC to corn. They suggest that EPTC-sulfone would not be a good herbicide. Kőmíves and Dutka (1980) rank the sulfone as the most toxic and EPTC as intermediate in phytotoxicity to corn.

In our studies, BAS-110 was synergistically toxic with EPTC in sorghum, but, antagonistic with EPTC in corn. BAS-110 inhibited uptake of EPTC in both species. However, BAS-110 did not affect the disappearance of the parent EPTC in either corn or sorghum. The various metabolites were not characterized. The differential action of BAS-110 as an EPTC antagonist in corn and as an EPTC synergist in sorghum may eventually help define the selective mechanism for this herbicide in plants. However, a greater understanding of the significance of these interactions in the selective mechanism for EPTC will require a better understanding of the identity and toxicity of the various oxidation products and conjugates of EPTC in corn and sorghum.

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[1]The original DPM values found in corn shoots are multiplied by 5 in Table 2 because [^{14}C]EPTC was only 20 % of the total EPTC treatment.

REFERENCES

Casida, J.E.; Gray, R.A.; Tilles, H. (1974) Thicarbamate sulfoxides: potent, selective and biodegradable herbicides. *Science 184,* 573-574.

Ekler, Z.; Stephenson, G.R. (1987) Physiological responses of corn and sorghum to BAS-145138, dichlormid and flurazole as antidotes for EPTC and metazachlor. Submitted to *Pesticide Biochemistry and Physiology.*

Fedtke, C.; Trebst, A. (1987) Advances in understanding herbicide modes of action. *In: Pesticide Science and Biotechnology, Proceedings 6th International Congress of Pesticide Chemistry,* IUPAC, Ottawa, Canada, Blackwell Scientific Publications, Oxford, U. K., pp. 161-168.

Horváth, L.; Pulay, A. (1980) Metabolism of EPTC in germinating corn: sulfone as the true carbamoylating agent. *Pesticide Biochemistry and Physiology 14,* 265-270.

Kőmíves, T.; Dutka, F. (1980) On the mode of action of EPTC and its antidotes on corn. *Cereal Research Communications 8,* 627-633.

Lamoureux, G.R.; Rusness, D.G. (1987) EPTC metabolism in corn, cotton and soybean: identification of a novel metabolite derived from the metabolism of glutathione conjugate. *Journal of Agricultural and Food Chemistry 35,* 1-7.

Lay, M.M.; Casida, J.E. (1976) Dichloroacetamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione S-transferase activity. *Pesticide Biochemistry and Physiology 6,* 442-456.

Lay, M.M.; Niland, M. (1985) Biochemical response of inbred and hybrid corn (*Zea Mays* L.) to R-25788 and its distribution with EPTC in corn seedlings. *Pesticide Biochemistry and Physiology 23,* 131-140.

Leavitt, J.R.C.; Penner, D. (1979) In vitro conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. *Journal of Agricultural and Food Chemistry 27,* 533-536.

Pallos, F.M.; Casida, J.E. (1978) *Chemistry and action of herbicide antidotes.* Academic Press: New York, p. 171.

Rademacher, W.; Jung, J. (1986) GA-biosynthesis inhibitors – an update. *Proceedings 13th Meeting Plant Growth Regulator Society of America,* St. Petersburg Beach, Florida, USA, pp. 1-13.

BIOCHEMICAL ASPECTS OF SAFENER ACTION: EFFECTS ON GLUTATHIONE, GLUTATHIONE
-S- TRANSFERASE AND ACETOHYDROXY ACID SYNTHETASE IN MAIZE

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ABSTRACT

The chemical safener N,N-diallyl -2,2 -dichloroacetamide (DDCA)
increased the glutathione content of root tissue, and
glutathione-S-transferase activity in the root and shoot tissues
of the treated maize (Zea mays) plants. Napthalic anhydride
(NA) also enhanced glutathione-S-transferase activity in root
and shoot, but had no effect upon glutathione content.

Both NA and DDCA enhanced the activity of acetohydroxy acid
synthetase (AHAS), the target site of sulfonylureas, in treated
plants. However, AHAS extracted from safened plants was more
sensitive to chlorsulfuron inhibition. NA in vitro did not
alter AHAS inhibition by chlorsulfuron.

INTRODUCTION

The herbicide safeners napthalic anhydride (NA) and N,N-diallyl -2,2
-dichloroacetamide (DDCA) enhance the tolerance of maize (Zea mays) to
thiocarbamate herbicides, and to a lesser extent, the sulfonylureas
(Hatzios 1983 Parker 1983).

Although much research has been directed towards their mode of action, the
biochemical basis for the protective action of these safeners in maize is
still not clearly understood.

DDCA enhances the level of glutathione-S-transferases (G-S-T) and reduced
glutathione (GSH) in treated tissue (Lay and Casida 1976, Mozer et al
1983, Komives et al 1985, Lay and Niland 1985), and hence the rate of
thiocarbamate detoxification via GSH conjugation of their sulfoxides.
G-S-T isoenzymes have been identified in maize (Guddewar and Dauterman
1979, Mozer et al 1983). These have different herbicidal specificities,
and the induction of isoenzymes particular to certain herbicide molecules,
may account for the specificity of safener action. (Edwards and Owen
1986).

Sweetser (1985) reported an enhanced rate of sulfonylurea metabolism in
NA/DDCA treated tissue, which was believed to be associated with an
induction of the cytochrome P-450 mixed function oxidase system (Fedtke
1987).

Because of the association between safener action and the
induction/enhancement of enzyme systems in treated tisue, it seemed
appropriate to investigate the effect of these safeners upon acetohydroxy
acid synthetase (AHAS) activity, the target site of the sulfonylurea

herbicides (Ray 1984). If either the total amount of AHAS is altered
considerably, or isoenzymes are induced with decreased sulfonylurea
sensitivity, then this might in part explain the safening action reported
by Parker (1983).

MATERIALS AND METHODS

Plant Material

Seeds of maize (var LG 11) were washed in water to remove fungicide
dressing, before applying either NA (97% w/w a.i.) or DDCA (20% w/w a.i.)
at rates of 0, 0.25, 0.5 and 1.0% by seed weight.

Three seeds were sown per 9 cm pot in Vermiculite and watered thoroughly
with 50% Hoaglands solution. Pots were placed in a light cabinet (16
hours light, 390 μ E M^{-2} S^{-1} 27/19°C), and watered every two days with 50
ml of nutrient solution.

GSH and G–S–T assays

Both assays were carried out using root and shoot tissue of 5 day old
plants. (2 leaf stage).

GSH was assayed spectrophotometrically by following the reduction of
5-5'-dithiobis-2-nitrobenzoic acid (DTNB) by GSH in the presence of NADPH
and glutathione reductase (Low et al 1983, Smith et al 1984).

G–S–T activity was measured spectrophotometrically using 1–chloro-2,4,
dinitrobenzene (CDNB) as a substrate. (Mozer et al 1983). Both assays
were carried out at 25°C.

AHAS extraction and assay

Root and leaf tissue was used from 7 day old plants (3 leaf stage). The
methods used were based upon those of Chaleff and Mauvais (1984) as
modified by R. Wallsgrove (personal communication).

(i)   Leaf Extraction

Approximately 0.8g of the youngest leaves of plants was homogenised on ice
in 5.5 ml of extraction buffer containing; 50 mM KH$_2$PO$_4$, 5 mM MgSO$_4$, 10 mM
pyruvate, 0.5 mM cocarboxylase, 10 μ M flavin adenine dinucleotide (FAD),
1 mM L–leucine, 1 mM L–valine, 10% ethanediol (v/v), 0.05% Triton X 100,
at pH 7.5, with 50% w/w polyvinyl polypyrrolidone (PVPP). The crude
extract was strained through 4 layers of muslin, and centrifuged at
11,600g for 8 minutes. 2ml was desalted on a Sephadex G 25 column
equilibrated with resuspension buffer containing 50 mM KH$_2$PO$_4$ pH 7.5, 5mm
MgSO$_4$, 10 mM pyruvate, 30% ethanediol.

(ii)   Root Extraction

Approximately 5g of root tissue was ground in a pestle and mortar in 10 ml
extraction buffer with 0.5g PVPP and a small amount of washed sand. The
resulting brei was strained through 4 layers of muslin, and spun at 4000g
for 5 minutes. AHAS was precipated from the supernatant solution using

$(NH_4)_2SO_4$ between 25 and 50% saturation. The pellet collected by centrifuging for 20 minutes at 10,000g was resuspended in 2ml of resuspension buffer and desalted as above.

Assay

In a total of 650 μ l, the assay contained; 50 mM KH_2PO_4 pH 7.5, 50 mM pyruvate, 10 mM $MgSO_4$, 250 μM cocarboxylase, 20 μ M FAD, 125 μ l enzyme, and as required, 125 μ l chlorsulfuron. Samples were incubated at $30^{\circ}C$ for 1 hour, when the reaction was stopped by the addition of 125 μ l of 3 M H_2SO_4. After incubation at $60^{\circ}C$ for 15 minutes, the acetoin content of samples was determined by the sequential addition of 125 μ l 20% NaOH, 187 μ l 0.5% Creatine, 187 μl 5% ∝ napthol. After 1 hour the samples were centrifuged and absorption at 530 nm recorded.

AHAS activity was expressed as a percentage values by comparison with the absorption values of treatments with samples incubated without chlorsulfuron. Where NA was used in vitro, it was first dissolved in acetone, before diluting in the phospate assay buffer. Final acetone concentration in the assay mixture was 0.25% v/v.

Protein assay

The protein content of enzyme extracts was determined using the method of Bradford (1976).

RESULTS

Treatment of maize seeds with NA and DDCA enhanced the activity of G-S-T in root and shoot tissue in a dose dependent manner (figure 1). The rsponse to DDCA treatments was greater than with equivalent NA treatments. Root and shoot tissue showed similar G-S-T enhancement with each safener (two to three fold at 1%).

No significant GSH response was found with DDCA in shoot tissue, or with NA treatment in root or shoot. However, DDCA did enhance the GSH content of root tissue in a dose dependent manner. (Figure 2).

At an application rate of 0.5% by seed weight, NA gave an increase in extractable AHAS activity of 1.25 to 1.5 fold, in both root and third leaf after 7 days. (Table 1). The response to DDCA was less however, with an increase of between 1.0 to 1.3 fold. These responses were less than those shown for G-S-T at the 0.5% application rate i.e. approximately a two fold increase in activity for both safeners.

To investigate the possible induction of a chlorsulfuron resistant isoenzyme of AHAS with safener treatment, inhibition curves were plotted for AHAS extracted from safened and unsafened tissue. Figures 3 a and b reveal that both NA and DDCA pretreatments at 0.5% decreased the activity of the extracted AHAS over a range of chlorsulfuron treatments, when compared with non-safened plants.

The addition of NA in vitro (10^{-5} -10^{-8} M), to AHAS extracted from unsafened plants, resulted in a 10 - 15% decrease in activity as compared with an NA free control (Figure 4). A similar result was found with the same NA concentrations in the presence of 10 ppb chlorsulfuron.
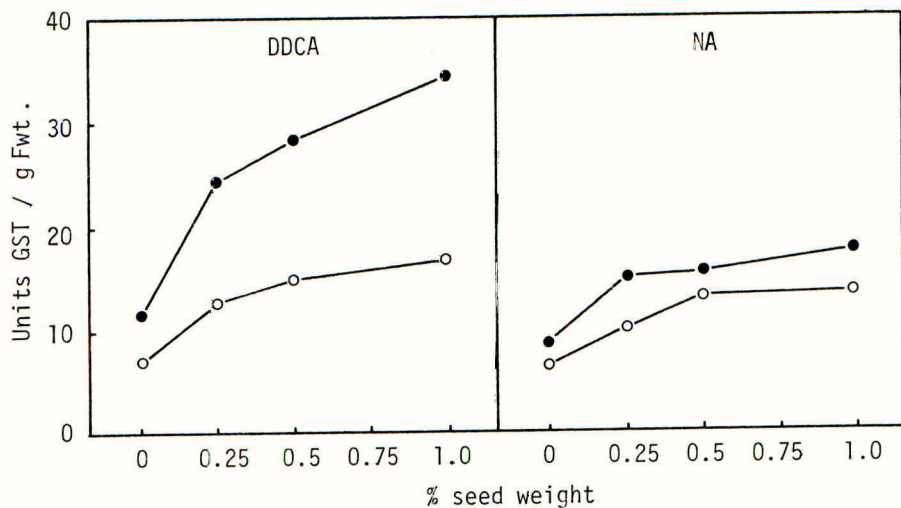
Fig. 1. Effect of DDCA and NA upon G-S-T activity in root (●——●) and shoot (O——O) tissue 5 days after treatment.
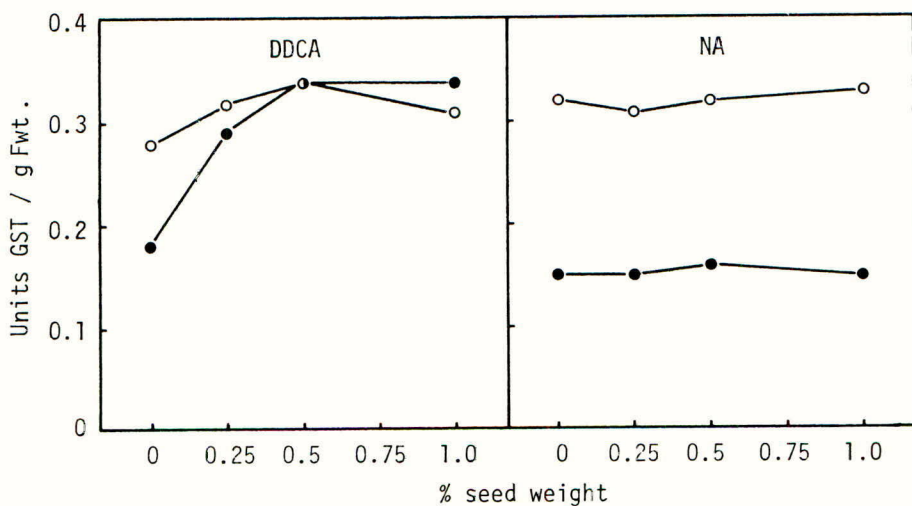

Fig. 2. Effect of DDCA and NA upon GSH levels in root (●——●) and shoot (O——O) tissue 5 days after treatment.

TABLE 1.

Effect of DDCA / NA as seed dressing at 0.5% by seed weight on extractable
AHAS activity 7 days after treatment.

Treatment	Leaf tissue		Root tissue	
	Abs./Protein	Abs./Fwt.	Abs./Protein	Abs./Fwt.
Control	7.72	0.66	9.59	10.1
DDCA	8.86	0.71	9.50	13.2
NA	9.91	0.87	12.43	15.2

Abs./Protein = Absorption 530nm / (ug Protein / 0.1ml enzyme extract).

Abs./Fwt.   = Absorption 530nm / g fresh weight extracted tissue.
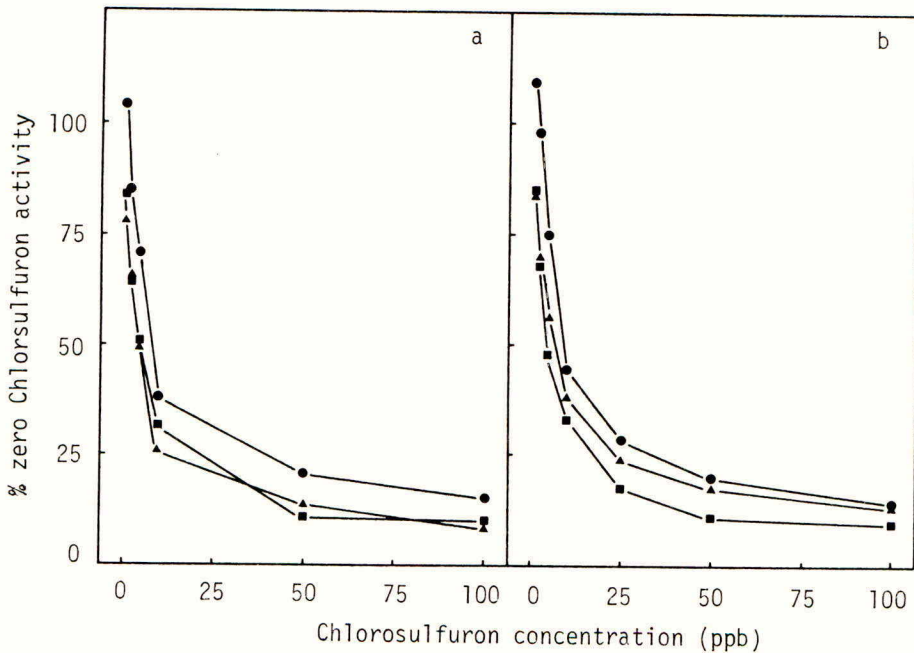
Fig. 3. AHAS / Chlorsulfuron inhibition curves with DDCA / NA pretreatments
in a) shoot and b) root. (●———●) control, (■———■) DDCA and (▲———▲) NA.
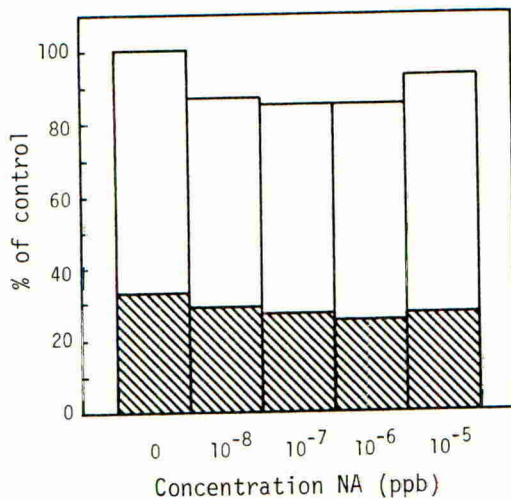
Fig. 4. Effect of NA in vitro upon AHAS activity $\pm$ Chlorsulfuron.

☐ 0 ppb Chlorsulfuron, ▨ 10 ppb Chlorsulfuron.

DISCUSSION

The conjugation of thiocarbamate herbicide sulfoxides with glutathione, mediated by glutathione-S-transferases, is an important detoxification mechanism in maize. (Hatzios 1982). Therefore, any increase in the endogenous levels of glutathione and glutathione-S-transferases is likely to increase the tolerance of maize to these herbicides, by enhancing the rate at which they are metabolised.

It has been reported that DDCA treatment enhanced root GSH content and the activity of G-S-T in root and shoot tissue. (Lay and Casida 1976, Mozer et al 1983). This response was confirmed in this work. (figure 1 and 2). NA treatment produced no change in endogenous GSH, but did increase G-S-T activity significantly (figure 1). This was similar to the findings of Mozer (1983), but opposed to those of Lay and Casida (1976), who found no G-S-T enhancement with NA. A possible explaination of these conflicting reports is the choice of substrate used to assay G-S-T activity. CDNB was used in this investigation and also by Mozer (1983), whereas EPTC sulfoxide was chosen as the subtrate by Lay and Casida (1976). G-S-T occurs as at least two isoenzymes (Guddewar and Dauterman 1979), and these have been shown to have different substrate specificities (Edwards and Owen 1986). Hence NA may enhance G-S-T isoenzymes which are active with CDNB, but not EPTC sulfoxide.

The use of other chemicals which modulate GSH levels and G-S-T activity could provide a means of modifying the sensitivity of both crop and weed plants to herbicides such as the thiocarbamates, thus extending their range of use. The herbicide tridiphane which inhibits G-S-T activity (Lamoureux et al 1986) has been used to synergise the activity of EPTC in the grass weed giant foxtail (Setaria faberi) (Ezra et al 1985). Other compounds, such methionine sulphoximine, which inhibit glutathione biosynthesis (Rennenberg and Uthemann 1980) may prove of value.

The safening of maize to sulfonylurea herbicides was reported to be associated with an increased rate of metabolism via the cytochrome P-450 mixed function oxidase system (Sweetser 1985). Since both this enzyme system and G-S-T activity are enhanced by safener treatment, the possibility that AHAS activity is also enhanced should not be overlooked as a possible mechanism of action.

The results presented here (figures 3 a and b, table 1) indicate that although AHAS activity was enhanced in safened plants, the sensitivity of AHAS to Chlorsulfuron was also increased. NA was also shown to have no effect in vitro upon AHAS inhibition by chlorsulfron (figure 4) and thus would be unlikely to alter the binding of the herbicide with the enzyme.

Hence, the safening of maize to chlorsulfuron by NA and DDCA, did not appear to be explained by the changes in AHAS activity observed.

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REFERENCES

Bradford, M.M. (1976)   A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254.

Chaleff, R.S; Mauvais, C.J. (1984)   Acetolactate synthetase is the site of action of two sulfonylurea herbicides in higher plants. Science 224, 1443-1445.

Edwards, R; Owen, W.J. (1986) Glutathione-S-transferases involved in herbicide detoxification in plant cell culture. Aspects of Applied Biology 11, 121-130.

Ezra, G; Dekker, J.H; Stephenson, G.R. (1985) Tridiphane as a synergist for herbicides in corn (Zea mays) and Proso Millet (Panicum miliaceum) Weed Science 33, 287-290.

Fedtke, C. (1987) Physiological activity spectra of existing graminicides and the new herbicide 2-(2-benzothiazolyl-oxy)-N-methyl-N-phenylacetamide (mefenacet). Weed Research 27, 221-228.

Guddewar, M.B; Dauterman, W.C; (1979) Purification of a glutathione-S-transferase from corn which conjugates S-triazine hebicides Phytochemistry 18, 735-740.

Hatzios, K.K. (1983) Herbicide antidotes: Development, chemistry, and mode of action. Advances in Agronomy 36, 265-316.

Hatzios, K.K; Penner, D. (1982) Metabolism of herbicides in higher plants Minneapolis, Burgess Publishing Company.

Kömives, T; Kömives, V.A; Balás, M; Dutka, F; (1985) Role of glutathione-related enzymes in the mode of action of herbicide antidotes. Proceedings 1985 British Crop Protection Conference Weeds 3, 1155-1162.

Lamoureux, G,L; Rusness D G (1986) Tridiphane 2-(3,5-Dichlorophenyl)-2-(2,2,2-trichloroethyl) oxirane an atrazine synergist: Enzymatic conversion to a potent glutathoine-S-transferase inhibitor. Pesticide Biochemistry and Physiology 26, 323-342.

Law, M,Y; Charles, S.A; Halliwell, B. (1983) Glutathione and ascorbic acid in spinach (Spinacia oleracea) chloroplasts. (Effect of H_2O_2 and paraquat). Biochemical Journal 210, 899-903.

Lay, M.M; Casida, J.E; (1976) Dichloroacetamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione-S-transferase activity. Pesticide Biochemistry and Physiology 6, 442-456

Lay, M.M; Niland, A.M; (1985) Biochemical response of in inbred and hybrid corn (Zea mays L) to R25788 and its distribution with EPTC in corn seedlings. Pesticide Biochemistry and Physiology 23, 131-140

Mozer, T.J; Tiemeier, D.C; Jaworski, E.G; (1983) Purification and characterisation of a corn glutathione-S-transferase. Biochemistry 22, 1068-1072

Parker, C; (1983) Herbicide antidotes - A review. Pesticide Science 14, 40-48

Ray, T.B; (1984) Site of action of chlorsulfuron. Plant Physiology 75, 827-831

Rennenberg H, Uthemann R (1980). Effects of L-methionine-s-sulfoximine on growth and glutathione synthesis in tobacco suspension cultures. Zeitschrift für Naturforschung 35, 945-951.

Smith, I.K; Kendall, A.C; Key, A.J; Turner, J,C; Lea, P.J; (1984) Increased levels of glutathione in a catalase - deficient mutant of barley (Hordeum vulgare L) Plant Science Letters 37, 29-33.

Sweetser, P.B; (1985) Safening of sulfonylurea herbicides to cereal crops. Mode of herbicide antidote action. Proceedings 1985 British Crop Protection Conference Weeds 3, 1147-1154.

THE USE OF FLUROCHLORIDONE FOR WEED CONTROL IN THE POTATO IN POLAND

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ABSTRACT

Small plot experiments and commercial trials on weed control with flurochloridone (3-chloro-4-chloromethyl-1-trifluro-tolyl)-2-pyrrolidone were carried out in Poland in the years 1981-1986.Pre-emergent applications of flurochloridone within 10 days after potato planting at the rate 0.5-0.75 kg/ha a.i. had given excellent control of annual broadleaved weeds.Tankmix of flurochloridone at the rate 0.375 kg/ha a.i. with pre-emergent herbicides (linuron or metribuzin) showed similar efficacy.When applied too late (shortly before potato emergence) flurochloridone caused injury to the crop.

INTRODUCTION

Potato yield reductions due to weeds reach 10-40% in Poland.The main weed species are *Chenopodium album*, *Sinapis arvensis*, *Viola arvensis*, *Anthemidae and Elymus repens*. The weeds are controlled on the acreage 150 thousand ha (7% of the total acreage) but the need for herbicide usage is increasing from year to year.

Broadleaved weeds are usually controlled with EPTC, linuron, monolinuron, cyanazine, metribuzin or prometryn. These chamicals show better efficacy when applied to soil with sufficient moisture content.

Flurochloridone (Stauffer Chemical Co.) is a novel herbicide selective for the potato. Commercial product is an emmulsifiable concentration marketed under the name "Racer 25EC".

This paper reports the study on the efficacy of flurochloridone against broadleaved weeds and its safety to the potato in field experiments and commercial trials in Poland.

MATERIAL AND METHODS

Flurochloridone was tested in field experiments at Bonin, Northern Poland, in the years 1981-1983 and in commercial trials located on 217 sites all over the country in the years 1983-1986.

In small plot trials (33 sq.m per plot) the treatments were randomized within 4 replicated blocks. Commercial trials were of 0.5-1 ha in large fields.

Flurochloridone (R-40244) was applied within 10 days after potato planting. The doses used ranged from 0.5 to 0.75 kg/ha a.i., the higher one being appplied on heavy soils. In the case of tankmixes the dose of flurochloridone was 0.375 kg/ha a.i. and the other herbicides were linuron (Afalon 50) at the rate 0.5 kg/ha a.i. or metribuzin (Sencor 70WP) at the rate 0.35 kg/ha a.i. All herbicide combinations were applied in 200-400 l of spray volume. Mechanical cultivation were the standard.

Efficiency of weed control in small plots was estimated by counting weeds in 3 x 1 m squares and in commercial trials - by visual estimation of soil surface covered with weeds.

Crop tolerance was assessed according to the EWRC scale, where 1 = no damage, 9 = plants killed.

RESULTS

In small plot trials good efficacy of weed control with flurochloridone has been confirmed against the following broadleaved weeds: *Gallium aparine*, *Thlaspi arvense*, *C.album*, *Gallinsoga parviflora* and *Stellaria media*.Reduced efficacy was seen against *Cirsium arvense*, *Convolvulus arvensis*, *Equisetum arvense* (table 1). Anthemis spp. occured mainly later in blanks (no potato plant), however in closed canopy these weeds were absent.

TABLE 1

Efficiency of the control of some weed species with flurochloridone in small plot trials (Bonin, mean for the years 1981-1983)

Weed species	Flurochloridone	
	0.5 kg/ha	0.375 kg/ha + 0.35 kg/ha metribuzin
Anthemis spp.	xx	xxx
C.arvense	0	0
G.aparine	xxx	xxx
T.arvense	xxx	xxx
Veronica sp.	xx	xx
Viola sp.	x	x
C.album	xxx	xxx
E.arvense	0	0
C.arvensis	0	0
G.parviflora	xxx	xxx
S.media	xxx	xxx

Efficiency: xxx - 95-99%; xx - 90-94%; x - 75-89%; 0 - 0-74%

The results of commercial trials indicate excellent control of
C.album,S.arvensis, S.media, Amaranthus retroflexus and *V.arvensis* (table 2).
Tankmixes of flurochloridone at a decreased rate plus linuron (0.5 kg/ha) or
metribuzin (0.35 kg/ha) showed similar efficiency of weed control to the high
rate alone (table 3).

TABLE 2

Efficiency of the control of some broadleaved weeds in commercial trials (mean
for the years 1983-1986)

Weed species	Frequency	Efficiency of the control/per cent of crops			
		full	good	medium	unsatisfactory
A.retroflexus	37.2	43.6	49.7	4.2	2.5
Anthemis spp.	57.2	32.1	27.6	33.8	6.5
C.album	96.8	64.8	28.8	5.3	3.1
S.arvensis	88.9	50.4	39.2	6.1	4.3
S.media	61.2	71.4	25.1	3.5	0
V.arvensis	45.3	41.6	46.2	10.1	2.1
E.arvense	61.3	18.4	2.6	29.0	5.0
C.arvensis	42.3	20.1	7.3	48.3	24.3
C.arvense	71.1	13.2	19.6	32.1	35.1
Echinochloa crus-galli	43.7	15.7	14.3	37.1	32.9
E.repens	73.4	0	1.7	41.7	56.6

TABLE 3

Efficiency of the control of some weed species with various rates of
flurochloridone

Weed species	Dose of flurochloridone/per cent of sites		
	0.75 kg*	0.5 kg	0.375 kg + other herbicide
G.aparine	100.0	76.7	95.9
Veronica sp.	98.4	90.9	94.3
V.spp.	85.4	71.9	82.4
T.arvense	100.0	96.4	98.1

*heavy soil

Weed control with flurochloridone was fairly stable from year to year
which confirms earlier research (Adamczewski *et al.*).

Flurochloridone alone or in mixture with other herbicides applied shortly before potato emergence or post-emergence injured potato plants (4-8 in EWRC scale) and the damage later resulted in a yield decrease (table 4). The herbicide applied 10 days after planting was selective for the potato.

TABLE 4

Influence of the date of flurochloridone application on tuber yield in the years 1983-1986

Days before emergence	No. of sites	Yield difference between treated and untreated - t/ha
0	12	- 0.3
2 - 5	28	+ 0.2
6 - 14	43	+ 9.7

Significant differences in the yields between treated and untreated fields were found. In commercial trials flurochloridone produced yields on average 5.1 t/ha higher. When decreased rates of flurochloridone in mixture with other herbicides were used the average yield was higher by 4.3 t/ha (table 5).

TABLE 5

Comparison of potato yields produced in commercial trials (mean for the years 1984-1985, n = 61)

Treatment	Rate per ha	Tuber yield in t/ha
flurochloridone	0.5-0.75 kg	27.6 a
flurochloridone+ other herbicide	0.375 kg +	26.8 a
control (mechanical cultivation till emergence)		21.5 b

DISCUSSION

The results of the experiments presented have shown that flurochloridone alone (0.5-0.75 kg/ha a.i.) or in mixture with linuron or metribuzin at a decreased rate (0.375 kg/ha a.i.) gives excellent control of broadleaved weeds. The level of the efficiency was not much changed from year to yearr. Some other weed species were controlled less effectively.

When applied early in the season i.e. within 10 days after potato planting the product is selective to the potato. The mechanism of this selectivity seems to be based on mechanical separation of the potato plant from the chemical (Forbes *et al*.1985).

As compared to other pre-emergent herbicides e.g.linuron, metribuzin, EPTC, flurochloridone reduces the need for mechanical cultivation. Sometimes only in heavy soils a need for additional cultivation may arise.

REFERENCES

Adamczewski K.A.; Kowanski K.; Pietkiewicz J.B. (1985) Zwalczanie chwastow preparatem Racer w pszenicy ozimej, zycie i ziemniakach w sezonie 1983/84. Ochrona Roslin, 11, 6-8.

Forbes G.R.; Mathews P.R. (1985) Pre-emergence R-40244 for early weed control in potato and carrots. Proceedings 1985 British Crop Protection Conference — Weeds, 3, 797-804.

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