SELECTIVITY AND METABOLISM OF DICLOFOP METHYL IN WHEAT, BARLEY, WILD OAT, AND GREEN FOXTAIL

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Barry Gordon Todd

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ABSTRACT

Todd, Barry Gordon. Ph.D., The University of Manitoba, October 1979. Selectivity and Metabolism of Diclofop Methyl in Wheat, Barley, Wild Oat, and Green Foxtail. Major Professor: Elmer H. Stobbe.

The contributions of spray retention, penetration, translocation, and metabolism to the selective toxic action of diclofop methyl {methyl 2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate} among wheat (*Triticum aestivum* L. 'Neepawa'), barley (*Hordeum vulgare* L. 'Bonanza'), wild oat (*Avena fatua* L.), and green foxtail [*Setaria viridis* (L.) Beauv.] were evaluated. On an ED_{50} basis, barley, wild oat, and green foxtail were 2, 190, and 1092 times more sensitive, respectively, to foliar-applied diclofop methyl than was wheat. Greater spray retention and more rapid penetration of diclofop methyl partially explained the susceptibility of green foxtail but did not explain selectivity among wheat, barley, and wild oat. Translocation of radioactivity following spot application to leaves was as great, or greater, in the tolerant species, wheat and barley, as in the susceptible species, wild oat and green foxtail. Hydrolysis of diclofop methyl to diclofop proceeded rapidly in all four

species studied. A log-log plot of percent diclofop methyl remaining versus time yielded linear plots, the slopes of which represented the relative abilities of the four species to degrade diclofop methyl. Degradation of diclofop methyl proceeded most rapidly in wild oat and least rapidly in green foxtail. Ability to degrade diclofop methyl was not correlated with species sensitivity to the herbicide.

Species tolerance to diclofop methyl was associated with an ability of the species to degrade the free acid, diclofop. The half-life of diclofop in wheat, barley, wild oat, and green foxtail leaf segments was calculated to be 6.1, 7.3, 15.0, and 9.3 hours, respectively. Degradation of diclofop by the tolerant species involved hydroxylation of the dichlorophenoxy ring. Barley also detoxified diclofop by a second pathway involving degradation of the propionic acid moiety. Diclofop and its degradation products were subject to conjugation to cell constituents.

Root uptake of ^{14}C-diclofop methyl by wheat, barley, wild oat, and green foxtail was proportional to the amount of solution absorbed during the treatment period and to the concentration of diclofop methyl in the treatment solution but was not related to species sensitivity to the herbicide. Translocation of radioactivity to shoots was greater in wheat, barley, and wild oat than in green foxtail. Tolerance of species to root applied diclofop

methyl was related to species ability to degrade diclofop. Differences in the abilities of roots and shoots of a given species to degrade diclofop were observed.

Foliar application of diclofop methyl in combination with 2,4-D (2,4-dichlorophenoxy acetic acid) resulted in reduced toxicity of diclofop methyl to wild oat. The free acid of 2,4-D was identified as the component of the 2,4-D formulation responsible for the reduction in diclofop methyl toxicity. Analysis of diclofop methyl emulsions with and without added 2,4-D revealed no degradation products of diclofop methyl nor any evidence of complexing between diclofop methyl and 2,4-D. Addition of 2,4-D to the diclofop methyl spray emulsion did not affect spray retention or penetration of diclofop methyl. Movement of radioactivity to roots and to shoot apices following spot application of ^{14}C-diclofop methyl to wild oat leaves was reduced by addition of 2,4-D to the treatment solution. As insufficient toxicant reached meristematic areas to permanently interrupt meristematic activity, the wild oat plants were able to outgrow the contact damage to their leaves.

Toxicity of diclofop methyl to susceptible species involves the combined toxic actions of the applied methyl ester and its free acid. Diclofop methyl causes membrane disruption, chlorosis, and necrosis. The free acid, diclofop, moves symplastically to meristematic areas where

it interferes with cell division and elongation processes. Treatment of corn (*Zea mays* L.) and oat (*Avena sativa* L.) seed with 1,8-naphthalic anhydride or R-25788 (N,N-diallyl-2,2-dichloroacetamide protected the plants against the systemic, toxic action of diclofop but did not overcome the contact action of diclofop methyl.

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FORMAT

This thesis has been written in manuscript style. Chapter 2 has been published in Weed Science (25:382-385, 1977). Chapters 1, 3, 4, 5, and 6 will be submitted for publication in Weed Science. Chapter 7 is intended as a research note for submission to the Canadian Journal of Plant Science.

INTRODUCTION

Wild oat is the most serious annual weed of cultivated fields in the prairie provinces (Sharma and Vanden Born, 1978). Control of wild oats in crops may, by reducing competition for moisture, nutrients, and sunlight, result in vigorous growth of green foxtail. Green foxtail may seriously reduce crop yields (Sturko, 1978). Where both wild oats and green foxtail are present, control of wild oats alone may not provide any crop yield incease, as the green foxtail population flourishes (Moyer and Dryden,1977). Thomas (1978) reported that green foxtail was present in 87%, and wild oats in 79%, of Manitoba grain fields. Clearly, there is a need for a herbicide, or herbicide mixtures, which will selectively control both of these grassy weeds in cereal and oilseed crops.

When applied as a post-emergence spray diclofop methyl selectively controls certain annual grassy weeds, including wild oats and green foxtail, in cereal and oilseed crops. An understanding of this selective toxic action would assist weed scientists in formulating recommendations for the use of diclofop methyl.

Selectivity of a herbicide may result from differential spray retention, penetration, translocation, and/or

metabolism by various species. Each of these processes represents a barrier which the applied herbicide must overcome to reach its site of action. Any, or all, of these processes may be modified by changing environmental conditions or by the physiological development of the plant. An understanding of these relationships may provide information on basic physiological processes within plants, information which in the future may provide the basis for the development of new weed control systems.

Diclofop methyl toxicity is reduced when applied in combination with 2,4-D for broad spectrum control of both grassy and broadleaved weeds. An understanding of the basis of this antagonistic interaction may suggest means by which this problem may be overcome. A means of overcoming the antagonistic interaction between 2,4-D and diclofop methyl would benefit western Canadian farmers through improved weed control programs and reduced application costs.

Studies were undertaken to evaluate: 1. the basis of selectivity of diclofop methyl among wheat, barley, wild oats, and green foxtail; and 2. the basis of the antagonistic effect of 2,4-D on diclofop methyl toxicity to wild oat.

LITERATURE REVIEW

1.Diclofop Methyl

1.1 Introduction. Diclofop methyl {methyl 2-(4-(2,4-dichlorophenoxy) phenoxy) propanoate} is a member of the phenoxy-phenoxy group of herbicides (Koecher and Lotzsch, 1975; Nestler *et al.*, 1978). Compounds within this group are effective only against monocot species at normal use rates. The spectrum of grassy weeds controlled is determined by the nature and position of the substituents on the phenoxy ring (Andersen, 1976b; Nestler *et al.*, 1978).

Diclofop methyl provided most effective grassy weed control when applied as an early post-emergence treatment (Wu and Santelmann, 1976). At equal rates diclofop methyl applied post-emergence gave better control of wild oats (*Avena fatua* L.) and green foxtail {*Setaria viridis* (L.) Beauv.}, and increased wheat yields more than did soil application (Chow, 1978). Preplant-incorporated applications of the herbicide provided more effective weed control than did pre-emergence applications (Todd and Stobbe, 1974b; Wu and Santelmann, 1976).

1.2 Selectivity. Andersen (1976b) evaluated the response of seedlings of 29 grasses to a post-emergence application

of diclofop methyl. Corn (*Zea mays* L.), goosegrass [*Eleusine indica* (L.) Gaertn.] witchgrass (*Panicum capillare* L.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], foxtails (*Setaria* spp.) and itchgrass (*Rottboellia exalta* L.f.) were all highly susceptible to diclofop methyl. Wild oat, large crabgrass [*Digitaria sanguinalis* (L.) Scop.], proso millet (*Panicum miliaceum* L.) sorghum [*Sorghum bicolor* (L.) Moench], Texas panicum (*Panicum texanum* Buckl.), field sandbur (*Cenchrus incertus* M.A. Curtis), shattercane [*Sorghum bicolor* (L.) Moench] and wooly cupgrass [*Eriochloa villosa* (Thunb.) Kunth.] were intermediate in response while johnsongrass [*Sorghum halepense* (L.) Pers.], quackgrass [*Agropyron repens* (L.) Beauv.], barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) downy brome (*Bromus tectorum* L.), hairy chess (*Bromus commutatus* Schrad.) and jointed goatgrass (*Aegilops cyclindrica* Host) were tolerant to the herbicide.

Diclofop methyl applied at a rate of 3.36 kg/ha had no effect on the yield of weed-free soybeans [*Glycine max* (L.) Merr.] (Andersen, 1976a). In the same study excellent control of volunteer corn and giant foxtail (*Setaria faberi* Herrm.) was achieved with 1.68 kg/ha of diclofop methyl applied early post-emergence.

Varietal differences in the susceptibility of corn inbreds to diclofop methyl have been observed (Andersen,

1976a). The inheritence of tolerance to diclofop methyl in corn was shown to involve additive gene action at several loci (Geadelmann and Andersen, 1977). Varietal differences in tolerence of barley to diclofop methyl have also been reported (Friesen *et al.*, 1976; Qureshi and Vanden Born, 1979).

Diclofop methyl (1.1 kg/ha) did not affect the growth of alfalfa (*Medicago sativa* L.), buckwheat (*Fagopyrum esculentum* Moench), fababeans (*Vicia faba* L.), flax (*Linum usitatissimum* L.), rapeseed (*Brassica napus* L.) soybeans, and sweet clover (*Melilotus alba* Desr.) but did reduce growth of yellow mustard (*Brassica hirta* Moench) slightly (Chow, 1978). Of 13 monocotyledonous species included in the same study, corn, green foxtail, oats (*Avena sativa* L.), wild oats, sorghum, and timothy (*Phleum pratense* L.) were susceptible to diclofop methyl. Tolerant grasses included wheat, barley, bromegrass (*Bromus inermis* Leyss.), intermediate wheatgrass [*Agropyron intermedium* (Host) Beauv.], Russian wild ryegrass (*Elymus junceus* Fisch.), and triticale (X *Triticosecale* Whittmack).

Selective removal of Italian ryegrass (*Lolium multiflorum* Lam.) from winter wheat has been achieved using diclofop methyl (Brewster *et al.*, 1977). This herbicide is also recommended for the selective control of the related species Persian darnel (*Lolium persicum* Boiss & Hoh.) in cereal and oilseed crops (Saskatchewan

Department of Agriculture, 1979).

1.3 Symptoms. Susceptible plants begin to turn chlorotic two to three days after diclofop methyl application. Within three to four days after application root and shoot growth ceases. During the next several days the chlorosis deepens, the affected tissues become necrotic, and complete collapse of the plant follows (Hoechst Canada Inc., 1979).

1.4 Translocation and Metabolism. Injection of diclofop methyl below the stem apex resulted in the death of both wild oat and barley plants. Application of the herbicide, as spots, to the leaves of wild oat and barley plants resulted in gradual necrosis of the leaf area above the point of application but growth of the plants was not inhibited (Friesen *et al.*, 1976). The authors concluded that movement of diclofop methyl in these two species was primarily acropetal, noting however, that rapid necrosis of the treated spot may have interfered with both absorption and downward movement of the applied herbicide.

Brezeanu *et al.* (1976) reported that translocation of ^{14}C-diclofop methyl was similar in wheat and wild oat. Translocation of ^{14}C out of the treated leaf amounted to only one to two percent of the absorbed radioactivity during a 96 hour period.

Extraction of wheat plants 18 days after treatment with ^{14}C-diclofop methyl yielded diclofop and ring-

hydroxylated derivatives of diclofop. Ring-hydroxylation resulted in the formation of 2-[4-(2,4-dichloro-5-hydroxyphenoxy) phenoxy] propionic acid. Lesser quantities of the 3-hydroxy and 6-hydroxy isomers were also present. Hydroxylation of the dioxy-phenoxy ring was not observed (Gorbach *et al.*, 1977).

1.5 Physiological effects. The effects of diclofop methyl on the gross levels of chlorophyll, starch, free sugars, protein, and RNA have been monitored in corn, a susceptible species (Koecher and Lotzsch, 1975). A 60% reduction in the chlorophyll content was observed within seven days after application. An increase in the content of free sugars was noted, but no significant changes in the gross levels of starch, protein, or RNA were reported.

The contents of chlorophylls a and b of wild oat shoots treated with diclofop methyl decreased after eight days by 41% and 56% respectively (Chow and LaBerge, 1978). A 63% inhibition of phloem translocation of photosynthates from shoots to roots of wild oats was also observed. The reduction in photosynthate translocation to the roots resulted in an accumulation of sugars (glucose, sucrose, fructose) in the shoots. Levels of ATP in shoots of diclofop methyl treated wild oats were 44% less than in the shoots of untreated wild oats. The authors suggest that reduced ATP levels may be responsible for the observed reduction in sugar transport to the roots.

Exposure of wild oat roots to diclofop methyl resulted in a marked reduction in the mitotic index of the coronal roots (Owino, 1977). Higher concentrations and/or longer exposure periods were required to achieve an equivalent effect in wheat coronal roots. Cell elongation processes were also sensitive to diclofop methyl. A 24 hour exposure to 0.05 ppm diclofop methyl in nutrient solution completely inhibited growth of the elongation region of wild oat and barley coronal roots. With wheat, a similar exposure to 0.10 ppm diclofop methyl initially reduced growth of the elongation region of the coronal roots but with time the coronal roots recovered from this effect. Severe root inhibition caused by exposure of wild oat roots to diclofop methyl has been associated with reduced uptake of ^{45}Ca from the root zone (Crowley *et al.*, 1978).

Ultrastructural modifications in wild oat leaves following diclofop methyl application have been described (Brezeanu *et al.*, 1976). In leaf tissue present at the time of application, extensive damage to the plasmalemma, cytoplasm, and chloroplasts was observed. Increased vacuolation, perhaps as a result of membrane disruption, was also reported. Mitochondria, in general, did not appear to be severely damaged. In new growth, abnormal development of chloroplasts was reported. Similar, although less severe, effects were observed in treated wheat plants.

Increased leaf-cell permeability, as measured by changes in the conductance of ambient solutions in which diclofop methyl treated leaf discs were floated, indicated that the effects of diclofop methyl on wild oat cell permeability occur even prior to the development of visual symptoms. Significant increases in wild oat leaf cell permeability were observed within 12 hours after foliar application of a 0.112 kg/ha dose of diclofop methyl. A significant increase in barley leaf cell permeability was observed 24 hours after the application, but in wheat no effects of diclofop methyl on cell permeability were observed (Crowley and Prendeville, 1979).

1.6 Interactions with other Herbicides. Tank-mixtures of diclofop methyl with 2,4-D (2,4-dichlorophenoxy acetic acid), MCPA (4-chloro-O-tolyl-oxy acetic acid), or dicamba (3,6-dichloro-O-anisic acid) provided less effective wild oat and green foxtail control than did diclofop methyl applied alone. The reduction in grassy weed control was greatest when diclofop methyl was tank-mixed with dicamba and least when mixed with MCPA. Diclofop methyl had no effect on the efficacy of the herbicides for broad-leaved weed control. Bromoxynil (3,5-dibromo-4-hyroxybenzonitrile) tank-mixed with diclofop methyl did not reduce its effectiveness against either wild oat or green foxtail (Todd and Stobbe, 1974c; Chow, 1974; O'Sullivan *et al.*, 1976).

The herbicidal activity of diclofop methyl was not

reduced when applied with MCPA solvent blank (i.e., the commercial formulation minus the active ingredient) indicating that the MCPA-diclofop methyl antagonism was not due to solvent incompatability (O'Sullivan *et al.*, 1976). Amine formulations of MCPA reduced wild oat control by diclofop methyl more than did ester formulations. Qureshi and VandenBorn (1979b) reported that uptake of ^{14}C-diclofop methyl by wild oat leaves was reduced by 52% when applied in combination with MCPA amine, but was reduced by only 10% when applied with MCPA ester.

Diclofop methyl toxicity to wild oat was reduced by more than 50% when applied as a tank mixture with either 2,4-D or dicamba. Application of 2,4-D or dicamba separately but within minutes of diclofop methyl application did not affect wild oat control by diclofop methyl (Owino *et al.*, 1975a, 1975b). MCPA reduced wild aot control by diclofop methyl when applied five seconds after diclofop methyl but had no effect when applied five seconds before diclofop methyl (Qureshi and VandenBorn, 1979b). Control of wild oats by diclofop methyl was reduced more when 2,4-D was applied 24 hours after diclofop methyl application than when applied minutes after diclofop methyl application (Hunter, 1975; Owino *et al.*, 1975a), and in general, applications of diclofop methyl and herbicides for the control of broadleaved weeds must be separated by at least four days if reductions in grassy weed control are to be avoided (Hoechst Canada Inc., 1979).

Other herbicides which have been reported to reduce diclofop methyl toxicity to grassy weeds include nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) (Chow, 1977; Delage and Coulthard, 1974; Vanstone and Stobbe, 1974), bentazon (3-isopropyl-1H-2,1,3,-benzothiadiazin-4-(3H)-one 2,2-dioxide) (Drew, 1974; Todd and Stobbe, 1974a), dinoseb (2-secbutyl-4,6-dinitrophenol) (Drew, 1974; Betts and Morrison, 1978), and MCPB [4-(4-chloro-O-tolyl-oxy) butyric acid] (Drew, 1974).

1.7 Behaviour in the Soil. Diclofop methyl has provided selective weed control when applied as a soil-incorporated treatment (Todd and Stobbe, 1974b; Wu and Santelmann, 1976). The efficacy of soil-incorporated diclofop methyl increased significantly as the soil moisture in a Tiffany sandy loam was increased from 75% to 125% of field capacity. Equivalent increases in efficacy were obtained when diclofop methyl treated soil at 50% of field capacity was watered to field capacity either at the time of herbicide application or 16 days later (Mulder and Nalewaja, 1979). In the field, the efficacy of soil-incorporated diclofop methyl would be dependent on the amount and time of rainfall.

Leachability of diclofop methyl decreased with increasing clay content of the soil suggesting that diclofop methyl was adsorbed onto clay particles. Movement of diclofop methyl in saturated soil columns increased as the water

volume applied increased. Even in soils with a high sand content more than 80% of the applied ^{14}C-diclofop methyl was recovered from the top 8 cm. of the soil column (Mulder and Nalewaja, 1979). The dependence of diclofop methyl on adequate soil moisture for activity, and its lack of mobility in the soil, may explain the need for incorporation of this herbicide.

Diclofop methyl was rapidly hydrolyzed (up to 90% in 24 hours) to its corresponding free acid, diclofop, in the soil (Smith, 1977). Under aerobic conditions diclofop was further degraded. During a 25 week period following application of ring-labelled ^{14}C-diclofop methyl to a sandy loam soil, 35% of the applied radioactivity was liberated as $^{14}CO_2$ (Martens, 1978). Both Smith and Martens have identified 4-(2,4-dichlorophenoxy) phenol as an intermediate in the degradation of diclofop in soils. Smith suggested that formation of this metabolite proceeds via a decarboxylation process yielding first 4-(2,4-dichlorophenoxy) phenetole which could then undergo dealkylation to give the corresponding phenol. A second possibility as suggested by Martens would involve the direct cleavage of the ether bond between the aromatic and aliphatic portions of the molecule. Degradation products other than the phenol were not present in sufficient quantities to permit characterization.

2. Herbicide Selectivity

2.1 Assessment of Relative Toxicity. To determine the relative toxicity of a given herbicide to a number of species it is necessary to develop dosage-response data. The dosage-response curve for a given species is typically sigmoidal in shape, making direct comparisons between species difficult. By plotting the data as the probit of the percentage growth reduction resulting from herbicide treatment versus the $\log_{10}$ of the herbicide dosage, a linear regression of response on dose can be obtained. From the linear regression the dosage required to reduce the growth of a given species by 50% (the ED_{50} level) can be calculated. The ED_{50} values obtained for various species may then be compared directly in order to establish the relative toxicity of the herbicide to those species (Blackman, 1952).

When assessing the relative toxicity of post-emergence herbicides, the mean dry weight of the species at the time of spraying must be subtracted from the mean dry weight obtained for each treatment at harvest. The net dry weight increase of the treated plants may then be expressed as a percentage of the net dry weight increase of the control plants. A plot of the probit of the relative growth values versus the $\log_{10}$ of the dosages will be linear, allowing calculation of the ED_{50} values. Failure to subtract the mean dry weight at the time of spraying

from the mean dry weight at harvest may introduce significant errors into the ED_{50} calculation (Hawton and Stobbe, 1971).

2.2 Spray Retention. Under a given set of spray parameters, spray retention by a given species will depend largely upon the morphology and leaf surface characteristics of the plant (Blackman *et al.*, 1958).

The tolerance of rapeseed to benazolin (4-chloro-2-oxobenzothiazolin-3-ylacetic acid) was associated with limited spray penetration. On a volume per unit dry weight basis, rapeseed retained less than half as much benazolin spray solution as did the susceptible species wild mustard (*Sinapsis arvensis* L.) (Schafer and Stobbe, 1973a). Similarly, tolerant soybean retained only half as much bentazone per unit leaf area as did susceptible Canada thistle [*Cirsium arvense* (L.) Scop.] (Penner, 1975).

Differential spray retention was an important factor in the selective control of broadleaved weeds in alfalfa and red clover (*Trifolium pratense* L.) with 2,4-DB [4-(2,4-dichlorophenoxy) butyric acid]. Common cocklebur (*Xanthium pensylvanicum* Wallr.) and redroot pigwood (*Amaranthus retroflexus* L.) retained three and six times as much spray solution, respectively, as did either of the legume species (Hawf and Behrens, 1974).

The grassy weed, green foxtail, retained only two-thirds as much nitrofen as did the broad-leaved species, rape and redroot pigweed (Hawton and Stobbe, 1971). In

this case spray retention was not directly correlated with selectivity as the susceptible species, green foxtail (ED_{50}= 115 g/ha), retained less spray solution per gram dry weight than did the tolerant species, rapeseed (ED_{50}=661 g/ha).

2.3 Penetration. Penetration of ^{14}C-ioxynil (4-hydroxy-3, 5-diiodobenzonitrile) into leaf discs of susceptible mustard was eight times greater during an eight hour period than was penetration into leaf segments of tolerant barley. More rapid penetration combined with a 26 fold retention difference largely accounted for the selective toxic action of ioxynil between these two species (Davies *et al.*, 1967, 1968).

Differential rate of penetration of ^{14}C-benazolin into leaves of wild mustard and rapeseed contributed to the selectivity of this herbicide. The three fold penetration difference was not sufficient, however, to account for the magnitude of selectivity observed, suggesting that other, more important, internal factors were involved in benazolin selectivity (Schafer and Stobbe, 1973a).

The penetration of herbicides into leaves of very closely related species may differ sufficiently to provide a basis for selective toxic action. Nitrofen absorption by leaves of the susceptible cabbage (*Brassica oleracea* L.) cultivar, Rio Verde, was twice as rapid as was absorption by leaves of the tolerant cultivar, Hybelle. Rubbing the leaf surfaces of Hybelle plants with glass wool to abrade the cuticle rendered them sensitive to nitrofen, an

indication that selectivity of nitrofen among these two cultivars was directly attributable to differential spray penetration (Pereira *et al.*, 1971).

2.4 Translocation. To be effective a herbicide must reach its site of action within the plant. Species differences in the transport of a herbicide from its site of absorption to its site of action may provide a basis for herbicide selectivity (Ennis, 1964).

The basis of selectivity of simazine [2-chloro-4,-6-bis-(ethylamino)-s-triazine] was studied in susceptible witchgrass and tolerant crabgrass (Robinson and Green, 1976). Root-uptake of ^{14}C-simazine by the two species was identical. Degradation of simazine also proceeded at similar rates in the two species. A major difference between the two species was observed in the distribution of the radioactivity within the plants. Witchgrass shoots contained 20% more simazine than did crabgrass shoots, a critical differential since simazine acts as a photosynthetic inhibitor.

Fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone], a herbicide which interferes with chlorophyll synthesis and/or stability, was readily translocated to shoots following root-uptake by susceptible corn and soybean but remained concentrated in roots of tolerant cotton (*Gossypium hirsutum* L). No species differences in uptake or metabolism were observed, leading

the authors to conclude that the tolerance of cotton to fluridone arises as a result of limited transport of the herbicide to its site of action (Berard *et al.*, 1978).

Susceptible wild mustard translocated foliar-applied ^{14}C-benazolin and/or its degradation products more readily than did tolerant rape species. While rates of metabolism of benazolin were similar in the three species studied, transport efficiences for ^{14}C of 83%, 28%, and 31% were calculated for wild mustard, turnip rape (*Brassica campestris* L.), and rape respectively (Schafer and Stobbe, 1973b).

Radiolabelled benzoylprop ethyl [ethyl-2-(N-benzoyl-3, 4-dichloroanilino) propionate], flamprop methyl {methyl-2-[N-3-chloro-4-fluorophenyl) benzamido] propionate} and flamprop isopropyl {isopropyl-2-[N-3-chloro-4-fluorophenyl) benzamido] propionate} moved primarily in the acropetal direction following spot application to leaves of wheat, barley, and wild oat (Jeffcoat and Harries, 1973, 1975; Jeffcoat *et al.*, 1977). The respective parent acids, however, moved basipetally in the phloem. Basipetal movement of benzoylprop was approxmately five times that of benzoylprop ethyl. Translocation of benzoylprop was limited, with only seven percent of the total applied radioactivity moving from the treated leaf during a three day period. Species differences with respect to the translocation of benzoylprop and flamprop were minor.

2.5 Herbicide Metabolism. Selective herbicidal action can

arise if metabolic changes to the applied herbicide occur in one species and not another, or where the metabolism of a herbicide proceeds via similar pathways but at different rates in different species. The selectivity of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-S-triazine], propanil (3,4-dichloropropionanilide), dicamba chloramben (3-amino-2,5-dichlorobenzoic acid), pyrazon [5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone] , and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] has been shown to relate to the relative abilities of various species to metabolize the applied compound (Shimabukuro and Swanson, 1969; Frear and Still, 1968; Chang and Vanden Born, 1971; Stroller and Wax, 1968; Stephenson and Ries, 1967; Frear *et al.*, 1972; Frear and Shimabukuro, 1970).

The degradation of benzoylprop ethyl involved enzymatic de-esterification, yielding the biologically active desethyl acid. Wheat, barley, and oat differed in their ability to de-esterify benzoylprop ethyl, the de-esterification rate being greatest in susceptible oat and least in tolerant wheat. Benzoylprop detoxification via conjugation to sugars was rapid enough in wheat to prevent accumulation of benzoylprop to phytotoxic levels. In susceptible oat the rate of conjugation was insufficient to prevent accumulation of the toxicant (Jeffcoat and Harries, 1973; Beynon *et al.*, 1974).

De-esterification of ^{14}C-flamprop isopropyl to the biologically active parent acid, flamprop, was more rapid in susceptible oat than in tolerant barley. After 14 days, 22% of the absorbed compound had been de-esterified by wild oat as compared to only 12% by barley. Conjugation of flamprop to sugars proceeded rapidly enough in barley to prevent any accumulation of toxicant. In wild oat the rate of de-esterification exceeded the rate of conjugation, the net result being an accumulation of the phytotoxic acid in this species (Jeffcoat *et al.*, 1977).

Rapid cleavage of the diphenyl ether linkage of fluorodifen (p-nitrophenyl, α, α, α -trifluoro-2-nitro-p-tolyl ether was catalyzed by a glutathione-S-transferase (Shimabukuro *et al.*, 1973). Fluorodifen resistant species including cotton, corn, peanut (*Arachis hypogaea* L.), pea and soybean generally contained higher levels of this glutathione-S-transferase than did susceptible species such as tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), and squash (*Cucurbita maxima* Ducheane).

Ring-hydroxylation of dicamba to yield the non-toxic metabolite, 5-hydroxy-dicamba, proceeded more rapidly in tolerant wheat and barley than in sensitive tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] and wild mustard (Chang and Vanden Born, 1971). While many species were able to hydroxylate the phenyl ring of 2,4-D the relative abilities of the species to catalyze the hydroxylation did not correlate

to species tolerance to 2,4-D (Fleeker and Steen, 1971).

Conjugation of free hydroxyl, carboxyl, or amino groups of herbicides to sugars or amino acids is generally considered to represent an important detoxification mechanism within plants (Frear, 1976). Conjugation of benzoylprop, pyrazon, dicamba, chloramben, dichlobenil (2,6-dichlorobenzonitrile), atrazine, fluorodifen, and other herbicides to natural plant constituents at varying rates in various species constitutes the basis for the selective toxic action of these compounds (Stoller and Wax, 1968; Stephenson and Ries, 1967; Chang and Vanden Born, 1971; Verloop and Nimmo, 1969; Jeffcoat and Harries, 1973; Frear *et al.*, 1972; Shimabukuro, 1975).

2.6 Differences at the Site of Action. The basis for chlorfenprop methyl [2-chloro-3-(4-chlorophenyl) methyl propionate] selectivity was studied in the oat cultivars Tiger (tolerant) and Flamings Krone (sensitive). Rates of penetration and metabolism were similar in both cultivars, but membrane damage to etiolated mesocotyl segments was greater in the susceptible cultivar than in the tolerant cultivar. Since equal concentrations of the biologically active acid were present close to, or at, the site of action in both cultivars, the basis of selectivity must have involved a difference at the site of action. As a result of this difference chlorfenprop would be capable of interacting with only the site of

action in the sensitive cultivar (Fedtke and Schmidt, 1977).

Studies to evaluate the basis of triazine resistance in lamb's quarters (*Chenopodium album* L.) and redroot pigweed biotypes have provided further evidence of herbicide selectivity based on differences at the site of action (Souza Machado *et al.*, 1977, 1978; Arntzen *et al.*, 1979).

Using chloroplasts isolated from lamb's quarters, Souza Machado *et al.* (1977) showed that atrazine inhibited electron transport only in chloroplasts derived from the sensitive biotype. Souza Machado *et al.* (1978) further demonstrated that there were no differences in atrazine penetration of the chloroplast envelopes isolated from resistant and susceptible biotypes. They concluded that chloroplasts from the tolerant lamb's quarters biotype contain one or more modified photosystem II components, a modification which decreases the binding affinity for triazines at their inhibition site in the photosystem II complex. A difference in photosystem II properties was detected in the absence of herbicide in chloroplasts derived from the resistant biotype, providing further evidence that a change in the composition of photosystem II components had indeed taken place.

Electrophoretic analysis of chloroplast membrane proteins derived from triazine sensitive and resistant redroot pigweed biotypes indicated small changes in the electrophoretic mobilities of two polypeptide species.

The two polypeptide species were absent from photosystem I fragments but were both present in photosystem II preparations, suggesting that a genetically controlled alteration in the target site had led to the differential toxicity of triazines to the two pigweed biotypes (Arntzen *et al.*, 1979).

3. Herbicide Interactions

3.1 Calculation of Antagonistic and Synergistic Responses.

Numerous authors have attempted to mathematically define herbicide-herbicide interactions (Bliss, 1939; Gowing, 1960; Tammes, 1964; Colby, 1967; Akobundu *et al.*, 1975; Rummens, 1975; Hamill and Penner, 1973). However, as emphasized in the recent review by Morse (1978), definition of interactions can be accomplished only by comparison of observed responses to an appropriate, well-defined, reference model. The reference model describes the joint action of the components of an herbicide mixture that is assumed to occur in the absence of any interaction. The absence of an interaction has been referred to as additive herbicide action (Colby, 1967). Deviations in the observed response relative to this reference model indicate non-additive herbicidal interactions. If the observed toxicity of the mixture is greater than the response predicted by the reference model the interaction could be termed synergistic. Where the observed response is less

than the predicted response, antagonism may be indicated.

The choice of an appropriate reference model is crucial to the subsequent interpretation of the data. Selection of a reference model requires some knowledge of the modes of action of the individual components of the mixture (Morse, 1978). Where the two components affect the same site(s) of action an additive dose model (ADM) may be appropriate. This model predicts that the response of a given herbicide mixture will equal the sum of the responses of the individual components (Tammes, 1964; Akobundu *et al.*, 1975). According to this model, a portion of the dosage of component 1 of the mixture can be replaced by the equi-effective dosage of component 2 with no overall effect on the response of the test species.

In the more general case, where the two components of the mixture exert their effects at independent sites of action, the multiplicative survival model (MSM) may be more appropriate (Morse, 1978). This model is described by the equation:

$$E = \frac{XY}{100} \quad \text{(Colby, 1967)}$$

where E = expected growth (survival) as a percent of control for a combination of herbicides A and B applied at dosages D_a and D_b respectively.

X = growth (survival) as a percent

of control resulting from application of herbicide A at dosage D_a.

Y = growth (survival) as a percent of control resulting from application of herbicide B at dosage D_b.

Differences between the expected response (E) and the observed response to a herbicide mixture can be statistically evaluated according to the methods of Hamill and Penner (1973).

3.2 Herbicide-Herbicide Antagonism. Herbicide-herbicide antagonism was first reported in 1936 by Crafts and Cleary who observed that mixtures of sodium chlorate and sodium tetraborate applied to soils were less phytotoxic to plants than equivalent doses of chlorate applied alone.

Dicamba, 2,4-D, and 2,4,5-T [(2,4,5-trichlorophenoxy) acetic acid], were antagonistic with respect to EPTC (S-ethyl dipropylthiocarbamate) toxicity to sorghum and giant foxtail (Beste and Schreiber, 1970). This antagonism was attributed to the opposing effects of EPTC and growth-regulator herbicides with respect to RNA synthesis (Beste and Schreiber, 1972). EPTC reduced RNA levels in soybean hypocotyl from 2.3 to 2.0 mg/g of tissue; 2,4-D increased RNA levels to 3.2 mg/g of tissue. The combination of 2,4-D and EPTC resulted in an RNA level of 2.6 mg/g, less than observed for 2,4-D applied alone but

significantly higher than either the control or the EPTC treatments.

Reduced toxicity of TCA (trichloroacetic acid) to wheat when applied in combination with either 2,4-D or MCPA has been reported (Chow, 1975). The phenoxy compounds reduced absorption, accumulation, and translocation of TCA by roots of intact wheat seedlings.

Combinations of herbicides for broadleaved weed control with the wild oat herbicide barban (4-chloro-2-butynyl m-chlorocarbanilate) reduced wild oat control by barban but had no effect on the efficacy of the herbicide for broadleaved weed control. Similar one-way antagonism was also observed when herbicides for broadleaved weed control were tank mixed with the wild oat herbicides benzoylprop ethyl, flamprop methyl, difenzoquat (1,2-dimethyl-3,5-diphenyl-1H-pyrazolium), and diclofop methyl (O'Sullivan and Vanden Born, 1975).

3.3 Herbicide Antidotes. Antagonism of herbicidal activity has been utilized in the development of crop 'protectants'. EPTC injury to corn was completely overcome through simultaneous application of R-25788 (N,N-diallyl-2,2-dicloroacetamide) (Chang *et al.*, 1972). The antidotal effect of R-25788 was species specific. R-25788 protected corn from the herbicidal effect of EPTC but did not reduce EPTC toxicity to green foxtail, lamb's quarters, or redroot pigweed. Chang *et al.*

(1973) speculated that because of its structural similarities to EPTC, R-25788 may bind inactively at the site for EPTC action in corn. This hypothesis was supported by the results of Stephenson *et al.* (1978) who, using a soil-free system, established that molecules structurally similar to EPTC were effective as antidotes. The greater the structural similarity between the test compound and EPTC, the greater were its antidotal properties.

Levels of glutathione-S-transferase, an enzyme implicated in the detoxification of EPTC in plants, increased nine fold during a 24 hour exposure of corn roots to R-25788 (Lay *et al.*, 1975). Much higher levels of R-25788 were required to induce increases in gluthathione-S-transferase levels in oat roots, and even then the level of the detoxifying enzyme remained so low that EPTC detoxification was not significantly affected. The differential stimulatory effect of R-25788 on glutathione-S-transferase levels in corn and oat may explain the selective antidotal action of this compound.

The chemical 1,8-naphthalic anhydride (NA) protected oats against barban toxicity. In this case treatment with NA afforded protection to both desirable tame oat and undesirable wild oat. Selective antidotal action was achieved by applying the protectant as a dust to the tame oat seeds prior to planting. Subsequent post-emergence barban application proved toxic to wild oats but not to the NA treated tame oats (Chang *et al.*, 1974).

CHAPTER 1

The Air Brush. A Technique for Applying Microliter Quantities of Pesticides to Plants

Abstract. The use of an air brush for the application of minute quantities of pesticides to plant surfaces is described. This technique permits radio-labelled pesticides to be applied in a manner closely simulating application from a conventional spray nozzle while at the same time directing the spray to specific portions of the plant. ^{14}C-diclofop methyl {methyl 2-[4-(2,4-dichloro-phenoxy) phenoxy] propanoate} applied using this technique penetrated wild oat (*Avena fatua* L.) leaves 2 to 3 times more rapidly than if applied as microdroplets with a syringe.

INTRODUCTION

In using radioactivily labelled pesticides to study the fate of pesticides applied to plants, a major difficulty arises in selecting a method of application which will simulate field spray application and yet conform to laboratory constraints. Some of the techniques which have been developed include: a) dipping of leaves into the solution (Blanchard, 1954); b) afixing an open ended tube to the leaf surface and filling this with pesticide solution (Sargent and Blackman, 1962); c) using a glass rod to spread treatment solution across the leaf surface (Ashton, 1958); d) placing a lanolin block containing

the pesticide on the leaf surface (Davis and Dusbabek, 1973); e) placing a wick having one end in the herbicide solution across the desired portion of the plant (Shimabukuro *et al.*, 1976) f) using a syringe to place µl quantities of the spray solution in droplet form on the leaf surface. A lanolin or silicone grease boundary may (Webster, 1962) or may not (Schafer and Stobbe, 1973a) be required to contain the µl droplets within the desired area. Currently, the most frequently utilized technique involves the use of a syringe to apply microdroplets of pesticide to the plant surface (Babiker and Duncan, 1975; Claus and Dusbabek, 1973; Jeffcoat and Harries, 1975; Mahoney and Penner, 1975; Richardson, 1975; Roberts and Stoydin, 1976; Springle *et al.*, 1975).

In this report a new method of application of µl quantities of pesticides to plants suggested to us by Dr. S. Gorbach[1] of Hoechst AG is described and compared to the syringe method of application.

MATERIALS AND METHODS

The air brush described in this report is a Binks Wren air brush model 59-10001A[2].

The plant material used in this study was grown in a growth chamber having an illumination of 4510 $\mu W/cm^2$

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[1]Personal Communication.

[2]Binks Manufacturing Company, 9201 West Belmont Avenue, Franklin Park, Illinois.

provided by wide spectrum fluorescent lamps on a 15-h photoperiod and a temperature regime of 25 C day and 15 C night. Wild oat plants were grown to the two-leaf stage in silica sand and watered as required with a half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Individual plants were then transferred to bottles containing nutrient solution. The plants were treated in the three-leaf stage. The treatment solution consisted of ^{14}C-diclofop methyl (9.6 µCi/mg) formulated with solvent blank and technical diclofop methyl to produce a 36% EC formulation. This formulation was diluted 1:100 (v/v) with water as might be done for foliar application. Approximately 2 µl of this emulsion containing 0.05 µCi/µl was applied to the adaxial surface near the middle of the second leaf with either a syringe or an air brush. Material was applied with the air brush, within the confines of a well ventilated fume hood, to a strip 0.3 by 3.0 cm. Each plant was placed horizontally on the counter top with the leaf to be treated positioned under an opening in a cardboard sheet. The air brush, operated at a pressure of 0.78 kg/cm^2 and held 2 to 3 cm above the plant surface, was then passed over the opening.

After various time intervals four plants treated by each technique were harvested. The treated zone was cut from the leaf and surface radioactivity was removed by rinsing with 5 ml of 30% ethanol as a directed pipette stream. The washings, in scintillation vials, were evaporated to dryness under an air stream. Ten ml

of scintillation solution containing 0.6% (w/v) PPO (2,5-diphenyl oxazole) and 0.01% (w/v) POPOP [1,4-bis-2 (5-phenyloxazolyl)-benzene] in toluene:methyl cellosolve (2:1 v/v) were added to each vial. Radioactivity was determined with a scintillation counter and all data was corrected for quenching, by the channels ratio method, and for background radioactivity. Radioactivity within the plant was determined by grinding the various plant parts to pass through a 0.508 mm (50 mesh) screen. Subsamples were then suspended in the scintillation solution to which had been added 4% (w/v) thixotropic gelling agent[3]. Radioactivity was assayed with the scintillation counter. Internal absorption of radioactivity by the tissue was determined to be approximately 52% by comparison to a set of subsamples which were combusted to $^{14}CO_2$ in a sample oxidizer. As preliminary experiments showed herbicide volatilization to be nonsignificant over the duration of the experiment, total applied radioactivity could be calculated as the sum of the radioactivity in the leaf washings plus radioactivity within the tissue.

RESULTS AND DISCUSSION

The air brush, as illustrated in Figure 1, can be powered by any regulated supply of compressed air. The spray characteristics can be altered as desired by adjusting

[3]Cab-O-Sil.

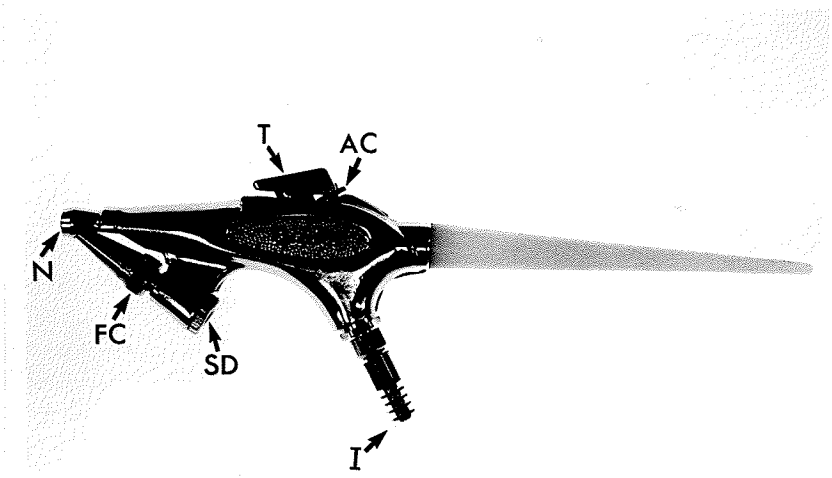

Figure 1. Photograph of an air brush showing trigger (T), air flow control (AC), fluid control (FC), spray solution duct (SD), air intake (I), and spray nozzle (N).

the air regulator, the air flow control, and/or the fluid control (Figure 1). The quantity of spray applied is adjusted by changing the rate at which the brush is passed over the target area. The manual operation of the air brush can lead to variability in the amount of spray applied. To overcome this variability the data were evaluated as percentages of the total applied dose. Approximately 0.25 ml of solution can be pipetted into the spray solution duct (Figure 1). This volume is sufficient to spray 15 to 20 plants.

The time course of diclofop methyl penetration into wild oat following application by either syringe or air brush is illustrated in Figure 2. Penetration occurred more rapidly and to a greater extent following air brush application. This rapid penetration is partially a function of the larger area contacted by the air brush spray. The leaf tissue underlying the syringe applied droplet rapidly became necrotic. Such physiological isolation of the syringe applied droplet would reduce penetration following that method of application. No necrosis was observed following the air brush application. Necrosis of wild oat plants normally does not develop until 4 to 7 days following field application of diclofop methyl (Hoechst Canada Inc., 1979).

The major advantage of the air brush technique is its similarity to a field spray application, thereby allowing a more realistic evaluation of pesticide-plant

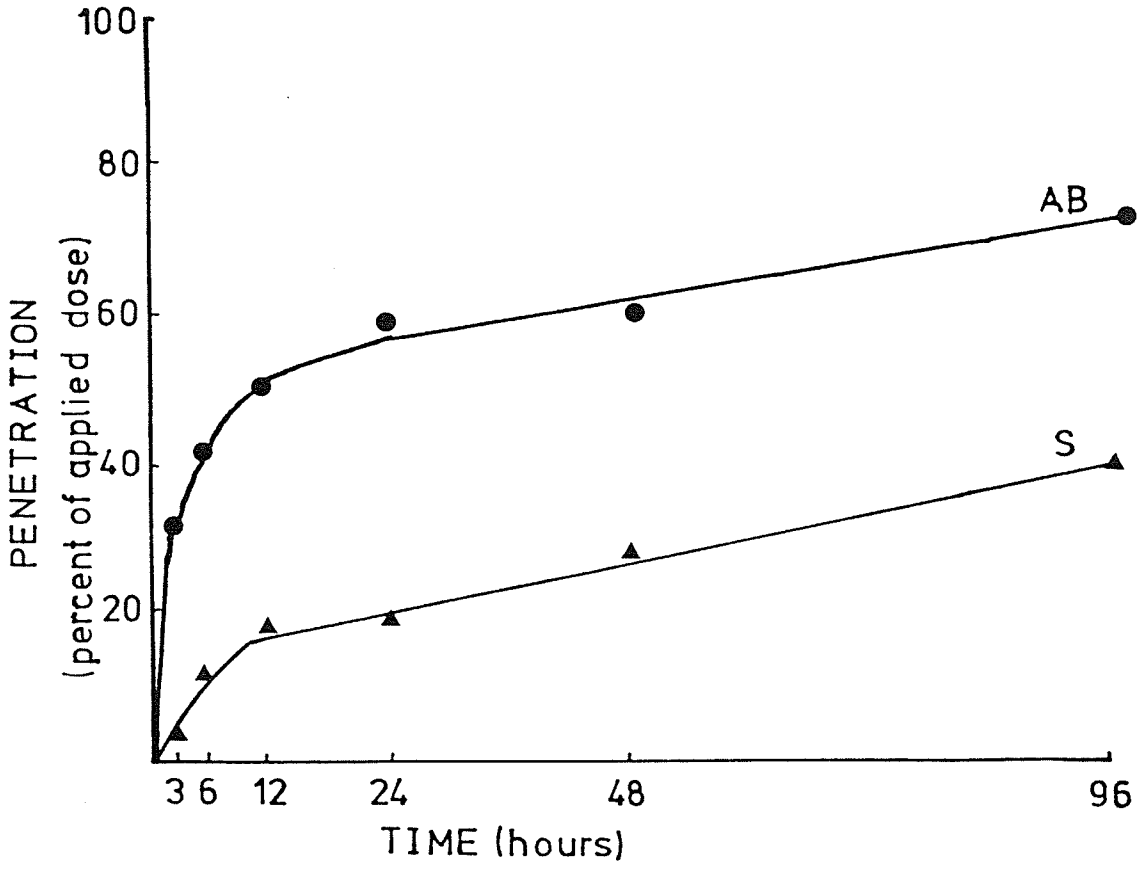

Figure 2. Time course of ^{14}C-diclofop methyl penetration into wild oat leaves following application with a syringe (S) or an air brush (AB).

interactions within the constraints of the laboratory.

The major disadvantage of the air brush technique is the wastage of a large proportion of the spray solution. For example, when the trigger is first pressed prior to moving the air brush over the target area 10 to 20 µl of solution may be lost. Approximately 20% of the available soltuion is deposited on the target area as opposed to 100% with a syringe. Because of the scarcity and cost of many radiolabelled pesticides wastages of this magnitude may be considered unacceptable.

CHAPTER 2

Selectivity of Diclofop Methyl Among Wheat, Barley, Wild Oat (*Avena fatua*) and Green Foxtail (*Setaria viridis*)

Abstract. The selectivity of {methyl-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate}, (hereinafter referred to as diclofop methyl), among wheat (*Triticum aestivum* L. 'Neepawa'), barley (*Hordeum vulgare* L. 'Bonanza'), wild oat (*Avena fatua* L.), and green foxtail [*Setaria viridis* (L.) Beauv.] was investigated. On an ED_{50} basis barley, wild oat, and green foxtail were 2, 190, and 1,092 times more sensitive, respectively, to foliar-applied diclofop methyl at the two-leaf stage than was wheat. Selectivity decreased with increasing maturity of the plant material with the ratio of selectivity between barley and wild oat decreasing from 67 at the two-leaf stage to three at the four-leaf-plus-one-tiller stage. Greater spray retention and more rapid penetration of diclofop methyl partially explained the susceptibility of green foxtail, but did not explain selectivity between wheat, wild oat, and barley. Root uptake of ^{14}C-diclofop methyl by the four species was proportional to the amount of solution absorbed during the treatment period and to the concentration of diclofop methyl in the treatment solution but did not relate to species sensitivity to this herbicide.

INTRODUCTION

Field studies have shown that diclofop methyl selectively

controls wild oats and green foxtail in wheat and barley when applied as a post-emergence treatment (Friesen *et al.*, 1977). Selective weed control also has been obtained from soil-incorporated treatments of this herbicide (Todd and Stobbe, 1974; Wu and Santelmann, 1977).

Herbicide selectivity can be evaluated by means of dosage-response experiments (Blackman, 1952). After establishing the magnitude of the selectivity its basis should be determined. This study was conducted to determine the contribution of spray retention and penetration to the selectivity of diclofop methyl.

MATERIALS AND METHODS

Dosage-response. Plants were grown in a very fine sandy loam soil in a greenhouse in which the temperature ranged from 16 to 28 C. Supplemental light of 2710 $\mu W/cm^2$ was provided by cool-white fluorescent lamps on a 16-h photoperiod. Wheat, barley, wild oat, and green foxtail plants in the two-leaf stage were sprayed with several rates of diclofop methyl (36% EC). The spray was applied at a volume of 134 l/ha and a pressure of 2.46 kg/cm^2. In wheat and barley, the dosages applied consisted of multiple applications of a spray emulsion designed to deliver a rate of 5.0 kg/ha (barley) or 15.0 kg/ha (wheat) on each individual pass of the sprayer. Sufficient drying time was allowed between passes of the sprayer to prevent

the emulsion from running down the plant leaves. Two weeks after spraying, the foliar plant parts were harvested and oven-dry weights obtained. The experiment consisted of a randomized complete block design with eight replicates. Four (wheat, barley, wild oat) or eight (green foxtail) plants in a 10-cm diameter plastic pot constituted a replicate.

The influence of leaf stage on the susceptibility of barley and wild oat to diclofop methyl was investigated by growing barley and wild oat plants to the two-leaf stage or four-leaf-plus-one-tiller stage and treating them as described above.

The selectivity of root-applied diclofop methyl was studied in a growth chamber having a light intensity of 4,500 $\mu W/cm^2$ provided by wide spectrum Grolux fluorescent lamps on a 16-h photoperiod and a temperature regime of 25 C day and 15 C night. Plants of the four species were grown in silica sand and watered with a modified half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) until they reached the two-leaf (wheat, barley, wild oat) or the four-leaf (green foxtail) stage. The plants then were transferred to bottles containing 180 ml of nutrient solution. At the three-leaf (wheat, barley, wild oat) or the four-leaf (green foxtail) stage the plants were transferred to fresh nutrient solution to which had been added 0.05 to 10.0 ppmw of technical diclofop methyl dissolved in sufficient ethanol to give a final

ethanol concentration of 0.1% (v/v). Following a 24 h exposure to the herbicide, and at 3 day intervals thereafter, the plants were transferred to fresh nutrient solution. Two weeks after initiating the treatment, the plants were harvested and root and shoot dry weights determined. A completely randomized experimental design having four replicates was employed.

Spray retention. Plants grown in the greenhouse to the two-leaf stage were sprayed with a diclofop methyl emulsion to which a water soluble dye[1] had been added. The spray emulsion, after drying on the plant surfaces, was washed off and quantified colorimetrically at 630 nm. the completely randomized experiment consisted of 16 replicates per treatment, each replicate being one 10-cm diameter pot containing four (wheat, barley, wild oat) or eight (green foxtail) plants.

Spray penetration. Diclofop methyl, uniformly labelled with ^{14}C in the dioxyphenoxy ring (sp. ac. 9.6 μCi/mg), was formulated with solvent blank and sufficient unlabelled technical diclofop methyl to produce an emulsion having a concentration equivalent to the 1.0 kg/ha spray emulsion under the spray parameters described above. The radio-labelled compound was applied to plants which had been grown first in silica sand and then in nutrient solution in the growth cabinet as previously described. Approximately

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[1]Niagra Sky Blue 6B. A product of the Allied Chemical Co.

2 µl of spray emulsion containing .005 µCi/µl was applied to the adaxial surface towards the center of the second leaf (wheat, barley, wild oat) or the fourth leaf (green foxtail) in a strip 0.3 cm. by 3.0 cm. using an air brush[2] operated at a pressure of 0.70 kg/cm^2. After various time intervals five plants of each species were harvested. The treated zone was cut from the leaf and surface activity removed by rinsing with 5 ml of 30% ethanol as a directed pipette stream. The washings, in scintillation vials, were evaporated to dryness under an air stream. Ten ml of scintillation solution containing 0.6% (w/v) PPO (2,5-diphenyl oxazole) and 0.01% (w/v) POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] in toluene:methyl cellosolve. (2:1 v/v) were added to each vial. Radioactivity was determined with a scintillation counter and all samples were corrected for quenching, by the channels ratio method, and for background radioactivity. Radioactivity within the plant was determined by grinding the various plant parts to pass through a 0.508 mm (50 mesh) screen. Subsamples then were suspended in the scintillation solution to which had been added 4% (w/v) thixotropic gelling agent[3]. Radioactivity was assayed with the scintillation counter and quench and background corrections were made. Internal

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[2]Binks model 10005 A. Binks Manufacturing Company, 9201 West Belmont Ave., Franklin Park, Illinois 60131.

[3]Cab-O-Sil.

absorption of radioactivity by these samples was determined to be approximately 52% by comparison to a set of subsamples which were combusted to $^{14}CO_2$ in a sample oxidizer. Preliminary experiments showed volatilization of this herbicide to be nonsignificant over the duration of the experiment, so total applied radioactivity was calculated as the sum of the radioactivity in the leaf washings plus the radioactivity in the tissue.

Root uptake. Individual plants of each of the four species were grown to the three-leaf (wheat, barley, wild oat) or the five-leaf (green foxtail) stage in the growth cabinet as described above. The plants were transferred to 20 ml of nutrient solution containing 0.1 or 1.0 ppmw technical diclofop methyl and 0.1 μCi ^{14}C-diclofop methyl dissolved in 0.1% ethanol (v/v). After 6 h the plants were transferred to fresh nutrient solution containing the same concentrations of unlabelled diclofop methyl for a further 18 h. The plants were then transferred into fresh nutrient solution. Aliquots of all the solutions were assayed for radioactivity by liquid scintillation methods. Total radioactivity remaining in the solutions was subtracted from the radioactivity initially present to determine uptake. Twelve plants of each species were exposed to each herbicide concentration.

RESULTS AND DISCUSSION

Dosage-response. Percent relative growth was calculated as

described by Hawton and Stobbe (1971) and plotted on a probit scale versus the log of the dosage to yield linear dosage-response curves (Figure 3) from which the ED_{50} concentrations were calculated (ED_{50}= the herbicide concentration required to reduce net dry weight increase to 50% of the control). The ED_{50} concentrations and ratios of selectivity are presented in Table 1. Based on the ED_{50} values, 1092 times as much herbicide would have to be applied to wheat as compared to green foxtail in order to achieve an equivalent growth reduction. The results illustrate the extreme sensitivity of green foxtail to this herbicide and the remarkable tolerance of the two crop species. The dosage-response curves for wheat and barley should not be interpreted as indicating that at low dosages, wheat is more susceptible to diclofop methyl than is barley. In fact the reverse is true. The overlapping of the curves at lower dosages arises from the fact that the two dosage response curves possess different slopes. This indicates a different mode of action of the herbicide in the two species (Sampford, 1952). Perhaps the true physiological action of the herbicide was masked by contact damage to the wheat leaves by the solvent.

At the four-leaf-plus-one-tiller stage ED_{50} values for barley and wild oat were determined to be 14.3 and 4.0 kg/ha, respectively. Thus the ratio of selectivity (barley:wild oat) decreased from 67 at the two-leaf stage to three at the four-leaf-plus-one-tiller stage.

Figure 3. Dosage-response of wheat (W), barley (B), wild oat (WO), and green foxtail to foliar-applied diclofop methyl.

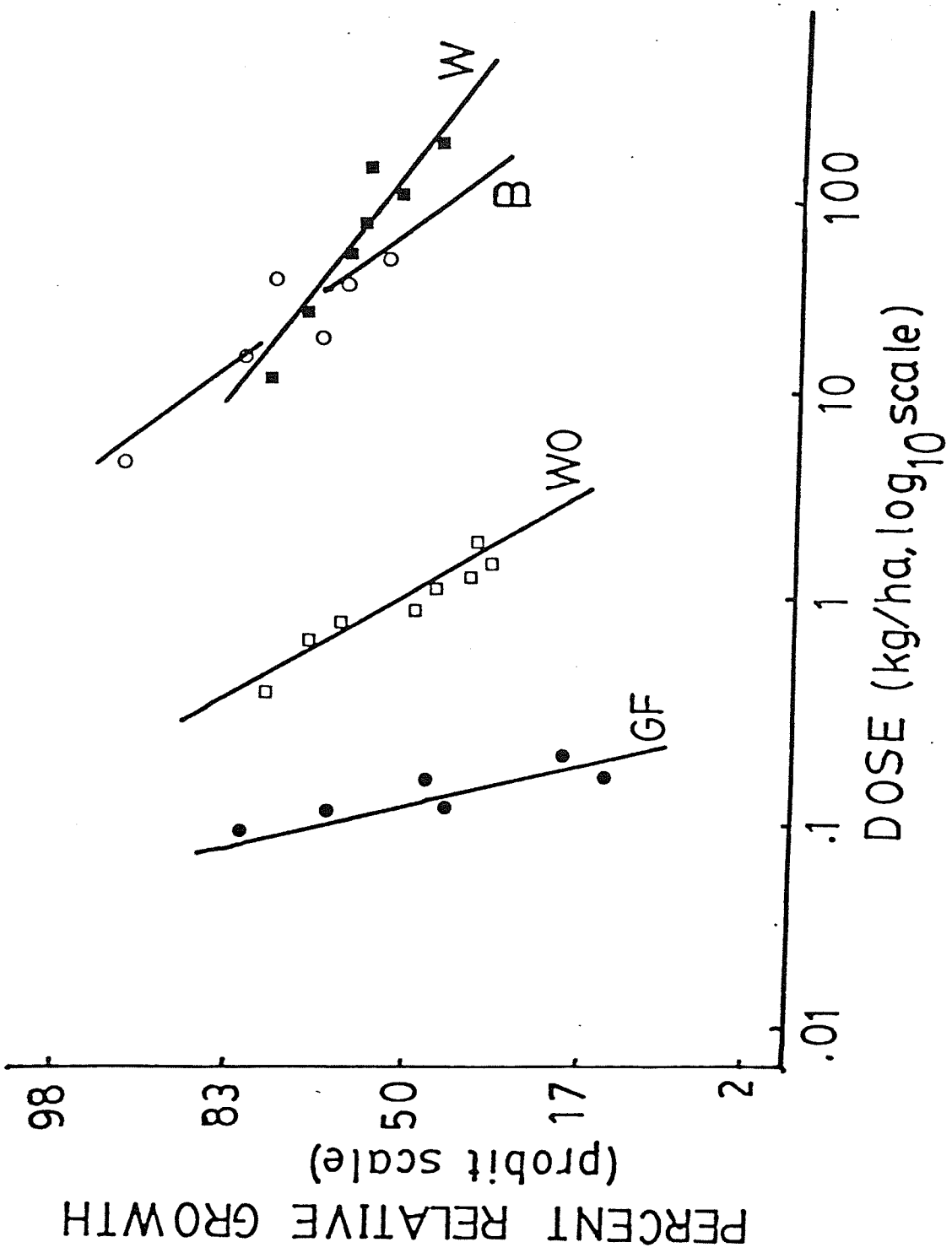

PERCENT RELATIVE GROWTH
(probit scale)
98
83
50
17
2
.01
.1
1
10
100
DOSE (kg/ha, log10 scale)
W
B
WO
GF

TABLE 1. ED_{50} levels and selectivity ratios for wheat, barley, wild oat, and green foxtail.

Species	ED_{50} (kg/ha)	Ratios of Selectivity		
Wheat	109.2	1		
Barley	67.0	2	1	
Wild Oat	1.0	109	67	1
Green Foxtail	0.1	1092	670	10

The decrease in the ratio of selectivity involved both increased susceptibility of barley and decreased susceptibility of wild oat at the later stage of application. Maximum selectivity between these two species can be achieved by spraying at the earlier leaf stage, substantiating field observations which noted damage to the barley crop following applications made at later leaf stages (Owino *et al.*, 1975c).

The concentrations of diclofop methyl required for 50% inhibition of shoot or root growth following root treatment are given in Table 2. Selectivity was maintained between species, with the exception of green foxtail, which was less sensitive to root treatment than was wild oat. In contrast, field results indicate more effective green foxtail control than wild oat control following soil-incorporated treatments (Todd and Stobbe, 1974). This discrepancy may relate to the shallow germination habit of green foxtail, resulting in growth of roots into the treated soil, while wild oat may germinate from greater depths below the treated layer of soil.

Spray retention. When expressed on a dry weight basis (Table 3) leaves of wheat and barley retained slightly more spray solution than wild oat. Green foxtail retained much larger amounts of the spray solution than the other three species, contributing to the sensitivity of green foxtail. The large volume of spray solution retained by

TABLE 2. Concentrations of diclofop methyl required for 50% inhibition of root and shoot growth following root uptake in wheat, barley, wild oat, and green foxtail.

	50% growth inhibition	
	root	shoot
Species	(ppmw)	(ppmw)
Wheat	22.50	90.0
Barley	3.50	64.0
Wild Oat	0.07	0.95
Green Foxtail	2.27	4.10

TABLE 3. Foliar spray retention by wheat, barley, wild oat and green foxtail.

Species	Volume Retained (µl/plant)	Volume Retained (µl/g dry weight of foliage)
Wheat	1.45	53.99
Barley	1.47	47.21
Wild Oat	.91	34.37
Green Foxtail	.51	272.08
LSD (0.05)	.28	14.44

green foxtail relates to the horizontal arrangement of its first two leaves as opposed to the upright growth habit of seedlings of the other three species. In green foxtail, the target area represents a large percentage of the total leaf area. In addition, there is less rebounding of spray droplets from a more horizontal leaf surface (Blackman *et al.*, 1958).

Penetration. The time course of ^{14}C-diclofop methyl penetration into leaves of each of the four species is illustrated in Figure 4. Uptake was rapid during the first 6 to 12 h and then continued at a slower rate for the remainder of the study. In contrast to reports (Blackman, 1952) of insignificant penetration subsequent to drying of the spray deposit, penetration of diclofop methyl continued long after drying of the spray deposit had taken place. More rapid penetration of diclofop methyl into green foxtail contributed to the susceptibility of this weed. Penetration of the herbicide into wild oat was less than into wheat and equal to that into barley. Penetration does not account for the selective toxic action of this compound among wild oat, wheat, and barley.

Root uptake. Percentage uptake of ^{14}C-diclofop methyl from nutrient solution by each of the four species is given in Table 4. Because all treatments contained 0.1 µCi, the identical uptake figures noted for the two concentrations

Figure 4. Penetration of ^{14}C-diclofop methyl into leaves of wheat (W), barley (B), wild oat (WO), and green foxtail (GF).

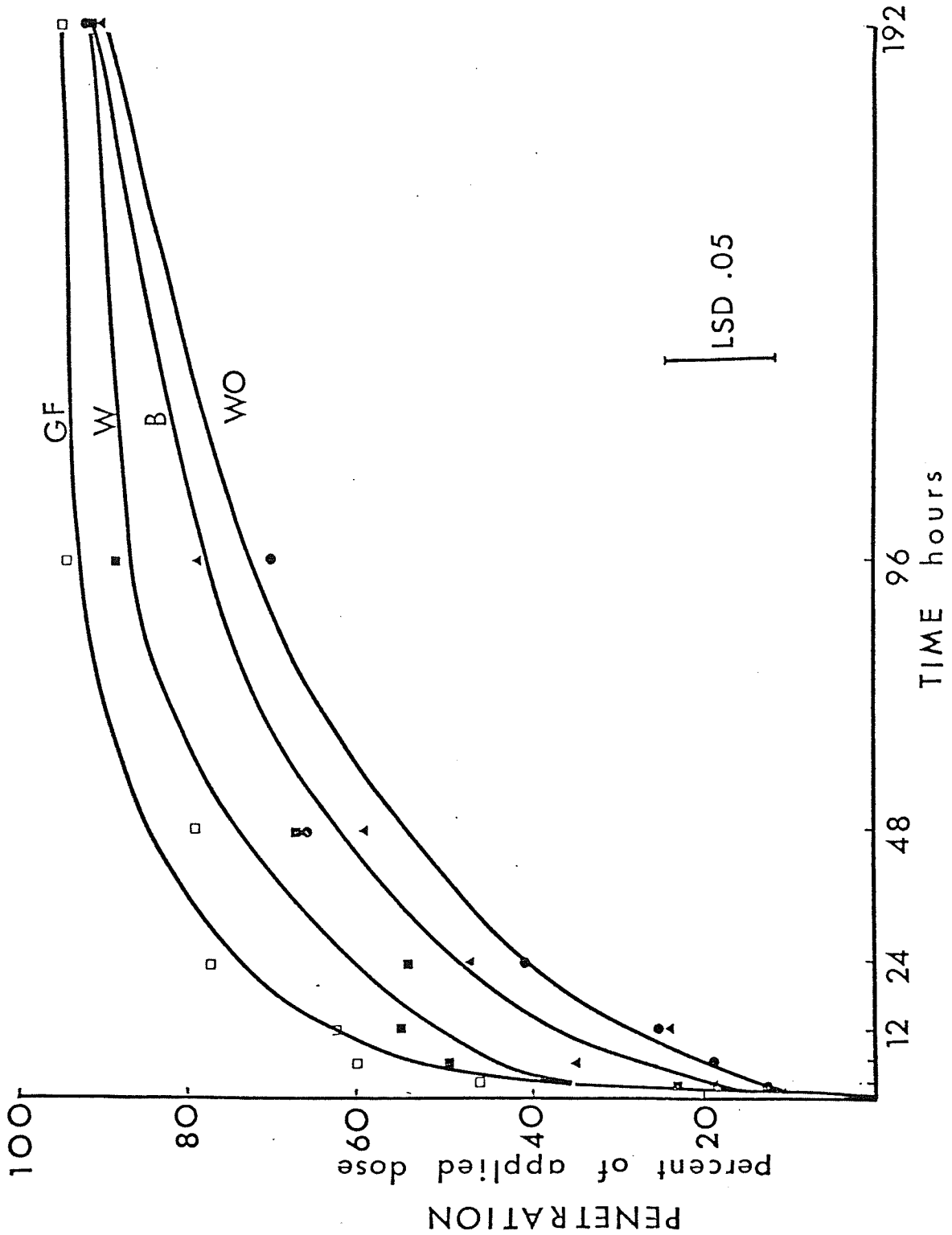

PENETRATION
percent of applied dose
100
80
60
40
20
12
24
48
96
192
TIME hours
GF
W
B
WO
LSD .05

TABLE 4. Uptake of ^{14}C-diclofop methyl by roots of wheat, barley, wild oat, and green foxtail.

Species	Solution absorbed (ml)	Percent of radioactivity absorbed 0.1 ppmw	1.0 ppmw
Wheat	2.0	42.99 ± 3.00	42.15 ± 4.23
Barley	1.0	27.82 ± 14.72	28.96 ± 5.13
Wild Oat	1.0	30.43 ± 3.62	34.32 ± 3.77
Green Foxtail	0.5	13.81 ± 5.98	11.68 ± 6.73

indicate that ten times as much diclofop methyl was absorbed from the 1 ppm solution as was absorbed from the 0.1 ppm solution. Uptake was proportional to herbicide concentration.

Approximately 2.0, 1.0, 1.0, and 0.5 mls of the radioactive treatment solution were absorbed through the roots of wheat, barley, wild oat, and green foxtail, respectively. Uptake of ^{14}C-diclofop methyl was proportional to the volume of treatment solution absorbed. While passive uptake of this herbicide by plant roots is suggested, the possibility of active uptake cannot be ignored. As the treatment solution was not aerated, the rate of uptake of herbicide may have been limited by the rate of diffusion of the herbicide to the root surface. Uptake would then be concentration dependent, and would increase with increasing water usage by the plants, as observed in these experiments. In either case it is clear that selective exclusion of diclofop methyl by roots of tolerant species does not occur. Differential uptake is not the basis for the selective toxic action of root-applied diclofop methyl.

CHAPTER 3

Translocation and Metabolism of Diclofop Methyl in Wheat, Barley, Wild Oat (*Avena fatua*), and Green Foxtail (*Setaria viridis*)

Abstract. Translocation and metabolism of diclofop methyl {methyl-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate was investigated in tolerant species, wheat (*Triticum aestivum* L. 'Neepawa'), and barley (*Hordeum vulgare* L. 'Bonanza'), and susceptible species, wild oat (*Avena fatua* L.) and green foxtail [*Setaria viridis* (L.) Beauv.] . Movement of radioactivity from treated leaves following ^{14}C-diclofop methyl application amounted to less than 4% of the radioactivity within the plants after 72 h. Differences between species in the movement of the radioactive label did not account for the selective toxic action of diclofop methyl. Wheat and barley were able to metabolize diclofop methyl and its free acid, diclofop, rapidly enough to prevent accumulation of either compound in the plant. In wild oat and green foxtail, however, more than 50% of the radioactivity extracted from the roots and from the shoot apex 72 h following application of ^{14}C-diclofop methyl to leaves was chromatographically identical to diclofop. Degradation of diclofop in wheat and barley occurred via dissimilar pathways. In wheat, hydroxylation of the chlorinated phenyl ring represented the primary detoxification reaction while in barley, degradation of the propionic acid moiety was observed. The susceptibility of grassy

weeds to diclofop methyl is related to their relative abilities to metabolize the applied herbicide.

INTRODUCTION

The herbicide diclofop methyl is used on the Canadian prairies as a post-emergence spray for the selective control of wild oats, green foxtail and other annual grassy weeds in cereal and oilseed crops.

Symptoms typical of diclofop methyl activity include chlorosis and inhibition of growth, both of which are observed within 3 to 4 days following application. With time the chlorosis deepens and the affected tissues become necrotic. Due to inhibition of meristematic activity no new growth is initiated and complete collapse of the plant occurs, usually within two weeks of application (Hoechst Canada Inc., 1979).

Microscopy of wild oat leaves treated with diclofop methyl has revealed extensive membrane disruption and structural damage in affected cells (Brezeneau *et al.*, 1976). Such membrane damage has been attributed to the action of the applied ester, diclofop methyl (Chapter 6). The observed inhibition of growth, however, has been attributed to the free acid, diclofop, which interferes with cellular division and elongation processes (Owino, 1977; Chapter 6).

The influence of spray retention and penetration on the selectivity of diclofop methyl among wheat, barley,

wild oat, and green foxtail has been investigated (Todd and Stobbe, 1977). Greater spray retention and penetration partially explained the sensitivity of green foxtail to diclofop methyl. These factors did not explain the selectivity of diclofop methyl among wheat, barley and wild oat. The purpose of this investigation was to evaluate the contributions of translocation and metabolism to the selectivity of diclofop methyl among wheat, barley, wild oat, and green foxtail.

MATERIALS AND METHODS

Wheat, barley, wild oat, and green foxtail plants were grown in silica sand and watered as required with a modified, half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Wheat, barley, and wild oat plants in the two-leaf, and green foxtail plants in the four-leaf, stage were transferred to bottles containing the half-strength nutrient solution. The plants were returned to the growth chamber for a 4 to 5 day equilibration period. A 25 C day and 15 C night were maintained in the growth room. A light intensity of 2550 $\mu W/cm^2$ was supplied during a 16-h photoperiod by cool-white fluorescent lamps.

Wheat, barley, and wild oat plants in the three-leaf, and green foxtail plants in the five-leaf, stage were selected for treatment. A 36% EC was prepared utilizing diclofop methyl uniformly labeled with ^{14}C in the dioxyphenyl ring (9.6 $\mu Ci/mg$), technical diclofop

methyl, and formulation blank. This EC then was diluted with water to yield an emulsion containing 0.75% (w/v) active ingredient. A total of 10 µl of radioactive emulsion containing 0.25 µCi was applied as 10 to 15 discrete droplets over a 3 cm length of the adaxial surface of the second (wheat, barley, wild oat) or fourth (green foxtail) leaf.

Three plants of each species were harvested 4, 8, 24, and 72 h following treatment. At harvest the plants were dissected into the following parts: treated zone of the treated leaf; treated leaf above the treated zone; treated leaf below the treated zone; leaves older than the treated leaf; leaves younger than the treated leaf; shoot apex; and roots. Leaf surface radioactivity was recovered from the treated zone of the treated leaf by washing with 5 ml of 30% (v/v) ethanol as a directed pipette stream. All of the plant parts were then frozen.

The leaf surface washings, in scintillation vials, were evaporated to dryness under an air stream and then were resuspended in 1 ml of 95% (v/v) ethanol. One µl of this solution was subsequently analyzed for metabolites of diclofop methyl. Ten ml of scintillation solution consisting of 0.6% (w/v) PPO (2,5-diphenyloxazole) and 0.01% (w/v) POPOP [1,4-bis-2-(5-phenyloxazolyl) benzene] in toluene:methyl cellosolve (2:1 v/v) were added to each vial. Radioactivity was quantified by liquid scintillation

spectrometry.

The internal radioactivity was extracted from the various plant parts in 1 ml 80% (v/v) aqueous acetonitrile using a glass tissue grinder. The apparatus was rinsed with four 1 ml aliquots of the aqueous acetonitrile and the washings were combined. The extract was centrifuged and the supernatant was decanted. Resuspension of the pellet in aqueous acetonitrile followed by centrifugation was repeated a further three times with all supernatants being combined. The volume of supernatant was adjusted to 8.0 ml and the radioactivity in a 1.0 ml aliquot was quantified by liquid scintillation methods. The remaining supernatant was evaporated to dryness under an air stream. The samples then were resuspended in 0.1 ml of 80% acetonitrile:95% ethanol (1:1 v/v).

Diclofop methyl metabolities in the tissue extracts and the leaf washings were separated by thin-layer chromatography on plastic backed silica gel TLC plates (0.25 mm thickness). The plates were developed to a height of 10 cm. in chloroform:acetic acid:hexane (80:15:5 v/v/v). Sections of the TLC plates corresponding to reference standards of diclofop methyl and its metabolites as observed under UV light were cut out and placed in scintillation vials. One ml of 95% ethanol followed by 10 mls of scintillation solution were added to each vial. Radioactivity was determined by liquid scintillation methods. Radioactivity on portions of the TLC plates not corresponding

to the reference standards was also determined. Due to limited movement of the herbicide in the plant the three replicates of each plant part other than the treated leaf zone were combined for metabolite identification.

RESULTS AND DISCUSSION

Analysis of the leaf surface washings recovered from each of the four test species revealed no significant degradation of the applied diclofop methyl on the leaf surface during the 72 h following application.

Only a small proportion of the applied radioactivity was translocated to other parts of the plants. Translocation of the radioactive label was greater in wheat than in barley and the susceptible species, wild oat and green foxtail (Table 5). Of the applied radioactivity, 1.73 and 0.74 percent had been translocated out of the treated zone of wheat and barley leaves, respectively, over a 72 h period, as compared to movement of 0.88 and 0.77 percent from the treated zone of wild oat and green foxtail leaves respectively. Brezeneau *et al.* (1977) have also reported limited translocation of radioactivity in wheat and wild oat treated with ^{14}C-diclofop methyl.

Both acropetal and basipetal movement of the radioactive label were observed in all four species (Figure 5). The radioactivity translocated out of the treated leaf accumulated in regions of high metabolic activity including the roots, new shoot growth, and the stem apical region.

TABLE 5. Translocation of radioactivity in wheat, barley, wild oat, and green foxtail following leaf application of ^{14}C-diclofop methyl.

Time (h)	% of applied radioactivity translocated from treatment zone				
	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
4	.08	.03	.02	.03	.04
8	.23	.09	.06	.14	.05
24	1.33	.39	.42	.28	.13
72	1.73	.74	.88	.77	.63

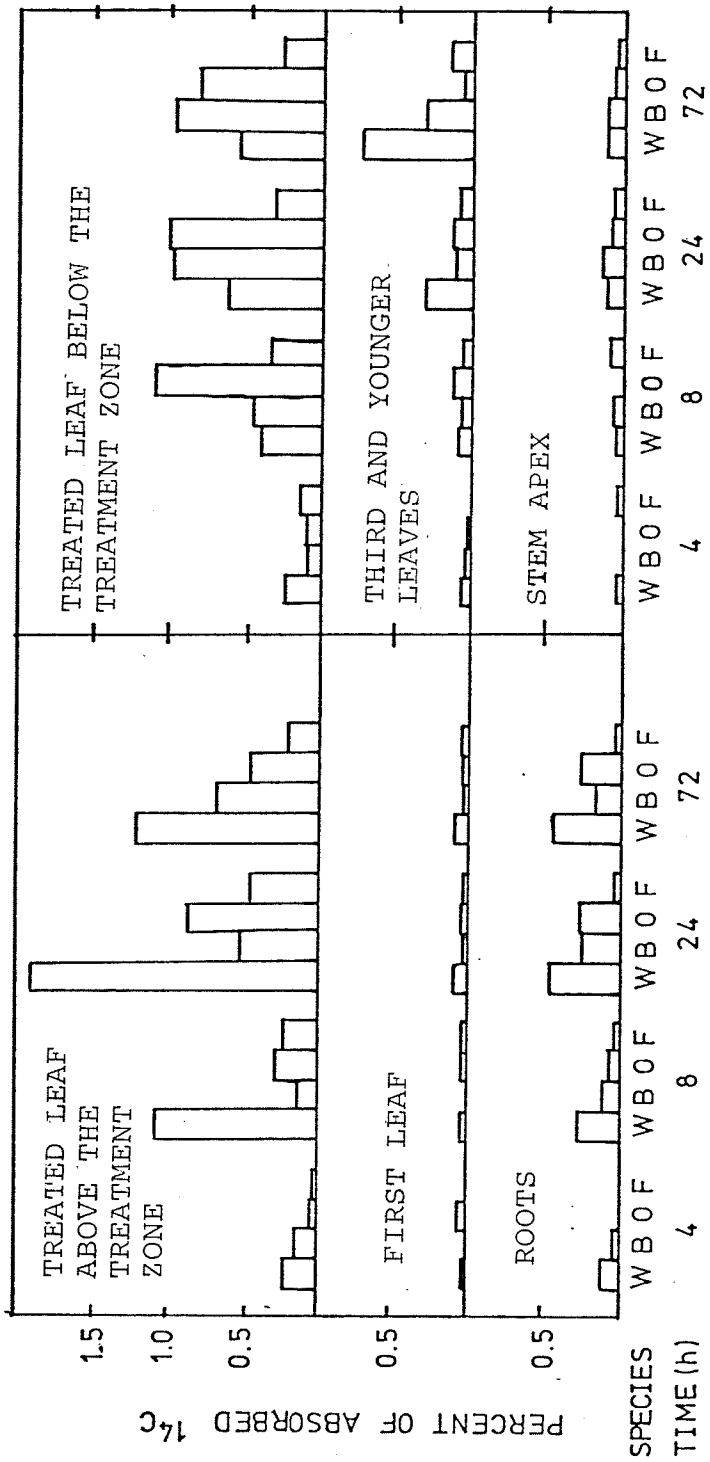


Figure 5. Distribution of radioactivity in wheat (W), barley (B), wild oat (O), and green foxtail (F) plants following leaf-spot application of ^{14}C-diclofop methyl.

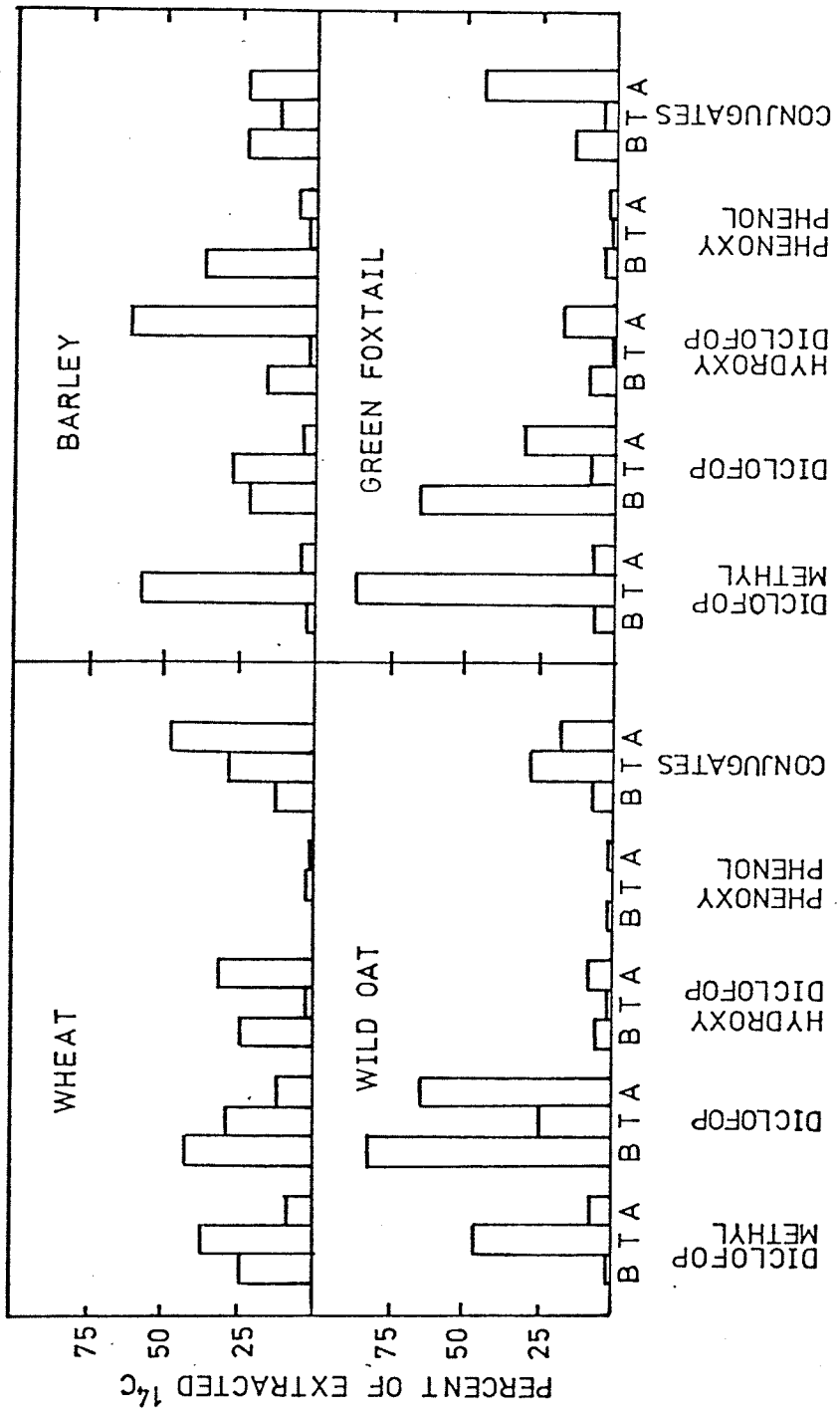
Very little radioactivity moved into the older leaves of the treated plants. Differences in the distribution of the absorbed radioactivity could not account for the selective toxic action of diclofop methyl among the four test species.

A comparison of diclofop methyl metabolites extracted from the treated zone, the treated leaf below the treated zone, and the treated leaf above the treated zone (Figure 6) suggests the relative apoplastic and symplastic mobilities of diclofop methyl and its metabolites within plants.

Diclofop methyl was so rapidly hydolyzed prior to transport that its movement within plants was negligible. More than half of the diclofop methyl vacuum infiltrated into leaf segments of wheat, barley, wild oat, and green foxtail was hydrolyzed within the first four h following treatment (Chapter 4). The large proportion of radioactivity retained as diclofop methyl in the treated zones of intact plants of these species probably represents herbicide which was absorbed into the cuticle but which did not partition into the aqueous environment of the underlying leaf tissue. In green foxtail, rapid cellular penetration of diclofop methyl resulted in disruption of the underlying leaf tissue, destroying its capacity for de-esterifying diclofop methyl. Consequently, 85% of the radioactivity extracted from the treated zone of green foxtail leaves 24 h following ^{14}C-diclofop methyl application was present as unaltered diclofop methyl.

The desmethyl acid, diclofop, exhibited both symplastic

Figure 6. Diclofop methyl metabolites present in the treated zone of the treated leaf (T), below the treated zone of the treated leaf (B), and above the treated zone of the treated leaf (A) 24 hours following spot application of ^{14}C-diclofop methyl to leaves of wheat, barley, wild oat, and green foxtail.


WHEAT
BARLEY
WILD OAT
GREEN FOXTAIL
PERCENT OF EXTRACTED ^{14}C
75
50
25
B T A
DICLOFOP METHYL
DICLOFOP
HYDROXY DICLOFOP
PHENOXY PHENOL
CONJUGATES

and apoplastic mobility. Basipetal movement of diclofop in the symplast exceeded acropetal movement in all four species. In wild oat and green foxtail diclofop transport resulted in the accumulation of diclofop in the leaf tissues above and below the treated zone. In wheat and barley accumulation of diclofop above and below the treated zone was not observed (Figure 6).

Wheat and barley leaves rapidly degraded diclofop. Seventy-two h following ^{14}C-diclofop methyl application diclofop accounted for 7%, 2%, 61% and 35% of the radioactivity extracted from the treated leaf below the treated zone in wheat, barley, wild oat, and green foxtail respectively (Table 6).

Degradation of diclofop by wheat and barley yielded a compound chromatographically identical to hydroxy-diclofop {2-[4-dichloro-3,5,6-hydroxyphenoxy) phenoxy] propionic acid}. The degradation of diclofop by barley yielded significant quantities of phenoxy-phenol [4-(2,4-dichlorophenoxy) phenol] , a metabolite not abundant in wheat, wild oat, or green foxtail. Both of these metabolic alterations would be expected to reduce the phytotoxicity of the parent molecule (Nestler *et al.*, 1978) and as such can be assumed to represent detoxification mechanisms.

Hydroxylation of diclofop by wheat has been reported (Gorbach *et al.*, 1977). Degradation of the propionic acid moiety of diclofop has not been observed in plants but does occur as a result of microbiological degradation

TABLE 6. Metabolites of ^{14}C-diclofop methyl extracted from the region of the treated leaf below the treatment zone.

Species	Time (h)	Percent of extracted radioactivty present as: diclofop methyl	diclofop	hydroxy[1] diclofop	phenoxy[2] phenol	conjugates
Wheat	24	23.53[3]	41.18	23.53	0.00	11.76
	72	3.64	6.89	24.96	14.72	49.78
Barley	24	1.62	21.06	17.63	37.71	21.98
	72	1.42	1.62	22.70	26.59	47.67
Wild Oat	24	1.21	80.81	7.61	1.74	8.62
	72	1.17	61.36	4.54	1.66	31.27
Green Foxtail	24	7.74	65.23	9.79	3.72	13.52
	72	20.51[3]	35.14	8.59	2.38	33.39

[1] 2-[4-(2,4-dichloro-3,5,6-hydroxy phenoxy) phenoxy] propionic acid.

[2] 4-(2,4-dichlorophenoxy) phenol.

[3] May represent leaf surface contamination.

in soils (Smith, 1977; Martens, 1978). Smith has postulated that degradation of the propionic acid moiety in soil proceeds via a decarboxylation mechanism yielding first 4-(2,4-dichlorophenoxy) phenetole which then undergoes direct conversion to the corresponding phenol. Studies of the degradation of phenoxy-alkanoic herbicides revealed that substitution of a methyl group for a hydrogen at the number 2 carbon atom of the acetic acid side chain, thereby creating an α-substituted propionic acid, blocked biological cleavage of the ether linkage (Alexander and Aleem, 1961). Removal of the methyl group via a decarboxylation reaction may be a prerequisite to cleavage of the phenoxy-propionic ether linkage of diclofop in plants.

Phenoxy-phenol was freely mobile within the symplast of barley plants. The proportion of ^{14}C present as phenoxy-phenol was much lower in the treated zone than in either the acropetal or basipetal portions of the treated leaf (Figure 6). Hydroxy-diclofop was also readily translocated. Both basipetal and acropetal transport of hydroxy diclofop were observed in all four species, but acropetal transport was favoured (Figure 6).

As both of the detoxification products were more readily translocated within plants than was diclofop, movement of radioactive label following ^{14}C-diclofop methyl application to plants would be expected to be greatest in tolerant species and least in susceptible species. This inverse relationship between ^{14}C translocation and susceptibility to diclofop methyl has been noted

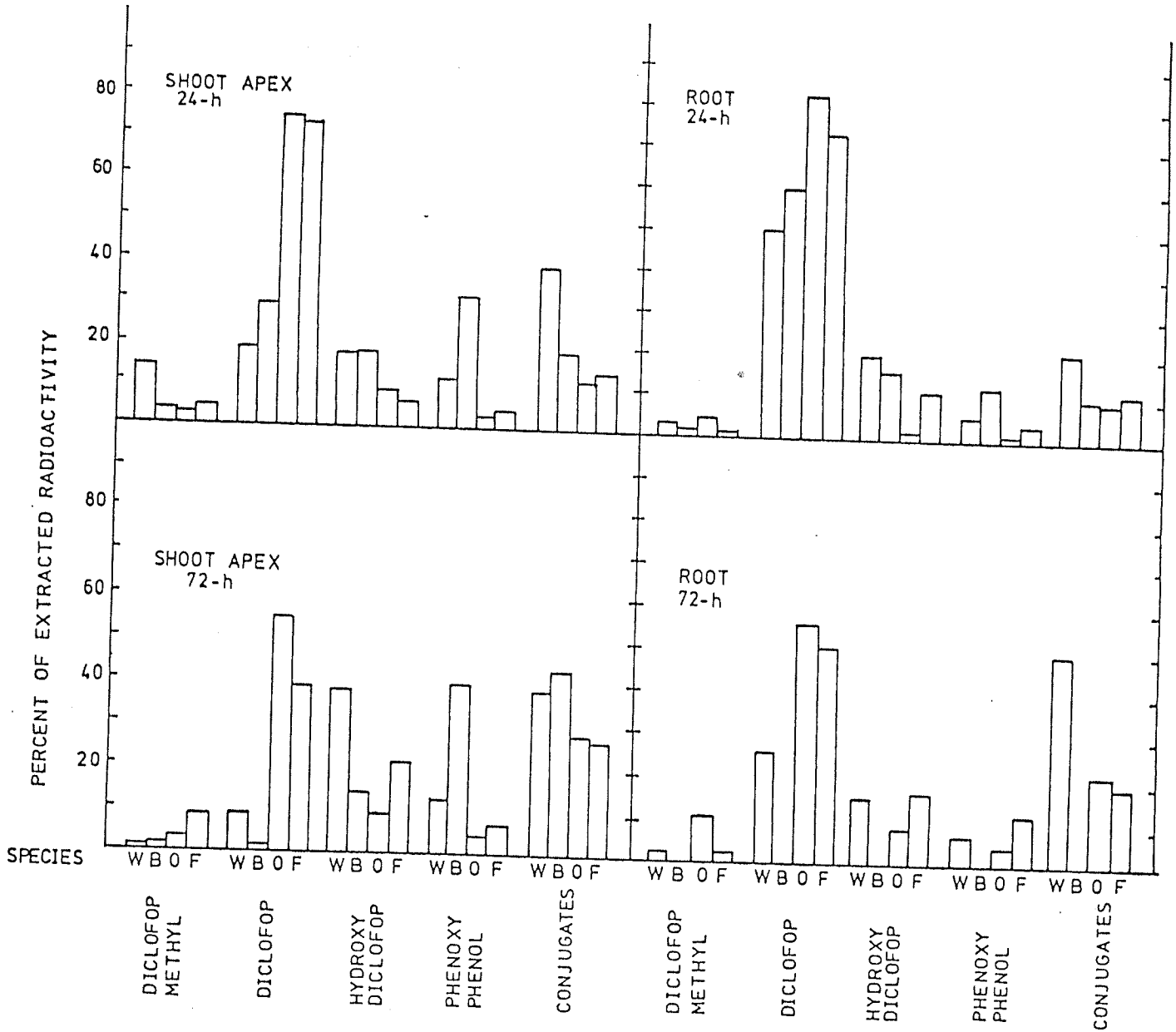
(Table 5).

Diclofop and hydroxy-diclofop may undergo conjugation to sugars and other plant constituents (Gorbach *et al.*,1977; Shimabukuro *et al.*, 1977). Conjugation of diclofop methyl metabolites to plant constituents was observed in all four species studied (Table 6). In wheat and barley hydroxy-diclofop, phenoxy-phenol, and diclofop may all have undergone conjugation reactions. Since little hydroxy-diclofop or phenoxy-phenol was formed in wild oat and green foxtail, diclofop conjugates would be expected to predominate. It is not known whether diclofop conjugates are subject to hydrolysis within plants. If so, the diclofop conjugates in wild oat and green foxtail may represent a pool of temporarily inactivated toxicant within the plant.

The toxic action of diclofop involves disruption of cell division and elongation processes in meristematic zones of roots and shoots (Owino, 1977). Analysis of extracts from roots and shoot apices of diclofop methyl treated plants revealed marked differences in the proportion fo the extracted radioactivity associated with diclofop in tolerant and susceptible species (Figure 7).

Diclofop accounted for 82% and 73% of the radioactivity extracted from wild oat and green foxtail roots, respectively 24 h after the ^{14}C-diclofop methyl application. In excess of 70% of the radioactivity extracted from wild oat and green foxtail shoot apices was associated with diclofop

Figure 7. Diclofop methyl metabolites extracted from the roots and the shoot apices 24 or 72 hours following spot application of ^{14}C-diclofop methyl to leaves of wheat (W), barley (B), wild oat (O), and green foxtail (F) [72 hour data for barley roots missing] .


SHOOT APEX
24-h
ROOT
24-h
SHOOT APEX
72-h
ROOT
72-h
PERCENT OF EXTRACTED RADIOACTIVITY
80
60
40
20
SPECIES
W B O F
DICLOFOP METHYL
DICLOFOP
HYDROXY DICLOFOP
PHENOXY PHENOL
CONJUGATES

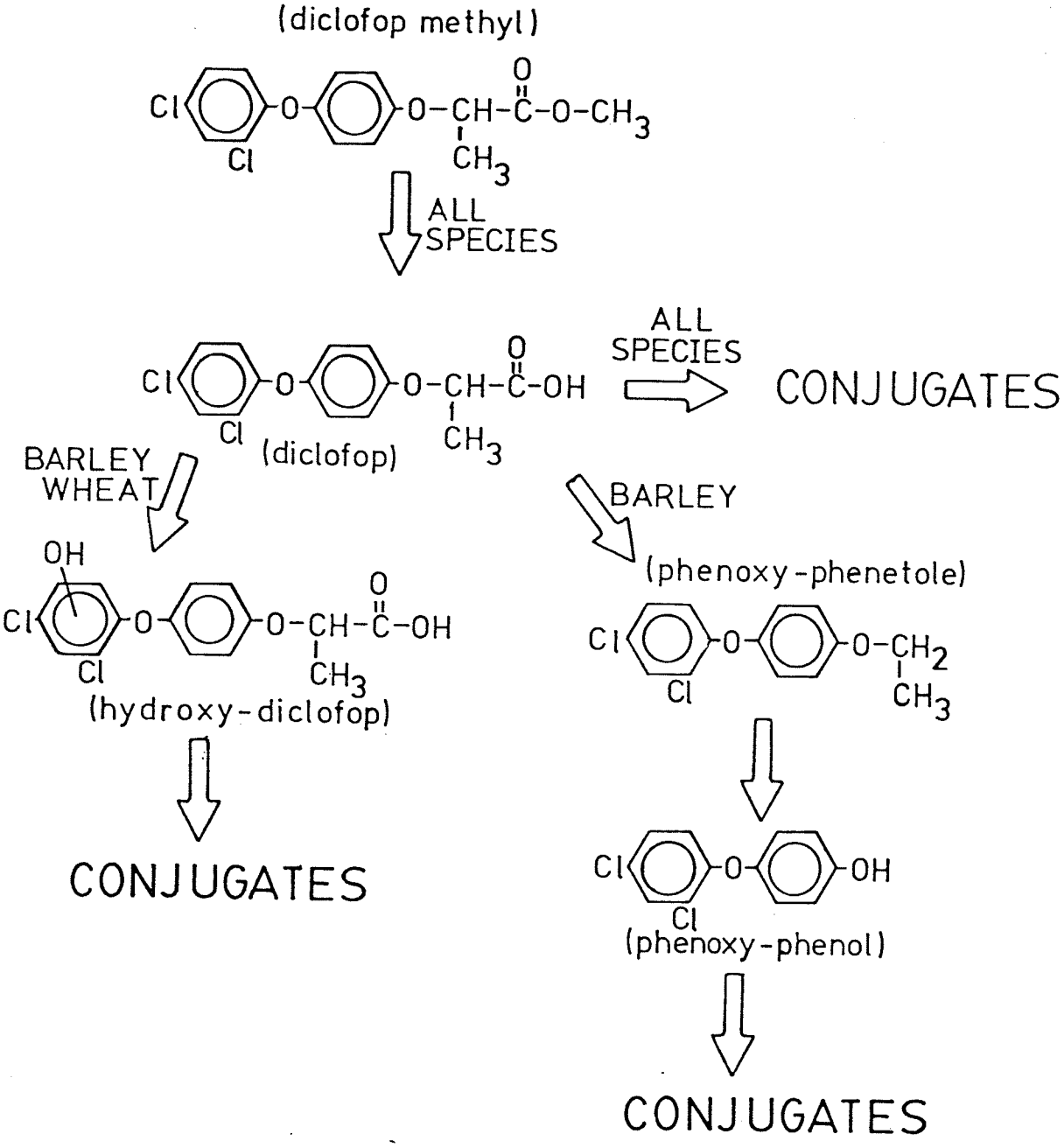


Figure 8. Schematic representation of diclofop methyl metabolism in wheat, barley, wild oat, and green foxtail.

24 h following treatment. By 72 h post-treatment, diclofop still accounted for 40% to 50% of the radioactivity extracted from wild oat and green foxtail roots and shoot apices. In contrast, radioactive extracts from wheat and barley shoot apices contained only 9% and 2% diclofop respectively 72 h following the diclofop methyl application. Although the proportion of diclofop in the wheat root extract was higher than in the shoot apex, it was still only half of that observed in the roots of the two susceptible species.

Wheat, barley, wild oats and green foxtail degrade diclofop via different metabolic pathways and at different rates (Figure 8). The ability of a given species to degrade diclofop to nonphytotoxic derivatives is a major factor determining species tolerance to diclofop methyl.

CHAPTER 4

Metabolism of Diclofop Methyl Vacuum Infiltrated into Leaf Segments of Wheat, Barley, Wild Oat (*Avena fatua*), and Green Foxtail (*Setaria viridis*)

Abstract. Diclofop methyl {methyl-2-(4-(2,4-dichlorophenoxy) phenoxy) propanoate} vacuum infiltrated into leaf segments of wheat (*Triticum aestivum* L. 'Neepawa'), barley (*Hordeum vulgare* L. 'Bonanza'), wild oat (*Avena fatua* L.), and green foxtail {*Setaria viridis* (L.) Beauv.} was rapidly hydrolyzed to the free acid, diclofop. Differences in the rate of diclofop methyl hydrolysis did not explain species sensitivity to the herbicide. Metabolism of the free acid exhibited a half-life of 6.1, 7.3, 15.0, and 9.3 hours in wheat, barley, wild oat, and green foxtail, respectively. Degradation of diclofop involved hydroxylation of the dichlorophenoxy ring, yielding a nonphytotoxic derivative. Barley also degraded diclofop *via* a second pathway in which the propionic acid side chain was removed from the molecule. The sensitivity of the grassy species studied was correlated to their relative abilities to degrade diclofop.

INTRODUCTION

The herbicide diclofop methyl selectively kills wild oats and green foxtail in wheat and barley (Friesen *et al.*, 1976; Todd and Stobbe, 1977). Previous reports have dealt with spray retention, penetration, translocation, and metabolism of this herbicide by wheat, barley, wild oat, and green foxtail (Chapters 2 and 3). Selectivity of diclofop methyl was related to the relative abilities of each of the species to metabolize both the applied ester and its

free acid, diclofop. Accumulation of diclofop in susceptible species results in an interruption of cell division processes, preceding death of the entire plant (Chapter 3; Owino, 1977).

It has been impossible to compare rates of diclofop methyl metabolism among species directly because of the confounding effects arising as a result of differential penetration and translocation. In order to overcome this problem, the study reported here was conducted utilizing leaf segments into which the radiolabelled herbicide was vacuum infiltrated (Chang *et al.*, 1971). With this procedure similar amounts of herbicide could be introduced into leaf tissue from each of the four species, allowing a direct comparison of the relative abilities of the various species to metabolize diclofop methyl.

MATERIALS AND METHODS

Wheat, barley, wild oat, and green foxtail plants were grown in a growth room in silica sand and watered as required with a half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). A light intensity of 2550 $\mu W/cm^2$ was maintained during a 16-h photoperiod using cool-white fluorescent lamps. Temperature was maintained at 25 C day and 15 C night.

Leaf segments 2 cm long were cut from the second leaves of wheat, barley, and wild oat plants in the three-leaf stage and from the fourth leaf of green foxtail

plants in the five leaf stage.

The ^{14}C-diclofop methyl (uniformly labelled in the dioxyphenyl ring) was dissolved in ethanol and added to 0.35 M mannitol to yield a 25 µM diclofop methyl solution (final ethanol concentration 0.1% v/v). The herbicide solution was vacuum infiltrated into the freshly cut leaf segments for 20 min (Chang *et al.*, 1971). The resultant concentration of herbicide within the tissue segments was approximately equal to the concentration of herbicide which would be found within a wild oat plant 24 h after application of a 1.0 kg/ha dosage (Chapters 2 and 3).

The leaf segments were removed from the treatment solution, rinsed for 1 min with running water, and placed on moist filter paper in petri dishes. Incubation of the leaf segments was carried out at 25 C in a growth chamber under continuous light (cool-white fluorescent plus incandescent lamps providing an intensity of 3800 µW/cm^2). Four samples of each species, each sample consisting of 20 leaf segments, were harvested 1, 2, 4, 8, 12, and 24 h after treatment and immediately frozen.

Samples were extracted with 80% aqueous acetonitrile as previously described (Chapter 3). Metabolite separation was achieved using thin-layer chromatography on silica gel (0.25 mm thickness) plastic backed plates developed to a height of 10 cm in chloroform:acetic acid:hexane (80:15:5 v/v/v). Localization of radioactive metabolites was achieved by cutting the plates horizontally into 0.5 cm strips. One ml

of 95% ethanol was added to each strip in individual liquid scintillation vials. Ten mls of scintillation liquid (Chapter 2) were added to each vial and the radioactivity was quantified by liquid scintillation methods. Once the Rf of each metabolite had been determined by the above procedure, subsequent sample TLC plates were cut into zones corresponding to each metabolite and the radioactivity in each zone was quantified.

Hydrophilic compounds (those exhibiting Rf values less than 0.1) were extracted from the TLC plate using 95% ethanol. The extract was spotted on silica gel TLC plates which had previously been developed to their full height in n-butanol:acetic acid: water (25:4:10 v/v/v). The plates then were developed to a height of 15 cm in the same solvent. The plates were cut horizontally into 0.5 cm strips and one ml of 95% ethanol was added to each strip in individual scintillation vials. Ten mls of scintillation liquid were added to each vial and radioactivity was quantified.

To determine whether significant radioactive residues were remaining associated with the cellular debris following the extraction procedure, the pellet obtained following centrifugation of the disrupted wild oat leaf tissue was degraded by a wet combustion technique (Claus and Behrens, 1976). The dried pellet was ground to a fine powder and digested in a closed container containing 20 ml of concentrated sulfuric acid and 10 ml 0.05 M $Na_2Cr_2O_7$-$2H_2O$.

The CO_2 evolved was recovered in one ml of ethanolamine: methyl cellosolve (1:1 v/v) and radioactivity was determined using liquid scintillation spectrometry.

An experiment to monitor $^{14}CO_2$ evolution from wild oat leaf segments infiltrated with the radioactive herbicide was conducted. The leaf segments were placed in respirometer flasks containing 0.3 ml of ethanolamine in the center well. After 4 or 24 h incubation, the ethanolamine was removed and the center well was washed with two 0.5 ml aliquots of methyl cellosolve. The ethanolamine and methyl cellosolve washes were combined for quantification of radioactivity by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Separation of radioactive metabolites by the TLC solvent system revealed five distinct peaks (Figure 9). The compounds designated I, II, and III in Figure 9 were chromatographically identical to diclofop methyl, diclofop, and phenoxy-phenol {4-(2,4-dichlorophenoxy) phenol}, respectively. The Rf value of compound IV corresponds to that of a hydroxylated derivative of diclofop identified by Gorbach *et al.* (1977) as 2-{4-(2,4-dichloro-3,5,6-hydroxy-phenoxy) phenoxy} propionic acid (hereinafter referred to as hydroxy-diclofop). The peak designated V represents polar compounds reported to be conjugates of diclofop methyl metabolites (Shimabukuro, 1977; Gorbach *et al.*, 1977). No degradation of the vacuum infiltrated herbicide to the

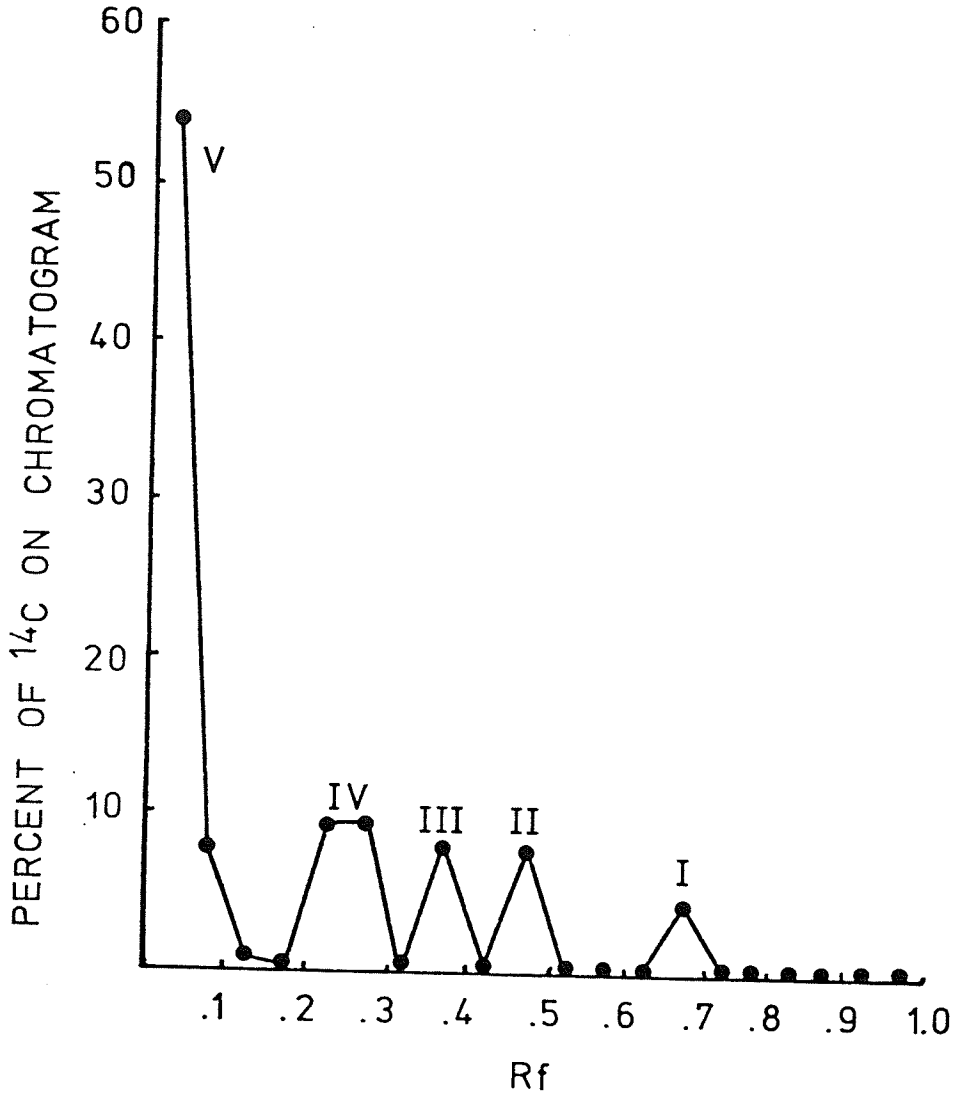


Figure 9. Thin layer chromatographic separation of diclofop methyl (I) and its metabolites extracted from wheat leaf segments 12 hours after vacuum infiltration of ^{14}C-diclofop methyl [solvent system chloroform:acetic acid:hexane (80:15:5 v/v/v)] .

level of free CO_2 was observed during the 24 h study and combustion of the cellular debris remaining following the aqueous acetonitrile extraction revealed that an extraction efficiency of approximately 99% had been achieved.

Hydrolysis of diclofop methyl to diclofop proceeded rapidly in all four species studied. A log-log plot of percent ester remaining *versus* time yielded linear plots, the slopes of which represent the relative abilities of the four species to degrade diclofop methyl. Degradation proceeded most rapidly in wild oat (slope = -1.02) and least rapidly in green foxtail (slope = -0.51) (Table 7).

The log-log relationship between diclofop methyl degradation and time may be the result of the combined activities of several relatively nonspecific esterase enzymes. Alternatively, the esterase enzyme involved may be allosterically regulated by its substrate with the result that at higher diclofop methyl concentrations de-esterification would proceed more rapidly than at lower diclofop methyl concentrations.

The contact damage observed following diclofop methyl application to susceptible species has been attributed to the action of the applied methyl ester (Chapter 6). The rate of degradation of diclofop methyl by susceptible green foxtail was only slightly slower than by tolerant wheat (Table 7). The extensive contact damage observed following diclofop methyl application to green foxtail does not result from an inability of this species to de-esterify the

TABLE 7. Degradation of ^{14}C-diclofop metyl vacuum-infiltrated into leaf segments of wheat, barley, wild oat, and green foxtail.

Species	equation describing diclofop methyl degradation	r^2
Wheat	log (y)[1] = 1.92-0.68 log (t)	.98
Barley	log (y) = 1.99-0.87 log (t)	.98
Wild Oat	log (y) = 1.72-1.02 log (t)	.98
Green Foxtail	log (y) = 1.87-0.51 log (t)	.91

[1] y = percent diclofop methyl remaining at time t (hours) where t is greater than or equal to 1.

applied diclofop methyl. Rather, greater diclofop methyl spray retention by, and penetration into, leaves of green foxtail results in such a rapid influx of diclofop methyl into the leaf tissue that membrane integrity is destroyed before the tissue is able to de-esterify the toxicant.

When the logarithm of the percent diclofop present in wild oat leaf segments was plotted *versus* time, a diphasic curve resulted (Figure 10). As diclofop methyl de-esterification proceeded more rapidly than did metabolism of the free acid formed, the level of free acid increased rapidly in the first phase. After four h very little ester remained and the descending phase, representing diclofop degradation, was linear. Similar diphasic curves representing the interaction between diclofop formation and degradation were observed for the other three species studied. As maximal levels of diclofop were attained approximately four h after treatment, linear regression analysis was applied to the means obtained from the 4, 8, 12, and 24 h samplings. From the equations derived, the half-life of diclofop in each of the four species was calculated (Table 8). The y-intercept calculated from the equations indicated time zero levels of ^{14}C-diclofop represented less than 100% of the extractable ^{14}C. This discrepancy between actual and theoretical y-intercept values results from the incomplete conversion of diclofop methyl to diclofop with time.

With the exception of green foxtail, the rate at which

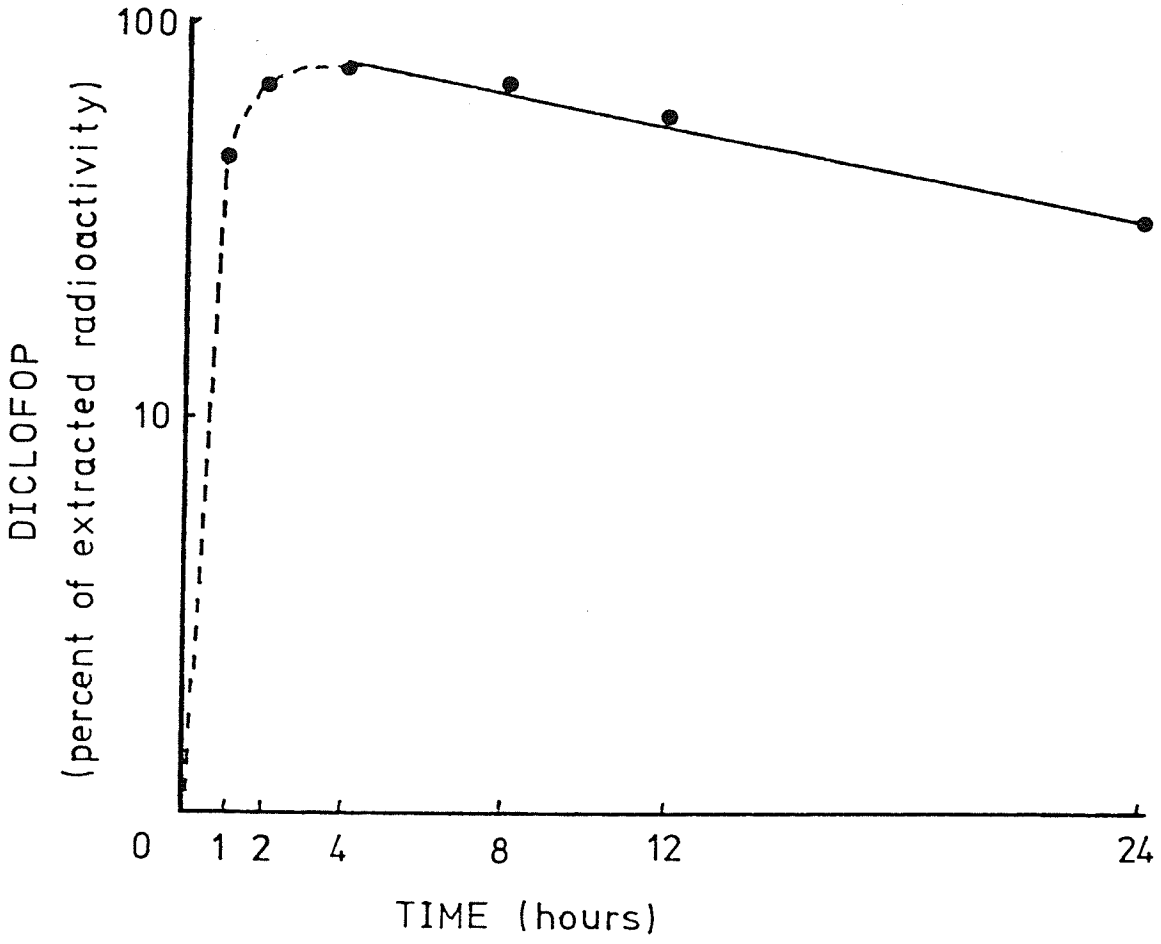


Figure 10. Time course of diclofop formation and degradation in wild oat leaf segments vacuum infiltrated with ^{14}C-diclofop methyl.

TABLE 8. The half-life of diclofop in wheat, barley, wild oat, and green foxtail.

Species	Equation describing diclofop degradation	r^2	$t_{1/2}$ (h)
Wheat	$\log (y)^1 = 1.98 - 0.046t$	.99	6.1
Barley	$\log (y) = 1.88 - 0.041t$	.98	7.3
Wild Oat	$\log (y) = 2.00 - 0.020t$	.99	15.0
Green foxtail	$\log (y) = 1.82 - 0.032t$	.97	9.3

[1] y=percent diclofop present at time t.

TABLE 9. Hydroxy-diclofop {2-[4-(2,4-dichloro-3,5,6-hydroxyphenoxy) phenoxy] propionic acid} formation in wheat, barley, wild oat, and green foxtail leaf segments.

Time	Hydroxy-diclofop (% of extracted radioactivity)				
(h)	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
1	0.6	0.4	0.2	0.5	0.3
2	1.0	1.6	0.3	0.7	0.5
4	2.7	4.3	0.6	2.3	1.4
8	7.3	10.7	1.2	4.6	2.8
12	11.2	12.9	0.6	4.6	1.7
24	11.0	14.9	4.4	4.5	2.9

a given species was able to metabolize diclofop was closely correlated to that species' tolerance to diclofop methyl. In the case of green foxtail, it has been previously shown that greater (10 fold) spray retention and penetration contribute to the susceptibility of this species to diclofop methyl (Chapter 2). The greater rate of degradation of diclofop by green foxtail as compared to wild oat is more than offset by the greater spray retention by, and more rapid penetration into, green foxtail leaves.

The difference between the diclofop half-life of 6.1 h in wheat and 15.0 h in wild oat may be critical with respect to the duration of exposure of the site of diclofop action to the toxicant. Thirty h following treatment the level of diclofop would be 800% higher in wild oat than in wheat.

Degradation of diclofop was accompanied by an increase in the proportion of radioactivity associated with hydroxy-diclofop (Table 9). Ring-hydroxylation of diclofop represents a detoxification mechanism (Chapter 3). Levels of hydroxy-diclofop were greater in tolerant wheat and barley than in susceptible wild oat.

Twelve h following treatment, 23% of the radioactivity extracted from barley leaf segments was present as phenoxy-phenol in contrast to levels of 3%, 2%, and 1% in wheat, wild oat, and green foxtail, respectively. These results confirm our previous finding (Chapter 3) that degradation of the propionic acid side chain represents an important degradative reaction in the detoxification of diclofop by

TABLE 10. Conjugation of diclofop methyl metabolites to cell constituents in wheat, barley, wild oat, and green foxtail leaf segments.

Time (h)	% of extracted ^{14}C associated with conjugates				
	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
1	0.7	0.5	1.0	0.5	0.2
2	1.2	1.8	2.7	1.6	0.5
4	6.2	7.6	6.6	8.1	3.0
8	21.3	20.6	19.5	32.4	10.7
12	48.6	32.8	28.5	40.2	8.3
24	74.8	49.7	59.2	63.6	6.9

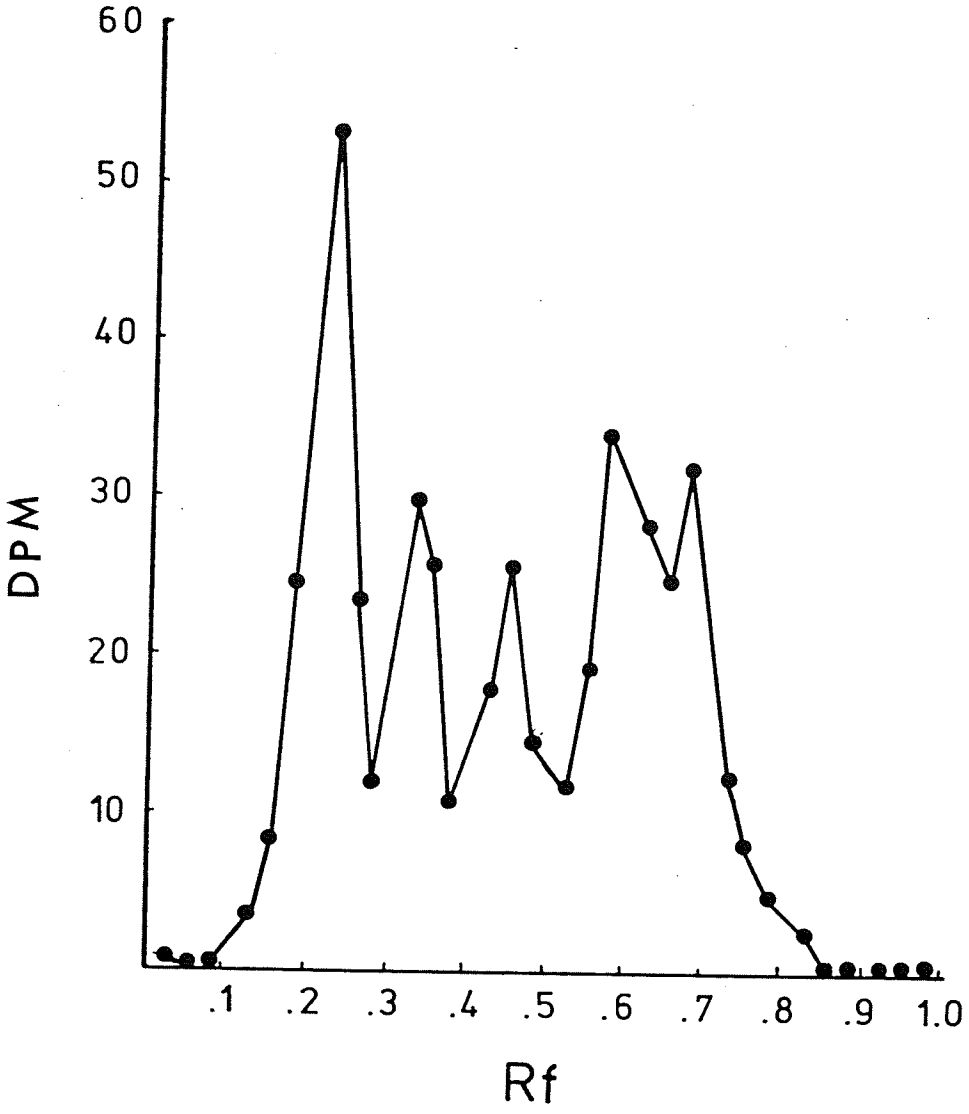


Figure 11. Thin layer chromatographic separation of diclofop conjugates extracted from wild oat leaf segments.

barley. Degradation of the propionic acid moiety by barley may be an indication that the hydroxylating enzyme present in barley either is not very abundant or else has a low affinity for diclofop. As such, the differential degradative pathways observed in wheat and barley may relate to the differential tolerance of wheat and barley to this herbicide.

Conjugation of diclofop methyl metabolites to plant constituents proceeded rapidly with time in all four species (Table 10). The conjugates present in the wild oat and wheat samples were incubated in 1 N HCl for 1 h. Wild oat samples released only diclofop. The hydrolysate obtained from wheat samples contained diclofop and hydroxy-diclofop in a ratio of approximately 1:9. Shimabukuro (1977) has reported that wheat has the ability to metabolize diclofop to hydroxy-diclofop and the lesser ability of wild oat to do so. Detoxification of diclofop *via* ring-hydroxylation is important with respect to species sensitivity to diclofop methyl.

TLC of conjugates isolated from wild oat leaf segments in n-butanol:acetic acid:water separated five radioactive peaks (Figure 11). Since acid hydrolysis of conjugates from wild oat yielded diclofop as the only major ^{14}C-metabolite, it appears that diclofop was conjugated to several different plant constituents. Further experiments are required to identify the constituents involved. It was not determined whether diclofop conjugation served as a detoxification mechanism or whether the conjugates represented a pool of temorarily inactivated toxicant.

CHAPTER 5

The Fate of Root-Applied Diclofop Methyl in Wheat, Barley, Wild Oat (*Avena fatua*) and Green Foxtail (*Setaria viridis*)

Abstract. The fate of root-fed ^{14}C-diclofop methyl {methyl-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate} was studied in wheat (*Triticum aestivum* L. 'Neepawa'), barley (*Hordeum vulgare* L. 'Bonanza'), wild oat (*Avena fatua* L.), and green foxtail (*Setaria viridis* (L.) Beauv.). Translocation of radioactivity from the root to the shoot was approximately twice as great in wheat, barley, and wild oat as it was in green foxtail. The tolerance of wheat to root-applications of diclofop methyl results from the ability of both roots and shoots of wheat to rapidly degrade the toxicant. Barley roots exhibited a lesser ability to metabolize the free acid, diclofop, than did barley shoots which may explain the relative sensitivity of barley roots to this herbicide. Wild oat shoots slowly metabolized the diclofop translocated to the shoot from the roots. More than 90% of the radioactivity in wild oat shoots was associated with diclofop or diclofop methyl following a 6 h exposure to the labelled herbicide. The inability of both wild oat roots and wild oat shoots to degrade diclofop explained the sensitivity of wild oat to hydroponic treatment with diclofop methyl. While green foxtail shoots were relatively ineffective in degrading diclofop, the ratio of diclofop to degradation products translocated to the shoot was much lower in green foxtail

than in the other species. Differential tolerance of not only species but also of individual organs was related to their ability to degrade diclofop.

INTRODUCTION

Diclofop methyl provides selective post-emergence control of annual grassy weeds in cereal, oilseed and other crops (Friesen *et al.*, 1976; Anderson, 1976; Todd and Stobbe, 1977). Soil-applied diclofop methyl also provides selective grassy weed control with pre-plant incorporated treatments providing more effective weed control than pre-emergence applications (Todd and Stobbe, 1974, Wu and Santelmann, 1976; Chow, 1978).

Mulder and Nalewaja (1979) reported that the soil moisture level at the time of application determined the degree of wild oat control achieved with soil-incorporated diclofop methyl. They found that wild oat control increased linearly as the soil moisture in a Tiffany sandy loam was increased from 75% to 125% of field capacity.

Green foxtail is reported to be more sensitive to soil-applied diclofop methyl than is wild oat (Todd and Stobbe, 1974; Wu and Santelmann, 1976). When grown in hydroponic culture wild oat was more sensitive to root-applied diclofop methyl than was green foxtail (Todd and Stobbe, 1977). Wheat has shown excellent tolerance to soil-applied diclofop methyl (Todd and Stobbe, 1974; Wu and Santelmann, 1976). Barley exhibited good tolerance to

diclofop methyl concentrations below 1.5 ppmw in the soil (Wu and Santelmann, 1976). Owino (1977) reported that diclofop methyl applied as a pre-planting soil-incorporated treatment at 1.5 kg/ha caused injury to barley and did not provide adequate control of either wild oats or green foxtail.

Differential effects of root-applied diclofop methyl on coronal root growth and on cell division in root tips of wheat, barley and wild oat have been reported by Owino (1977). Most severe and lasting effects on cell division and root growth were observed in wild oat. Effects on barley roots were only slightly less severe than those observed on wild oat. Wheat roots were initially affected by the treatment but rapidly recovered.

Absorption of root-applied ^{14}C-diclofop methyl by wheat, barley, wild oat, and green foxtail was proportional to water use by each species and was not correlated with species sensitivity to the herbicide (Todd and Stobbe, 1977).

The study reported here was undertaken to determine if translocation or metabolism of root-applied diclofop methyl contribute to the selectivity of this herbicide among four grassy species.

MATERIALS AND METHODS

Wheat, barley, wild oat, and green foxtail plants were grown in silica sand in a growth room having a temperature

regime of 25 C day and 15 C night. A light intensity of 2550 $\mu W/cm^2$ was provided during a 16-h photoperiod by cool-white fluorescent lamps. The plants were watered as required with a half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Wheat, barley and wild oat plants in the two-leaf stage and green foxtail plants in the four-leaf stage were transferred to bottles containing 180 ml of nutrient solution. Wheat, barley, and wild oat plants in the three-leaf stage and green foxtail plants in the five-leaf stage were transferred from the bottles to test-tubes containing 20 ml of the treatment solution. The treatment solution consisted of nutrient solution to which had been added 1.0 ppmw technical grade diclofop methyl (including 0.25 μCi uniformly ring-labelled ^{14}C-diclofop methyl; 9.6 $\mu Ci/mg$) dissolved in 0.1% ethanol (v/v). After 6 h the plants were transferred to fresh nutrient solution containing the same concentration of nonlabelled diclofop methyl. After an additional 18 h the plants were transferred into fresh nutrient solution.

Three plants of each species were harvested 6, 24, and 72 h following initial exposure to the radioactive herbicide. Roots were washed for 1 min under running water and blotted dry. The shoots were then severed from the roots and both plant parts were immediately frozen.

Radioactivity in the roots and in the shoots was extracted using 80% aqueous acetonitrile (Chapter 3).

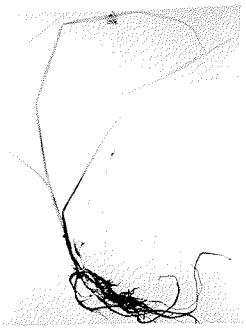
Radioactivity in the extracts was quantified using liquid scintillation spectrometry. Separation of diclofop methyl metabolites was achieved using thin-layer chromatography (TLC). After spotting, the plastic-backed silica gel TLC plates were developed to a height of 10 cm in chloroform:acetic acid:hexane 80:15:5 (v/v/v). Zones corresponding to diclofop methyl and its metabolites were cut from the TLC plates and radioactivity was quantified.

Two plants of each species were harvested 96 h following initiation of the treatment. These plants were frozen and pressed and then the whole plants were autoradiographed using Kodak no-screen X-Ray film. The plants were maintained frozen during the three-week exposure period to overcome the possibility of spurious results due to uneven drying.

RESULTS AND DISCUSSION

Autoradiographs (Figure 12) developed from wheat, barley, wild oat, and green foxtail plants following root-treatment with ^{14}C-diclofop methyl revealed movement of the radioactive label throughout the foliage of all four species. The bulk of the radioactivity, however, remained in the root system. Some accumulation of the radioactivity in the root tips and in the crown region of the plants was observed. Accumulation of the herbicide and/or its metabolites in these regions is noteworthy in view of the reported effects of diclofop methyl on cell division

Figure 12. Autoradiographs (left) developed from wheat (W), barley (B), wild oat (WO), and green foxtail (GF) plants (right) following root-exposure to ^{14}C-diclofop methyl.



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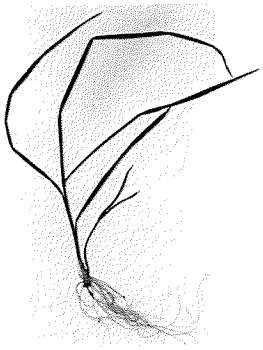

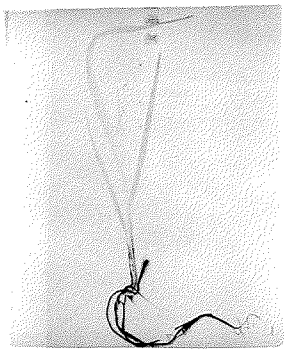

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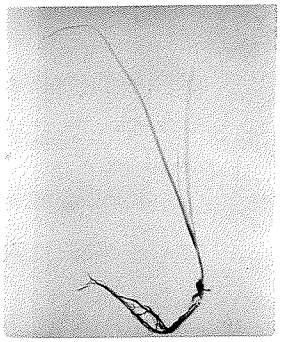
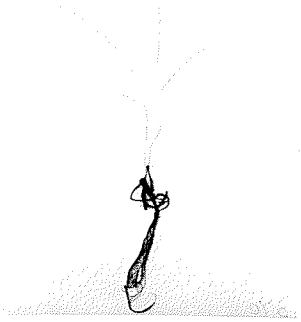


WO

GF



(Owino, 1977).

The tolerant species wheat and barley absorbed as much or more radioactivity from the treatment solution as did the susceptible species wild oat and green foxtail, confirming our earlier finding that selective exclusion of diclofop methyl by roots of tolerant species does not occur (Todd and Stobbe, 1977).

During the first 24 h following initiation of the treatment all four species translocated approximately 10% of the absorbed radioactivity from the roots to the shoots (Table 11). During the following 48 h the percentage of the absorbed radioactivity present in the shoots rose to approximately 20% in wheat, barley, and wild oat, but did not increase in green foxtail. This result may be an indication of an early herbicidal effect in green foxtail. Field studies have indicated that green foxtail was more sensitive to soil-incorporated diclofop methyl than were wheat, barley, or wild oat (Todd and Stobbe, 1974).

In the roots of those species sampled immediately following the 6-h treatment with radioactive diclofop methyl solution, approximately 15% of the extracted radioactivity was present as diclofop methyl (Table 12). Levels of diclofop methyl in the shoots were as low as, or lower than, levels of diclofop methyl in the roots. The level of diclofop methyl in the roots was further reduced to about 4% of the extractable radioactivity 72 h after initiation of the treatment, indicating that

TABLE 11. Translocation of radioactive label to shoots of wheat, barley, wild oat, and green foxtail following root-application of ^{14}C-diclofop methyl

Time (h)	Percentage of absorbed radioactivity translocated to shoot				
	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
6	2.7	4.3	4.6	2.0	3.1
24	8.3	4.6	9.6	6.5	3.2
72	18.3	20.8	16.9	9.6	5.8

TABLE 12. Diclofop methyl present in roots and shoots of wheat, barley, wild oat, and green foxtail at the termination of a 6 h root exposure to ^{14}C-diclofop methyl solution.

Species	Diclofop methyl (% of extracted radioactivity)	
	Root	Shoot
wheat	13.9 ± 3.6	7.8 ± 4.9
barley	13.9 ± 0.7	17.1 ± 2.2
wild oat	16.2 ± 0.3	8.6 ± 1.5
green foxtail	15.8 ± 2.2	9.8 ± 1.6

roots of all four species were able to deesterify diclofop methyl. Absorption of diclofop directly from the treatment solution may also have occurred. Hydrolysis of diclofop methyl to diclofop proceeds rapidly in soils (Smith, 1977; Martens, 1978) and in aqueous systems[1].

Diclofop methyl is not likely to be the toxicant responsible for inhibiting root growth as its presence was so transient in the roots of all four species. Shimabukuro *et al.*, (1978) have reported that roots of wild oat are more sensitive to diclofop than to its methyl ester.

Six h after initiating the ^{14}C-diclofop methyl treatment 68%, 76%, 77%, and 70% of the extracted radioactivity was associated with diclofop in wheat, barley, wild oat, and green foxtail, respectively. If diclofop is the toxicant responsible for the interruption of root meristematic activity, then a decline in root tip cell divisions would be expected in all four species. Owino (1977) reported that exposure of wheat, barley, and wild oat plants to nutrient solution containing 0.5 ppmw diclofop methyl resulted in a reduction in cell division and elongation of coronal roots of all three species. Wheat roots recovered from the effects of the diclofop methyl treatment but roots of barley and wild oat did not. Apparently both wheat and barley are physiologically sensitive to

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[1]Hoechst AG, personal communication.

diclofop if it reaches its site of action in sufficient quantity. Wheat roots were able to metabolize diclofop more rapidly than were roots of the other two species (Figure 13). Metabolism of diclofop by wheat roots reduced the amount of toxicant at the site of action, allowing cell division and elongation processes to recover.

Wheat roots degraded diclofop rapidly, decreasing the level of toxicant (ester plus acid) by 40% during the 18 h period following termination of the radioactive treatment (Figure 13). Degradation of diclofop yielded two compounds. The first of these co-chromatographed with 4-(2,4-dichloro-phenoxy) phenol (hereinafter referred to as phenoxy-phenol). The second compound had an Rf value corresponding to hydroxy-diclofop {2-(4-(2,4-dichloro-3,5,6-hydroxyphenoxy) phenoxy) propionic acid} (Gorbach *et al.*, 1977). Wheat roots subsequently conjugated these compounds, and diclofop, to plant constituents yielding polar compounds which remained at the origin following development of the TLC plates. The radiolabelled metabolites of diclofop could be released from these conjugates by acid hydrolysis. In wheat roots, phenoxy-phenol was more abundant than was hydroxy-diclofop (Table 13). This contrasts with the situation in wheat shoots (Table 13, and Chapters 3 and 4), where formation of hydroxy-diclofop was favoured.

Wheat shoots also degraded diclofop rapidly. Thirty percent of the toxicant present in wheat shoots at the end of the treatment was degraded during the following

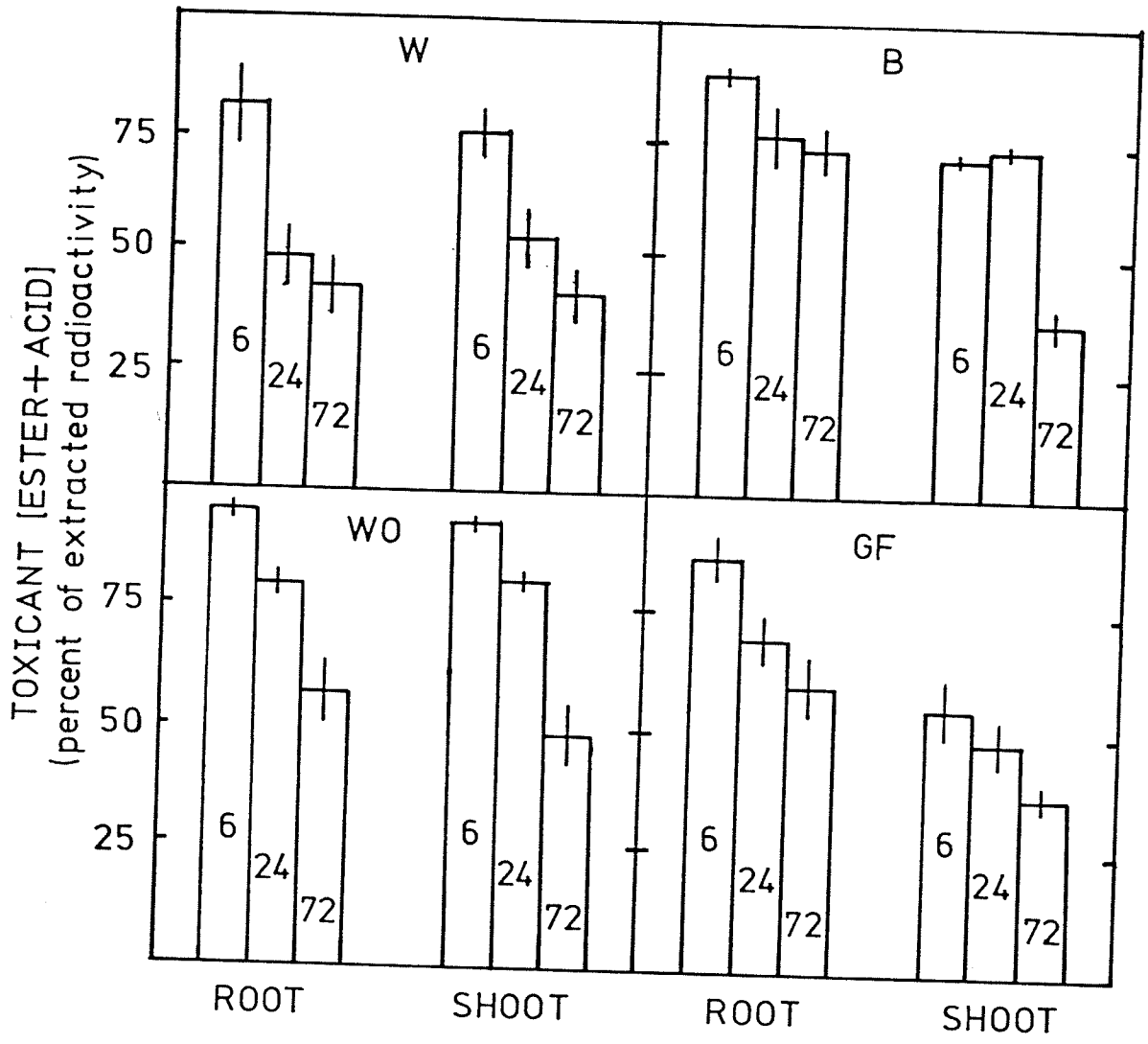

Figure 13. Degradation of toxicant (diclofop methyl plus diclofop) by roots and shoots of wheat (W), barley (B), wild oat (WO), and green foxtail (GF) 6, 24, and 72 hours following initial exposure of roots to ^{14}C diclofop methyl (vertical line associated with each bar represents the standard error of the mean).

TABLE 13. Relative abundance of hydroxy-diclofop {2-[4-(2,4-dichloro-3,5,6-hydroxyphenoxy) phenoxy] propionic acid} and phenoxy-phenol [4-(2,4-dichlorophenoxy) phenol] extracted from wheat roots and shoots following root-uptake of ^{14}C-diclofop methyl

	(h)	Hydroxy Diclofop	Phenoxy Phenol	Ratio (HD/PP)
		(% of extracted radioactivity)		
Root	6	3.8 ± 1.0	4.9 ± 1.6	0.78
	24	10.6 ± 1.4	16.0 ± 3.7	0.65
	72	10.2 ± 3.5	18.9 ± 1.7	0.54
Shoot	6	8.0 ± 1.4	5.7 ± 0.4	1.40
	24	22.7 ± 4.7	11.5 ± 2.1	1.98
	72	15.6 ± 3.0	12.8 ± 1.5	1.22

18 h (Figure 13) and by 72 h following treatment initiation diclofop accounted for less than 40% of the extractable radioactivity. The ability of both wheat roots and wheat shoots to degrade diclofop contributes to the tolerance of this species to diclofop methyl.

Barley roots degraded diclofop very slowly (Figure 13). Seventy-two h following treatment initiation, diclofop accounted for more than 70% of the extractable radioactivity. As diclofop was not rapidly degraded by the roots, it remained available for translocation to the shoots. The rate of translocation initially exceeded the rate of degradation by barley shoots with the result that toxicant accumulated in the shoots, reaching a peak level 24 h after beginning the dose period. Thereafter barley shoots degraded the toxicant very rapidly (Figure 13). It is clear that not only different species but also different organs of one given species may differ in their ability to degrade diclofop. These results may explain why barley, a species tolerant to foliar applications of diclofop methyl is relatively sensitive to soil applications of this herbicide. The differential abilities of barley roots and barley shoots to degrade diclofop also explains why barley roots are 18 times more sensitive to root-applied diclofop methyl than are barley shoots (Todd and Stobbe, 1977).

Degradation of diclofop by wild oat roots occurred at a rate intermediate between the rapid rate observed

in wheat roots and the slow rate observed in barley roots (Figure 13). More than 90% of the radioactivity extracted from wild oat shoots at the end of the 6 h dose period was associated with toxicant. This level of toxicant, much higher than observed in any of the other species, is an indication that wild oat shoots possess only a very limited ability to degrade diclofop. Consequently the toxicant translocated from the roots to the shoot accumulates. The toxicant was degraded slowly during the subsequent 18 h period and more rapidly thereafter. By 72 h after treatment initiation, toxicant accounted for 50% of the extractable radioactivity. Rather than having been degraded the diclofop may have been directly conjugated to plant constituents. Wild oat shoots formed hydroxy-diclofop more slowly than did wheat and barley shoots (Table 14) and yet wild oat shoots contained proportionally more polar conjugates 72 h after treatment initiation than did wheat and barley shoots (Table 15). It is not known whether or not this 'bound' diclofop can be released by subsequent hydrolysis reactions within the plant, but if so it may represent a potential source of toxicant within the plant. Slow release of this material could serve to maintain the titre of toxicant above the critical level for toxic action for a longer duration.

Toxicant represented a smaller proportion of the radioactivity extracted from green foxtail shoots relative to the other species studied (Figure 13). The level of

TABLE 14. Hydroxy-diclofop {2-[4-(2,4-dichloro-3,5,6-hydroxyphenoxy) phenoxy] propionic acid} formation in shoots of wheat, barley, wild oat, and green foxtail following root uptake of ^{14}C-diclofop methyl

Time	Hydroxy-diclofop (% of extracted radioactivity)				
(h)	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
6	8.0	13.8	2.9	39.6	3.9
24	22.7	11.3	8.5	20.6	5.6
72	15.6	23.8	18.1	24.3	4.8
LSD (0.05)	7.1	3.5	2.9	4.1	

TABLE 15. Formation of conjugates of diclofop methyl metabolites in wheat, barley, wild oat, and green foxtail shoots following root-uptake of ^{14}C-diclofop methyl

Time (h)	Conjugates (percent of extracted radioactivity)				
	Wheat	Barley	Wild Oat	Green Foxtail	LSD (0.05)
6	5.8	6.8	0.5	6.9	1.9
24	12.4	9.7	7.1	22.5	5.7
72	17.3	17.6	25.7	35.2	7.2
LSD (0.05)	4.3	4.0	5.8	7.8	

hydroxy-diclofop in green foxtail shoots at the 6 h sampling was three times that observed in any of the other species (Table 14). Hydroxylation of diclofop to yield hydroxy-diclofop did not proceed rapidly in the shoots, however, as the level of toxicant in the shoots declined only slightly with time. Furthermore, the percentage of hydroxy-diclofop in the shoot decreased with time rather than increasing as in the other species. The high proportion of hydroxy-diclofop in the shoots must have arisen as a result of hydroxylation in the root followed by translocation of the hydroxy-diclofop to the shoot. These results indicate that green foxtail roots possess a greater ability to degrade diclofop than do green foxtail shoots. Green foxtail was more tolerant to root-applied diclofop methyl than was wild oat (Todd and Stobbe, 1977). In the same study, it was observed that, in contrast to the other species studied, the concentration of diclofop methyl required to inhibit shoot growth by 50% following root-application was only marginally higher than the concentration required to inhibit root growth by 50%. The ratios I_{50} (ppmw) root:shoot were 0.25, 0.05, 0.07, and 0.55 for wheat, barley, wild oat, and green foxtail, respectively. The differential tolerance of green foxtail roots and shoots to root-applied diclofop methyl is based on the differential abilities of these organs to degrade diclofop.

The results of this study indicate that tolerance to root-applied diclofop methyl is related to the abilities of both the roots and the shoots to degrade the biologically active acid, diclofop. Species whose roots and shoots are both able to rapidly degrade diclofop will be tolerant to root-applied diclofop methyl; species whose roots are unable to degrade diclofop but whose shoots are effective in detoxifying diclofop will be marginally tolerant; species whose roots actively degrade diclofop but whose shoots are unable to do so will be marginally susceptible; and those species where neither the roots or the shoots are effective in degrading diclofop will be susceptible to root-applied diclofop methyl. These four conditions are represented by wheat, barley, green foxtail, and wild oat respectively. These relationships suggest that diclofop toxicity to shoots is a more important factor in the overall toxicity of this chemical to plants than is toxicity to roots.

CHAPTER 6

The Basis of the Antagonistic Effect of 2,4-D on Diclofop Methyl Toxicity to Wild Oat

Abstract. The interaction between diclofop methyl {2-[4-(2,4-dichlorophenoxy) phenoxy] methyl propanoate} and 2,4-D [2,4-dichlorophenoxy) acetic acid] was evaluated. Foliar-applications of tank mixtures of the two herbicides were antagonistic with respect to wild oat (*Avena fatua* L.) control. Application of mixtures of the two herbicides to wild oat roots resulted in an additive herbicidal effect. The free acid of 2,4-D was identified as the component of the 2,4-D formulation responsible for the reduction in toxicity of foliar-applied diclofop methyl to wild oat. Analysis of diclofop methyl solutions with and without added 2,4-D revealed no degradation products of diclofop methyl nor any evidence of complexing between diclofop methyl and 2,4-D. Addition of 2,4-D to the diclofop methyl spray solution did not affect spray retention or penetration of diclofop methyl by wild oat. Movement of radioactivity to roots and to shoot apices following spot application of ^{14}C-diclofop methyl to wild oat leaves was reduced by addition of 2,4-D to the treatment solution. Deesterification of diclofop methyl was inhibited by 2,4-D. The resulting level of diclofop methyl in wild oat leaves caused membrane damage and leaf necrosis. Consequently, symplastic movement of the des-methyl acid, diclofop, to sensitive meristematic areas was reduced. As

insufficient toxicant reached meristematic areas to permanently interrupt meristematic activity, the wild oat plants were able to outgrow the contact damage to their leaves.

INTRODUCTION

Diclofop methyl is a post-emergence herbicide which selectively controls wild oats, green foxtail, and other annual grassy weeds in cereal and oilseed crops (Friesen *et al.*, 1976; Todd and Stobbe, 1977). Application of tank mixtures of diclofop methyl plus 2,4-D for broad spectrum weed control results in reduced grassy weed control by diclofop methyl. Control of broad-leaved weeds by the mixture is equivalent to that obtained using 2,4-D alone (Todd and Stobbe, 1974; O'Sullivan *et al.*, 1977).

The objectives of this study were to evaluate the interaction between 2,4-D and diclofop methyl with respect to wild oat control and to determine the basis of this interaction.

MATERIALS AND METHODS

Evaluation of the diclofop methyl -2,4 D interaction. Wild oat plants were grown in silica sand in styrofoam cups and watered as required with a modified half-strength Hoagland's solution (Hoagland and Arnon, 1950). Plants in the two-leaf stage received foliar or root applications of diclofop methyl and/or 2,4-D. Combinations of the two

herbicides evaluated were: (a) foliar-application of tank mixtures of the two herbicides; (b) root-application of tank mixtures of the two herbicides; (c) foliar-application of diclofop methyl and root-application of 2,4-D; and (d) root-application of diclofop methyl and foliar-application of 2,4-D. Rates of 0.0 or 1.12 kg/ha of diclofop methyl (36% EC) and 0.0, 0.6, 1.2, or 1.8 kg/ha of 2,4-D amine were applied to the foliage in a spray volume of 134.1/ha at a pressure of 2.46 kg/cm^2. During spraying, the sand surface was covered by a layer of vermiculite which was subsequently removed, preventing root-uptake of the foliar-applied treatments. Root applications consisted of 15 ml of 0 or 100 µM diclofop methyl solution and 15 ml of 0, 1, 10, or 100 µM 2,4-D acid solution. The respective herbicide solutions were prepared with technical grade active ingredient dissolved in ethanol (0.1% of final solution volume) and added to nutrient solution. The rates of diclofop methyl were chosen to provide approximately 50% inhibition of wild oat shoot growth.

Three weeks after treatment the plants were harvested and root and shoot dry weights were obtained. An expected dry weight reduction for each diclofop methyl-2,4-D combination was calculated, based on the dry weight resulting from application of each component of the mixture alone. The expected dry weight for the mixture then was compared to the actual dry weight obtained. Actual dry weight reductions greater than the expected dry weight reduction indicate synergism; actual dry weight reductions

less than expected dry weight reductions indicate antagonism; and actual dry weight reductions equal to expected dry weight reductions indicate additive herbicide action (Colby, 1967). Differences between actual and expected dry weight reductions were subjected to statistical analysis (Hamill and Penner, 1973).

The experiment was conducted in a growth room having a temperature regime of 25 C day and 15 C night. Cool-white fluorescent lamps provided a light intensity of 2500 $\mu W/cm^2$ during a 16-h photoperiod. The completely randomized experiment consisted of four replicates, each replicate being one cup containing three plants.

Analysis for diclofop methyl-2,4-D interaction in the spray tank. ^{14}C-diclofop methyl, uniformly labelled in the dioxyphenyl ring (9.6 $\mu Ci/mg$), and technical diclofop methyl were added to the formulation blank[1] to make a 36% EC formulation. This formulation was diluted with distilled water to make a spray solution designed to deliver 1.0 kg/ha if applied in a spray volume of 134 l/ha. To a portion of the diclofop methyl solution, 2,4-D amine was added in sufficient quantity to apply a 0.8 kg/ha rate based on the above spray volume. After standing for 24 h at room temperature, 5 µl aliquots of the diclofop methyl and diclofop methyl

[1]Solvent plus emulsifiers utilized in the commercial formulation as supplied by Hoechst AG.

plus 2,4-D amine solutions were spotted on plastic-backed silica gel (0.25 mm thickness) TLC plates[2]. After development to a height of 10 cm in chloroform:acetic acid: hexane (80:15:5 v/v/v), the chromatographs were cut horizontally into 0.5 cm strips. One ml of 95% ethanol was added to each strip in individual scintillation vials. Ten ml of scintillation liquid (Chapter 2) were added to each vial and radioactivity was quantified by liquid scintillation spectrometry.

Determination of the component of the 2,4-D formulation responsible for the diclofop methyl-2,4-D interaction. Wild oat plants were grown in a very fine sandy loam soil in a greenhouse in which the temperature ranged from 16 to 28 C. Supplemental light of 2710 $\mu W/cm^2$ was provided by cool-white fluorescent lamps during a 16-h photoperiod. Wild oat plants in the two-leaf stage were sprayed with diclofop methyl (36% EC) at a rate of 1.2 kg/ha alone or in combination with the various components of a 2,4-D isooctyl ester formulation. Components of the 2,4-D formulation were added in the quantities which would be present in a 0.8 kg/ha application of 2,4-D. The spray was applied at a volume of 134 l/ha and a pressure of 2.46 kg/cm^2. Two weeks after spraying, the foliar plant parts were harvested and oven dry weights were obtained. The experiment consisted of a randomized complete block design with eight replicates.

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[2]Bakerflex Silica Gel IB2F.

Four wild oat plants, in a 10-cm diameter plastic pot, constituted a replicate.

Spray retention. Wild oat plants were grown in soil in the green house as described above. Spray solutions of diclofop methyl (1.0 kg/ha) and diclofop methyl plus 2,4-D amine (1.0 + 0.8 kg/ha) containing a water soluble dye[3] were prepared. The treatments were applied to wild oat plants in the two-leaf stage in a spray volume of 134 l/ha at a pressure of 2.46 kg/cm^2. After drying on the plant surfaces, the spray deposit was washed off and the dye was quantified colorimetrically at 630 nm. The completely randomized experiment consisted of 16 replicates. Four wild oat plants, in a 10-cm diameter plastic pot, constituted a replicate.

Spray penetration. Wild oat plants were grown in silica sand and watered with half-strength nutrient solution. Plants in the two-leaf stage were transferred to bottles containing half-strength nutrient solution. Once the plants had reached the three-leaf stage, approximately 2 µl of ^{14}C-diclofop methyl or ^{14}C-diclofop methyl plus 2,4-D amine (prepared as described above) was applied to the adaxial surface towards the middle of the second leaf using an air brush (Chapter 1). After various time intervals the treated zone was cut from the leaf and

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[3]Niagara Sky Blue 6B. A product of the Allied Chemical Co.

leaf surface radioactivity was removed by rinsing with 5 ml of 30% ethanol. The washings, in scintillation vials, were evaporated to dryness under an air stream. Ten ml of scintillation liquid was added to each vial and radioactivity was quantified. Radioactivity within the plant was determined by suspending the ground plant tissue in scintillation solution to which had been added 4% (w/v) thixotropic gelling agent[4]. As preliminary experiments showed volatilization of diclofop methyl to be nonsignificant over the duration of this experiment, total applied radioactivity was calculated as the sum of the radioactivity in the leaf washings plus the radioactivity in the plant tissue.

The completely randomized experiment was conducted in a growth room having a temperature regime of 25 C (day) and 15 C (night). A light intensity of 2500 $\mu W/cm^2$ was provided by fluorescent lamps during a 16-h photoperiod. Each treatment was replicated four times.

Translocation and Metabolism. Wild oat plants were grown and transferred to bottles containing nutrient solution as described above. Ten µl of ^{14}C-diclofop methyl emulsion with or without added 2,4-D amine were applied by syringe to the adaxial surface of the second leaf blade of plants in the three-leaf stage. After 4, 8, 24 and 72 h three plants from each treatment were harvested. Leaf

[4]Cab-O-Sil.

surface activity was recovered and quantified. The plants were divided into the following parts: treated zone; second leaf above the treated zone; second leaf below the treated zone; root; shoot apex; first leaf; and third and younger leaves. The tissues were then frozen. The various plant parts were extracted in 80% acetonitrile using a glass tissue grinder (Chapter 3).

Diclofop methyl metabolites in the tissue extracts and the leaf washings were separated by thin-layer chromatography. The plastic-backed TLC plates were developed to a height of 10 cm in chloroform:acetic acid:hexane (80:15:5 v/v/v). Sections of the TLC plates corresponding to reference standards of diclofop methyl and its metabolites as observed under UV light were cut out and placed in scintillation vials. One ml of 95% ethanol was added to each vial. Ten mls of scintillation liquid was then added and radioactivity quantified using liquid scintillation spectrometry. Radioactivity on portions of the TLC plates not corresponding to the reference standards was also quantified. Due to limited movement of the herbicide in the plant the three replicates of each plant part other than the treated leaf zone were combined for metabolite identification.

RESULTS AND DISCUSSION

Evaluation of the diclofop methyl - 2,4-D interaction. An

antagonistic interaction was observed when tank mixtures of diclofop methyl and 2,4-D amine were applied foliarly (Table 16). However, application of tank mixtures of diclofop methyl and 2,4-D to the roots resulted in additive herbicide action (Table 17). Foliar-application of diclofop methyl to plants which had received root-treatment with 2,4-D also resulted in additive herbicide action (Table 18), while synergism was noted when root-application of diclofop methyl was combined with a foliar treatment of 2,4-D amine (Table 19). Antagonism of diclofop methyl toxicity to wild oat by 2,4-D was unique to the situation where both herbicides were applied to the foliage.

Diclofop methyl exerts two distinct toxic actions within the wild oat plant. First, chlorosis and necrosis develop in leaf tissues as a result of membrane damage (Brezeanu *et al.*, 1976). Secondly, cell division in meristematic areas is inhibited (Owino, 1977). In wild oat plants foliarly treated with tank mixtures of diclofop methyl plus 2,4-D, leaf chlorosis developed but the plants outgrew this damage as meristematic activity continued.

In reducing diclofop methyl toxicity to wild oat, 2,4-D could (a) act in a physiologically opposite manner to diclofop methyl by stimulating cell division in meristematic areas; (b) compete, on the basis of structural similarities, for binding sites at the meristem essential

TABLE 16. The interaction between diclofop methyl and 2,4-D when applied as a tank-mixture to the foliage of wild oats.

Rate (kg/ha)		Net dry weight increase (% of control)			
diclofop methyl	2,4-D amine	Expected	Actual	Difference (E - A)	Interaction
		Shoot Growth			
1.2	0.6	50.7	100.9	-50.2 **	antagonistic
1.2	1.2	49.9	96.1	-46.2 **	antagonistic
1.2	1.8	37.4	87.2	-49.8 **	antagonistic
		Root Growth			
1.2	0.6	-17.7	45.7	-63.4 **	antagonistic
1.2	1.2	-20.1	13.7	-33.8 **	antagonistic
1.2	1.8	-24.4	-18.2	- 6.2 NS	additive

** Significant difference (p = .01)

TABLE 17. The interaction between diclofop methyl and 2,4-D when applied as a tank mixture to the roots of wild oats.

diclofop methyl (μM)	2,4-D (μM)	Net dry weight increase (% of control) Expected	Actual	Difference (E - A)		Interaction
		Shoot Growth				
100	1	57.9	44.9	+13.0	NS[1]	additive
100	10	57.2	49.6	+ 7.6	NS	additive
100	100	33.2	48.3	-15.1	NS	additive
		Root growth				
100	1	- 7.7	-12.7	+ 5.0	NS	additive
100	10	-10.9	-17.5	+ 6.6	NS	additive
100	100	- 6.4	-13.7	+ 7.3	NS	additive

[1]Nonsignificant interaction (p=0.01).

TABLE 18. The interaction between diclofop methyl applied to the shoots and 2,4-D applied to the roots of wild oats.

diclofop methyl (kg/ha)	2,4-D (μM)	Net dry weight increase (% of control) Expected	Actual	Difference (E - A)		Interaction
		Shoot Growth				
1.2	1	62.5	91.7	-29.2	NS[1]	additive
1.2	10	75.1	90.7	-15.6	NS	additive
1.2	100	31.7	40.7	- 9.0	NS	additive
		Root Growth				
1.2	1	2.2	- 1.4	+ 3.6	NS	additive
1.2	10	3.9	- 2.1	+ 6.0	NS	additive
1.2	100	5.6	4.4	+ 1.2	NS	additive

[1]Non-significant interaction (p=0.01).

TABLE 19. The interaction between diclofop methyl applied to the roots and 2,4-D applied to the shoots of wild oats.

diclofop methyl (μM)	2,4-D amine (kg/ha)	Net dry weight increase (% of control) Expected	Actual	Difference (E - A)	Interaction
		Shoot Growth			
100	0.6	63.1	21.9	+41.2 **	synergistic
100	1.2	63.4	41.1	+22.3 NS	additive
100	1.8	54.0	39.6	+14.4 NS	additive
		Root Growth			
100	0.6	0.0	-46.2	+46.2 **	synergistic
100	1.2	0.0	-47.2	+47.2 **	synergistic
100	1.8	0.0	-43.3	+43.3 **	synergistic

** Significant difference (p = 0.01).

for diclofop methyl toxicity; (c) reduce the quantity of toxicant reaching the meristematic sites of action.

Foliar applications of diclofop methyl severely inhibited root growth of wild oat (Table 20). If 2,4-D exerts its antagonistic effects at the meristem, then root applications of 2,4-D would be expected to be highly antagonistic to foliar applications of diclofop methyl. However, the herbicidal effects of foliar-applied diclofop methyl in combination with root-applied 2,4-D were additive with respect to wild oat root growth (Table 18). Similarly, when tank mixtures of diclofop methyl and 2,4-D were applied to wild oat roots no antagonism was noted (Table 17) even though root-applied diclofop methyl inhibits wild oat root growth (Table 20). The antagonistic effect of 2,4-D on diclofop methyl toxicity to wild oat is not exerted at the meristematic sites of diclofop methyl action. Interaction of diclofop methyl and 2,4-D in leaf tissue must act to reduce the amount of toxicant reaching the root and shoot meristems.

Analysis for diclofop methyl - 2,4-D interaction in the spray tank. TLC analysis of ^{14}C-diclofop methyl solutions with and without added 2,4-D amine revealed no degradation products of diclofop methyl nor any evidence of complexing between diclofop methyl and 2,4-D.

TABLE 20. The effect of diclofop methyl on wild oat root growth.

Shoot-applied diclofop methyl		Root-applied diclofop methyl	
Rate (kg/ha)	Net dry weight increase (% of control)	Rate (μM)	Net dry weight increase (% of control)
0.0	100.0	0	100.0
0.6	2.3	1	93.6
1.2	- 13.7	10	71.9
1.8	- 4.8	100	- 9.6

Determination of the component of the 2,4-D formulation responsible for antagonism. The free acid of 2,4-D reduced wild oat control to the same extent as did the complete 2,4-D formulation (Table 21). Addition of solvent and/or emulsifier from the 2,4-D formulation to the diclofop methyl solution did not reduce wild oat control by diclofop methyl. The free acid of 2,4-D is the component of the 2,4-D formulation responsible for reducing diclofop methyl toxicity to wild oat.

Spray retention. Wild oat plants retained 48.6 µl spray solution per g dry weight of foliage when sprayed with diclofop methyl. When sprayed with diclofop methyl plus 2,4-D amine 48.2 µl spray solution per g dry weight of foliage was retained. Addition of 2,4-D amine to the diclofop methyl spray solution did not affect spray retention by wild oats.

Spray penetration. Addition of 2,4-D amine to the diclofop methyl spray solution did not affect the uptake of ^{14}C-diclofop methyl by leaves of wild oat (Figure 14).

Translocation. Translocation of ^{14}C-diclofop methyl and/or the products of its metabolism was very limited (Table 22) with only 1.56% of the absorbed radioactivity recovered from plant parts other than the treated zone 72 h after application. Addition of 2,4-D amine did not alter the quantity of radioactivity translocated away from the treatment

TABLE 21. The effect of various components of a 2,4-D ester formulation on the toxicity of diclofop methyl to wild oat.

Treatment	Net dry weight increase (mg/pot)	Percent Control
control	924.2	
diclofop methyl	293.6	68.23
diclofop methyl + 2,4-D acid	617.9	33.14
diclofop methyl + 2,4-D acid + ester	443.9	51.97
diclofop methyl + 2,4-D acid + solvent	680.9	26.33
diclofop methyl + 2,4-D acid + emulsifier	605.1	34.53
diclofop methyl + 2,4-D acid + ester + emulsifier	548.6	40.64
diclofop methyl + 2,4-D acid + ester + solvent + emulsifier	642.9	30.44
diclofop methyl + solvent	282.6	69.42
diclofop methyl + emulsifier	248.4	73.12
diclofop methyl + solvent + emulsifier	343.6	62.82
diclofop methyl + 2,4-D acid + solvent + emulsifier	685.3	25.84
LSD (0.05)	135.6	

Figure 14. Penetration of ^{14}C-diclofop methyl (DM) with and without added 2,4-D amine into wild oat leaves [differences between treatments were not significant ($p = 0.05$)].

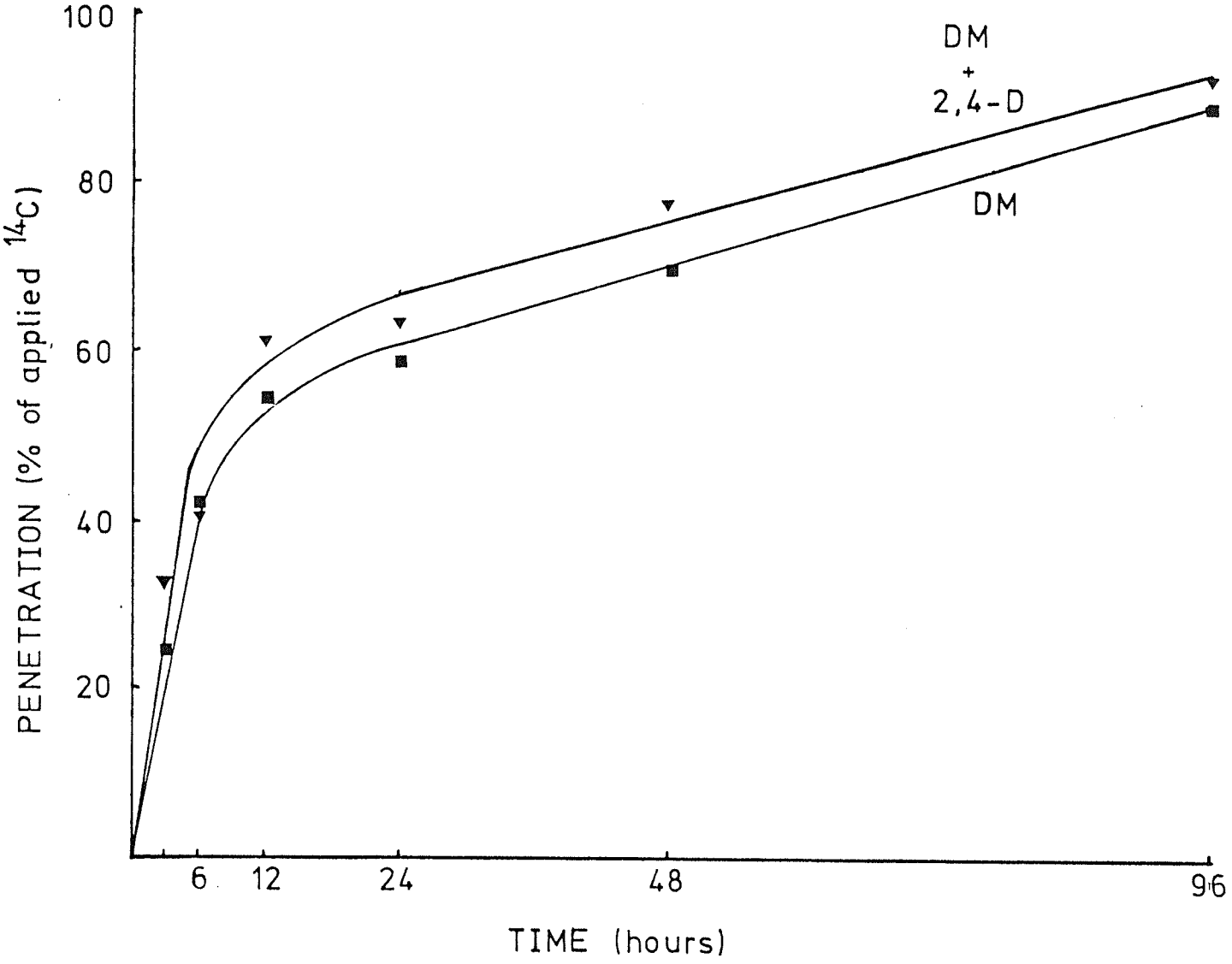
100
80
60
40
20
PENETRATION (% of applied ^{14}C)
DM
+
2,4-D
DM
6
12
24
48
96
TIME (hours)

TABLE 22. Translocation of radioactivity from the treated zone of wild oat leaves following spot application of ^{14}C-diclofop methyl with and without added 2,4-D amine.

Time (h)	Diclofop methyl (% of internal radioactivity)	Diclofop methyl + 2,4-D amine (% of internal radioactivity)
4	.08	0.26
8	.92	1.00
24	2.39	1.30
72	1.56	1.71

LSD (0.05) = 0.96

zone (Table 22) but did affect the direction of translocation. Translocation towards the tip of the treated leaf was enhanced by the addition of 2,4-D amine to the diclofop methyl spray solution (Table 23). The quantity of radioisotope reaching the root and the shoot apex was significantly less in plants treated with diclofop methyl plus 2,4-D than in plants treated with diclofop methyl alone (Table 24). Although measurable radioactivity had reached the shoot apex within 24 h in diclofop methyl treated plants, radioactivity was not detectable in the shoot apex of plants treated with diclofop methyl plus 2,4-D prior to the 72 h sampling.

Metabolism. Analysis of material recovered from the leaf surface revealed that addition of 2,4-D amine to the ^{14}C-diclofop methyl solution had not resulted in diclofop methyl degradation on the leaf surface. Greater than 98 per cent of the radioactivity recovered 72 h following application was present as diclofop methyl.

Previous studies have shown that wild oat readily de-esterifies diclofop methyl. The free acid, diclofop, is then slowly conjugated to various plant constituents (Chapters 3 and 4).

The rate of de-esterification of diclofop methyl in the treated zone of wild oat leaves was reduced by the addition of 2,4-D amine (Table 25). High levels of diclofop methyl and low levels of the free acid in the treated

TABLE 23. Percentage of ^{14}C-translocate moving acropetally following leaf spot treatments of wild oats with ^{14}C-diclofop methyl alone or tank-mixed with 2,4-D amine.

Time (h)	Acropetal translocation as a percentage of total translocation	
	diclofop methyl	diclofop methyl plus 2,4-D
4	10	59
8	27	57
24	35	59
72	26	68

LSD (0.05) = 22

TABLE 24. Translocation of radioactivity to root and shoot apex of wild oat following leaf spot treatment with ^{14}C-diclofop methyl alone or tank mixed with 2,4-D amine.

	Translocation			
	Root		Shoot Apex	
Time (h)	DM[1] (DPM)	DM + 2,4-D (DPM)	DM (DPM)	DM + 2,4-D (DPM)
4	0	3	0	0
8	85	77	3	0
24	310	47	109	0
72	642	249	147	47

[1]DM = diclofop methyl.

TABLE 25. Analysis of extract from treatment zone of wild oat leaves for metabolites of diclofop methyl.

Time (h)		Percent of extracted radioactivity present as: conjugates[1]	hydroxy diclofop[2]	phenoxy phenol[3]	diclofop	diclofop methyl
4	DM[4]	4.72 ± 5.22	0.56 ± .04	0.33 ± .49	18.15 ± 7.39	76.05 ± 4.35
	DM + 2,4-D	0.78 ± .64	0.24 ± .05	0.31 ± .09	6.87 ± 1.24	91.83 ± 1.91
8	DM	4.12 ± .69	0.64 ± .27	0.48 ± .21	20.63 ± 11.89	74.13±12.98
	DM + 2,4-D	2.35 ± 1.10	0.68 ± .21	0.27 ± .08	14.31 ± 2.31	82.40 ± 2.08
24	DM	28.46 ± 3.52	1.07 ± .53	0.48 ± .10	23.82 ± 2.69	46.18 ± 4.73
	DM + 2,4-D	17.27 ± 4.91	0.57 ± .21	0.37 ± .05	12.08 ± 5.48	69.70 ± 8.17
72	DM	54.69 ± 3.19	0.99 ± .15	0.40 ± .07	12.28 ± .46	31.64 ± 3.73
	DM + 2,4-D	25.63 ± 9.41	0.68 ± .04	0.47 ± .10	6.29 ± 1.56	66.92 ± 8.39

[1] water soluble conjugates of diclofop methyl and its metabolites.

[2] 2- [4-(2,4-dichloro-3,5,6-hydroxyphenoxy) phenoxy] propionic acid.

[3] 4-(2,4-dichlorophenoxy) phenol.

[4] diclofop methyl

zone of plants treated with a tank mixture of diclofop methl plus 2,4-D amine resulted in severe necrosis of the leaf tissue (Figure 15). These results show that diclofop methyl is the toxicant responsible for chlorosis and necrosis of leaf tissue in diclofop methyl treated plants. Destruction of the leaf tissue in plants treated with a mixture of diclofop methyl and 2,4-D reduces basipetal symplastic translocation and allows diclofop methyl and its degradation products to be swept acropetally in the transpiration stream.

Deesterification of the isopropyl ester of 2,4-D was shown by Crafts (1960) to be a prerequisite for symplastic translocation of 2,4-D. Similarly Jeffcoat and Harries (1973), Jeffcoat and Harries (1975) and Jeffcoat *et al.*, (1977) have shown that benzoylprop ethyl [ethyl-2-(N-benzoyl-3,4-dichloroanilino) propionate], flamprop methyl {methyl-2-[N-(3-chloro-4-fluorophenyl) benzamido] propionate}, and flamprop isopropyl {isopropyl-2-[N-(3-chloro-4-fluorophenyl) benzamido] propionate} move primarily in the acropetal direction while their respective parent acids move basipetally in the symplast. Basipetal movement of benzoylprop acid was approximately five times that of benzoylprop ethyl. The correlation between reduced symplastic movement of the radioactive label and reduced de-esterification of diclofop methyl in leaf tissue observed in this study strongly suggest that while diclofop methyl moves acropetally, diclofop moves basipetally in the symplast.

Control DM DM + 2,4-D

Figure 15. Development of necrosis on wild oat leaves treated with diclofop methyl (DM) alone or in combination with 2,4-D amine.

TABLE 26. Analysis of extracts from roots and shoot apices for diclofop methyl metabolites.

Plant Part	Time (h)	Treatment	Percent of extracted radioactivity present as: conjugates[1]	hydroxy[2] diclofop	phenoxy[3] phenol	diclofop	diclofop methyl
Root	24	DM[4]	8.31	1.99	2.66	82.06	4.98
		DM + 2,4-D	9.33	8.00	0.00	68.00	14.67
	72	DM	20.32	7.65	4.63	55.13	12.27
		DM + 2,4-D	16.14	3.94	12.99	61.81	5.12
Shoot Apex	24	DM	11.60	8.80	2.80	74.00	2.80
		DM + 2,4-D	*	*	*	*	*
	72	DM	28.31	9.04	4.82	54.82	3.01
		DM + 2,4-D	35.13	4.05	0.00	52.70	8.11

[1]water soluble conjugates of diclofop methyl and/or its metabolites.

[2]2-[4-(2,4-dichloro-3,5-6-hydroxyphenoxy) phenoxy] propionic acid.

[3]4-(2,4-dichlorophenoxy) phenol.

[4]diclofop methyl.

*insufficient radioactivity for analysis.

In the root and in the shoot apex the predominant metabolite was the free acid (Table 26). The free acid appears to be the toxicant responsible for the inhibition of meristematic activity as virtually no diclofop methyl was recovered from either the roots or the shoot apex. Shimabukuro *et al.* (1978) have reported that the free acid is more inhibitory to root growth than is diclofop methyl itself.

The antagonistic effect of 2,4-D on diclofop methyl toxicity to wild oat involves reduced movement of diclofop to meristematic areas. With little free acid present in the meristematic areas, meristematic activity continues and the plant rapidly outgrows the contact damage of diclofop methyl to its leaves.

CHAPTER 7

1,8-Naphthalic Anhydride and R-25788: Antidotes Against Diclofop Methyl Toxicity to Oats and Corn

R-25788 (N,N-diallyl-2,2-dichloroacetamide) and 1,8-naphthalic anhydride (NA) were evaluated as protectants against diclofop methyl {methyl-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate} toxicity to oats (*Avena sativa* L.) and corn (*Zea mays* L.). Both chemicals overcame the systemic toxic action of the free acid, diclofop, but neither prevented the contact activity of the applied ester, diclofop methyl. As a result the treated plants became chlorotic but new growth continued to be produced. With time the plants have the potential to largely outgrow the herbicidal effect. Attempts to utilize root-applied 2,4-D (2,4-dichlorophenoxy acetic acid) as a protectant against diclofop methyl toxicity to oats were unsuccessful.

Diclofop methyl provides selective, post-emergence, annual grass control in a wide variety of grassy and broad-leaved crops (Andersen, 1976b; Friesen *et al.*, 1976; Todd and Stobbe, 1977; Chow, 1978). Corn and tame oats are two important crops which are not tolerant to this herbicide (Andersen, 1976b; Chow, 1978). Diclofop methyl does control certain weeds which remain as serious problems in corn and/or oats, including foxtails (*Setaria* spp.), witchgrass (*Panicum capillare* L.), and barnyardgrass [*Echinochloa*

crus-galli (L.) Beauv.] (Andersen, 1976b). It would be useful if diclofop methyl could be utilized in corn or oats to control these and other grassy weeds.

R-25788 has been utilized to protect corn against damage by dithiocarbamate herbicides such as EPTC (S-ethyl dipropylthiocarbamate) and butylate (S-ethyl diisobutyl-thiocarbamate). The antidotal action of R-25788 was selective. When R-25788 was uniformly mixed with the soil, corn was protected from EPTC injury but weed species were not (Chang *et al.*, 1973).

Protection of tame oats against barban (4-chloro-2-butynyl m-chlorocarbanilate) injury has been achieved using 1,8-naphthalic anhydride (NA) (Chang *et al.*, 1974). Selective protection was achieved by applying NA to the oat seed prior to planting. In this way it was possible to achieve wild oat control in a tame oat crop.

Addition of 2,4-D to diclofop methyl spray emulsions reduces diclofop methyl toxicity to wild oat (*Avena fatua* L.) (Todd and Stobbe, 1974c; O'Sullivan *et al.*, 1977).

This report concerns studies undertaken to evaluate NA, R-25788, and 2,4-D as antidotes against diclofop methyl toxicity to corn and oats.

Corn and oat plants were grown in soil in a growth room having a temperature regime of 28 C day and 20 C night. Light energy of 6600 $\mu W/cm^2$ was provided by a mixture of fluorescent and incandescent lamps during a 16-h photoperiod.

Seed treatments of NA or R-25788 were applied by shaking corn or oat seeds in a plastic bag to which the seed treatment had been added at a rate of 1% of seed weight. Diclofop methyl (36% EC) was applied at rates of 1.25 to 1.75 kg/ha in a spray volume of 225 l/ha at a pressure of 3.0 kg/cm^2 when the oat and corn plants were in the 2 to 3 leaf-stage. Two weeks after spraying, the plant shoots were harvested and oven dry weights were obtained.

Preliminary experiments indicated that 2,4-D rates as low as 0.25% of seed weight delayed germination and inhibited growth of oats. Lower rates of 2,4-D were tested by incorporating 2,4-D into the nutrient solution supplied to the oat plants growing in sand-culture. Pre-germinated oat seeds were planted in silica sand and watered as required with a modified half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) containing 2,4-D concentrations of 10^{-5} to 10^{-9}M. The plants, in the 3 leaf-stage, were sprayed with diclofop methyl (1.75 kg/ha) as described above. The shoots were harvested 2 weeks after spraying and oven-dry weights were obtained.

All experiments employed a completely randomized design with 4 replicates. Each replicate was one pot containing 4 uniform plants.

NA provided oats protection against diclofop methyl toxicity (Table 27). The net shoot dry weight increases 2 weeks after spraying with diclofop methyl were 86% and 57% of the control for oats with and without NA seed

TABLE 27. Interaction between seed-applied 1,8-naphthalic anhydride (NA) or N,N-diallyl-2,2-dichloroacetamide (R-25788) or root-applied 2,4-D and foliage-applied diclofop methyl.

Antidotal Compound	Antidote Rate	Test Species	Diclofop Methyl (kg/ha)	Predicted[1] Growth (% of control)	Actual Growth	Difference[2] (P-A)	Nature of Interaction
NA	1% of seed weight	oats	1.40	56	86	-30*	antagonistic
		corn	1.40	17	40	-23*	antagonistic
R-25788	1% of seed weight	corn	1.25	18	45	-27*	antagonistic
			1.50	16	46	-30*	antagonistic
			1.75	15	42	-27*	antagonistic
2,4-D	10^{-9}M	oats	1.75	45	44	+ 1	additive
	10^{-8}M		1.75	57	49	- 2	additive
	10^{-7}M		1.75	42	40	+ 2	additive
	10^{-6}M		1.75	43	46	- 3	additive
	10^{-5}M		1.75	11	22	-11	additive

[1] Predicted growth calculated according to Colby (1967).

[2] Values designed * indicate a statistically significant ($p = 0.05$) interaction. Analysis according to Hamill and Penner (1973).

treatment respectively. Statistical analysis of the data (Hamill and Penner, 1973) indicated a significant ($p=0.05$) antagonistic interaction. All of the oats treated with diclofop methyl exhibited chlorosis at the bases of the second and third leaves within 2 days after treatment and growth appeared to be halted. However, within 5 days following diclofop methyl application, those oats which had received the NA treatment had resumed growth and were producing new growth. Those oats which had received no NA treatment remained stunted following the diclofop methyl application. The only growth observed in these plants was a broadening at the base of the leaves.

NA also afforded protection of corn against diclofop methyl injury. Corn was more sensitive to diclofop methyl than was oat. The net shoot dry weight increases 2 weeks after spraying with diclofop methyl were 40% and 18%, respectively,for corn with and without NA seed treatment. This antagonistic interaction proved to be statistically significant ($p=0.05$). Those corn plants which had received NA seed treatment were still alive 2 weeks after spraying with diclofop methyl while those which had received no NA were dead.

The difference between the predicted growth and the actual growth of plants treated with both NA and diclofop methyl was greater for oat than for corn, indicating that NA was more effective in reducing diclofop methyl injury to oats than to corn (Table 27).

R-25788 seed-treatment reduced the toxicity of foliage-applied diclofop methyl to corn. Again the interaction proved statistically significant, an indication of an antidotal effect. The reduction in diclofop methyl toxicity to corn was marginally greater when corn seed was treated with R-25788 than when treated with NA. As was observed with NA, R-25788 did not afford corn complete protection against diclofop methyl toxicity. Corn plants which received no R-25788 pretreatment turned chlorotic following diclofop methyl application, growth stopped, and the plants collapsed completely prior to the planned end of the experiment. Chlorotic symptoms also developed in those corn plants which had received R-25788 seed treatment but the interruption in growth was only temporary. Two weeks after spraying these plants were producing new growth of normal appearance. Because growth of these plants had been temporarily interrupted and because much of the growth present at the time of spraying had become chlorotic and necrotic, the plants were unable to achieve the same growth as the control plants over the 2 week duration of the experiment. Had the experiment been extended over a longer period of time much of this growth difference would have disappeared.

NA prevented death of oat and corn plants, and R-25788 prevented death of corn plants, following diclofop methyl application but neither compound prevented development of the chlorotic symptoms typical of diclofop methyl activity.

Development of chlorosis following diclofop methyl application has been attributed to the contact action of the applied methyl ester (Chapter 6). Inhibition of growth in susceptible species following diclofop methyl application has been attributed to an interruption of cell division and elongation by the desmethyl acid, diclofop (Chapters 3, 5, and 6; Owino, 1977). The diclofop methyl antagonists, NA and R-25788, prevented the systemic action of diclofop but did not prevent the contact action of diclofop methyl. As such, NA and R-25788 may provide a means for studying the individual modes of action of diclofop and diclofop methyl.

The degree of protection afforded corn by R-25788 was similar regardless of the rate of diclofop methyl applied (Table 27). The lowest rate of diclofop methyl applied (1.25 kg/ha) provided maximal contact damage and raising the rate of diclofop methyl added no further effect. These results indicate that while the contact damage of diclofop methyl may contribute to the control of susceptible species, if the herbicide is applied after the plants have well established root systems capable of regenerating new shoots, complete death of the plant cannot be achieved without the systemic action of the free acid.

Incorporation of 2,4-D into the nutrient solution used to water oat plants did not reduce diclofop methyl toxicity to oats (Table 27). Subsequent studies have shown that 2,4-D is antagonistic to diclofop methyl only when both herbicides are applied foliarly, with the 2,4-D acting to interrupt the

movement of diclofop to sensitive meristematic areas (Chaper 6). Consequently 2,4-D has no potential application as a seed treatment for selective protection of crops from diclofop methyl damage.

Although none of the treatments employed in these studies were successful in protecting either corn or oat from the herbicidal effect of diclofop methyl, success was achieved in protecting these species from the systemic phytotoxic effects of diclofop. The potential remains for finding compounds which could offset the effects of both diclofop and diclofop methyl and thereby allow the use of this herbicide as a weed control tool in crops such as corn and oats.

GENERAL DISCUSSION

Selective chemical weed control is an essential element in modern agricultrual production. However, the compexity of the herbicide-plant interaction is such that the basis of the selective toxic action of most herbicides is, at best, only poorly understood. An understanding of the basis of selectivity of herbicide action assists in the development of specific weed control recommendations which maximize weed control and minimize crop injury. Knowledge of herbicide selectivity promotes efficiency in the use of the toxicant, thereby minimizing risks to the environment. Additionally, studies into the basis of selective toxic action provide information essential to the quest for new herbicides and new weed control systems.

In order to evaluate the contributions of various factors to the selectivity of a given herbicide, some understanding of where and how the toxicant affects susceptible plants is required. While the intent of the reported investigations was not to elucidate the mechanism of action of diclofop methyl, the observations do provide basic information on the mode of action of this herbicide.

Diclofop methyl and its analogues have been described as members of a unique herbicidal chemistry, and have

been referred to as phenoxy-phenoxy compounds (Koecher and Lotzsch, 1975; Nestler *et al.*, 1978). Based upon the observed biological activity of this compound in relationship to its structure, diclofop methyl can be more meaningfully classified as an α-substituted propionic acid. Besides the phenoxy-phenoxy compounds, other herbicides which should be included in this group include dalapon, benzoylprop ethyl, flamprop methyl, flamprop isopropyl, and chlorfenprop methyl. The structural similarities of these compounds are illustrated in Figure 16. When applied at recommended field application rates, all of these herbicides provide control of certain grassy weeds with little or no effect on most broadleaved species. All of these compounds do exhibit contact activity, but in all cases effective grassy weed control is dependent on systemic movement to meristematic regions within the plant. With the exception of dalapon, all of the herbicides listed above provide selective control of wild oats in cereal crops.

The relationship between the α-substituted propionic acid structure and toxicity to grasses suggests that grasses are inherently sensitive to propionic acid derivatives. The substitution dictates the spectrum of sensitive species. This spectrum ranges from no grasses controlled where the substitution is 2,4-dichlorophenoxy as in 2,4-DP [2-(2,4-dichlorophenoxy) propionic acid] ; 2,4,5-

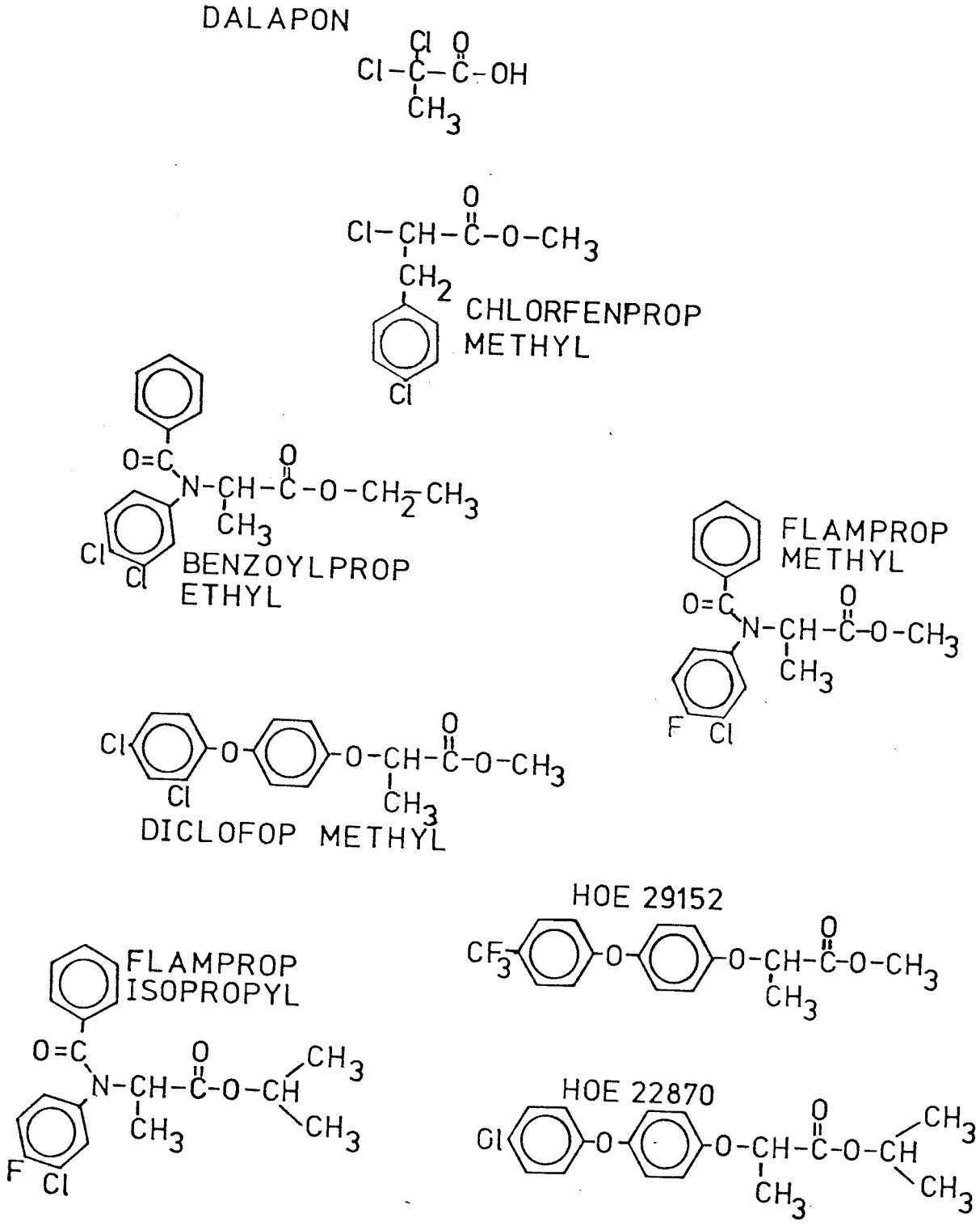

Figure 16. Examples of α - substituted propionic acid herbicides.

trichlorophenoxy as in silvex [2-(2,4,5-trichlorophenoxy) propionic acid] ; or 2-methyl-4-chlorophenoxy as in mecoprop [2-(4-chloro-O-tolyl oxy) propionic acid] to virtually complete control of all grasses where the substitution is chlorine as in dalapon or (4-trifluoromethylphenoxy) phenoxy as in HOE 29152 {2-[4-trifluoromethylphenoxy) phenoxy] methyl propanoate}. These substitutions undoubtedly interfere with the ability of the propionic acid moiety to participate normally in cellular metabolism. Even the grassy weed herbicide TCA (trichloro-acetic acid) fits into this generalized structure-activity relationship. Although TCA is an acetic acid derivative the trichloro substitution renders this molecule almost identical sterically to dalapon.

Following diclofop methyl treatment, susceptible plants exhibit leaf chlorosis and necrosis (Brezeanu *et al.*, 1976). Subsequently cell division and elongation processes are affected, inhibiting root and shoot growth (Owino, 1977). These symptoms represent two distinct physiological actions of this herbicide within plants. Effects of the herbicide on cell division and elongation processes were prevented by treating oat and corn seed with R-25788 or NA prior to planting (Chapter 7). Development of leaf chlorosis and necrosis was not prevented by either chemical. The magnitude of the chlorotic effect was correlated with the level of diclofop methyl in the tissue (Chapter 6), leading to the conclusion that

leaf chlorosis and necrosis develop as a result of contact action by the applied ester. This contact action results in a loss of cell-membrane permeability and ultimately in a disruption of cell membrane integrity (Crowley and Prendeville, 1979). While such effects on cell membrane integrity are typical of diphenyl ether activity, the contact action of diclofop methyl appears to be associated with the propionic acid moiety. The results of Crowley and Prendeville (1979) indicated a distinct difference in the modes of action of diclofop methyl and diphenyl ether herbicides with respect to effects on cell membrane integrity. Furthermore, the α-substituted propionic acid herbicides, dalapon and chlorfenprop methyl, exhibit contact activity involving membrane disruption. Neither of these compounds possesses a diphenyl ether moiety. The greater toxicity of diclofop methyl in the disruption of cellular membranes as compared to diclofop may relate to the lipophilic nature of the ester. Such lipophilic character would facilitate interaction with lipo-protein membrane components.

Inhibition of meristematic activity and cell elongation following diclofop methyl application can be attributed to the action of the free acid, diclofop. Inhibition of wild oat root growth following foliar application of diclofop methyl occurred even though root-uptake was prevented by covering the soil surface with vermiculite

(Chapter 6), indicating that the toxicant was translocated from the shoot to the root. Little or no diclofop methyl was translocated to the root following leaf application of ^{14}C-diclofop methyl. Accumulation of diclofop in the roots and in the shoot apices of susceptible species was observed (Chapter 3).

Root growth inhibition was observed when green foxtail was planted into soil sprayed 5 weeks previously with diclofop methyl (Chow, 1978). As complete hydrolysis of diclofop methyl to diclofop would be expected to occur in a 5 week period in the soil (Smith, 1977), the observed effect on green foxtail root growth must have arisen as a result of the action of the free acid. Shimabukuro *et al.* (1978) have reported that diclofop is a more potent inhibitor of wild oat root growth than is diclofop methyl.

The conclusion that diclofop methyl activity results in membrane disruption while diclofop interferes with cell division and elongation processes is in disagreement with the conclusions of Shimabukuro *et al.* (1978). These authors concluded that inhibition of stem growth was the result of diclofop methyl action while ultrastructural damage and cell destruction were attributed to the action of the free acid. This conclusion was based on the finding that diclofop methyl was a stronger auxin antagonist than was diclofop in an oat coleoptile straight growth test. The

authors could not explain why diclofop, the weaker auxin antagonist, was a more effective inhibitor of root growth than was diclofop methyl.

The systemic action of diclofop is a more important factor in the death of susceptible plants than is the contact action of the applied methyl ester. When movement of diclofop to meristematic areas was interrupted through the action of 2,4-D, diclofop methyl treated wild oats became chlorotic but continued to produce new growth and ultimately outgrew the herbicidal effect (Chapter 6). Similarly, corn plants grown from R-25788 treated seed exhibited complete destruction of foliage following diclofop methyl application but the systemic action of diclofop was prevented. Subsequently, the plants produced new foliage and appeared to be outgrowing the herbicidal effect (Chapter 7). It can be concluded that the contact action of diclofop methyl will kill only plants whose root systems are not yet well enough established to support regrowth of the shoot.

The contact action of diclofop methyl does represent an important facet in the overall mode of action of this compound. Destruction of leaf tissue including the photosynthetic apparatus of susceptible weed species drastically reduces the photosynthetic capacity of the plant within a few days of spraying. Weed competition for water and nutrients is quickly halted even though the systemic action of diclofop may require 10 to 14 days before resulting in

the complete collapse of the plant. Through the combined toxic actions of diclofop and diclofop methyl weed competition is removed quickly and completely.

From the slopes of the dosage-response curves (Figure 3) it can be concluded that the mode of action of diclofop methyl differs between wild oat and green foxtail (Blackman, 1952). Observations recorded during the experiment indicated severe contact damage to green foxtail. While wild oat did exhibit leaf chlorosis and necrosis, growth stagnation followed by collapse of the plant was a more important factor contributing to the observed growth reductions. The similar slopes of the dosage-response curves for wild oat and barley indicates that the mode of action of diclofop methyl is similar in both species but that a proportionately larger dose is required to achieve the same growth reduction in barley as in wild oat. The slope of the dosage-response curve for wheat was different from those for wild oat and barley. The damage to wheat was primarily contact damage which was attributed to the drying effect of the formulation solvent, xylene. As the contact activity of xylene was much less than the contact activity of diclofop methyl, much larger dosage increments were required with xylene to produce an effect equal to a smaller dose of diclofop methyl. Consequently, the slope of the dosage-response curve for wheat is not as steep as that for green foxtail.

The dosage-response curves illustrate the tolerance of

the two crop species and the susceptibility of the weed species to diclofop methyl. The magnitude of selectivity among these four Gramineae species is remarkable. One thousand times as much herbicide must be applied to achieve a 50% growth reduction in wheat as is required to achieve a 50% growth reduction in green foxtail.

The sensitivity of green foxtail to diclofop methyl is due, in part, to greater spray retention by, and more rapid spray penetration into, leaves of this species as compared to wheat, barley, and wild oat. Relative to the other species studied, more than 20 times as much herbicide would be present within green foxtail leaves 6 hours after diclofop methyl application. The rapid influx of diclofop methyl into leaves of green foxtail resulted in extensive contact damage to the plant. Disruption of the leaf tissues prevented further metabolism of the herbicide within the plant. The half-life of ^{14}C-diclofop methyl vacuum infiltrated into green foxtail leaf segments was only marginally longer than the half-life calculated for the same process in wheat (Chapter 4). The inability of intact green foxtail plants to deesterify diclofop methyl (Chapter 3) must have arisen as a result of cellular disruption by diclofop methyl and not as the result of an inherent inability of this species to catalyze the reaction involved.

The contact activity of diclofop methyl represents an important component in the mode of action of this herbicide

in green foxtail. However, if the contact action of diclofop methyl were solely responsible for the death of treated green foxtail plants, addition of 2,4-D to the diclofop methyl spray solution should not result in any reduction in diclofop methyl toxicity to green foxtail. Tank-mixing 2,4-D with diclofop methyl does reduce diclofop methyl toxicity to green foxtail (Delage and Coulthard, 1974). Since 2,4-D reduces the amount of diclofop reaching sensitive meristematic areas (Chapter 6), the antagonistic effect of 2,4-D on diclofop methyl toxicity to green foxtail is an indication that complete destruction of green foxtail plants is dependent on the systemic action of diclofop. The inability of green foxtail to degrade diclofop and the subsequent accumulation of this toxicant in the roots and in the shoot apex were documented in Chapter 3. The accumulation of diclofop in the roots and in the shoot apex ensures complete destruction of the green foxtail plant.

The selectivity of foliar-applied diclofop methyl among wheat, barley, and wild oat was not related to species differences with respect to spray retention or penetration (Chapter 2). Translocation of radioactive label following ^{14}C-diclofop methyl application to leaves was limited. Only one to two percent of the absorbed radioactivity was exported from the treated leaf in all four species studied. The tolerant species wheat and barley translocated as much,

or more, radioactive label as did the susceptible species wild oat and green foxtail.

Hydrolysis of diclofop methyl occurred so rapidly that there was little opportunity for diclofop methyl movement in plants. The free acid, diclofop, exhibited symplastic mobility. In this regard, the movement of diclofop in the plant was consistent with the translocation patterns observed for the α-substituted propionic acid herbicides, benzoylprop ethyl and flamprop methyl (Jeffcoat and Harries, 1973; 1975), and flamprop isopropyl (Jeffcoat *et al.*, 1977). Ester hydrolysis was a prerequisite for symplastic translocation of these herbicides.

Peterson and Edgington (1976) have proposed that the property which allows a pesticide to be transported in the symplast is its ability to be retained by the symplast following its entry through the plasmalemma. Chemicals which are able to move freely back out of the cell following uptake would be swept away by the transpiration stream. As the pesticide was swept away, a concentration gradient favouring further movement out of the cell would be maintained. Such pesticides would exhibit primarily acropetal translocation. Only if a pesticide, once having entered the symplast, had difficulty recrossing the plasmalemma would symplastic transport predominate. The observed transport pattern of diclofop is consistent with these postulates. Diclofop methyl, a lipophilic molecule,

would readily cross the plasmalemma in both directions. However, on entering the symplast, diclofop methyl would be subject to ester hydrolysis. The diclofop so formed, being less lipophilic than diclofop methyl would not be able to cross the plasmalemma as readily. Consequently, diclofop would be retained in the symplast and transported accordingly.

Alternatively, the translocation of diclofop may relate to phloem-loading phenomena. Endogenous phloem sap constituents include carbohydrates, sugar alcohols, sugar phosphates, organic acids, organic phosphates, amino acids, nucleic acids, vitamins, and plant growth regulators (Crafts and Crisp, 1971). These materials represent a broad spectrum of chemical configurations, polarities, solubilities, molecular weights and chemical reactivities, and yet all are mobile in the phloem. One property shared by these compounds is acidity. All contain free carboxylic acid groups or at least acidic hydroxyl groups. Similarly, herbicides which contain free carboxylic acid or hydroxyl groups exhibit symplastic transport (Ashton and Crafts, 1973). Peterson *et al.* (1977) reported that translocation velocities of sucrose and amino acids in oats were similar but that the mass transfer rates of amino acids were much lower than for sucrose, indicating that the rate limiting step for amino acid transport was entry into the phloem. Several studies have indicated that an energy-dependent carrier

mechanism was involved in the uptake of amino acids (Birt and Hird, 1958; Nobel and Cheung, 1973; Shtarkshall and Reinhold, 1974). Phloem loading of sucrose has been attributed to a carrier mechanism coupled to the co-transport of protons (Giaquinta, 1977). An acidic grouping on the herbicide molecule might be a requirement for binding to a non-specific carrier molecule involved in the phloem-loading process. Masking the acidic group through esterification would prevent binding and reduce symplastic transport. Alternatively, the lipophilic nature of the ester may decrease its solubility in the cytoplasm and increase its tendency to remain associated with the plasmalemma.

The diclofop methyl metabolites, hydroxy-diclofop and phenoxy-phenol, both of which possess a free hydroxyl group, exhibited symplastic mobility. Hydroxy-diclofop also moved acropetally in the transpiration stream. As these compounds were more mobile than diclofop, translocation of radioactive label following ^{14}C-diclofop methyl application was greater in tolerant than in susceptible species.

The selectivity of foliar-applied diclofop methyl among wheat, barley, wild oat, and green foxtail was not related to species differences with respect to de-esterification of diclofop methyl. Hydrolysis of diclofop methyl proceeded more rapidly in wild oat than in the tolerant species, wheat and barley. Hydrolysis of diclofop methyl vacuum infiltrated into leaf segments of green foxtail proceeded more slowly than in the other three species. The longer time

required for de-esterification of the applied diclofop methyl in green foxtail may have contributed to, but was not the basis of the rapid contact activity associated with diclofop methyl toxicity to this species.

De-esterification of diclofop methyl did not proceed as rapidly following spot application to leaves of intact plants as it did in the vacuum infiltrated leaf segments (Chapter 3). Ester hydrolysis in the intact plants proceeded rapidly during the first 4 hours following herbicide application but only slowly thereafter. Seventy-two hours after diclofop methyl application to intact plants, 32%, 52%, 32%, and 87% of the absorbed radioactivity was present as diclofop methyl in wheat, barley, wild oat, and green foxtail respectively. These figures compare to the 1 to 2 hour half-life of diclofop methyl vacuum infiltrated into leaf segments of these species. Vacuum infiltration brought the diclofop methyl into intimate contact with the leaf cells. Application to the leaf surface in intact plants requires that the herbicide move through the cuticle before reaching the underlying tissue. Lipophilic compounds may remain trapped in the cuticle if they do not possess sufficient hydrophilic character to allow for their partitioning into the aqueous environment of the underlying leaf tissue. The fact that diclofop methyl was not completely hydrolyzed in intact plants indicates that significant quantities of the herbicide dissolved into the cuticle but

did not pass through it. Barley cuticle retained more of the absorbed herbicide (52%) than did wild oat cuticle (32%), contributing to the relative tolerance of barley. In green foxtail,rapid tissue destruction as a result of the contact action of the herbicide rendered the tissue incapable of hydrolyzing diclofop methyl. The percentage of the extracted radioactivity present as diclofop methyl rose with time as penetration into the leaf continued in the absence of ester hydrolysis. The confounding effect of tissue destruction on diclofop methyl de-esterification makes it impossible to estimate the percentage of the absorbed diclofop methyl trapped in the green foxtail cuticle. These results indicate the importance of evaluating herbicide metabolism in systems in which the toxicant is brought into close proximity to the metabolizing cells as well as in intact plant systems.

The selectivity of diclofop methyl was closely related to species differences in metabolism of the free acid, diclofop. In the susceptible species degradation of diclofop did not proceed rapidly enough to prevent accumulation of the toxicant in sensitive root and stem apex regions. In tolerant wheat and barley, diclofop represented a much smaller percentage of the radioactivity extracted from the roots and from shoot apices than in susceptible wild oat and green foxtail. Wheat and barley shoots degraded much of the diclofop within the plant before it could reach target

tissues, and any diclofop which reached the root or the shoot apex of these species was rapidly degraded. Phytotoxic levels of diclofop did not persist at the site of action long enough to cause death of wheat or barley plants.

The studies in which ^{14}C-diclofop methyl was vacuum infiltrated into leaf segments of each of the four species provided further evidence in support of the relationship between inability to degrade diclofop and susceptibility to foliar-applied diclofop methyl. The diclofop half-life of 6.1, 7.3, 15.0, and 9.3 hours in wheat, barley, wild oat, and green foxtail respectively were in accordance with the relative sensitivities of these species to the herbicide. Green foxtail (ED_{50} = 0.1 kg/ha) exhibited a greater capacity to metabolize diclofop than did wild oat (ED_{50}=1.0 kg/ha). Due to spray retention and penetration factors,approximately 20 times as much toxicant penetrates green foxtail leaves as penetrates wild oat leaves. Based on equivalent internal concentrations of herbicide green foxtail is more tolerant to diclofop methyl than is wild oat. This tolerance is based on the greater ability of green foxtail to degrade diclofop.

The difference in diclofop half-life in wheat (6.1 h) and wild oat (15.0 h) would result in a level of diclofop 800% higher in wild oat than in wheat 30 hours after application. Even relatively small differences in the

degradation half-life of diclofop may be critical with respect to the duration of exposure of the site of action to lethal concentrations of toxicant.

Further support for a metabolic basis for selectivity of diclofop methyl comes from the studies of Owino (1977) who observed that toxic effects on cell division similar to those observed in susceptible wild oat could be generated in tolerant wheat or barley by raising the concentration of toxicant or extending the duration of exposure to the herbicide. These procedures undoubtedly overwhelmed the ability of wheat and barley to detoxify diclofop, resulting in a prolonged exposure of the site of action to lethal concentrations of toxicant. Clearly, tolerant species do possess the site for diclofop action. Inherent differences in the ability of diclofop to combine with its site of action may exist. In species where the site of action had low affinity for diclofop higher concentrations of diclofop would be required to generate toxic effects. Determination of the relative contributions of differential metabolism and differential affinity of the site of action for the toxicant awaits characterization of the site of action of diclofop at a subcellular level.

Detoxification of diclofop in plants involved degradation via two major pathways. In wheat the major degradative pathway involved hydroxylation of the dichlorophenyl moiety, yielding hydroxy-diclofop. Hydroxy-diclofop was subject to conjugation to plant constituents. The major degradation pathway in

barley involved removal of the propionic acid moiety, yielding phenoxy-phenol. Phenoxy-phenol was also subject to conjugation with free sugars or other compounds.

The ring hydroxylation reaction which yields hydroxy-diclofop was observed in wheat (Gorbach *et al.*, 1977). Degradation of the propionic acid side chain by plants has not been reported. Smith (1977) and Martens (1978) have reported metabolism of diclofop to phenoxy-phenol in soils. Smith postulated that the reaction proceded via decarboxylation yielding 4-(2,4-dichlorophenoxy) phenetole as an intermediate. The phenetole was then postulated to undergo direct conversion to the corresponding phenol. The phenetole was not persistent enough in the soil to permit accumulation to levels which were sufficient to confirm its presence in the soil. Martens suggested that direct cleavage of the phenoxy-propionic acid ether linkage may occur. Studies of the biological degradation of phenoxy-alkanoic herbicides indicate that when the alkanoic side chain was acetic acid, the ether linkage between the phenyl ring and the side chain was rapidly cleaved. Substitution of a methyl group for a hydrogen at the number 2 carbon atom of the acetic acid side chain, thereby creating an α-substituted propionic acid, blocked cleavage of the ether linkage (Alexander and Aleem, 1961). These observations support the two step degradation scheme for the removal of the propionic acid side chain of diclofop as postulated by Smith.

Differential degradation of diclofop by wheat and barley may relate to the relative tolerance of these species to diclofop methyl. The observation that in barley, degradation of diclofop involved metabolism of the aliphatic side chain may indicate that the enzyme which catalyzes ring hydroxylation of diclofop is of limited abundance in barley, or, if present has a lower affinity for diclofop than does the analagous enzyme in wheat. Such differences would cause the level of diclofop in barley to rise until the threshold level required for the action of the propionic acid decarboxylation enzyme was reached. The decarboxylation enzyme in barley would have a lower affinity for diclofop than would the hydroxylation enzyme in wheat. The resultant higher levels of diclofop in barley would be associated with a lower tolerance of this species to diclofop methyl.

Wild oat and green foxtail were able to metabolize diclofop, but at a rate much lower than that observed in wheat and barley. The observed reduction in diclofop methyl toxicity to wild oat at higher temperatures (Chow, 1978) may have been the result of a temperature induced increase in the rate of metabolism of diclofop by the wild oat plants. Such observations provide further support for the hypothesis that tolerance to diclofop methyl is primarily related to species ability to degrade diclofop.

Conjugation of diclofop and its degradation products

to cell constituents was observed in all four species. The extent of conjugate formation was almost as great in wild oat and green foxtail as it was in wheat and barley (Chapters 3, 4, and 5). The precursor-product relationships suggest that in susceptible species diclofop was conjugated directly to plant constituents. In tolerant species degradation of diclofop to hydroxy-diclofop or phenoxy-phenol precedes conjugation.

Conjugation of herbicides to sugars, amino acids, and other plant constituents is considered to be a detoxification reaction (Frear, 1976). However it has not been established whether or not herbicide conjugates are subject to hydrolysis within plants. If the diclofop-conjugate is subject to hydrolysis within plants, it could represent a pool of temporarily inactivated toxicant within the plant. Release of this toxicant could extend the duration of exposure of the site of action to the herbicide.

The selectivity of root-applied diclofop methyl among wheat, barley, wild oat, and green foxtail was also related to species differences with respect to diclofop degradation (Chapter 5). Within species, differences in the sensitivity of roots and shoots to diclofop methyl were related to the relative abilities of these organs to degrade diclofop. Metabolism of diclofop by shoots was a more important factor in the overall tolerance of a given species to root-applied diclofop methyl than was metabolism of diclofop by the roots. Death of the shoot apical meristem is

required if regrowth of the plant is to be avoided. Grassy weeds which have tillered (developed additional shoot meristems) are not controlled by post emergence applications of diclofop methyl (Hoechst Canada Inc., 1979).

Reductions in the translocation of diclofop to the shoot apical meristem reduce diclofop methyl toxicity to wild oat (Chapter 6). Although an effect of 2,4-D on the de-esterification of diclofop methyl was observed, it was not established whether the increased diclofop methyl levels in the tissue were responsible for reduced diclofop translocation or *vice versa*. Hill *et al.* (unpublished data), working with a crude esterase enzyme preparation, found no effect of 2,4-D on diclofop methyl de-esterification *in vitro*. This result suggests that a 2,4-D related inhibition of translocation represents the basis of the antagonistic effect of 2,4-D on diclofop methyl activity. Pretreatment of plants with phenoxy-acid herbicides including 2,4-D, MCPA, and MCPB reduced translocation of subsequently applied aminotriazole and maleic hydrazide. Transport of sugars synthesized from $^{14}CO_2$ released from urea was also reduced (Robertson and Kirkwood, 1970). Reduced transport of these phloem-mobile compounds was attributed to an uncoupling of oxidative phosphorylation by 2,4-D.

The reduced activity of other post-emergence wild oat herbicides when applied in combination with phenoxy-

herbicides may also be attributable to reduced translocation. Barban, benzoylprop ethyl, flamprop methyl, difenzoquat, and diclofop methyl all affect apical function in susceptible species, all exhibit limited transport from the leaves to the apical region, and the activity of all of these herbicides is reduced to some extent when they are applied in combination with 2,4-D, MCPA, or dicamba.

SUMMARY AND CONCLUSIONS

The selectivity of diclofop methyl among wheat, barley, wild oat, and green foxtail depends primarily upon the differential metabolism of diclofop in these species. In wheat and barley,degradation of diclofop proceeds rapidly enough to prevent accumulation of the toxicant in sensitive meristematic areas. In wild oat diclofop degradation proceeds slowly and consequently toxic levels of the herbicide accumulate within the plant. Green foxtail is able to detoxify diclofop more rapidly than is wild oat, but greater spray retention by and penetration into green foxtail leaves overwhelms the detoxification mechanism. Rapid membrane disruption precludes metabolism of the herbicide by green foxtail. Consequently, green foxtail is more sensitive to foliar-applied diclofop methyl than is wild oat.

Detoxification of diclofop proceeds via dissimilar metabolic pathways in wheat and barley. In wheat, diclofop undergoes ring-hydroxylation yielding hydroxy-diclofop. In barley, degradation of the propionic acid moiety represents an inportant detoxification reaction.

Root absorption of diclofop methyl was dependent upon the concentration of herbicide in the treatment solution and the uptake of water by the plants. Selectivity

of root-applied diclofop methyl is the result of species differences with respect to diclofop degradation. Roots and shoots of a given species may differ with respect to their ability to degrade diclofop.

The herbicidal action observed following diclofop methyl application to susceptible species represents the combined toxic effects of the applied methyl ester and its free acid, diclofop. Diclofop methyl is a contact herbicide which destroys cell membrane integrity, resulting in chlorosis and the development of necrotic lesions on the plant foliage. In plants diclofop methyl is rapidly hydrolyzed to diclofop. Diclofop is a systemic toxicant which inteferes with cell division and elongation processes in meristematic zones. Interruption of cell division and elongation processes by diclofop results in growth stagnation and ultimately in the complete collapse of the plant.

Effective grassy weed control following diclofop methyl application depends upon the systemic transport of diclofop. Interruption of diclofop transport to shoot and root meristems as a result of 2,4-D application results in reduced toxicity of diclofop methyl to wild oats.

R-25788 and NA(1,8-naphthalic anhydride) protect corn and oats from the sytemic toxic action of diclofop but do not prevent the contact action of the methyl ester. These compounds may represent a tool for investigating the modes of action of diclofop methyl and diclofop individually.

SUGGESTIONS FOR FURTHER WORK

In order to evaluate the relative contributions of differential metabolism and differential binding at the site of action to diclofop methyl selectivity among grasses, further work is required to establish the site of diclofop action at a subcellular level.

As less than two percent of the diclofop deposited on the plant reaches sensitive meristematic areas, a considerable increase in herbicidal efficiency could be achieved if a larger proportion of the applied dose could be delivered to the site for diclofop action within the plant. Such improvements in the efficiency of symplastic transport of diclofop in plants await a clearer understanding of transport processes within plants.

Further work is required to establish whether the observed antagonistic effect of 2,4-D on diclofop methyl de-esterification preceeds the interruption in basipetal diclofop transport. It is possible that the effect of 2,4-D on diclofop methyl metabolism results from reduced transport of diclofop away from the site of application.

Further studies are required to identify the conjugation products of diclofop and its metabolites within plants. Determination of the biological activity of diclofop conjugates

and measurements of the rate of hydrolysis of diclofop conjugates would further our understanding of the contribution of differential metabolism to the selective toxic action of diclofop methyl.

An evaluation of the basis of selectivity of diclofop methyl among a susceptible monocot species and a tolerant dicot species would indicate whether the tolerance of broadleaf species to diclofop methyl arises as a result of the ability of these species to degrade diclofop. Perhaps three-carbon-compounds such as propionic acid play a more important role in the primary metabolism of monocots, thereby rendering monocots inherently more sensitive than dicots to metabolic disruption by propionic acid derivatives. Dicots are generally more sensitive to acetic acid derivatives than are monocots. Basic physiological studies are required to clarify these relationships.

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