SESSION 5

CROP SAFENING: THE CONCEPT AND THE PRACTICE

THE EVOLUTION OF PRACTICAL CROP SAFENERS

R. A. Gray and L. L. Green
Stauffer Chemical Company, Mountain View Research Center, Mountain View, CA 94042

P. E. Hoch and F. M. Pallos
Stauffer Chemical Company, de Guigne Technical Center, Richmond, CA 94804

Summary. Herbicide safeners are an effective means of expanding the utility of active herbicides. The initial attempts at identifying practical products revolved around physical shielding of crops from herbicide contact with activated charcoal. Observations of pesticide antagonism lead to the development of systematic research targeted at a chemical safener. 1,8-Naphthalic anhydride (NA) was the first product to demonstrate the practical utility of chemical safening agents. The events associated with R-25788 (N,N-diallyl-dichloroacetamide) discovery and development are described. Observations on the trends leading to successful safener development are forwarded.

THE NEED FOR HERBICIDE SAFENERS

We in agriculture must continue to seek new methods of increasing crop production if we are to meet the challenge of feeding the world. Our ability to meet this commitment, by forcing land with marginal production potential into use, is limited. Instead, we must look for new technology which will allow us to better utilize the potential of lands already under cultivation.

The introduction of new herbicide products is an essential element in this technology search. Since their introduction more than forty years ago, herbicides have improved crop yields and quality as well as reduced costs through savings in labor.[1] However, as the costs and restrictions associated with new agricultural chemicals increases, the frequency of such introductions has dropped. This drop has encouraged researchers to investigate ways to better utilize the products already available. One novel approach to this has been to employ herbicide antidotes, or safening agents.

Safening agents benefit a herbicide by increasing the ratio of crop selectivity to weed control. This is achieved by enhancing the crop's ability to withstand herbicide exposure. The advantages of such activity can be the ability to increase the use rate in an already tolerant crop, and thereby achieve better weed control, or the ability to introduce the herbicide into a new crop in which entry was barred by poor selectivity. Both of these result in the farmer having more effective chemical products for combating weed pests.

EARLY WORK WITH HERBICIDE SAFENERS

The initial scientific research into herbicide safeners was confined to a physical shielding of the crop from herbicide contact. Arle, Leonard and Harris[2] first accomplished this effect more than thirty years ago by applying a coating of activated charcoal to sweet potato slips which were then exposed to 2,4-D. The limited success achieved with this treatment has continued to draw the attention of the agricultural community. Current work, by a number of researchers, has shown practical potential for activated charcoal with a number of crops, herbicides and cultural practices.[3,4]

The main drawback in activated charcoal use has been the inability to maintain the physical barrier between crop and herbicide. As crop roots grow into the zone of herbicide absorption, contact and injury result.

The first indications of a potential for chemical safening agents emerged from observations of antagonism between pesticides. Though many researchers had observed this phenomenon, Otto Hoffman was the first to develop its potential as a safening agent. His early observation of antagonism between 2,4-D and 2,4,6-T on tomato plants (1947),[5] was the stimulus for his research. His efforts to employ this new concept lead to the first systematic research directed at identifying chemical safening agents.

Hoffman's earliest efforts were directed at developing a safening agent for foliar application of 'Barban'® herbicide to wheat. Workers in England were also active in this research. Hoffman noted that 2,4-D, 2,3,6-TBA and MCPA as post-emergent sprays with Barban reduced activity of the combinations on wheat. This led to the thought by Hoffman of seed treating wheat with various growth regulants. The best material found was 4-chloro-2-hydroximinoacetanilide, coded S-449 (1962). Two other compounds 2,4-dichloro-9-xanthenone and N-methyl-3,4-dichlorobenzene sulfonamide were found about 1/4 as active. Expansion of Hoffman's effort to investigate the predisposition of various herbicide/crop combinations to safening agents, lead him to work with EPTC and corn.[5] He found that 4-chloro-2-hydroximinoacetanilide is also active as a safener on corn against EPTC injury.[5]

Stauffer researchers R. Gray, et al in the early 1960's were also investigating seed treatment of corn with various chemicals to reduce the injurious effects of 'Eptam'® herbicide. Gibberellic acid, indole acetic acid, naphthalene acetic acid were found to give slight but not practical protection. Charcoal was found effective but was not persistant enough and late injury occurred.

The observation of Hoffman that S-449 protected corn from EPTC injury led Stauffer Research personnel R. Gray, et al to repeat this work and intensify their efforts for other more effective safeners. Other compounds were found about equally effective as seed treatments but like S-449 they did not give long enough protection at 1248 ppm of seed and were not commercially developed.

At the 1969 Weed Society of America, Hoffman announced the discovery of a more effective corn seed treatment safener to reduce injury by EPTC. The chemical was 1,8-naphthalic anhydride (NA). Other compounds such as N,N'-dialkyloxamide, N,N'-dipropargylmalonamide and propargyl amine were also mentioned but were less active.

The 1,8-naphthalic anhydride was effective at 0.5% by weight on seed preventing EPTC injury even at rates as high as 9 kg ai/ha in the greenhouse.

Commercialization of this compound followed the announcement in 1972 when Gulf introduced 'Protect'® antidote into the marketplace.

Though a practical success, NA achieved only marginal consumer acceptance. The product failed because of the need for seed treatment application. This application method was dictated by a need for intimate seed contact, in order to elicit the desired safening response, and a potential need to avoid safener contact with weed seeds, so as to avoid herbicide antagonism. This application requirement proved to be a significant drawback for consumer acceptance of NA.

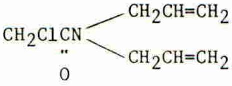
Stauffer Chemical Company's interest in safener research was enhanced by Hoffman's success in identifying NA. Hoffman's work demonstrated that safening agents had the potential to increase the usefulness of Stauffer's proprietary thiocarbamate herbicides. Our initial investigations were limited to evaluating the potential of certain compounds already synthesized, and available on the shelf. Research biologist Duane Arneklev at Stauffer's Mountain View, California, Research Center became involved at this point. The testing procedures employed were similar to those developed and disclosed by Hoffman's EPTC/NA corn work. These evaluations were restricted to corn; he examined safener potential with 6.7 kg ai/ha EPTC preplant incorporated, and used seed treatment applications of 0.05% to 0.5% safener weight/seed weight.

Several marginally active safening agents were identified by this approach. In January 1970, Ferenc Pallos at Stauffer's Richmond, California, Research Center started a tailored synthesis program to pursue various hypotheses concerning the structural requirements for safener action. In the meanwhile, by April 1970, Arneklev's efforts pinpointed R-6869, a methanesulfonate, as an antidote for corn with activity superior to NA. It showed higher activity by seed treatment but it also was found to be active by soil application. R-6869 applied at 1 kg ai/ha to the soil protected corn from EPTC at 6 kg ai/ha. The structure of R-6869 suggested to us at that time that a possible alkylating effect could be responsible for the antidotal action.

$$CH_3SO_2OCH_2CH_2Br$$

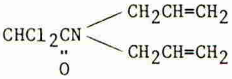
R-6869

The pursuit of the alkylating hypothesis led us to evaluate haloacetamides such as CDAA with its active halogen. Indeed it was found to be an active safener for EPTC on corn.



CDAA

Since the monochloroacetamide halogen is quite chemically labile, it was theorized that if we could reduce the reactivity of the chlorine in the molecule, we also would reduce its activity, thus providing an added measure of evidence for the alkylating hypothesis. To test the idea, the dichloro compound was synthesized in October 1970 and submitted for testing with the code number R-25788.



R-25788

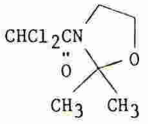
The results disproved our predictions but good science is designed to challenge hypothesis. R-25788 was found to be a much stronger antidote than CDAA and has resulted in the commercialization of several products by Stauffer Chemical Co.

At that time Stauffer's biological screen routinely alternated rows of safener treated and untreated corn seed. Because of this procedure, we were able to make two important observations of R-25788 safener activity. The first was that seed application of R-25788 showed complete reduction of EPTC injury to corn. The second, and most surprising, was that untreated corn seed, in adjacent rows, also showed a marked reduction in EPTC injury. The implication of this finding was that R-25788, like R-6869, had the potential of working as a soil applied safener.

Greenhouse evaluations, performed in late 1970, were designed to fully characterize the safener activity of R-25788. Rate studies showed that soil incorporation of one part antidote tank-mixed with 100 parts EPTC was sufficient to provide complete corn protection. In addition, it was established that while R-25788 was a highly active safener on corn, it had no effect on EPTC control of weeds. Further, it was found that the excellent safener activity discovered with EPTC could be transferred to other thiocarbamate herbicides such as butylate and vernolate.

Field evaluations of R-25788, begun in 1971, soon established that the soil activity observed in the greenhouse could be easily translated to field plots where typical user cultural practices were employed.

As the soil activity of R-25788 was investigated, an accelerated synthesis effort produced hundreds of analogs for screening. The result of this effort was the identification of several new compounds and chemical series which exhibited safener activity comparable to R-25788. One compound developed during this period was 2,2-dimethyl-3-dichloroacetyl oxazolidine (R-29148).



R-29148

Commercialization of R-25788 was aimed at tank-mixed combinations with various thiocarbamates. Marketing decisions to pursue combinations with EPTC and butylate were established during this time. 'Eradicane'® (EPTC + R-25788) would be developed as a product aimed at the corn market in which hard-to-control weeds, such as wild cane (Sorghum bicolor), rhizome johnsongrass (Sorghum halepense), quackgrass (Agropyron repens), seedling johnsongrass (Sorghum halepense) and wild proso millet (Panicum miliaceum), were major problems. 'Sutan$^+$'® (Butylate + R-25788) would be developed for general weed control in corn. Both products would offer excellent weed control with total elimination of corn injury.

Stauffer's evaluation of R-25788 was not limited to testing as a safening agent with corn. Once soil activity was established, an effort was launched to evaluate safener activity with numerous crops. Though a marginal response could be elicited from some crops, none displayed the dramatic response to soil applied R-25788 that was seen with corn.

Commercial introduction of R-25788 in combination with EPTC (Eradicane) and butylate (Sutan$^+$) was achieved in 1973. The success of these products may be best demonstrated by the greatly increased usage of these herbicides over the subsequent years.

Since the development of R-25788, Stauffer has continued to refine and improve its procedures for identifying safening agents. Our initial efforts were directed at finding antidotes for corn only. Upon realizing that activity was not generally transferable between different crops, Stauffer Chemical Company's screening procedures were changed to include those crops with which success was desired.

This research effort continues and Stauffer has an extensive library of safeners for corn and other crops for thiocarbamate herbicides. Exciting leads for new safeners for other crops and other classes of herbicides are also under development.

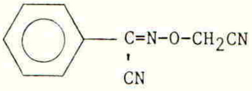
Needless to say, it has been found easier to find crop safening action when the herbicide is somewhat selective to the crop. It's also apparent that more safening compounds "fall out" for certain crops especially corn, sorghum and more readily for certain classes of herbicides than others. This reflects a need for more fundamental knowledge on mode of action of the phenomenon of herbicide antagonism or safeners action. Some excellent work has been done and is being done in this area at universities and in the private sector.

Basic research in the interactive biochemistry of the plant, herbicide, and safener is only part of the need. One must add an understanding of all other variables affecting such compounds relating to volatility, leaching, route of entry into the plant, soil types, moisture, and plant variety differences.

RECENT DEVELOPMENTS IN HERBICIDE SAFENERS

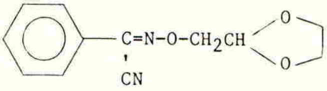
As the practical and economic success of herbicide safener research has been realized, the competition for new products and discoveries has intensified. Today more than seventeen companies and research organizations worldwide have published patents in this area. The research success embodied in these patents covers a large spectrum of herbicides, crops and use methods.

Recent successes by Monsanto and Ciba-Geigy corporations have shown sufficient activity to warrant the initial steps of commercial development. Both of these companies have isolated seed treatment safeners which reduce acetanilide herbicide injury to grain sorghum. These compounds are somewhat reminiscent of NA in that seed treatment is necessary in order to assure good crop protection while avoiding antagonism of weed control. Ciba-Geigy's entry into this market was prompted by their 1977 discovery of alpha-[(cyanomethoxy)imino]benzeneacetonitrile (CGA-43089) (cyoxymetrinyl)[8].



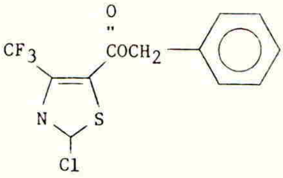
CGA-43089

This compound was demonstrated to be an effective seed treatment safener of metolachlor injury to grain sorghum. Though the safening performance of this compound is substantial, development has been hindered by adverse effects on crop seed vigor and viability. Attempts to ameliorate this effect have apparently lead Ciba-Geigy to begin development of a new safener, CGA-92194, which has comparable safener activity without producing effects on sorghum seed viability.



CGA-92194

Monsanto's most prominent success to date has been their discovery of 2-chloro-4-trifluoromethyl-5-thiazole carboxylic acid, benzyl ester, (Mon-4606) (flurazole).[9] Seed treatment applications will effectively reduce alachlor injury to grain sorghum. Continued development of Mon-4606 by Monsanto could lead to full commercialization in the next one to two years.



MON-4606

HISTORICAL PERSPECTIVE

In looking back over the past 10 to 15 years of safener research, distinct trends in the development of the safener concept can be observed. The initial concept relied on a physical barrier to separate the crop from herbicide contact. Though marginally successful, this failed because of our inability to maintain the barrier around the growing plant.

Observations of pesticide antagonism were to precipitate the first major change in the safener concept. If an immunity to herbicide injury could be elicited by a chemical reaction between a safener and a crop, then the problems of barrier maintenance could be overcome. This idea led to the development of the first chemical safener, 1,8-naphthalic anhydride.

The major drawback of the chemical safener concept was not in its technical aspect, but its lack of marketing sophistication. As with all products, it is necessary to mesh new technical advancements with the established use patterns of the market. NA failed to accomplish this because of the necessity of seed treatment application.

R-25788 was the first safener product to combine a sound technical and marketing concept into one package. This was achieved by developing a highly active safener which was sufficiently selective in its spectrum as to allow broadcast application. The result was a quick and widespread market acceptance of the product.

Though probably the most technically challenging solution to meshing new technology with market needs, the route taken by Stauffer with R-25788 is not the only solution available. The more recent attempts at safener development have again returned to seed treatment application. In order to avoid market rejection of this methodology, the seed treatment is being applied by either the chemical manufacturer or seed distributor. The potential advantages of this solution are reduced requirements for safener activity and selectivity, and a possibility for increased sale of proprietary seed hybrids.

FUTURE PROSPECTS FOR SAFENERS

It's apparent from the extensive research activity reflected in patents and the literature and in commercial pronouncements of safeners that many of us feel that this is a viable field for research, and safeners have a future.

As was stated earlier in the paper, a definitive understanding of the mechanisms involved in the antidotal action of established safeners would be most useful in conceiving of other combinations.[10,12] Major discoveries in the safener area as well as most of the crop protection chemical areas have been made via targeted but generally empirical approaches. This certainly will yield safeners of the future.

Tissue culture, suspension and callus, has been established as a valuable tool to supplement the search for herbicidal activity. Perhaps more important for the future, use of tissue culture will permit a more definitive approach to basic biochemical plant cell interactions with herbicides and safeners.[11]

The enormous library of knowledge on crop protection chemicals has led to increasing attention by researchers on quantitative structure activity relations (QSAR). Obviously Stauffer and other company researchers have applied such thinking to safeners. Stauffer's simplest case is with the safener R-25788. As can be imagined, hundreds of such analogues have been prepared. Many such structures were designed with targeted structure activity thinking and many active compounds resulted. Among the most active is R-29148 but I must confess serendipity also played a key role in its discovery. G. R. Stephenson's research in this area is also quite significant.[13] Stephenson's research suggested that designing safeners close in structure to the herbicide was a viable approach to new safener leads.

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CROP SAFENING IN JAPAN

Ko Wakabayashi and Shooichi Matsunaka

Pesticide Science Society of Japan (Mitsubishi Chem. Ind. Ltd., Research Centre,
1000 Kamoshida-cho, Midori-ku, Yokohama 227; and Kobe University,
Faculty of Agriculture, Rokkodai-cho, Kita-ku, Kobe 657, Japan)

Summary. In their improved and expanded uses, herbicides should show enhanced selectivity to prevent crop injury. From this standpoint, we considered and reviewed the present Japanese situation of the research and development regarding the following subjects: modification of agricultural techniques in herbicide application, improved formulations of herbicides, structural modification to obtain new selective herbicides, use of crop safeners together with herbicides, and recent studies concerning the genetic enhancement of crop resistance against herbicides.

INTRODUCTION

In approximately 40 years of rapid advance in herbicide research and development all over the world, we are, at present, in a steady state situation in chemical weed control. Because of increasing costs and risks in research and development which include extensive toxicological evaluations, new herbicides are discovered less frequently than in the past and relatively few new chemicals are coming into commercial use. Furthermore, any indication of unfavourable toxicological properties will result in current herbicides being discontinued. This situation concerning herbicide research and development urges us to reconsider the use of current herbicides. Improved and expanded use of these herbicides to enhance selectivity and prevent crop injury has been one of the aims of this reconsideration. This may be accomplished by modifications of agricultural techniques in herbicide application, by the use of improved formulations of herbicides, by structural modification to obtain new selective herbicides, by the use of crop safeners or antidotes together with herbicides, or by enhancement of crop resistance against herbicides using modernized breeding technology.

In this paper, we will consider and discuss the practice of selective weed control in Japan and the general principles underlying this control, review the recent progress of the research and development of crop safening in herbicide uses, and evaluate its role in world agriculture.

CROP SAFENING IN JAPAN

(1) Improvements in agricultural weeding practices

In Japan, rice is still the most important crop, having a cultivation area of 2.4 million ha in 1980. Weed control in rice paddy fields is achieved by the use of herbicides in two or three sequential applications. In 1946 weed control in rice was done by hand or mechanically. About 500 h of manpower per ha were required for weeding at that time, but only 66 h are calculated for this at present. Taking the costs of herbicides and their application into consideration, the economic effect of herbicide use (hand weeding cost minus chemical weeding cost) may be calculated to be 1,000 US $/ha; a total of 2.4 billion US $ for Japan.

An important factor in chemical weed control is that two different growing techniques are used in Japanese rice cultivation. One is the transplanting of rice seedlings, and the other is the flooding of the fields. In this country, both techniques have a long history and, harmonized with herbicide applications, are contributing to good weed control practice. Transplanting of somwhat bigger seedlings has a great advantage in the competition with the emerged weeds. Flooding has two benefits; the first is the decreasing of the total amount of weeds and the second is the selective suppression of C_4-weeds which are rampant in upland conditions in the hot summer season and severely compete with rice, a C_3-plant. Arai et al. (1955) surveyed the species and amounts of weeds in three different humid soil conditions. Tanaka (1976) has recently reconsidered their results from the concept of C_3-C_4 plant classification and found that C_3-weeds dominate C_4-weeds under flooded conditions. On the other hand, Vega et al. (1969) have indicated the difference of grain yields in the non-weeded and hand weeded plots in their experiments in Philippines. In flooded rice cultivations the grain yield is much higher and losses through weed damage (36%) are lower than those for upland rice (84%). This information shows us that the flooding practice contributes to giving rice plants an advantage in competition with weeds.

Table 1

Summary of data on distribution of summer weeds in different humid soil conditions in rice culture (after Tanaka, 1976)

	Soil conditions		
Weed type	Submerged	Water saturated	Upland
Total for C_3 weeds (g)	8.65 (89%)	1.70 (6%)	3.85 (7%)
Total for C_4 weeds (g)	1.05 (11%)	29.10 (94%)	54.00 (93%)

(2) Improvements of herbicide formulations for crop safening

Granular formulations of herbicides

During the 30 year history of modern herbicide use in this country many compounds were introduced and developed for paddy rice culture. The most remarkable achievement in this respect is the utilization of granular formulations of herbicides. Initially, 2,4-D and MCPA were structurally modifed to ester forms and their granules were applied in the paddy field to avoid injury to neighbouring useful plants, especially mulberry trees. The purpose for the formulations was to restrict the herbicides to a very thin layer on the soil. Next came the development of PCP (pentachloro-phenol) granules. If PCP-Na solutions were applied to the transplanted rice plants, the rice leaves would suffer severe injury. However, PCP granules did not adhere to the foliage of the plants and caused no injury to the crop, but sank on to the mud of the paddy fields, forming a uniform herbicide layer on the soil surface. Thus they proved reasonably effective in killing weeds, mainly barnyard grass whose growing parts are always concentrated in the region of the herbicide layer, and prevented crop injury through the root (Takematsu, 1968).

Now, almost all herbicides used in paddy fields have been formulated in granular form, even though they have a few disadvantages, such as somewhat higher cost of formulation, storage and transportation. These can be compensated by the advantages of their easy application and the above-mentioned crop safening effect to rice plants. Nowadays, the granular formulations of thiobencarb and CNP have been markedly successful in paddy rice culture in Japan. When sales of herbicides in Japan in 1980 are classified from the standpoint of formulations, the percentages are:- granules (56%), emulsifiable concentrates (30%), wettable powders (13%) and others (1%). Among the ten most successful rice herbicides, only one, oxadiazon, which will be described in the next section, is formulation as an emulsifiable concentrate, the others are as granules.

Emulsifiable concentrate herbicide formulations

Oxadiazon emulsifiable concentrate: formulation and application timing. An unusual herbicide formulation available for paddy rice culture is the emulsifiable concentrate of oxadiazon. This concentrate is applied by hand directly from special holes in a container like a Coca-Cola bottle, on to the muddy water surface at the time of puddling. The floating soil particles adsorb the herbicide and then settle to form a firm soil-herbicide layer on the bottom. This gives good weed control and does not cause injury to transplanted rice seedlings. If the concentrate is applied at other times before or after transplanting, it causes crop damage and the selectivity is not maintained. The granules of oxadiazon also form a similar thin herbicide layer, but some of the herbicide dissolves, is absorbed by the stems, and causes crop injury. This explains why the emulsifiable concentrate formulation of this herbicide can only be used safely in muddy water conditions (Kawamura, 1971).

Propanil emulsifiable concentrate: enhancement of differential herbicide absorption between crops and weeds. Propanil shows a considerable selectivity between rice plants and barnyard grass. Its selectivity results from the presence or absence in the plants of an aryl acylamidase which can detoxify the parent herbicide to 3,4-dichloroaniline. In addition to this biochemical aspect, Konnai (1967) also researched improved formulations of this herbicide, and reported that the selectivity of propanil is intimately connected with the hydrophilic-lipophilic balance of the formulation and the difference between the fine leaf structures of both plants. Further physiological studies revealed that the surfaces of rice leaves are relatively more lipophilic than those of barnyard grass. Much empirical work was necessary to optimize the propanil formulation using surface-active agents, solvents and other substances in order to achieve maximum selectivity. As a result, 2-(1-cyclohexenyl)-cyclohexanone (CHCH), a more hydrophilic solvent, was found to increase the selectivity of propanil. The typical formulation for propanil now used in paddy rice culture consists of: propanil (35%), isophorone (25%), CHCH (7%), xylene (25%) and Solpol (surface-active agent) (8%).

(3) Use of controlled release herbicides

Controlled release pesticide technology promises to improve the efficacy of some existing pesticides and solve the environmental problems associated with others. Several parameters, including volatility, photodecomposition, leaching, microbial metabolism, binding, and accumulation, determine the persistence, distribution, and indirectly the efficacy of pesticides. A major benefit that controlled release technology can bring to weed control is to expand herbicide selectivity to additional crops.

Microencapsulation of herbicides

Microcapsules are 1-200 micron particles of a solid or liquid core surrounded by a wall. The wall is usually polymeric in nature and protects the core material (pesticide) in storage, but is designed to release the core in a controlled fashion when the microcapsules are used. The core material can be released by crushing the wall, breaking the wall by pressure from within, dissolving the wall, hydrolysing the wall or diffusing through the wall (Scher, 1977). Microencapsulation of herbicides using many types of wall materials is being investigated in Japan. Yamamoto and Katsuda (1980) have indicated that pyrethroid insecticides become more stable when included in β-cyclo-dextrin, particularly because photodecomposition is reduced. Yamamoto (1982) has recently included the thiocarbamate herbicides, such as molinate, using β-cyclo-dextrin and starch-xanthate in his research. He has demonstrated great improvements by reducing the volatility of the herbicide.

Control of herbicide movement in soil: modification of herbicide leaching

Modification of the leaching properties of soil-applied herbicides has great significance in selective weed control. Reduced leaching of a relatively mobile herbicide could lead to better selectivity if the selectivity depends largely upon the depth at which herbicide is located. Moreover, keeping the herbicide in the target area could also reduce the dosage required, thus decreasing phytotoxicity. The use of certain food-grade surface active agents or latex emulsions as adjuvants is being investigated in Japan and is resulting in additional benefits, such as the decrease of soil residue problems.

Use of surfactants. Saito and Hayabe (1962) reported that the leaching of simazine was greatly reduced when used with the surfactant oxyethylene docosanol (OED).

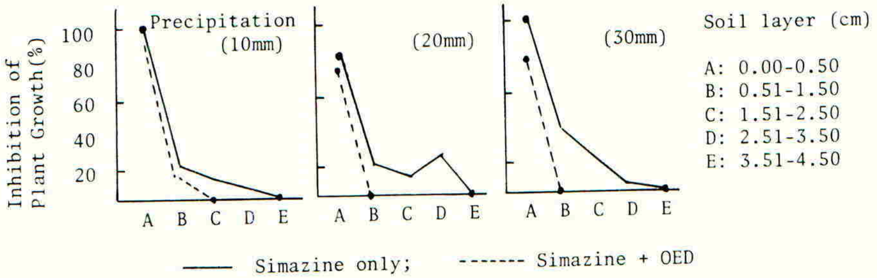


Figure 1. Effect of OED on leaching of simazine

Use of latex emulsions. Takematsu et al. (1978) studied the effects of latex emulsions on the leaching and adsorption of many types of soil-applied herbicides and found that a urethane type of latex emulsion, Sanpex CE-15, was the most promising. Simazine is normally leached to a depth of 3-4 cm but (with the addition of Sanpex CE-15) forms a firm herbicide-soil layer at a depth of 0-2 cm. Sanpex CE-15 also prevents leaching of many other herbicides such as diuron, atrazine, MK-616 (N-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide), and reduces their phytotoxicity as shown in Table 2 (Watanabe, 1982).

Table 2. Crop safening effect of Sanpex CE-15. Scale: 0-5 (plants dead)

Herbicide dosage (100g/ha)	Sanpex CE-15 dosage (kg/ha)	Phytotoxicity	
		Wheat	Radish
Diuron	0	1	2
	2.5	0	1
	5.0	0	0
Atrazine	0	1	1
	2.5	0	0
	5.0	0	0
Trifluralin	0	2.5	0
	2.5	1	0
	5.0	0	0
MK-616	0	2	1*
	2.5	2	0.5*
	5	1	0*

TEST: 400 l/ha of the mixture of Sanpex CE-15 and herbicide is sprayed in upland conditions. Effect is evaluated 20 days after application.

* Plant = Soybean

Crabgrass and amaranthus were completely killed in all application plots, showing a score of 5.

(4) Structural modification of herbicides

The presence of a special reactive or functional moiety in the molecule of a herbicide may render it more mobile in soil, more easily absorbed by crops, and more phytotoxic. A derivative of such a molecule may possess more favourable mobility, absorption and selective properties, although in some cases the herbicidal spectrum of the original compound may be slightly altered.

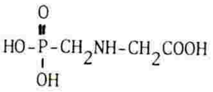
Masked herbicides

With 'masked herbicides', the intention is that the original herbicidal molecule should be liberated in a range of soil-layers or within weed plants but less so within crops, and preferably only in the target area or within the target weeds. Several new selective herbicides have been based on this concept in Japan.

Esters of 2,4-D and MCPA in paddy rice culture. 2,4-D and MCPA are much more active against dicotyledonous than monocotyledonous plants, including rice. However, the vapours of these chemicals may cause phytotoxicity to rice plants and also to nearby mulberry trees. Esters normally exhibit greater herbicidal activity than the parent acids and appear to widen the difference between the susceptibility of weeds and the tolerance of useful plants (Takematasu, 1968). The ethyl and allyl esters of 2,4-D or MCPA may be cited as examples.

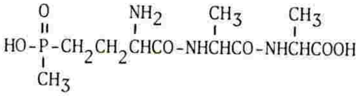
Applying the granular formulation of these herbicides 25 to 30 days after transplanting rice seedlings catches most of the weeds at their more susceptible stage, while inflicting minimal injury on rice and mulberry.

Herbicidal antibiotic, bialaphos. A herbicidal antibiotic, bialaphos, is obtained from the fermentation broth of *Streptomyces hygroscopicus*. The chemical structure is identified as L-2-amino-4-(hydroxy)(methyl)phosphinoyl)-butyryl-L-alanyl-L-alanine and resembles the L-alanyl-L-alanine conjugate of glyphosate, although it does not correspond precisely. Bialaphos controls both dicotyledons and monocotyledons, and is useful for the control of many kinds of weeds of agricultural importance such as barnyard grass, lambs quarter, purple nutsedge, curly dock and water hyacinth. Bialaphos is slower acting than paraquat, and considerably faster acting than glyphosate. It controls the regrowth of perennials longer than paraquat, and has a more lasting effect like glyphosate. The herbicide acts on any growth stage of weeds by foliar application, but shows little or no activity by soil application, so that it may be suitable for orchards or mulberry fields (Sekizawa *et al*., 1982).



$$\mathrm{HO{-}\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}{-}CH_2NH{-}CH_2COOH}$$

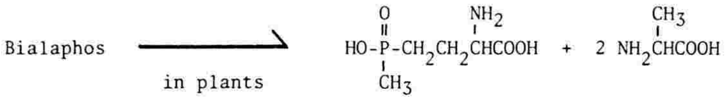
Glyphosate



$$\mathrm{HO{-}\underset{\underset{CH_3}{|}}{\overset{\overset{O}{\|}}{P}}{-}CH_2CH_2\overset{\overset{NH_2}{|}}{C}HCO{-}NH\overset{\overset{CH_3}{|}}{C}HCO{-}NH\overset{\overset{CH_3}{|}}{C}HCOOH}$$

Bialaphos

Sekizawa *et al*. (1982) have recently observed that a main metabolite in sensitive plants, e.g. yellow nutsedge or crabgrass, is L-2-amino-4-(hydroxy)-(methyl) phosphinoyl)-butyric acid which strongly inhibits the glutamine synthetate in such plants. A synthetic racemic mixture, D,L-2-amino-4-((hydroxy)(methyl)-phosphinoyl)-butyric acid (HOE-661) has also shown very similar herbicidal activity to bialaphos and its metabolite. These facts suggest that the herbicidal action of this antibiotic is concerned with the metabolism of susceptible plant species and that its herbicidal principle is based on the metabolite through bioactivation. Bialaphos may be roughly considered a kind of masked HOE-661 herbicide, which liberates the original herbicide molecule only within the plants.



$$\text{Bialaphos} \xrightarrow[\text{in plants}]{} \mathrm{HO{-}\underset{\underset{CH_3}{|}}{\overset{\overset{O}{\|}}{P}}{-}CH_2CH_2\overset{\overset{NH_2}{|}}{C}HCOOH} + 2\ \mathrm{NH_2\overset{\overset{CH_3}{|}}{C}HCOOH}$$

(L-form) L-alanine

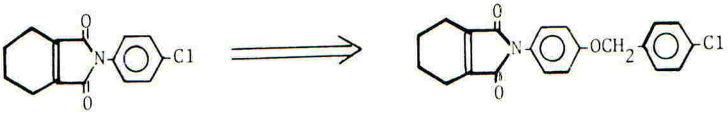
Structural modification to give selective properties

N-(4-(4-chlorobenzyloxy)phenyl)-3,4,5,6-tetrahydrophthalimide (MK-129). One of the authors (1979) has already reported that N-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide (MK-616) is a potent herbicidal compound belonging to the diphenylether category of herbicides, having o-substituent(s) such as CNP or nitrofen. This compound was originally too toxic to rice plants to use in paddy rice. Subsequent research with a series of analogues had three objectives:-

I. To find out what structural modification could be made to the parent compound without losing its unique biological properties. Such information could elucidate a molecular feature, either in whole or in part, associated with herbicidal activity.

II. To find out whether the parent compound and its analogues are active _per se_, or whether their activity results from modification within, or in the vicinity of, the plants.

III. To design new compounds with improved selectivity making use of the information obtained.



(MK-616) (MK-129)

N-(4-(4-chlorobenzyloxy)phenyl)-3,4,5,6-tetrahydrophthalimide (MK-129) was found to be selective enough to use in paddy rice (Table 3).

Table 3

Herbicidal activity of MK-129 in pre-emergence application 3 days after transplanting (Wakabayashi and Watanabe, 1982a). Scale: 0-10 (complete kill)

Plants	MK-129 (1.5 kg/ha)	MK-616 (1.0 kg/ha)
Echinochloa crusgalli	10	7
Lindernia procumbens	10	10
Rotala indica	10	9
Alisma canaliculatum	10	10
Cyperus difformis	10	10
Monochoria vaginalis	10	8
Eleocharis acicularis	7	5
Eleocharis kuroguwai	10	8
Rice plants	0	5

Butachlor herbicide in paddy rice culture. Alachlor is a selective pre-emergence herbicide acting for 10 to 12 weeks. It is used for the control of annual grasses and many broad-leaved weeds in such crops as maize, soybean, cotton, sugar cane, peanuts, radish, rapeseed and other brassicas in upland conditions, but causes severe crop injury in paddy rice culture. Butachlor, being only slightly changed with the methoxy of alachlor replaced by butoxy, has been used for rice culture in Japan and Korea. It exhibited good selectivity, controlling grasses and certain broadleaf weeds in transplanted rice grown under flooded conditions; also in rice drilled directly into the soil (Selleck, 1969).

(5) Use of crop safeners or antidotes together with herbicides

There are many groups of chemical herbicides that have desirable attributes in weed control but do not always possess a sufficient margin of selectivity. An added degree of selectivity can often be achieved by suitable placement of the herbicide, by critical timing of application, by controlled release of the herbicide, or now the fourth approach, with chemical safening agents.

Twenty years ago, Matsunaka (1963) in his review "Development of antidotes for herbicides" classified antidotes (crop safeners) into five types:-

1. Binding type) Inactivation type
2. Degradation type)
3. Competition type
4. Compensation type
5. Exciting type

A typical example of binding antidote is the use of activated charcoal to inactivate herbicide residues, such as simazine (Anderson, 1968), linuron (Anderson, 1968), and diuron (Bayer, 1967) in soil. In Japan, Yamane *et al.* (1962) reported the utilization of charcoal for dichlobenil in wheat cultivation. Within crop plants, herbicides are often inactivated by conjugation with endogenous metabolites. Some compounds which can conjugate with the herbicide, or increase the amount of the metabolites, may thus be utilized as a binding type of antidote. In the maize plant, simazine may be inactivated by endogenous 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one, to hydroxy-simazine. Outside plants, pyridine, hydroxylamine or calcium polysulfide can degrade simazine (Castelfranco and Brown, 1962). These compounds are referred to as the 'degradation type' of antidotes, along with riboflavin which can accelerate the photodegradation of amitrole (Castelfranco *et al.*, 1963). One of the authors (Matsunaka, 1963) confirmed that the pretreatment of rice seedlings with riboflavin solution before transplanting produces tolerance to amitrole applied to the soil. If the glutathion and glutathion-*S*-transferase theory for EPTC-antidote, R-25788 (N,N-diallyl-2,2-dichloroacetamide) is correct, this compound may be classified a degradation type of antidote. A classical study of many antidotes for barban was the first example, using a 'competition type' of antidote (Hoffman, 1962). The herbicidal symptoms of Hill reaction inhibitors, phenylureas and *s*-triazines, can be relieved by the addition of sugars (Gentner and Hilton, 1960; Moreland, 1959). In this case, sugars may be referred to as 'compensation type' antidotes, but this has not found practical uses. Purine derivatives, such as adenine or guanine (for amitrole ; Sund *et al.*, 1960) and pantothenic acid (for dalapon; Hilton *et al.*, 1959) may also be of this type. 'Exciting type' antidotes 'excite' or promote the total activities of crop plants for detoxification or recovery from the injury of herbicides.

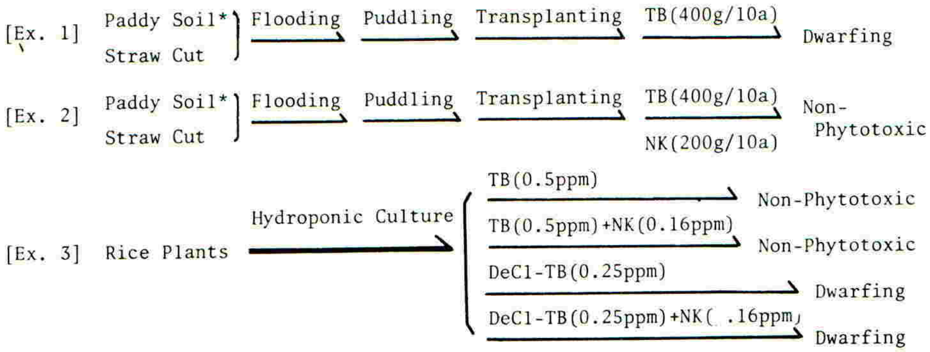
Ichizen (1980) has also reviewed "Development of antidotes for herbicides", in which he discussed recent advances in chemical and physical safening.

Reduction of the phytotoxicity of thiobencarb by methoxyphenone

Thiobencarb is sufficiently non-phytotoxic to rice to be used in paddy fields. Nevertheless, during the past few years, a dwarfing injury to rice plants has occurred in paddy fields under special conditions in Japan, and this has been attributed to the use of thiobencarb (Shigematsu *et al.*, 1980). A dechlorinated metabolite of thiobencarb, S-benzyl-N,N-diethylthiocarbamate, has been shown to be the cause of the dwarfing (Yamada *et al.*, 1979; Ishikawa *et al.*, 1980; Tatsuyama *et al.*, 1981). After further biological and biochemical studies using rice seedlings, it has been found that dechlorinated thiobencarb is formed only in soils under strongly reducing conditions, but not in sterilized soil even in the presence of organic matter. This fact suggests that the dechlorination of thiobencarb is promoted by certain soil microorganisms (Abe, 1981; Kuwatsuka, 1982).

If we could suppress the soil microorganism which is responsible for the dechlorination of thiobencarb or find the competitive substrate for thiobencarb, we might safely use the herbicide without any risk of adverse effects in paddy rice. There has already been one example in which methoxyphenone, a commercial herbicide, has been shown to act as the inhibitor of the dechlorination of thiobencarb. Shigematsu *et al.* (1981) have reported the antidoting activity of methoxyphenone in their model experiments, shown in Figure 2. In rice plants grown hydroponically, dechlorinated thiobencarb was not detected by residue analysis, although it caused plant injury when added to the hydroponic solution. Also under these conditions no antidoting effect of methoxyphenone for either thiobencarb or dechlorinated thiobencarb was found. The remarkable antidoting effect of methoxyphenone is observed only when it is applied with thiobencarb in paddy cultural conditions. It has now been concluded that this antidote may inhibit the microbial dechlorination of thiobencarb.

Chisaka (1981) studied the behaviour of methoxyphenone in combination with thiobencarb and observed that methoxyphenone was rapidly reduced in soil under reducing conditions to form the alcohol. This fact may suggest that methoxyphenone serves as an alternative substrate like methylenedioxy phenyl compounds in the synergism of pyrethroid insecticides. The discovery of such a biochemical effect of methoxyphenone has given a new impetus to research to develop more effective and cheaper compounds having this property. At present, we have several registered thiobencarb formulations combined with methoxyphenone.



*In which dwarfing was recorded in the previous year; TB: Thiobencarb, NK: Methoxyphenone, and DeCl-TB: Dechlorinated thiobencarb

Figure 2. Model experiment of antidoting action of methoxyphenone (Shigematsu et al, 1981)

$$\text{(3-Me)(4-MeO)}C_6H_3\text{-CO-}C_6H_4\text{(Me-3)} \longrightarrow \text{(3-Me)(4-MeO)}C_6H_3\text{-}\underset{\text{OH}}{\text{CH}}\text{-}C_6H_4\text{(Me-3)}$$

Methoxyphenone

Reduced Product

Improved selectivity of thiobencarb between rice plants and barnyard grass

Ichizen (1976) has investigated the phytotoxicity of thiobencarb to improve its selectivity between rice plants and barnyard grass and to identify effective antidotes. 1-naphthoxyacetic acid, 2,6-dichlorophenoxyacetic acid, 2,4,6-trichlorophenoxyacetic acid and 4-methyl-3-chloroaniline were all active thiobencarb-antidotes. 1,8-naphthalic anhydride and N,N-diallyl-2,2-dichloroacetimide (R 25788) were less active.

1,8-octamethylene diamine as the antidote for urea herbicides

N-(3,4-dichlorophenylcarbamoyl)-N-methylglycine monohydrate and N-(3,4-dichlorophenylcarbamoyl)-N-methyl β-alanine methylester are new urea herbicides and are mainly effective against broad-leaved weeds. Okii et al. (1979) studied their selectivity for wheat and weeds and found that 1,8-octamethylene diamine acts as an antidote for both compounds. This chemical can be used as a foliar application and its antidoting effects may be attributed to the prevention of the foliar uptake of herbicides. This chemical also reduces the phytotoxicity of fenac to rice and wheat plants and that of amiben to soybean.

1,8-naphthalic anhydride as the antidote for MK-616

As mentioned earlier, MK-616 is one of the most potent and cheapest compounds in the imide series of herbicides. Wakabayashi and Watanabe (1982b) screened many chemicals for a suitable antidote to MK-616: 1,8-naphthalic anhydride was found to be the most active. Its improved selectivity for corn is shown in Table 4.

Table 4. Antidoting activity of 1,8-naphthalic anhydride for MK-616

Application	Dosage Kg(ai)/ha	Crops		Weeds		
		Corn	Soybean	Crabgrass	Lambsquater	Velvet-leaf
MK-616wp alone	1	3	1	10	10	7
	2	5	3	10	10	9
Mk-616wp + NA	1	0	1	10	10	7
	2	0	3	10	10	9

NA: 1,8-naphthalic anhydride, 0.5% seed dressing
EVALUATION: 10 plants dead ~ 0 non-phytotoxic

(6) Use of crops resistant to herbicides

The introduction of herbicide tolerance genes into economic crops may be utilized to increase crop safety and promote improved crop husbandry practices. In the present decade, several possibilities for breeding crop cultivars with improved resistance to herbicides have been investigated by means of increased mutations in selection systems. It has already been indicated that induced mutation may provide a tool for breeding crop cultivars with increased resistance to certain herbicides.

Mutant selection at the whole-plant level

Pinthus *et al*. (1972) have selected wheat mutants with increased seedling resistance to terbutryne and tomato mutants resistant to diphenamid in experiments using populations grown from seeds treated with ethyl methanesulfonate as a mutagen. Souza Machado *et al*. (1980) reported the potential for transferring triazine herbicide resistance from a weed into economic crops. In that case, the cytoplasmically inherited resistance of an atrazine resistant weed biotype of *Brassica campestris* (2n=20) was intraspecifically incorporated into *B. campestris* cultivars (rapeseeds, 2n=20) through hybridization and backcrossing using the weed biotype of *B. campestris* as the female parent and cultivated rapeseeds as recurrent pollen parents.

Although plant breeders have achieved major improvements in many species using such classic techniques, conventional selection and subsequent breeding is a slow process. It is now believed that recent genetic engineering, supported by advances in several associated fields of plant tissue culture and genetic manipulation of plant cells, will superimpose powerful new techniques on classic breeding methods.

Mutant selection using tissue cultures: Callus or cell-suspension culture

The use of tissue cultures of flowering plants in mutant selection has proved very attractive in recent years. Manipulating large populations of cells, understanding the direct interaction between cells and herbicides, and regenerating plants from selected lines are all facets of this research. Many attempts to select for herbicide resistance have already been made by the use of calluses; these have revealed the main drawbacks impeding further developments but also indicated the future applications of this new technology to plant breeding. Examples of its value include the development of tobacco plants resistant to amitrole (Barg and Umiel, 1977), bentazone (Radin and Carlson, 1978), paraquat (Miller and Hughes, 1979), phenmedipham (Radin and Carlson, 1978) and picloram (Chaleff and Parsons, 1978), and the clovers resistant to phenoxyacetic acid herbicides (Oswald *et al*., 1977).

A combination of mutation studies at plant level and tissue culture has also been initiated. One example is the isolation of a herbicide-resistant mutant of Nicotiana tabacum by the regeneration of plants from green areas of leaves bleached by herbicides (Radin and Carlson, 1978).

Mutant selection using protoplasts

If a herbicide-resistant mutant is selected from a callus or suspension culture, not all cells are always resistant to the herbicide. Selection however using protoplasts isolated from plants may be superior because selection is done at the single cell level. Procedures such as protoplast cloning, which have the objective of enhancing a desirable variety rather than creating a new one, have become particularly favoured by Japanese breeders for the production of herbicide-resistant varieties (Uchimiya, 1981).

Herbicide-resistant varieties developed by somatic hybridization or gene manipulation

Because protoplasts have no cell wall, they can be fused with other protoplasts, thereby providing opportunities for somatic hybridization. Fusion of protoplasts of different species is now providing an additional method of hybridization. Sexually incompatible species can be hybridized and agriculturally useful hybrids can be selected for several characteristics including herbicide resistance. Many possibilities exist for hybridization assessments, both nuclear and cytoplasmic, between various crop species.

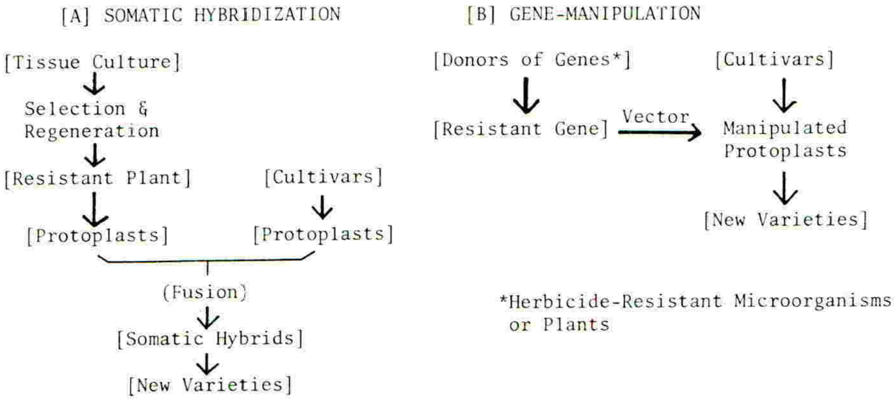


Figure 3. Production of herbicide-resistant varieties through Somatic hybridization and Gene-manipulation

The absence of the cell wall in protoplasts also facilitates the uptake of DNA and enables the transformation to be carried out at almost the same level as microbiological transformations. These manipulations are not restricted to the cellular level because sometimes whole plants can be regenerated by suitable tissue culture procedures, and then subjected to a conventional breeding programme. Thus, isolated plant protoplasts are providing an opportunity for the transfer of genes between different species. This may be accomplished by fusion with a mutant protoplast system or by direct transformation. Transfer of genes using Agrobacterium plasmid as a vector appears to be promising, and fusion with wild-type protoplasts will ensure the regeneration of non-tumorous plants. Figure 3 shows the procedure to produce herbicide-resistant varieties through somatic hybridization or gene-manipulation (Uchimiya, 1981; Cocking, 1981).

The Meiji Seika Kaisha Co. is now investigating the production of bialaphos resistant crops through the gene-manipulation method, while Mitsubishi Plantech Research Institute has begun the search for imide-herbicide resistant crops through a cell fusion method. Monsanto Co. has been successful in producing glyphosate resistant crops through the gene-manipulation method in the United States.

Due consideration of the mechanisms of selective action of herbicides makes us confident that resistant traits of herbicide insensitive plant species may reside in specific dominant genes. This is critical in experiments on somatic hybridization or gene-manipulation where the genes are expected to be expressed in the recipient cells. The target resistant mechanisms proposed in the recent investigations may be as follows (Nielson et al., 1979):-

1. Inactivation or destruction of the herbicide
2. Control of herbicide uptake
3. Modification of enzyme properties: Mutation of target enzyme
4. Overproduction of enzyme to decompose the herbicide
5. Oversynthesis of substrates to reverse phytotoxicity

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THE MODE OF ACTION OF HERBICIDE SAFENERS

G. R. Stephenson and G. Ezra
Department of Environmental Biology, Ontario Agricultural College
University of Guelph, Guelph, Ontario, Canada

Summary. The modes of action of NA, cyoxymetrinil, flurazole and R-25788 as safeners for thiocarbamate and other herbicides in plants are reviewed. Although not selective in its action unless applied selectively to crop seeds, NA is the most versatile safener with good activity for a number of different herbicides in a wide range of crop species. Its biochemical action as a safener is not fully understood but can be partially explained by overcoming herbicidal inhibition of lipid synthesis, enhancing herbicide detoxication, inhibiting herbicide accumulation at the shoot apex of grasses and interacting with the action of plant hormones. Chloracetamide safeners such as R-25788 are highly selective for protecting maize but not other plant species from thiocarbamates and other herbicides. They may be antagonistic to herbicidal effects on lipid or GA synthesis. They may enhance herbicidal metabolism or they may competitively inhibit the uptake or action of closely similar herbicides. Many of the practical aspects for the use of cyoxymetrinil or flurazole as seed applied safeners have been defined. However there is as yet little knowledge of their biochemical action as safeners for chloracetamide herbicides in sorghum.

INTRODUCTION

The concept of using chemical safeners to improve herbicide selectivity in crops was first developed by Hoffman of Gulf Chemical Company. The idea originated in 1947 when he observed an antagonistic interaction between 2,4,6-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid on tomatoes (Hoffman, 1978). However, his first publication did not appear until 1962, when he established that various chemical seed treatments were effective as safeners for barban in wheat (Hoffman, 1962). NA (1,8-naphthalic anhydride) became the first commercially used herbicide safener after Hoffman showed that when used as a seed treatment, it effectively prevented EPTC injury to maize (Hoffman, 1969). The next major discovery was that of Pallos and other researchers of Stauffer Chemical Company who found that various chloroacetamides were highly effective as safeners for thiocarbamate herbicides in maize (Pallos et al., 1972, 1978). In early studies, Chang et al. (1972) confirmed the physiological selectivity of R-25788 (N,N-diallyl-2,2-dichloroacetamide) as either a seed applied or soil applied safener for EPTC or butylate in maize but not other plant species. This property enabled R-25788 to be marketed as a formulation additive in new "safer" or more "selective" formulations of EPTC and butylate and potentially in other thiocarbamate herbicides as well.

These discoveries have led to a new optimism within the agricultural chemical industry. Researchers now realize that if an existing herbicide lacks adequate selectivity for use in a major crop, searching for a different herbicide is not their only alternative. Scientists at Ciba-Geigy (1977, 1978) have recently developed cyoxymetrinil and their counter parts at Monsanto (Sacher et al., 1982) have developed flurazole as seed applied safeners to extend the use of their respective chloroacetamide herbicides to include sorghum. Furthermore it is increasingly common to hear that researchers in industry are searching for safeners for candidate herbicides that are not yet registered. In these instances, developing selectivity for at least one major crop may be the key factor in the success of a new compound.

While industrial researchers continue their search for new chemical safeners, considerable research is being conducted to elucidate the mechanisms involved for the safeners already developed. NA and particularly R-25788 have been studied by several groups of investigators but research on cyoxymetrinil and flurazole is just now appearing in the literature. There is as yet no single theory on the mode of action of any safener that is fully accepted by all researchers involved. However, much of what has been learned could be useful to those attempting to develop new safeners for other herbicides in various crops.

SEED APPLIED HERBICIDE SAFENERS

NA (1,8-naphthalic anhydride)

Alachlor, barban, EPTC and the related herbicides that can be antagonized by NA, all have quite similar inhibitory effects on grasses such as maize, sorghum, oats, barley and rice. Injured plants are often dark green, twisted and bent and the leaves are often tightly rolled and brittle. Hickey and Krueger (1974) have suggested that alachlor and other herbicides with these effects may inhibit the emergence of the primary leaves from the coleoptile. Treatment with NA may have a loosening effect on the coleoptile which prevents this mechanical injury and the subsequent retardation of grass growth. However the biochemical aspects of this loosening effect have not been fully defined.

NA seed treatments have not been shown to inhibit the uptake of herbicides by protected grass species (Holm and Szabo, 1974; Thiessen, 1978). However, Thiessen (1978) did observe that NA prevented the accumulation of foliarly applied ^{14}C-barban at the shoot apex of protected oat seedlings. While NA has been shown to enhance the metabolism of cisanilide in maize (Holm and Szabo, 1974) significant effects of NA on the metabolism of other herbicides have not been reported.

Wilkinson (1978) has proposed that one of the most important toxic effects of EPTC is its inhibition of lipid synthesis. He has also shown that these effects on lipid synthesis can be reversed by treatment with NA. Wilkinson employed isolated spinach chloroplasts in these studies of lipid synthesis thus their relevance to the safening action in intact maize seedlings is uncertain.

There are also several indications that the safening action of NA involves interactions with plant hormones. The safening effect of NA was discovered by Hoffman (1978) in his testing of various auxins for suitability as seed applied safeners. The auxin herbicide, 2,4-D, is itself antagonistic to either EPTC or barban (Best and Schrieber, 1972), EPTC is known to block some steps in gibberellin synthesis (Wilkinson and Ashley, 1978) and NA plus GA often has a greater safening effect than NA alone (Stephenson and Chang, 1978).

Despite its potential use as a seed applied safener for many different herbicides in many different crops, NA has not emerged as a commercially important safener. This is most likely due to its variable effectiveness (Thiessen and Stephenson, 1980). However research interest seems to have declined along with the decline in its commercial importance and we may never have a precise understanding of its biochemical action.

Cyoxymetrinil

Cyoxymetrinil (α-[(cyanomethoxy)imino]benzacetonitrile) has been developed by Ciba-Geigy (1977, 1978) as a seed applied safener for the herbicide metolachlor in sorghum. It is effective against rates of metolachlor as high as 4 kg/ha when applied to sorghum seed at a rate of 1.25 to 1.5 g/kg of seed. Tolerance to metolachlor is maintained even in combination with various s-triazine herbicides. Several species of annual grasses are controlled and increases in sorghum yield have been observed. Cyoxymetrinil is not specific for sorghum and some protection from metolachlor for weeds has been observed when it wasn't applied selectively to the seed. This property may make it or one of its analogues useful for protecting other crops such as proso millet, rice or wheat from various herbicides (Nyffeler et al., 1980).

The site of uptake for both cyoxymetrinil and metolachlor appears to be the coleoptide of sorghum (Nyffeler, 1980). Metolachlor applied to the root zone of sorghum seedlings had no effect but applications in the shoot zone were highly toxic. Under these latter conditions cyoxymetrinil applications to the seed gave significant protection. It has been suggested that cyoxymetrinil may act by inhibiting the uptake of metolachlor or by enhancing its degradation during the 2 or 3 day period when the coleoptile is in contact with the herbicide.

Researchers at Ciba-Geigy (Turner et al., 1982) have also reported on the safening activity of CGA-92194 [N-1,3-dioxolan - 2 -yl-methoxy)-imino-benzacetonitrile] an analogue of cyoxymetrinil. This new safener may be more effective than cyoxymetrinil because it is itself less toxic to sorghum when applied as a seed treatment.

Flurazole

Flurazole [5-thiazole carboxylic acid, benzyl ester, 2-chloro-4-(trifluoromethyl)] is one of a series of thiazole carboxylic acids that have been discovered by Monsanto (Sacher et al., 1982) to have activity as safeners for alachlor in sorghum. When applied as a seed treatment or in furrow treatment, flurazole effectively protected sorghum from alachlor applied preplant incorporated, preemergence or early post emergence. Flurazole has exhibited good activity as a safener for various hybrids of grain sorghum. It has also given slight protection from thiocarbamate herbicides in maize (Hatzios, 1982). However, there is as yet very little known about its activity as a safener for other classes of herbicides or about its mechanism of action.

Influence of soil type on the activity of seed applied safeners

Ketchersid et al. (1981) did not observe any reduced effectiveness of cyoxymetrinil when treated sorghum seed was exposed to metolachlor in heavier or more adsorptive soils. However, in excessively moist soils protection was less than complete, possibly because of differences in the mobility or availability of metolachlor compared to cyoxymetrinil. Flurazole has also been observed to have good activity in a range of different soil types (Sacher, 1982). In these two instances seed treatments are employed to protect the plant from a soil active herbicide, thus soil factors that might reduce the availability or uptake of the safener might have similar effects on the herbicide. In contrast, when a seed applied safener is employed for a herbicide which is applied after the crop has emerged, variations in soil type can be extremely important. Thiessen et al. (1980) found this to be the major explanation for the variable effectiveness of NA as a safener in oats for post emergence applications of barban.

CHLOROACETAMIDES AS SELECTIVE, SOIL ACTIVE SAFENERS

R-25788 (N,N-diallyl-2,2-dichloracetamide) is the most widely used herbicide safener, simply because it is a component in commercial formulations of EPTC and butylate, two widely used thiocarbamate herbicides. Its physiological selectivity, as a safener for maize but few other plant species has prompted most investigators to conclude that its mode of action is quite different from that of NA. Several theories have also been proposed for its mode of action but more research is certainly needed before its biochemical effects are fully understood.

Effects of R-25788 on herbicide uptake and translocation

In studies with intact maize seedlings exposed to ^{14}C-EPTC in nutrient culture, R-25788 has not been observed to inhibit either the uptake or translocation of EPTC

(Chang et al., 1974). In fact, greater rates of EPTC uptake and translocation can be observed in maize seedlings protected with R-25788. Furthermore, Chang et al. (1974) observed significant reductions in EPTC injury to maize when the seedlings were not treated with R-25788 until after removal from a two day pretreatment with EPTC. These studies led to early conclusions that R-25788 did not act by inhibiting the uptake of EPTC or other herbicides by maize seedlings. However, recent investigations with R-25788 and ^{14}C-EPTC in maize cell cultures have reopened this question. Ezra et al. (1982a) observed that in cell culture, one of the earliest effects of R-25788 was a competitive inhibition of ^{14}C-EPTC uptake by the maize cells. Moreover, CDAA (N,N-diallyl-l-chloro acetamide) a related but less active safener also inhibited ^{14}C-EPTC uptake but to a lesser extent than did R-25788. These latter results indicate that while R-25788 may not inhibit the rather passive process of uptake and apoplastic movement of EPTC in the transpiration stream of intact plants, a part of its protective action may be due to a reduction of EPTC uptake by living cells in the sympasts.

Effects of R-25788 on herbicide metabolism

Lay et al. (1975) were the first to suggest that R-25788 may protect maize by enhancing the rate of EPTC detoxication. Lay and his co-workers (1975, 1976, 1978, 1982) have proposed that EPTC is first oxidatively activated to EPTC-sulfoxide and that this sulfoxide metabolite is the major toxic moeity in plants. They have established that R-25788 elevates root glutathione (GSH) levels in maize seedlings. They have also shown that R-25788 pretreatment enhances the *in vitro* rate of EPTC-sulfoxide carbamoylation to water soluble conjugates--most likely the S-(N,N-dialkyl carbamyl) derivatives of GSH. They suggest that the greater rate of EPTC-sulfoxide conjugation is due to an elevation of the activity of the appropriate GSH-s-transferase enzyme in response to R-25788. Many aspects of this theory are quite speculative, however in recent studies with $^{35}SO_4$ (1982) they have observed that R-25788 enhances the actual biosynthesis of GSH and they propose that it acts at a very early stage in sulfate metabolism. Dutka and Komives (1982) don't agree with the importance or the toxicity that Lay et al. ascribe to the sulfoxide metabolite of EPTC. In their studies, simultaneous stem injections of GSH with EPTC did reduce EPTC damage to maize seedlings. However in their system, EPTC-sulfoxide (EPTC-SO) was not as toxic as unaltered EPTC and EPTC-sulfone (EPTC-SO_2), another potential metabolite of EPTC, was the most toxic of all. They speculated that mixed function oxidase (mfo) enzymes may oxidize EPTC to its sulfoxide and they found that injections of piperonyl butoxide, an mfo inhibitor was actually synergistic in combination with EPTC in maize seedlings. Hatzios (1981) has also observed that tebuthiuron, another suspected mfo inhibitor, is synergistically phytotoxic in combination with EPTC. Neither of these investigators have conducted actual metabolic studies but if the EPTC-sulfoxide is as toxic as proposed by Lay et al. and if it is produced by mfo enzymes, mfo enzyme inhibitors should act as safeners not as synergists as observed by Dutka, Komives (1982) and Hatzios (1981). In other studies Lamoureux and Rusness (1982) have shown that EPTC and other herbicides that may be converted to GSH-conjugates are subsequently converted to malonyl-cysteine conjugates. They suggest that these latter conjugates may be terminal metabolites but their phytotoxicity or lack thereof has not yet been examined.

Obviously the identity and phytotoxicity of EPTC and all of its plant metabolites need to be clearly defined and the enzymes better characterized before the role of metabolic effects in the safening action of R-25788 can be fully understood.

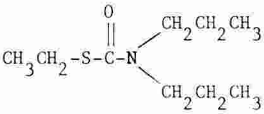
Antagonistic effects of R-25788 and EPTC on the biosynthesis of lipids and plant hormones

Possibly the strongest challenge to Lay and Casida's theory of R-25788 action as a herbicide safener is the work with cell cultures of Ezra et al. (1982). They point out that it is much easier to observe the kinetics of biochemical effects in maize cell suspension cultures than it is in intact plants. In addition to the very early effects of R-25788 on ^{14}EPTC uptake, mentioned earlier, they have also observed antagonistic effects of EPTC and R-25788 on lipid biosynthesis in the cell cultures as early as one hour after treatment. In fact R-25788 counteracted the inhibitory effect of EPTC on lipid biosynthesis within 2 hr of treatment. Although GSH levels were also elevated in their system, they didn't start increasing until 12 hr after treatment of the cultures with R-25788. Furthermore ^{14}C-EPTC was quickly metabolized to water soluble products within 8 hr. They suggest that this early antagonistic effect with EPTC on lipid biosynthesis is a more likely mechanism for the safening action than its later effects on GSH biosynthesis. Since maize is a plant species that normally has rather high levels of GSH it is difficult to understand why it would ever be a limiting factor in EPTC metabolism, even in the absence of R-25788. In other studies, Wilkinson and Smith (1975) have also observed that R-25788 can counteract the inhibitory effects of EPTC on lipid biosynthesis in spinach chloroplasts. Leavitt and Penner (1979) have shown that R-25788 is also antagonistic to the effects of EPTC on epicuticular waxes in maize.

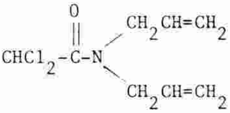
More recently, Wilkinson has confirmed the long suspected link between R-25788-EPTC interactions and GA (gibberellic acid) (1982). In a cell free system from etiolated sorghum coleoptiles, Wilkinson observed an inhibition of the incorporation of ^{14}C-mevalonic acid(MVA)into kaurene, a GA precursor, with levels of EPTC as low as 10^{-7} M. Moreover, simultaneous treatment with R-25788 restored the rate of MVA incorporation to normal. He has proposed that the EPTC induced morphological effects in plants are all consistent with an inhibition of GA action or biosynthesis. However in early studies on the selectivity of R-25788 as a safener, Chang et al. (1972) observed that R-25788 had only slight activity as a safener for EPTC in sorghum. Thus, it is difficult to conclude just how important these antagonistic interactions in GA biosynthesis are to the total toxic action of EPTC and the safening action of R-25788. Comparative studies of this nature with both maize and sorghum would be most helpful, indeed.

Structure-activity-relationships for chloroacetamide safeners

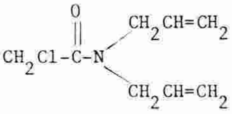
In one of their earliest studies, Chang et al. (1973) noted the close structural similarities between the herbicide EPTC, its safener, R-25788 and CDAA, a herbicide with considerable activity as an antagonist or safener for EPTC (see below).



EPTC



R-25788



CDAA

They weren't surprized to observe that R-25788 was also an effective safener for most other related thiocarbamate herbicides. In subsequent studies, Stephenson et al. (1978, 1979) synthesized and examined the safening activity of many different analogues of R-25788 in a soil-free, quartz sand nutrient culture, bioassay system. Closely related series were developed to determine the importance of acyl or amine chain lengths, acyl chlorination, and saturation of amine chains. With this soil-free bioassay system, they observed that acetamides and even some carbamates that were more similar in structure to EPTC than R-25788 could have greater activity as safeners for EPTC in maize. Such results led to the speculation that the acetamide safeners may act as competitive inhibitors at some site(s) of EPTC action. However in more recent studies, Stephenson et al. (1982) have observed nearly identical structure-activity-relationships between safening activity and the elevation GSH levels in maize roots with two related series of acetamide safeners. CDAA, one of the safeners in the series tested, is also known to be conjugated with GSH (Shimabukuro et al., 1978). Thus it is tempting to extend the theory of Lay et al. and suggest that thiocarbamate herbicides and chloroacetamide safeners are metabolized in maize by very similar pathways, involving GSH. Similar but less phytotoxic molecules may act as safeners by elevating the same substrates and the activity of the same enzymes as required for the metabolism of the herbicide.

Clearly, these many theories are a long way from coming together to form a convincing although multifaceted scenario for the mode of action of chloracetamides as herbicide safeners. However, the one effect that correlates with safener activity which has been observed by most investigators, is the elevation of GSH levels in maize roots. Timing is of course important, but if effects on GSH levels aren't directly responsible for the protective action against herbicides, variations in GSH levels must at least reflect the degree of protection that has occurred.

THE SEARCH FOR NEW SAFENERS

Most of the herbicides that have thus far been successfully antagonized by the safeners discussed herein, are primarily active on grassy weeds and are moderately toxic to grass crops. They aren't usually acutely toxic but more often they act as severe inhibitors of grass growth. It is very likely that any new herbicide or growth regulator with similar effects on grasses could be at least partially antagonized by one or more of the safeners already developed.

Any antagonistic combination of two herbicides observed in field or greenhouse studies should be viewed as a possible lead to the discovery of a chemical safener. The antagonism between 2,4-D and 2,4,6-T was Hoffman's initial observation which eventually led to the development of NA. An observed antagonistic interaction between EPTC and a chloroacetamide herbicide such as CDAA could also have led to the development of R-25788. If such a combination is selectively antagonistic for some crop species and not weeds, the two chemicals or analogues thereof could be formulated together. If the antagonism occurs for most crop and weed species, the next step is to determine if one of the chemicals (or analogues thereof) can be developed as a seed applied safener for the other herbicide.

Studies of structure-activity-relationships indicate that closely similar but less phytotoxic analogues of herbicides should be viewed as potential safeners for these same herbicides in crops. This list of chemicals could include many of those that were "returned to the shelf" because they were much less active than their more active, more successful analogues. These similar analogues could act as competitive inhibitors or they could stimulate the metabolism of their more phytotoxic analogues in moderately tolerant crop species. This approach could also have led to the discovery of the chloroacetamide safeners for thiocarbamate herbicides.

Although research on mode of action has already given us considerable insight it would be unwise to abandon the pure impirical approach in the search for effective safener-herbicide-crop combinations. Parker et al. (1980) at the Weed Research Organization routinely examine the efficacy of existing safeners for most new herbicides in maize. Their group (Blair et al., 1976) has certainly documented a number of potentially effective combinations and we can expect many other successes with this approach by other investigators.

As patents expire on existing herbicides and as development costs soar for chemically new herbicides, there will be more freedom and more incentive to develop and to employ chemical safeners to improve herbicide selectivity and to solve new weed problems.

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CGA 92194, A NEW SAFENER TO PROTECT SORGHUM FROM INJURIOUS EFFECTS OF METOLACHLOR

J. Rufener and A. Nyffeler
CIBA-GEIGY Limited, Agricultural Division, CH-4002 Basle, Switzerland

J.W. Peek
CIBA-GEIGY Corporation, Agricultural Division, Greensboro, NC 27409, USA

Summary. Concep II® (CGA 92194; N-(1,3-dioxolan-2-yl-methoxy)-imino-benzene-acetonitrile, a new safener which protects sorghum from metolachlor injury, is currently being developed by CIBA-GEIGY Ltd. This new safener has proved to be safe and effective on standard grain sorghum varieties of different seed qualities as well as on various yellow endosperm, sweet sorghum and sudangrass varieties. 1-2 g a.i. of CGA 92194 per kg seed give full protection from injury by metolachlor applied at rates which provide broad spectrum grass control. Sorghum tolerance is maintained when metolachlor is applied in combination with s-triazines (atrazine, propazine, terbuthylazine or terbutryn) for additional activity on broadleaved weeds. Full safening efficacy of CGA 92194 is obtained over a broad range of temperature and soil moisture conditions and no significant activity loss is observed after long-term seed storage.

INTRODUCTION

Because of the changes in weed populations which occur in today's crop production systems, weed control can become increasingly difficult. Important adverse changes which have occurred are:

- An increase of perennial weeds as a consequence of reduced tillage
- The selection of genetically resistant strains of weeds through repeated application of a single herbicide in monocultures
- The establishment of weeds which are closely related to the cultivated species, equally well adapted to the specific growing conditions and having a very similar sensitivity to herbicides.

Adoption of suitable cultural methods, crop rotations, chemical weed control programmes and the use of new techniques for herbicide application may, in many instances, prevent the spread of perennial weeds or strains with genetic herbicide resistance. The possibilities of controlling weeds that are closely related to cultivated species are, however, more limited. Until recently, the prospects of finding chemicals with the high degree of selectivity required to control Brassica kaber (wild mustard) in rapeseed, Beta vulgaris (weed beet) in sugar beets, Oryza rufipogon (red rice) in dry sown rice or Sorghum bicolor (shattercane) in grain sorghum were far from bright.

However, a most promising method to control such weeds was made available with the introduction of safeners, which can be applied to crop seeds and which provide protection of the crop against otherwise marginally or insufficiently selective herbicides.

CIBA-GEIGY's CONCEP® (code number CGA 43089) was introduced in 1980 as a safening agent protecting grain sorghum from metolachlor injury (Ellis et al., 1980). With seed treatments of 1.25 g a.i. of CGA 43089 per kg seed, adequate sorghum protection was achieved, even when metolachlor was applied at rates above those required for acceptable suppression of related grassy weeds belonging to the same tribe, the Andropogoneae, such as S.bicolor or Sorghum halepense (seedling johnsongrass).

Concep made possible the use of metolachlor in sorghum, to obtain significantly better and more reliable control of a broad spectrum of grass weeds than that obtained with the traditionally-used herbicides.

Limitations of the use of CGA 43089 have been its occasional inhibitory effect on germination of seed lots of marginal quality and a restriction excluding its application on sudangrass and certain yellow endosperm hybrids.

Recently, a new safener protecting sorghum from chloroacetanilide herbicide damage has been discovered in the laboratories of CIBA-GEIGY Basel, Switzerland, and given the code number CGA 92194. This new compound, which is chemically related to CGA 43089, also has an outstanding safening effect, but without many of the above mentioned limitations (Dill et al., 1982). This report describes the characteristics of this new safener and summarized the results obtained from laboratory, growth chamber and field experiments in 1981/82.

METHODS AND MATERIALS

Seed treatments with CGA 92194: A water slurry containing selected rates of CGA 92194 WP 70 and a fungicide/insecticide combination (containing 75 % captan and 3 % methoxychlor) were mixed with the seeds in a blender, tumbler or other seed dressing apparatus. The rates of CGA 92194 varied from 0.5 to 3.0 g a.i./kg seed and the rate of the fungicide/insecticide mixture was a constant 2.0 g formulated material/kg seed, including the controls.

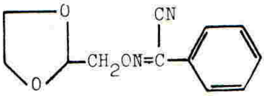
Germination tests. A germination test on vertical, rolled paper towels, as described by Blankendaal et al., (1972), was used to evaluate the effect of safener treatments on sorghum germination. Each treatment comprised 100 seeds x 4 replicates and evaluation of seed germination took place 5 days after test initiation.

Growth chamber tests. Studies on the prevention of metolachlor injury to sorghum by CGA 92194 were carried out in growth chambers. Safener pre-treated sorghum was grown in pots containing 500 cm3 of a sandy clay loam soil. Each treatment was replicated 5 times. Standard growing conditions (unless otherwise stated) were: 13 h daylength with a light intensity of 24 klux; day temperature 22°C and night temperature 20°C; relative air humidity 70 %. For the visual evaluation of crop phytotoxicity a 9 to 1 scale was used where 9 indicates no injury, 7 is maximum tolerated injury and 1 is complete kill.

Field trials. The performance of CGA 92194 was tested in field trials over a period of 2 years in the Northern and Southern hemispheres. The experimental design was always a randomized complete block with 20 - 30 m^2 plots and 4-6 replicates.

Data on CGA 92194.

Trade name: Concep II®
Formulation: 70 % WP:, can be used for dry seed dressing, water slurry treatment or with Mist-O-Matic systems.
Chemical name: N-(1,3-dioxolan-2-yl-methoxy)-imino-benzene-acetonitrile.
Structural formula:



Empirical formula: $C_{12}H_{12}N_2O_3$
Molecular weight: 232.24
Melting point: 77.7°C
Solubilities at 20°C: water, 20 ppm; hexane, 0.56 %; methanol, 3 %; acetone, 25 %; methylene chloride, 45 %
Vapour pressure at 20°C: 3.9×10^{-6} Torr

Toxicity of the technical material:

Acute oral	LD_{50}	(rats)	> 5000 mg/kg
Acute dermal	LD_{50}	(rats)	> 5000 mg/kg

Eye and skin irritation (rabbits) minimal

RESULTS AND DISCUSSION

Tolerance of sorghum to CGA 92194 (germination tests).

High quality sorghum seed maintains its good germination capacity when treated with CGA 92194 at rates between 0.5 and 3.0 g a.i./kg seed. (Table 1). Germination reduction was consistently less than 5 %, whereas CGA 43089 at 1.25 g a.i./kg seed gave a reduction of about 8 %.

On low quality seed lots, yellow endosperm varieties and sudangrass, where CGA 43089 has not been sufficiently safe, germination tests again show good tolerance to CGA 92194 (Table 2).

Table 1

Influence of safener treatment on germination of high quality sorghum seed
(Average of 4 trials, 2 on 'G 522' and 2 on 'G 623'

Safener	Formulation	Rate [g a.i./kg seed]	Germination [%]
None	--	--	95.4
CGA 92194	WP 70	0.5	93.6
CGA 92194	WP 70	0.75	93.8
CGA 92194	WP 70	1.0	93.3
CGA 92194	WP 70	1.25	93.3
CGA 92194	WP 70	2.0	92.7
CGA 92194	WP 70	3.0	91.2
CGA 43089	SO 250	1.25	87.6

Table 2

Influence of safener treatment on germination of problem sorghum varieties

		Germination [%]		
Safener	Rate [g a.i./kg seed]	'G 623 GBR' Low vigor lot	'G 624' Yellow endosperm	'FS 506' Sudangrass
None	--	90.3	87.5	87.5
CGA 92194	1.0	89.5	87.5	84.8
CGA 92194	2.0	87.8	85.3	83.0
CGA 43089	1.0	80.5	50.8	68.0
CGA 43089	2.0	50.3	27.5	59.3

The safening effect of CGA 92194 (growth chamber tests).

As previously observed with CGA 43089, tankmix applications of metolachlor with CGA 92194 protected not only sorghum but also the grassy weeds of the Andropogoneae tribe. Therefore only trials with seed treatments of CGA 92194 followed by broadcast applications of metolachlor are reported here.

In our growth chamber tests, where safener-treated sorghum seeds were planted in pots and treated pre-emergence with metolachlor, crop tolerance to the herbicide was greatly improved (Table 3). A pre-emergence application of metolachlor at 2 kg a.i./ha severely injured unsafened sorghum plants, but on seeds pre-treated with CGA 92194 at 1-2 g a.i./kg seed, the same metolachlor application caused at most very slight injury to sorghum. The safener remained active even after a long seed storage period.

Table 3

Protection of sorghum from metolachlor injury in a growth chamber test
(Influence of storage time on safening)

Safener	Rate [g a.i./kg seed]	Phytotoxicity to sorghum 'G 623' at 20-30 DAS*) on the 9 - 1 scale sown at 20 DAT*) Rate of metolachlor [kg a.i./ha] 1.0	 sown at 20 DAT*) 2.0	 sown at 170 DAT 1.0	 sown at 170 DAT 2.0	 sown at 536 DAT 1.0	 sown at 536 DAT 2.0
None	--	1	1	2	1	5	2
CGA 92194	1.0	9	8	9	9	8	8
CGA 92194	2.0	9	9	9	9	9	9
CGA 43089	1.0	9	9	9	6	6	6
CGA 43089	2.0	8	8	8	8	7	7

*) = Days After Treatment
= Days After Sowing

In other growth chamber tests, the safening response of CGA 92194 was studied under different temperature regimes and soil moisture conditions. Between 14 and 26°C very little effect of temperature on safening efficacy of CGA 92194 was observed (Table 4). Sorghum was also well protected from metolachlor injury when soil moisture was maintained at 50 % or 60 % of field capacity. Only with 4 kg a.i./ha of metolachlor and with the 80 % field capacity regime was a retardation of sorghum growth observed. But this is twice the normal use rate of metolachlor, and such moisture conditions would be exceptional in sorghum-growing areas (Table 5).

Table 4

Influence of temperature on safening effect of CGA 92194

Day/night temperature [°C]	Rate of CGA 92194 [g a.i./kg seed]	Phytotoxicity to sorghum 'G 522' at 31 DAS*) on the 9 - 1 scale Rate of metolachlor [kg a.i./ha] 0	 1.0	 2.0	 4.0
18/14	0	9	5	2	1
18/14	2.0	9	9	9	8
22/18	0	9	3	1	1
22/18	2.0	9	9	9	7
26/22	0	9	8	5	1
26/22	2.0	9	9	9	9

*) = Days After Sowing

Table 5

Influence of soil moisture on safening effect of CGA 92194

Soil moisture [% field capacity]	Rate of CGA 92194 [g a.i./kg seed]	Phytotoxicity to sorghum 'G 522' at 20 DAS*) on the 9 - 1 scale			
		Rate of metolachlor [kg a.i./ha]			
		0	1.0	2.0	4.0
50	0	9	8	7	2
50	2.0	9	9	9	9
60	0	9	4	3	2
60	2.0	9	9	9	8
80	0	9	2	1	1
80	2.0	9	8	8	5

*) = Days After Sowing

Field experiments.

In an intensive field trial programme, excellent tolerance of sorghum to the highly active herbicide metolachlor was consistently confirmed when crop seeds had been pre-treated with CGA 92194. Of the large number of trials carried out, only a few can be reported here.

The level of the safening effect of CGA 92194 under field conditions proved to be very acceptable, even under conditions where metolachlor caused exceptionally severe injury to unsafened sorghum, i.e. when rainfall occurred shortly after planting (Table 6).

Table 6

Safening effect of CGA 92194 in a trial where 37 mm of rain fell between herbicide treatment and crop emergence

Pre-emergence herbicide treatment	Rate [kg a.i./ha]	Phytotoxicity to sorghum 'PNR 8311' at 40 DAT*) [%]				
		Rate of CGA 92194 [g a.i./kg seed]				
		0	1.0	1.25	1.5	2.0
metolachlor	1.0	75	6	3	9	3
metolachlor	2.0	83	8	8	10	8

*) = Days After Treatment

The control of weeds obtained with metolachlor in sorghum trials was closely similar to that previously reported in maize and other crops (Gerber et al., 1974). CGA 92194 applied to sorghum seed had no antagonistic effect on the control of grasses by metolachlor.

Combinations of metolachlor with s-triazines (atrazine, propazine, terbuthylazine or terbutryn) can successfully be applied on safened sorghum, whenever this is necessary due to the presence of broadleaved weeds inadequately controlled by metolachlor alone. (Tables 7 and 8). With such combinations, the application recommendation of both, metolachlor and the selected s-triazine, should be closely observed.

Table 7

Sorghum tolerance and weed control of chloroacetanilide-triazine combinations; sorghum protected with 1.5 g CGA 92194/kg seed; average of 3 trials with different cultivars

Pre-emergence herbicide treatment	Rate [kg a.i./ha]	Phytotox. to sorghum at 25 DAT*) [%]	Weed control at ~ 95 DAT*) [%] Eleusine indica (3 trials)	Amaranthus hybridus (3 trials)	Cleome monophylla (2 trials)
metolachlor + propazine	1.2 1.2	3	96	100	95
metolachlor + terbuthylazine	1.2 1.2	3	97	99	92
metolachlor + propazine + terbuthylazine	1.2 0.6 0.6	4	98	97	96
propachlor + propazine	2.4 1.2	5	77	99	95

*) = Days After Treatment

Table 8

Yield of sorghum treated with chloroacetanilide-triazine combinations; sorghum protected with 1.5 g CGA 92194/kg seed; 3 trials with different cultivars on weed-free sites

Herbicide treatment	Rates [kg a.i./ha]	Application timing	Sorghum Yield [t/ha] 'PNR 8311'	'NK 283'	'NK 283'
metolachlor + propazine	1.5 1.5	pre pre	4.40	4.50	5.80
metolachlor + terbuthylazine	1.5 1.5	pre pre	4.53	4.31	5.67
metolachlor + atrazine + bromofenoxim	1.5 0.5 0.4	pre E-post E-post	4.72	4.29	5.70
propachlor + propazine	3.2 1.8	pre pre	3.87	3.93	5.33

pre = pre-emergence
E-post = early post-emergence

CONCLUSIONS

Selective broad-spectrum weed control in grain sorghum using metolachlor or metolachlor/s-triazine combinations is made possible by the new seed-applied safener CGA 92194. Yellow endosperm varieties, sudangrass and low-quality seed tolerate CGA 92194 better than CGA 43089.

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THE EFFECTIVENESS OF MON-4606 AS SEED SAFENER AGAINST ALACHLOR AND ACETOCHLOR IN GRAIN SORGHUM

R. Brinker, D. Schafer and R. Radke
Research Dep. Agric. Div. Monsanto, St-Louis, MO 63166

G. Boeken and H. Frazier
Research Dep. Agric. Div. Monsanto, Louvain-La-Neuve, Belgium

Summary. Grain sorghum possesses marginal tolerance to acetanilides such as alachlor and acetochlor and crop safety becomes inadequate when significant precipitation is received prior to crop emergence. This has precluded its use for control of grasses and certain broadleaf weeds in grain sorghum. Monsanto has recently discovered a safener encoded MON-4606 (5-thiazolecarboxylic acid, benzyl ester, 2-chloro-, 4-(trifluoromethyl)-,) which permits the use of alachlor and acetochlor in grain sorghum.

Studies were conducted from 1978 through 1980 to characterize performance and efficacy of this safener under field conditions. The preferred application methods identified were seed dressings and in-furrow treatments. Tank-mixtures of alachlor plus MON-4606 (1:1 ratio) as broadcast treatments were also effective. Seed and in-furrow treatments of grain sorghum with MON-4606 did not significantly alter weed control provided by alachlor. MON-4606 was effective across the normal range of sorghum seeding depths and across a wide range of commercial sorghum hybrids. Grain sorghum, acetanilides, alachlor, acetochlor, crop safety, safener.

INTRODUCTION

In spite of intensive research, present day herbicides do not provide consistent and selective broad spectrum grass control in grain sorghum. It is estimated that 50% of the treated sorghum acreage (6 M̄ ha in USA) is infested with annual grasses (Hoffman, 1962) and the benefit from chemical grass control in this crop has been demonstrated by different researchers (Burnside and Wicks, 1967, 1969 ; Wiese *et al*, 1964).

Alachlor has gained a wide acceptance for its effective control of grasses and certain broadleaf weeds in major crops such as soybeans, corn and peanuts.

However, the inherent phytotoxicity of alachlor against grain sorghum has precluded its use for weed control in this crop. Monsanto (Howe and Lee, US Pat, N° 4199506) has recently discovered a safener encoded MON-4606 (5-thiazolecarboxylic acid, benzyl ester, 2-chloro-, 4-(trifluoromethyl)-) which permits the use of alachlor in grain sorghum.

The experiments reported herein, were conducted to characterize the spectrum activity, rate response, preferred application method of MON-4606, plus alachlor and acetochlor. Additional investigations were made on thirteen sorghum hybrids.

CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES

MON-4606 in the pure state is a white crystalline solid with low water solubility and low vapor pressure (3.8 by 10^{-5} mm Hg at $25^{\circ}C$). Experimental formulations have included a 3 lb/US gallon emulsifiable concentrate, an 80% powder and a 1% granular formulation. Preliminary toxicology studies indicate an acute oral LD_{50} of 7700 mg/kg for rats and an acute dermal LD_{50} of > 3160 mg/kg for rabbits. It is considered a slight eye irritant and non-irritating to skin. No special handling procedures beyond normal precautions should be necessary.

MATERIAL AND METHODS

MON-4606 has been evaluated in field tests in U.S.A. since 1978. Field studies conducted at St. Charles (Missouri) were conducted on a Ray Silt Loam (1% organic matter, 6% clay, 74% silt and 19% sand).

The safener MON-4606 was applied to the sorghum seeds at rates of 1.25-2.50 g ai/kg and in-furrow treatments (0.125-0.25 kg ai/ha).

Seed dressings of MON-4606 were applied utilizing an 80% powder formulation or methylene chloride solutions.

In-furrow treatments utilized a 1% granular formulation, applied through granular insecticide attachments on the John Deere planter. Tank mixtures of alachlor plus MON 4606 (1:1 ratio) as broadcast treatments were applied in 225 l/ha water.

Grain sorghum seeds were planted with a Planet J.R. seeder or with a John Deere Model 71 planter. Herbicides [alachlor (2-chloro-2', 6'-diethyl-N-/methoxymethyl) acetanilide and acetochlor (acetamide, alpha-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) were applied pre-emergence, at 3 to 4 rates, in a water volume of 225 to 330 l/ha with a back-pack CO_2 sprayer or a tractor mounted plot sprayer. Field trials received precipitation or irrigation within one week after treatment.

The experimental design was a randomized complete block with a split-plot treatment arrangement consisting of main plots (safener) and subplots (herbicide treatments). The experiment was replicated three times. Individual plot size was 3 m x 6 m.

Visual plot ratings of sorghum injury and weed control were made 6 weeks after test initiation. (6 WAT).

The rating scale used was 0 to 100 with 0 = no injury to any of the plants/ weeds and 100 = complete kill.

APPLICATION METHODS AND RATE RESPONSE

Preliminary greenhouse experiments (table 1) indicated that the safener MON-4606 was highly effective against alachlor when applied as a seed treatment (1.25-2.5 g ai/kg), in-furrow (0.125-0.25 kg ai/ha) or tank-mixed (2.0-4.0 kg ai/ha). However, low use rates of seed and in-furrow treatments dictated these to be the preferred application methods.

Table 1

Grain sorghum responses to MON-4606 application systems with alachlor at 1.1 kg ai/ha under greenhouse conditions (% inhibition)

		MON-4606	
		+	-
Seed treatment	0.125%	10	85
In-furrow	0.1 kg/ha	12	90
Broadcast	2.2 kg/ha	20	40

Greenhouse data also indicated that acetochlor was more phytotoxic than alachlor against unprotected sorghum and associated weeds. Under these conditions, MON-4606 increased sorghum tolerance to alachlor by 125 fold and to acetochlor by 33 fold.

Extensive field investigations with alachlor and acetochlor were conducted from 1978 to 1980 at St. Charles, Missouri. Consistent crop injury with alachlor and acetochlor was ensured by timely precipitation or irrigation, between the time of treatment and seedling emergence. Sorghum that emerged prior to receiving rainfall escaped herbicidal injury.

Data reported from 9 field tests indicated that under previous field conditions, grain sorghum treated with MON-4606 tolerated more than 4 kg ai/ha alachlor and 3 kg ai/ha acetochlor, which is more than twice that needed for effective weed control.

RESPONSE OF SORGHUM HYBRIDS

The responses of various sorghum hybrids to MON-4606 and alachlor were investigated in a series of field studies (Table 2).

Table 2

Phytotoxicity of alachlor with and without MON-4606 (0.125% w/w) to various sorghum cultivars in field tests conducted at St.Charles (Missouri) (% phytotoxicity at 6 WAT)

		Pioneer					De-kalb	Northrup -king		Funks				
Treatment	kg ai/ha	894	8272	8442	8451	8475	E59+	2778	2884	G522 DR	G611	G550	G499	G623 BR
Alachlor	2.0	23	23	17	23	62	42	20	23	78	35	40	37	37
Alachlor + MON-4606	2.0+0.011	10	0	2	3	5	0	0	2	8	2	8	8	25

The tolerance of the various cultivars to alachlor showed a normal variation ranging from the highly susceptible FUNKS G522 DR to the most tolerant (Pioneer 8442). The sorghum inhibition averaged, across hybride and alachlor rates was reduced from 35% to 6% by MON-4606 seed treatment (0.125%).

The data indicated that although good safety is observed with alachlor plus MON-4606, a minimum of variation occurs in the safening response.

WEED CONTROL

Data from field tests conducted at St. Charles (Missouri) indicated that alachlor at 2 kg/ha provided selective control of major grassy and small seeded broadleaf weeds with the exception of velvet leaf (Abutilon theophrasti), Jimson weed (Datura stramonium) and morning glory (Ipomoea spp.). However, the introduction of the herbicide safener MON-4606 allowed safe use of alachlor at higher rates (4 kg ai/ha) in sorghum. The seed safener was highly effective irrespective of the application method (PE, PPI or post-emergence) of alachlor. (Table 3).

Table 3

Weed control and crop tolerance of protected grain sorghum seed (MON-4606 at 0.125% w/w) to pre-emergence applications of alachlor (2 and 4 kg ai/ha) at St. Charles (Missouri) (% inhibition)

Chemical	Rate in kg ai/ha	Sorghum		Giant Foxtail	Barnyard grass	Yellow nutsedge	Common lambs-quarter	Redroot pigweed	Velvet leaf
		MON-4606 -	 +	+MON-4606					
Alachlor	2	53	5	99	100	90	94	99	33
Alachlor	4	71	9	99	100	85	99	100	54

Tank-mixtures of alachlor plus MON-4606 applied as pre-plant incorporated or pre-emergence treatments also effectively reduced sorghum injury and displayed selective control of major weeds such as giant foxtail (Setaria viridis), barnyardgrass (Echinochloa crus-galli), redroot pigweed (Amaranthus retroflexus) and common purslane (Portulaca oleracea).

However, reduced activity against seedling Johnson grass and shattercane (Sorghum bicolor) occurred with broadcast (tank-mix) applications.

In greenhouse experiments, alachlor and acetochlor controlled selectivity prevalent weeds such as green foxtail (Setaria viridis), seedling Johnson grass (Sorghum halepense) and common lambsquarter (Chenopodium album).

CONCLUSIONS

Greenhouse and field investigations indicated that MON-4606 [5-thiazolecarboxylic acid, benzyl ester, 2-chloro-4-(trifluoromethyl)-] is an effective antidote for safening acetanilide herbicides such as alachlor and acetochlor on grain sorghum. It was effective and reliable as a seed and in-furrow safener treatment.

As seed treatment, MON-4606 was very effective in safening all tested grain sorghum hybrids with alachlor and acetochlor. At the use rate of 1.25 g/kg seed MON-4606 reduced alachlor (2 kg ai/ha) injury to commercially acceptable levels, when adequate irrigation and/or precipitation occurred within 4-7 days after seeding. In-furrow treatments of sorghum seeds with MON-4606 granules (0.12 to 0.25 kg/ha) were as effective as seed treatments for all tested sorghum varieties and method of alachlor application.

With the exception of broadcast applications, treatments do not adversely affect weed control provided by both alachlor and acetochlor.

Due to its low toxicology, no special handling procedure for MON-4606 beyond normal precautions should be necessary.

Monsanto will proceed towards commercialization of MON-4606 in 1982(1)

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(1) Screen®

Proceedings 1982 British Crop Protection Conference - Weeds

FURTHER STUDIES WITH HERBICIDE SAFENERS ON RICE AND MAIZE

C.Parker
ARC Weed Research Organization, Begbroke Hill, Yarnton, Oxford OX5 1PF

Summary Seed dressing with the herbicide safener 1,8-naphthalic anhydride (NA) enabled rice to tolerate a four-fold increase in the dose of diclofop-methyl pre-emergence, and a two-fold increase in the dose early post-emergence. Protection against NC 20484 (2,3-dihydro-3,3-dimethyl-5-benzofuranyl ethane sulphonate) was at least two-fold under ideal warm conditions but less than two-fold at lower temperatures. Protection was less than two-fold against oxadiazon and negligible against pendimethalin.

Maize was well protected by both NA seed dressing and R 25788 (N,N-diallyl-2,2-dichloroacetamide) sprayed pre-emergence at 1 kg/ha against diclofop-methyl applied pre-emergence. NA also protected maize against early post-emergence application of diclofop-methyl. There was no useful additive or synergistic interaction between the two safeners when applied together. As with rice there were indications that NA protected maize better under warm than under cool conditions.

INTRODUCTION

Certain weeds, closely related to crop species present particular difficulty for selective chemical weed control. Some of these, namely *Rottboellia exaltata* as a weed of maize and wild rice (*Oryza punctata*) and red rice (*O. rufipogon*) in rice, have been the subject of study at the Weed Research Organization (WRO) for some years. Improved selectivity by using herbicide safeners, particularly 1,8-naphthalic anhydride (NA) has been obtained with alachlor (Parker and Dean, 1976), perfluidone (Blair and Dean, 1976), chlorsulfuron (Parker *et al*, 1980) and diclofop-methyl (Parker, 1981). This report gives further detail of the studies with diclofop-methyl and of experiments to determine the influence of temperature on safener action; also possible interactions between NA and R 25788 (N,N-diallyl-2,2-dichloroacetamide), and between NA and calcium peroxide (as a seed treatment to improve rice germination under wet conditions as described by Yoshida and Parao, 1981).

METHODS AND MATERIALS

All experiments were carried out in glasshouses at WRO, using 10 cm or 12 cm diameter pots and a sandy loam soil containing additional complete fertilizer appropriate to the test species. Unless otherwise indicated seeds were sown at 2 cm depth, four or five per pot for maize and 10 per pot for rice.

NA (technical ca 98%) was applied as a seed dressing at a nominal 0.5% ie 0.5 g NA was shaken with 100 g seed, but the actual retention of NA on the seed was not measured. R 25788 at 1 kg/ha was applied as a spray of the emulsifiable concentrate over the soil or plants. For calcium peroxide treatments seeds were wetted with 4% polyvinylacetate, and stirred in dry calcium peroxide powder to build up a layer equivalent to 30-40% of the seed weight. For combined NA/calcium peroxide treatments the seeds were first dressed with NA.

Herbicides were applied in approximately 400 litres of water per ha by a laboratory sprayer fitted with a single nozzle travelling at 0.55 m/sec. Herbicides were in all cases normal commercial formulations without further added surfactant. Pots were watered daily from above as required and in winter months supplementary heating and lighting (12 hours per day) were provided. Contrasting temperature conditions were achieved by using greenhouses with different ventilation/heating regimes. Where necessary further details are given as footnotes to the tables.

Assessment was by weighing of shoot material cut at soil level three to six weeks after sowing. Treatments were replicated three times and arranged in complete randomised blocks. Results were subjected to analysis of variance and standard errors are presented in the tables.

RESULTS

Rice

NA protected rice from diclofop-methyl (Tables 1, 2 and 3) allowing an approximate four-fold increase in dose for equivalent effect pre-emergence. Although rice was less susceptible to treatment post-emergence there was some protection in both experiments, approximately two-fold where this could be assessed (Table 3). In contrast R. exaltata was more susceptible to post-emergence treatment. There was negligible protection of rice by R 25788 (results not presented) but R. exaltata was protected by both NA (four-fold) and R 25788 (two-fold).

The protective effect of NA against diclofop-methyl pre-emergence was confirmed in a further four rice varieties (Table 2). The protection factor was at least two-fold in each case, though the level of sensitivity of the varieties varied somewhat.

Table 1

Effects of NA seed dressing on the susceptibility of maize and rice to diclofop-methyl

Herbicide	kg/ha	Shoot fresh wts as % of untreated Rice Nil	 + NA	 Maize Nil	 + NA
Pre-emergence					
diclofop-methyl	0.25	69.7	71.3	91.1	94.0
	0.5	33.9	73.2	103.8	81.1
	1.0	10.9	69.0	76.8	91.3
Post-emergence (11 days)					
diclofop-methyl	0.25			8.1	95.1
	0.5	55.5	73.7	0.2	69.6
	1.0			0.0	53.2
No herbicide	0	100	94.5	100	93.7
S.E. of means		8.7		7.1	

Rice IR 298, maize Julia, sown 25.9.80, assessed 21-30.10.80
Stages of growth at 11 days - maize $2\frac{1}{2}$-$3\frac{1}{2}$ leaves, 10-15 cm
- rice 1-$1\frac{1}{4}$ leaves, 3-6 cm
Average daily maximum/minimum 26.6°C/13.7°C

Table 2

Effects of NA seed dressing on the susceptibility of five rice cultivars to diclofop-methyl applied pre-emergence

		Shoot fresh wts as % of untreated											
Cultivar Sowing depth		IR 298 (1 cm)		IR 298 (2 cm)		IR 28 (2 cm)		IR 8 (2 cm)		Starbonnet (2 cm)		Blue Bonnet (2 cm)	
Herbicide	kg/ha	Nil	+ NA	Nil	+ NA	Nil	+ NA	Nil	+ NA	Nil	+ NA	Nil	+ NA
Pre-emergence													
diclofop-methyl	0.25	60.2	92.4	106.0	84.7	93.5	92.4	71.1	75.6	82.3	70.1	116.1	97.6
	0.5	35.3	82.2	61.5	81.0	56.7	93.3	74.3	78.0	5.5	67.6	59.7	96.6
	1.0	0.9	63.3	5.1	71.9	37.9	92.4	34.3	77.1	1.9	51.6	26.7	80.6
No herbicide	0	100.0	89.6	100.0	86.3	100.0	93.0	100.0	87.7	100.0	89.6	100.0	100.2
S.E. of means		8.0		8.0		10.1		7.8		7.7		8.5	

Sown 20.8.81, assessed 18.9.81.
Average daily maximum/minimum 28.5°C/17.1°C

Table 3

Effects of NA seed dressing on the susceptibility of rice and R. exaltata to diclofop-methyl

Herbicide	kg/ha	Shoot dry wts as % of untreated Rice Nil	 + NA	 R. exaltata Nil	 + NA	 + R 25788
Pre-emergence						
diclofop-methyl	0.5	2.5	64.2	38.5	88.6	71.1
	1.0	0.9	61.7	13.6	81.7	36.6
	2.0	0	42.0	0.7	55.7	11.1
Post-emergence (11 days after sowing)						
diclofop-methyl	0.25	77.8	72.8	5.0		
	0.5	71.6	70.6	0		
	1.0	34.6	65.4	0		
No herbicide	0	100	83.3	100	102.2	94.5
S.E. of means		5.7			6.3	

Rice IR 298, R. exaltata sown 1.4.81, assessed 8.5.81
Stages of growth at 11 days - rice 2 leaves, 5-12 cm
- R. exaltata 2-3 leaves, 5-12 cm
Average daily maximum/minimum 26.4°C/18.1°C

In the fourth experiment (Table 4) the protection was a little less than fourfold with NA but was not influenced by the simultaneous treatment of the seed with calcium peroxide. Calcium peroxide alone caused a slight increase in susceptibility of rice to the herbicide. Similar results (ie slight increase in damage with calcium peroxide, and lack of interaction with NA) were obtained in several other experiments with diclofop-methyl and in one with alachlor (results not presented).

Table 4

Effects of NA and/or calcium peroxide seed dressings on the susceptibility of rice to diclofop-methyl

Herbicide	kg/ha	Nil	Shoot fresh wts as pre-emergence % of untreated + NA	 + CaO_2	 + NA + CaO_2
diclofop-methyl	0.25	95.0	88.4	69.3	94.2
	0.5	33.1	85.5	23.5	85.7
	1.0	11.6	57.1	8.5	58.2
No herbicide	0	100	77.3	108.5	81.5
S.E. of means			7.3		

Rice IR 298, sown 17.8.81, assessed 14.9.81
Average daily maximum/minimum 28.5°C/17.1°C

In experiments with other herbicides, pendimethalin was well tolerated by rice planted at 2 cm but not at 0.5 cm and the damage was not alleviated by NA (Table 5).

Damage from oxadiazon (again more serious with shallow planting) was somewhat alleviated by NA but the protection factor was only about two-fold. Other results not presented, showed that there was a small degree of protection against fluazifop-butyl and sethoxydim applied to soil and incorporated before planting rice but the protection factor was less than two-fold.

Table 5

Effects of NA seed dressing on susceptibility of rice to pendimethalin and oxadiazon pre-emergence

Herbicide	sowing depth kg/ha	Shoot fresh wts as % of untreated 2 cm Nil	 2 cm + NA	 0.5 cm Nil	 0.5 cm + NA
pendimethalin	2	117.2	125.8	81.0	91.5
	4	124.7	119.4	45.1	54.2
oxadiazon	1	90.3	91.4	52.8	57.0
	2	65.6	79.6	35.2	56.3
No herbicide	0	100	120.4	100	86.6
S.E. of means			9.5		

Rice IR 298, sown 4.11.81, assessed 9.12.81
Average daily maximum/minimum 23.7°C/17.7°C

Table 6

Effects of NA seed dressing on the susceptibility of rice and maize to NC 20484 pre-emergence under two temperature regimes

Herbicide	Temperature kg/ha	Shoot fresh wts as % of untreated Warm Nil	 Warm + NA	 Cool Nil	 Cool + NA
Rice					
NC 20484	0.25	91.7	101.5	115.9	93.8
	0.5	31.0	86.7	78.7	105.2
	1.0	3.9	43.0	4.3	19.0
No herbicide	0	100	100.3	100	91.7
S.E. of means			7.0		
Maize					
NC 20484	0.25	37.4	122.5	74.6	95.3
	0.5	2.6	95.6	66.3	76.7
	1.0	1.1	38.7	0	16.9
No herbicide	0	100	108.9	100	93.9
S.E. of means			13.0		

Rice IR 298, sown 14.7.81, assessed 12.8.81 and 1.9.81
Maize Julia, sown 14.7.81, assessed 3 and 14.8.81
Average daily maximum/minimum "warm" 28°C/16.6°C "cool" 25°C/15.6°C

Table 6 shows at least two-fold protection by NA against NC 20484 in the warmer of two glasshouses. Under cooler conditions there was less damage without NA, but very little protection by the seed dressing.

Maize

In the first experiment (Table 1) maize was protected by NA against diclofop-methyl - over four-fold in the case of post-emergence application.

Table 6 indicates a comparable effect of temperature to that observed on rice, with over four-fold protection of maize by NA against NC 20484 under warm conditions but less than two-fold protection under cooler conditions when susceptibility of the crop was in any case lower.

A further experiment (Table 7) suggested an influence of temperature also on the effectiveness of NA against diclofop-methyl and chlorsulfuron and that the protective effect of NA applied to the seed before sowing persisted to provide good protection at least to 12 days after sowing. In a further experiment (not presented) some protective effect against diclofop-methyl persisted to 19 days from sowing but was less complete even at the lowest dose of 0.25 kg/ha. It was also shown that R 25788 provided good protection when applied simultaneously with the herbicide pre-emergence, but when applied post-emergence with the herbicide, protection was much reduced. In the final experiment (Table 8) combinations of the two safeners appeared to be less than additive, ie R 25788 treatment, although providing protection on its own, did not significantly enhance the protection provided by NA.

Table 7

Effects of NA seed dressing on susceptibility of maize to three herbicides under two temperature regimes

		Shoot fresh wts as % of untreated			
Herbicide	Temperature	Warm		Cool	
	kg/ha	Nil	+ NA	Nil	+ NA
Pre-emergence					
diclofop-methyl	0.5	93.0	75.5	77.3	65.7
	1	39.1	74.0	80.4	60.9
	2	14.7	85.1	22.4	63.3
chlorsulfuron	0.005	9.2	63.7	35.8	63.5
	0.01	5.9	45.7	14.5	15.8
	0.002	1.0	24.5	2.7	13.1
NC 20484	0.25	9.3	44.0	48.8	98.0
	0.5	6.1	15.1	15.1	51.2
	1.0	0.2	19.4	0.7	15.0
Post-emergence (12 days after sowing)					
diclofop-methyl	0.25	5.0	85.4		
	0.5	0.7	63.2		
	1.0	0	48.5		
No herbicide		100	82.8	100	100.6
S.E. of means		9.4		14.3	

Maize Julia, sown 4.2.82, assessed 2 and 26.3.82
Stage of growth at 12 days 3-3½ leaves, 15-30 cm
Average daily maximum/minimum "warm" 24°C/20°C "cool" 19.5°C/10.3°C

Table 8

Effects of NA seed dressing and/or R 25788 spray on the susceptibility of maize to chlorsulfuron pre-emergence

Herbicide	kg/ha	Shoot dry wts as % of untreated Nil	+ NA	+ R 25788	+ NA + R 25788
Caldera					
chlorsulfuron	0.01	24.1	68.6	69.4	60.8
	0.02	10.7	49.3	40.1	58.4
No herbicide	0	100	70.0	92.3	63.6
Julia					
chlorsulfuron	0.01	31.9	67.3	66.1	61.3
	0.02	8.8	56.7	32.1	53.9
No herbicide	0	100	69.1	82.0	61.3
S.E. of means	Caldera 6.9		Julia 7.0		

Maize sown 24.3.81, assessed 21.4.81
Average daily maximum/minimum 27.3°C/18°C

DISCUSSION

The levels of protection of rice and maize reported in this paper are barely adequate to ensure perfect selectivity of diclofop-methyl, or the other herbicides, against R. exaltata in maize, or against red rice in rice. Protection factors in excess of four-fold are probably required where there is not already some selectivity in favour of the crop. For less stringent selectivity problems, however, the results may be worth following up, as for instance in the control of R. exaltata in rice. Available treatments do not always give reliable control of this weed in rice and as it is very sensitive to post-emergence application of diclofop-methyl the use of NA in conjunction with an early post-emergence treatment of about 0.5 kg/ha could provide excellent selectivity: although maize may not tolerate an overall post-emergence application of diclofop-methyl, the use of NA might provide sufficient tolerance to allow an inter-row directed spray of the herbicide.

It is of interest that protection is possible against a member of the new phenoxy-phenoxy group of herbicides, but while good protection is demonstrated against diclofop-methyl, very much smaller levels of protection have been observed against the chemically related fluazifop-butyl or against sethoxydim (Richardson et al, 1981).

The possibility that NA and R 25788 might interact synergistically was not unfortunately confirmed, but their lack of interaction may possibly throw light on their respective modes of action.

The effects of temperature condition on the effectiveness of NA were far from consistent. While the two experiments in which contrasting conditions were imposed both suggested more effective protection under warmer conditions, the results from other experiments were not always in accordance. In particular the protection factor for NA on rice in Table 4 was less than in Tables 1 and 3 despite relatively high temperatures. Also with maize the "cool" conditions under which poor protection against NC 20484 were obtained in Table 6 were in fact warmer than the "warm" conditions under which good protection was obtained in Table 7. It is therefore probable that other environmental factors contributed, such as soil moisture and atmospheric humidity, both of which would be influenced by different temperature and ventilation conditions. Temperature *per se* is therefore probably not the sole factor involved in the differing levels of protection provided but the results do strongly suggest a tendency for better performance of NA under warmer conditions, which will deserve more systematic study wherever variable performance is a problem. As temperatures were relatively low in the experiment with oxadiazon (Table 5) this result deserves re-examination.

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SAFENING RYEGRASSES AGAINST PRE-EMERGENCE HERBICIDES BY SEED DRESSINGS OF 1,8-NAPHTHALIC ANHYDRIDE

F.W. Kirkham, W.G. Richardson and T.M. West
ARC Weed Research Organization, Begbroke Hill, Yarnton, Oxford OX5 1PF

Summary. In pot experiments seed dressings of 1,8-naphthalic anhydride (NA) safened ryegrass cultivars against pre-emergence surface sprays of alachlor, NC 20484 and, to a lesser extent, MBR 18337. A dressing of 1.0% by weight of seed was optimal for safening S.23 perennial ryegrass. Perennial ryegrass showed a greater degree of safening with NA than the hybrid or the two Italian cultivars tested, although the latter three showed more innate resistance to the herbicides. The safening of perennial ryegrass indicated a potential selective control of *Poa annua* by alachlor, and of *P. annua* and *Bromus sterilis* by MBR 18337. This herbicide also showed promise for controlling *Alopecurus myosuroides*, both in NA-treated and untreated ryegrass. NC 20484 was highly selective against both *P. annua* and *Stellaria media* in unsafened ryegrass, and controlled volunteer barley more reliably when NA treatment allowed the use of higher doses. Selectivity, cultivars, rates of safener.

INTRODUCTION

Newly sown ryegrass leys are frequently invaded by indigenous species and large increases in ryegrass production can be achieved by their early control (Kirkham *et al.*, 1982). In herbage seed crops unsown species, particularly weed grasses, can affect profitability, not only by reducing yields through competition, but also by increasing seed cleaning costs (Evans and Yates, 1981). Moreover the crop may fail to reach certification standard, possibly preventing sale of the seed.

Few herbicides are available for grass-weed control in establishing ryegrass and successful use of crop safeners could widen the choice. Preliminary results by two of the present authors (Richardson and Kirkham, 1982) are the only report of safening ryegrass against herbicides; the tolerance of S.23 perennial ryegrass to 7 of the 16 herbicides tested was significantly increased by dressing seed with 1,8-naphthalic anhydride (NA). Of these herbicides, alachlor (α-chloro-2',6'-diethyl-*N*-methoxymethyl acetanilide) responded best to the safener, followed by NC 20484 (2,3-dihydro-3,3-dimethyl-5-benzofuranyl ethane sulphonate), MBR 18337 (*N*-(4-ethylthio)-2-(trifluoromethyl) phenyl) methanesulphonamide) and perfluidone (1,1,1-trifluoro-N-(4-phenylsulphonyl-*O*-tolyl) methanesulphonamide).

The first two experiments reported here show that dressing S.23 ryegrass seed with NA affects the selectivity of the above-mentioned herbicides between ryegrass, white clover and certain grass weeds. The third experiment investigated the effects of different rates of NA seed dressing on the growth of S.23 ryegrass and on its subsequent response to alachlor, NC 20484 and MBR 18337.

The final experiment compared the sensitivity of three perennial (S.23, Melle and S.24), one hybrid (Sabel) and two Italian (RvP and Sabalan) ryegrass cultivars to spray applications of alachlor and NC 20484 after seed treatment with NA.

METHODS AND MATERIALS

All experiments were conducted in the glasshouses. Plastic pots of 9.0 cm diameter were used and all treatments were replicated three times. NA seed dressings were applied by shaking the appropriate amounts of seed and safener together in a glass jar. Approximately 80-85% of the safener, measured by weight, was retained by the seed at each rate of NA.

All herbicides were applied within a few hours of sowing to the soil surface in water at a rate equivalent to 386 l/ha, using a laboratory pot sprayer consisting of a single Teejet 8002E nozzle travelling at a constant speed and height above the pots.

Two sandy loam soils were used in the experiments viz. 'Begbroke' (Experiments 1, 3 and 4) or 'Yarnton' (Experiments 1 and 2) soil. The only notable difference between these soils was in their organic matter contents (Begbroke 3.8%, Yarnton 2.2%). Each was supplemented with sulphate of ammonia (21% N) at 1.0 g per kg of soil, sulphate of potash (48% K_2O) at 1.2 g/kg, superphosphate (18% P_2O_5) at 2.0 g/kg and magnesium sulphate at 0.8 g/kg.

Table 1

Plant raising details

Species/ cultivar	Planting date	Harvest date	No. of seeds/pot	Depth of sowing (cm)	Temperature range and mean (°C)
Expt. 1					
Perennial ryegrass, S.23	2/12/81	18/1/82	15	0.5	1-25, 13
Alopecurus myosuroides	"	"	20	0.25	
Avena fatua	"	"	15	1.0	
Bromus sterilis	"	"	7	1.0	
Expt. 2					
Perennial ryegrass, S.23	16/3/82	5/5/82	8	0.5	5-27, 15
White clover, cv. Grasslands Huia	"	"	15	0.5	
Winter barley, cv. Sonja	"	21/4/82	8	0.5	
Poa annua	"	5/5/82	15	0.5	
Stellaria media	"	"	25	0.5	
Expt. 3					
Perennial ryegrass, S.23	22/1/82	3/3/82	12	0.5	9-24, 16
Expt. 4					
Ryegrass cultivars	12/5/82	16/6/82	12	0.5	9-34, 19

Plants of each species, cultivar, or NA treatment were grown in separate pots. One pot per relicate of each species was left unsprayed while each of the remainder received one herbicide treatment. In all the experiments, NA-dressed and undressed ryegrasses were compared and no other species received a dressing of NA. In experiment 1, ryegrass was grown in both soil types while the weed species were grown in Begbroke soil only. A randomised block design was used in each experiment with the position of each herbicide x safener combination randomised within each replicate. However, there were differences between Experiments 1, 2 and 4 in the way the species or cultivars were laid out, affecting the way the results were analysed. In Experiment 2 the position of each species x treatment x safener combination was completely randomised within each replicate. In Experiments 1 and 4 all the pots of each cultivar or species were grouped together in separate blocks for each species/ cultivar, with the replicates split for soil type in the case of ryegrass in Experiment 1.

All pots were watered from overhead as necessary, and at the completion of each experiment plants were cut at soil level and the foliage fresh weights per pot were recorded. Table 1 shows details of sowing and growing conditions for each experiment.

RESULTS

Experiment 1. Selectivity of two herbicides between ryegrass and three weed grasses as influenced by NA

Dressing seed with NA tended to stimulate the growth of ryegrass (Table 2). This effect was significant in Yarnton soil but just failed to reach significance in Begbroke soil ($P < 0.05$). Furthermore, NA produced significant safening against all but the highest dose of each herbicide in Begbroke soil, although in Yarnton soil the difference also failed to reach significance with the middle dose of MBR 18337. Comparing the soil means, the herbicides were significantly more active against ryegrass in Yarnton than in Begbroke soil but the analysis of variance showed no significant interaction between soil and safener effects.

Table 2

Effect of two herbicides applied at 3 rates on yield of ryegrass with and without 1,8-naphthalic anhydride (NA) and 3 weeds (% of untreated controls)

		Perennial ryegrass				Alopecurus myosuroides*		Avena fatua	Bromus sterilis*	
		Begbroke soil		Yarnton soil						
Herbicide	(kg a.i./ha)	- NA	+ NA	- NA	+ NA	- NA		- NA	- NA	
Alachlor	0.10	29.6	96.4	7.9	79.7	52.5	(47.7)	87.3	72.2	(58.4)
	0.18	5.7	73.7	5.2	73.1	58.3	(49.8)	80.3	57.2	(49.2)
	0.25	0.1	24.0	1.8	16.3	47.5	(43.9)	22.2	28.5	(31.6)
MBR 18337	0.10	75.6	108.6	24.5	81.2	4.4	(9.1)	76.6	38.0	(37.6)
	0.18	35.8	66.4	16.5	31.6	3.2	(7.5)	69.8	8.7	(13.7)
	0.25	26.8	44.7	1.2	8.8	0.3	(1.7)	56.6	0.4	(2.9)
Unsprayed control		100.0	126.6	100.0	127.7	100.0		100.0	100.0	
Soil means		58.1		41.1						
S.E. soil means (ryegrass)			±1.83							
S.E. herbicide treatments (weed species)							(±8.09)	±7.92		(±6.00)
S.E. herbicide treatment x safener x soil (ryegrass)			±9.52							

* Analyses performed on Arcsin transformed data for these species (bracketed figures)

All three weed grasses were much more resistant to alachlor than untreated ryegrass and the degree of safening achieved by NA was not sufficient to reverse these trends. By contrast, all MBR 18337 treatments gave over 95% control of A. myosuroides, whilst NA-treated ryegrass was unaffected by the lowest dose and only reduced by 34% by the middle dose. B. sterilis was slightly less susceptible than A. myosuroides to MBR 18337, but was still reduced by 62%, 91% and nearly 100% by the low, middle and high doses respectively. A. fatua was not controlled selectively by any of the herbicide treatments.

Experiment 2. Selectivity of four herbicides between ryegrass, white clover, barley and two weeds as influenced by NA.

The fresh weights of ryegrass seedlings from untreated seed were significantly reduced by the higher dose of alachlor ($P < 0.001$) and by perfluidone at both doses ($P < 0.05$) (Table 3). In contrast to the results of Experiment 1, NA seed dressing produced no stimulatory effect on the growth of unsprayed ryegrass.

Table 3

Foliage yield of five species, including perennial reygrass with and without naphthalic anhydride (NA) seed dressing, following pre-emergence spraying with four herbicides at 2 doses. (% of untreated controls)

Herbicide (kg a.i./ha)		Perennial ryegrass		White clover	Barley	*P. annua*	*S. media*
		- NA	+ NA	- NA	- NA	- NA	- NA
Alachlor	0.08	91.1	102.8	55.2	95.9	74.5	94.5
	0.15	49.3	104.0	8.6	106.5	13.8	86.9
NC 20484	0.20	95.0	89.8	0	65.9	0	0
	0.40	80.4	92.6	0	19.9	0	0
MBR 18337	0.08	89.4	90.4	66.9	99.7	44.0	122.9
	0.15	87.0	52.9	19.6	86.8	0	135.7
Perfluidone	0.10	72.1	49.1	74.9	102.8	59.0	130.5
	0.20	68.0	61.1	96.2	118.4	50.2	90.5
Unsprayed control		100.0	96.3	100.0	100.0	100.0	100.0
	S.E. (not applicable to zero values)			±9.62			

Moreover, not only did NA fail to safen ryegrass against MBR 18337 in this experiment, but the effects of both this herbicide and perfluidone were increased slightly by NA treatment, reaching statistical significance with the higher dose of MBR 18337.

White clover was killed by both doses of NC 20484 and was significantly more susceptible to all the herbicides used, except perfluidone, than were either NA-treated or untreated ryegrass.

S. media was completely controlled by NC 20484 at both doses but was not damaged by any other treatment. Indeed, the high dose of MBR 18337 and the low dose of perfluidone increased growth of this species significantly. *P. annua* was killed by both doses of NC 20484 and by MBR 18337 at the highest dose. Alachlor at the lower dose caused only a modest reduction in *P. annua* growth but the higher dose achieved an 86% reduction. At this dose alachlor more than halved the weight of untreated ryegrass without affecting NA-treated plants.

Barley (*Hordeum distichon*) was not damaged by any of the herbicides except NC 20484, both doses of which significantly reduced weights of barley, the higher dose giving over 80% control.

Experiment 3. The effects of different rates of NA seed dressing

The growth of unsprayed ryegrass was inhibited by NA seed dressing and this inhibition increased with increasing rates of safener, reaching significance at the highest rate ($P < 0.05$). However, all the herbicide treatments overcame this trend with NA rates up to 1.0% w/w. Thus, mean foliage weights for each rate of safener, i.e. mean of all herbicide treatments, increased significantly stepwise up to the 1.0% rate, but declined significantly for the 2.0% treatment.

NA at 0.5% safened ryegrass significantly against the lowest dose of alachlor and all three doses of NC 20484. Increasing the NA rate to 1.0% further increased safening against all herbicide treatments, reaching significance compared with the

0.5% rate with the middle doses of both alachlor and NC 20484. NA was less effective against MBR 18337 in this experiment and there was no significant difference between safener rates with this herbicide.

Table 4

Foliage yield of perennial ryegrass treated with various rates of 1,8-naphthalic anhydride (NA) seed dressing and sprayed pre-emergence with three herbicides (% of untreated controls)

Herbicide (kg a.i./ha)		No safener	0.5% NA	1.0% NA	2.0% NA
Alachlor	0.13	4.4	58.2	64.9	70.6
	0.25	0.9	4.7	45.8	26.3
	0.50	0	1.8	12.4	2.7
MBR 18337	0.13	49.4	55.1	62.9	60.5
	0.25	8.1	10.8	18.0	11.8
	0.50	1.2	0.1	5.6	1.8
NC 20484	0.20	32.1	80.7	80.9	61.5
	0.40	1.2	51.7	73.8	69.5
	0.80	0	20.0	30.1	29.9
Unsprayed control		100.0	89.5	82.7	59.1
	Mean	19.7	37.3	46.7	39.4

S.E. herbicide x safener (not applicable to zero values) ± 7.35

S.E. safener means ± 2.33

Experiment 4. Comparison of the effects of NA on six ryegrass cultivars

NA treatment appeared to stimulate the growth of unsprayed S.23 perennial ryegrass slightly and to inhibit the growth of all the other cultivars, these effects being significant only with Sabalan (Table 5).

Table 5

Foliage yield of six ryegrass cultivars either treated (+ NA) or untreated (- NA) with 1,8-naphthalic anhydride seed dressing and sprayed pre-emergence with two herbicides. (% of untreated controls)

			Perennial			Hybrid	Italian	
Herbicide (kg a.i./ha)		Seed treatment	S.23	Melle	S.24	Sabel	RvP	Sabalan
Alachlor	0.13	+ NA	101.9	94.7	76.0	107.8	85.2	82.9
	0.25	"	87.7	86.8	61.4	93.5	95.8	73.6
NC 20484	0.25	"	113.2	101.8	74.5	104.8	103.5	84.5
	0.50	"	88.0	83.7	66.9	68.9	88.8	82.7
Unsprayed control		"	108.0	97.4	81.2	91.8	96.5	71.6
+ NA Mean			99.8	92.8	72.0	93.4	93.9	79.1
Alachlor	0.13	- NA	43.6	47.1	47.2	64.6	81.2	79.6
	0.25	"	5.1	6.8	9.7	16.7	32.4	31.3
NC 20484	0.25	"	78.8	61.8	50.6	61.5	81.7	77.7
	0.50	"	12.2	27.7	9.4	28.9	63.5	48.3
Unsprayed control		"	100.0	100.0	100.0	100.0	100.0	100.0
- NA Mean			47.9	48.7	43.4	54.3	71.8	67.4

S.E. Safener x herbicide treatment x cultivar means ± 7.79
S.E. Safener x cultivar means ± 4.10

Comparing the overall treatment means of the plants with and without NA, all the cultivars were safened significantly by the seed dressing ($P < 0.001$, except Sabalan, = $P < 0.05$). The comparatively small difference shown by Sabalan can be attributed largely to the significant depression caused by NA, combined with a slightly lower degree of protection than was achieved with the perennial and hybrid cultivars.

All three perennial cultivars and Sabel hybrid ryegrass were safened significantly against each herbicide treatment individually. However, for RvP the effect failed to reach significance with the lower dose of alachlor, while Sabalan showed significant differences only with the higher dose of each herbicide.

There were noticeable differences between cultivars in their tolerance of each herbicide in the absence of NA treatment. Comparing the overall means for each cultivar without NA treatment, both Italian ryegrasses (RvP and Sabalan) were significantly less affected than the hybrid (Sabel) or any of the perennial cultivars. However, the only individual treatment to show this significantly was the lower dose of alachlor. S. 24 was depressed significantly by the lower dose of NC 20484 when compared both with the Italian cultivars and with S. 23, whilst all the perennial cultivars except Melle were significantly depressed by the higher dose compared with the two Italian ryegrasses.

DISCUSSION

These results confirm that seed dressings of NA safen perennial ryegrass against both alachlor and NC 20484 (Richardson and Kirkham, 1982). Moreover, protection was not confined to one cultivar or species of ryegrass, and although perennials responded slightly better to NA than the Italian (RvP and Sabalan) and hybrid (Sabel) ryegrasses, the latter three cultivars showed more innate tolerance of the herbicides.

With alachlor and NC 20484, the protection factor was usually well in excess of x 2, i.e. more than twice the dose was required to produce the same damage on safened as on unsafened ryegrass. In Experiment 3, NA at 1.0% gave over x 4 protection against alachlor and nearly achieved this level against NC 20484. Rarely have such encouraging results been achieved in temperate crops (Parker, in press).

With MBR 18337, protection was smaller, more variable and sometimes non-existent (Experiment 2). Results with perfluidone, too, were much less promising than in earlier work (Kirkham and Richardson, 1981; Richardson and Kirkham, 1982).

Alachlor was not sufficiently active against the three weed grasses tested in Experiment 1, but in Experiment 2 the higher dose controlled P. annua without damaging NA-treated ryegrass, even though the weight of untreated ryegrass was halved by this dose. NC 20484 was not included in Experiment 1 so its effects on A. fatua, B. sterilis and A. myosuroides were not recorded. However, reference to earlier screening work (Richardson et al., 1982) suggests that NC 20484 could control a wide range of both grass and broad-leaved species in NA-treated ryegrass, including A. myosuroides and possibly B. sterilis, although A. fatua was more resistant. Both P. annua and S. media could be controlled safely in untreated ryegrass by NC 20484 but the control of volunteer barley by this herbicide could be much more reliable in NA-treated than untreated ryegrass. On the other hand, the tolerance of barley to both alachlor and MBR 18337 suggests a possible use for these herbicides in undersown cereals.

The margin of selectivity between untreated ryegrass and the weed grasses was higher with MBR 18337 than with alachlor in Experiment 1 and MBR 18337 was particularly effective againt both A. myosuroides and P. annua. Moreover, the level of protection achieved against MBR 18337 in Experiment 1 was sufficient to suggest a possible selective B. sterilis control i.e. less than 20% reduction in ryegrass growth with at least 80% control of the weed (Kirkham and Richardson, 1981), at a dose between 0.1 and 0.18 kg a.i./ha.

Except for perfluidone, all the herbicides were too damaging to white clover, precluding their use in clover-based leys. An attempt to safen this species against alachlor using NA has failed (Kirkham, unpublished). NA has generally been effective only in graminaceous crops (Parker, in press), although Blair (1979) reported success in field beans (*Vicia faba*).

NA on its own usually depressed ryegrass growth but sometimes stimulated it and further work is needed to explain these variations.

In conclusion, this work shows clearly the potential of NA for protecting ryegrass against several herbicides, particularly alachlor and NC 20484. Both NC 20484 and MBR 18337 show considerable potential for weed control in untreated ryegrass and this potential could be extended to a wider range of species in NA-treated crops.

If the level of protection against alachlor achieved in these experiments can be repeated under field conditions, this herbicide could be used for seedbed control of *P. annua* during the establishment of ryegrass swards, or even to control volunteer ryegrass seedlings in newly-sown ryegrass seed crops.

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