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

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Barry Gordon Todd

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

Department of Plant Science

October 1979

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

BY

BARRY GORDON TODD

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGMENTS

I extend my sincere thanks to my advisor, Dr. Elmer Stobbe, who seemed always to know when a few words of encouragement were needed. Elmer unselfishly arranged and re-arranged his personal and work schedules in order to assist me in the preparation of this thesis. The interest Elmer has taken in my personal, as well as my academic, development has been greatly appreciated.

I would like to thank Dr. Gerald Stephenson for his advice and support during my stay at the University of Guelph. I only wish I had stayed longer!

I have greatly appreciated the advice and efforts of my committee members, Drs. J. Jamieson, L. LaCroix, and W. Woodbury.

The friendship and cooperation of my fellow students will never be forgotten. Barrie Forbes, Bernie Hill, Elaine Johnson, Henry Nelson, Martin Owino, Mervin Pritchard, Ross Rankin, Allan Sturko, Larry Taylor, and David Vanstone all enriched my term at the University of Manitoba.

Thanks to Allan Brown, Yvonne Dirkes, Nancy Eisner, Ed Makowski, Ruth Makowski, and Elise Pelly for their help and friendship during my stay at the University of Guelph.

Radioactive and technical diclofop methyl, reference

standards of diclofop methyl metabolites, and financial assistance were generously supplied by Hoechst AG. The continued interest of Hoechst personnel in this project provided an important source of moral support.

The financial assistance provided by a National Research Council of Canada post-graduate scholarship is gratefully acknowledged.

Above all, I would like to thank my wife, Sandra, for her patience, understanding, love, and continual encouragement, without which the completion of this thesis would not have been accomplished.

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.