methrin than from those treated with *cis*-permethrin. Overall, the synergist pretreatments reduced metabolism of all permethrin preparations. The figure also indicates that synergist pretreatments had less effect on metabolism of *cis*-permethrin than on metabolism of *trans*-permethrin and less effect on metabolism in the bollworm than with the tobacco budworm.

DISCUSSION

Both hydrolytic and oxidative reactions are involved in permethrin metabolism by the bollworm and the tobacco budworm. The use of synergist pretreatments reduced permethrin metabolism in both species. The effect was greater in the tobacco budworm than in the bollworm and greater with *cis*- than with *trans*-permethrin.

The present study shows that in *Heliothis*, as in rats, *cis*-permethrin is more resistant to metabolism than *trans*-permethrin. Tobacco budworm larvae metabolized both isomers more effectively than did bollworm larvae.

Elliot et al. (1976) and Gaughan et al. (1977) concluded that permethrin is highly biodegradable in mammals. The present study indicates a much greater degree of permethrin stability in *Heliothis*. This differential stability between insect and mammal is an appropriate property for a candidate insecticide.

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Wild Oat Herbicide Studies. 2. Physiological and Chemical Changes in Barley and Wild Oats Treated with Diclofop-methyl Herbicide in Relation to Plant Tolerance

P. N. P. Chow* and D. E. LaBerge

Diclofop-methyl [methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate], a selective, postemergence herbicide for the control of annual grassy weeds, was studied, using greenhouse seedlings of moderately resistant barley (*Hordeum vulgare* L.) and susceptible wild oats (*Avena fatua* L.). More physiological and chemical changes were noted in wild oats treated with diclofop-methyl than in barley, including reduced growth of shoots and roots, decreased chlorophyll content and photosynthetic activity, inhibition of photosynthate translocation to the roots, reduction of adenosine 5'-triphosphate (ATP) content and accumulation of sugars in the shoots. These differential changes were closely related to the tolerance of barley and wild oats to diclofop-methyl. The changes associated with herbicidal action are discussed.

Wild oats are extensively distributed on the agricultural lands of the northern temperate regions of the world (Thurston and Phillipson, 1976). Herbicides are widely used to control this pest in crops. Diclofop-methyl was recently registered in Canada and several European countries for the control of wild oats and other annual grassy weeds. This herbicide not only has good selectivity between mono- and dicotyledonous families but also adequate selectivity within monocotyledons (Chow, 1978). The latter selectivity is sufficient to control wild oats in cereals. The differential retention of applied diclofop-methyl on the leaves of four gramineous species was a contributing factor in the selectivity (Todd and Stobbe, 1977). The damaged chloroplasts in wild oats treated with diclofop-methyl were observed (Brezeanu et al., 1976). The metabolism and residues of this herbicide in wheat were reported (Gorbach et al., 1977).

Field observations indicated that a week or so after application of diclofop-methyl the leaves of susceptible species became chlorotic, mottled, then wilted and the plant had very little root development. Thus, we undertook to study the changes of the parameters such as

Research Station, Research Branch, Agriculture Canada, Brandon, Manitoba R7A 5Z7, Canada (P.N.P.C.) and the Grain Research Laboratory, Agriculture Canada, Winnipeg, Manitoba R3C 3G9, Canada (D.E.L.).

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Table I. Effect of Diclofop-methyl on Tolerance of Barley and Wild Oats 11 Days after the Application

diclofop-methyl, kg/ha	fresh weight, g					
	total		shoots		roots	
	barley[a]	wild oats	barley	wild oats	barley	wild oats
0 (control)	15.6 a	10.7 a	10.7 a	7.1 a	4.9 a	3.6 a
0.84	14.4 a	7.4 b	9.9 a	5.0 b	4.5 a	2.4 b
	(92.3)[b]	(69.2)	(92.5)	(70.4)	(91.8)	(66.7)
1.12	14.9 a	6.9 b	10.4 a	4.8 b	4.5 a	2.0 b
	(95.5)	(64.5)	(97.2)	(67.6)	(91.8)	(55.6)

[a] In each column, values (fresh weight) followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). [b] In each column, relative values in parentheses represent changes against the control.

chlorophylls, photosynthesis, phloem translocation, ATP, and sugars in six-row barley (cv. Conquest), a moderately resistant crop, and wild oats, a susceptible grassy weed, treated with diclofop-methyl. These changes may be related to plant tolerance to diclofop-methyl. This may also lead to a better understanding of the physiological reactions of gramineous species to this new class of herbicides.

MATERIALS AND METHODS

Plant Cultures and Herbicide Application. Seeds of barley and wild oats were germinated and grown in washed white quartz sand in the greenhouse for 9 to 12 days. Uniform seedlings were transferred to nutrient solutions for growth to the three-leaf stage for treatments. The aerated nutrient solutions (pH 5.5 for barley and pH 5.0 for wild oats) were prepared daily. Three seedlings were selected per treatment, and each treatment was replicated three times, except in the plant tolerance study where six replicates were used. The seedlings were grown under a light intensity of 16500 lx with a 16-h photoperiod. Temperatures ranged from 22 to 26 °C during the day and 14 to 22 °C at night. Herbicide solutions, prepared with diclofop-methyl (36% emulsifiable concentrate) without a wetting agent, were applied at a rate of 200 L/ha at a pressure of 2.1 kg/cm^2 on the foliage of seedlings at the three-leaf stage of growth as applied in the field. Control plants were not sprayed. The plants were harvested and analyzed at the sixth to eighth day after application of the herbicide, except in the plant tolerance experiment where the plants were harvested at the eleventh day during the early spring.

Measurement of Plant Tolerance. When the treated and control plants were harvested, the roots were rinsed with tap water (20 s) and then with distilled water (10 s). Excess water was removed by blotting the roots on a paper towel. Seedlings were separated into shoots and roots, and the weights of the tissues were determined.

Determination of Chlorophylls. Ten grams of the apical portion of fresh shoots of the treated and control plants were blended for 2 min with cold 80% acetone, and the extract was filtered. The chlorophyll filtrate was diluted with 80% acetone to a volume equivalent of 1 g of fresh weight to 100.0 mL of acetone and measured at 645 and 663 nm using a Hitachi-Perkin-Elmer UV-VIS spectrophotometer (Arnon, 1949; Bruinsma, 1963). The whole procedure was performed in dim light to minimize breakdown of chlorophylls (Bruinsma, 1963).

Estimation of $^{14}CO_2$-Photosynthesis and ^{14}C-Phloem Translocation. The method described by Chow et al. (1966) was employed using $^{14}CO_2$ to measure photosynthetic activity and phloem translocation in the plant. One hundred μCi $^{14}CO_2$ (sp act. 100 μCi/mmol) was generated from sodium [^{14}C]bicarbonate in a transparent plexiglass chamber (0.8 m^3) at 21 °C and 8300 lx. A small fan in the chamber circulated the $^{14}CO_2$ to the plants for 0.5 h. The plants were removed from the chamber and exposed to light for 1.5 h to permit further photosynthesis and ^{14}C-photosynthate translocation. Seedlings were then segmented into shoots and roots. The tissues were extracted with 80% ethanol. After decoloration with 0.5% benzoyl peroxide (Chow, 1977), the radioactivity of the ethanolic solutions was measured by liquid scintillation counting in a toluene–Triton scintillation solution, which consisted of two volumes of toluene scintillation solution and one volume of purified Triton X-100 (octylphenoxypolyethoxyethanol) (Patterson and Greene, 1965).

Table II. Effect of Diclofop-methyl on Chlorophyll Content in Shoots of Barley and Wild Oats 8 Days after the Application

diclofop-methyl, kg/ha	Chl a, mg/L	Chl b, mg/L	Chl a + b, mg/L	Chl a/b
		barley		
0 (control)	7.47 ± 1.5[a]	2.38 ± 0.3	9.85	3.14
1.12	7.37 ± 1.8	2.42 ± 0.6	9.79	3.05
	(98.7)[b]	(101.7)	(99.4)	
		wild oats		
0 (control)	7.14 ± 2.9	2.29 ± 4.1	9.43	3.12
1.12	4.21 ± 3.3	1.42 ± 1.9	5.63	2.96
	(59.0)	(43.7)	(59.7)	

[a] In each column, values are the treatment mean ± coefficient of variability (%). [b] In each column of each species, relative values in parentheses represent changes against the control.

Analysis of ATP level. After harvesting, fresh tissues of treated and control plants were weighed, frozen in liquid nitrogen, freeze-dried, and then ground to a particle size passing through a 420-μ sieve. Adenosine 5′-triphosphate (ATP) was extracted from dried tissue by boiling in distilled water at 100 °C for 1 min, followed by rapid cooling with ice (Stewart and Guinn, 1969; Ching and Ching, 1972). ATP was determined by using the luciferin–luciferase technique (St. John, 1970). Luciferin–luciferase from fireflies (Sigma Fle-50) was diluted in HEPES–$MgSO_4$ buffer (pH 7.5) containing bovine albumin and PVP-10. When ATP in aqueous extracts reacted with this enzyme preparation, the emitted light was measured over a 15-s interval with a Chem-Glow photometer (American Instrument Co.). A standard curve was prepared relating intensity of light emission to ATP levels in the range of 0.1 to 1.5 μg/mL of ATP.

Assay of Sugar Contents. Sugars were extracted from fresh tissues of treated and control plants with 80% ethanol. The extracts were dried in a rotory evaporator, and the residues were dissolved in 10.0 mL of distilled water. The aqueous extracts were filtered through a millipore filter (pore size, 0.8 μm). Sugars were separated as borate derivatives by anion-exchange column chromatography, and the separated sugars were determined quantitatively by continuously monitoring the column

Table III. Effect of Diclofop-methyl at 1.12 kg/ha on Photosynthesis and Photosynthate Translocation in Barley and Wild Oats 6 Days after the Application

diclofop-methyl, kg/ha	$^{14}CO_2^-$ photosynthesis in shoots		^{14}C translocation to roots	
	barley	wild oats	barley	wild oats
	radioactivity dpm/g fr. wt. ± c.v. %	radioactivity dpm/g fr. wt. ± c.v. %	radioactivity dpm/g fr. wt. ± c.v. %	radioactivity dpm/g fr. wt. ± c.v. %
0 (control)	292 703 ± 1.5	759 638 ± 1.8	84 181 ± 1.4	200 723 ± 0.7
1.12	294 758 ± 1.8 (100.7)[a]	576 075 ± 2.7 (75.8)	71 184 ± 0.7 (84.6)	74 434 ± 1.8 (37.1)

[a] In each column, relative values in parentheses represent changes against the control.

Table IV. Effect of Diclofop-methyl on ATP Level in the Shoots of Barley and Wild Oats 7 Days after the Application

diclofop-methyl, kg/ha	ATP, μg/g dry wt ± c.v. (%)	
	barley	wild oats
0 (control)	103.8 ± 1.6	826.6 ± 1.5
0.84	106.0 ± 5.2 (102.1)[a]	721.5 ± 2.8 (87.3)
1.12	105.0 ± 1.1 (101.2)	461 ± 3.4 (55.9)

[a] In each column relative values in parentheses represent changes against the control.

effluent at 420 nm following addition of 0.1% orcinol in 70% sulfuric acid and heating to develop the color (LaBerge et al., 1973).

RESULTS

Plant Tolerance. The application of diclofop-methyl at rates of 0.84 and 1.12 kg/ha to plants demonstrated that 11 days after treatment the shoots and roots of barley were affected only slightly, while wild oats were stunted and chlorotic resulting in marked weight reduction (Table I). It is clear that there is a differential response in these two gramineous species to diclofop-methyl.

Chlorophyll Content. The content of chlorophylls a and b of wild oat shoots treated with diclofop-methyl at 1.12 kg/ha decreased after 8 days by 41% and 56%, respectively, with a coincident appearance of chlorosis, whereas the chlorophyll content in treated barley virtually did not change (Table II). The ratio of chlorophylls a to b decreased slightly indicating a similar influence of diclofop-methyl on conversion of chlorophyll a to chlorophyll b in both species.

Photosynthesis and Phloem Translocation. There was no change in photosynthesis in barley shoots for 6 days following herbicide appication at a rate of 1.12 kg/ha (Table III). By contrast, the photosynthetic rate in wild oat shoots was inhibited by 24% at corresponding days. Based on ^{14}C activity detected in roots, there was a 15% inhibition of phloem translocation of photosynthates from shoots to roots in barley and a 63% inhibition of translocation in wild oats.

ATP Production. Virtually there were no changes of ATP in barley shoots 7 days after treatment with diclofop-methyl as compared to the control plants. In contrast with barley, levels of ATP in shoots of wild oats decreased sharply with the greatest decrease (44%) occurring at 1.12 kg/ha of herbicide application (Table IV).

Sugar Content. The content of sucrose, glucose, fructose, raffinose, maltose, and ribose in the shoots of barley generally increased after 7 days following treatment with diclofop-methyl when compared to control plants (Table V). By contrast, the increase in sugar content in treated wild oat shoots was greater than in barley. The sugars, sucrose, glucose, and fructose, were greatly increased but glucose and fructose levelled off at the higher herbicide rate (1.12 kg/ha).

DISCUSSION

This study shows that there is a pronounced reduction in chlorophyll content and inhibition of photosynthesis in wild oats treated with diclofop-methyl (Tables II and III). These are in agreement with electron microscopic result that a major effect of this herbicide on wild oats is an apparent destruction of the chloroplasts (Brezeanu et al., 1976). Our data also indicate that there is an accumulation of sugars in treated wild oat shoots (Table V) and reduced translocation of photosynthates to the roots (Table III) resulting in poor development of the root system (Table I). The adverse effects were less in the treated barley, which is coincident with the higher tolerance to diclofop-methyl. These results suggest that diclofop-methyl may damage the normal functioning of phloem cells resulting in plugging of the cell causing sucrose accumulation in the shoots. Several herbicides including 2,4-D (Eames,

Table V. Effect of Diclofop-methyl on Sugar Content in Shoots of Barley and Wild Oats 7 Days after the Application

diclofop-methyl, kg/ha	sugar content, μg/g fresh wt					
	sucrose	glucose	fructose	raffinose	maltose	ribose
			barley			
0 (control)	446	429	248	115	52	27
0.84	461 (103.4)[a]	651 (151.8)	413 (166.5)	147 (127.8)	53 (101.9)	34 (125.9)
1.12	639 (143.3)	666 (155.2)	400 (161.3)	339 (294.8)	80 (153.8)	34 (125.9)
			wild oats			
0 (control)	601	882	402	Tr[b]	54	Tr
0.84	1590 (264.6)	1833 (207.8)	769 (191.3)	Tr	Tr	Tr
1.12	1989 (330.9)	1491 (169.0)	698 (173.6)	Tr	Tr	Tr

[a] In each column of each species, relative values in parentheses represent changes against the control. [b] Trace amount measured.

1950; Leonard et al., 1967; Swanson, 1946), picloram (Leonard et al., 1967), and maleic hydrazide (Currier et al., 1951) also affect the phloem transport system.

Furthermore, treatment of wild oats with diclofop-methyl results in a large decrease in ATP production in the shoots (Table IV). Reduction in ATP production in the shoots of treated plants may restrain a number of physiological activities in the tissues. ATP is required to energize the movement of photosynthates in phloem tissues (Kursanov and Brochenko, 1961; Rathnam and Das, 1975). Reduced translocation of photosynthates to roots by reduced ATP may retard root development (Table I).

Sucrose is a major carbohydrate translocated by several plant species including wheat and barley (Edelman et al., 1959) and sugarcane (Hartt et al., 1963). Sucrose accumulated in the shoots of wild oats treated with diclofop-methyl (Table V), and this may be expected if sucrose is also the major carbohydrate translocated in wild oats. The conversion of glucose to sucrose also requires ATP (Edelman et al., 1959). Therefore, an accumulation of glucose in shoots of treated wild oats may be partly caused by a reduction in ATP synthesis.

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Residues of Ethylenebis(dithiocarbamate) and Ethylenethiourea in Treated Tomatoes and Commercial Tomato Products

Brian D. Ripley* and Diane F. Cox

Residues of ethylenebis(dithiocarbamates) (EBDC) and ethylenethiourea (ETU) were monitored in tomatoes after application of several EBDC formulations from 1973 to 1977. After spraying at recommended rates, residues of EBDC on tomatoes were below the current Canadian tolerance of 4 ppm at the recommended harvest interval. ETU was detected during the analysis period at levels of <0.05 ppm on tomatoes whereas ETU residues in tomato juice and whole pack products, prepared from the treated tomatoes in the first 3 days after EBDC application, ranged from not detected (<0.01) to 0.17 ppm. Commercial tomato products contained traces of EBDC (<0.2 ppm) and ETU residues of ≤0.03 ppm. Boiling of some samples demonstrated additional ETU formation.

The ethylenebis(dithiocarbamates) (EBDC) are an agriculturally important group of fungicides, but their continued use is in jeopardy because ethylenethiourea (ETU; 2-imidazolidinethione) has been shown to be associated with these compounds. ETU is a degradation product in EBDC formulations, may be formed from EBDC by aeration or during cooking, and is present in most EBDC-treated crops (Federal Register, 1977). There is also evidence to suggest that long-term consumption of foods containing ETU could be inimical to health although Graham et al. (1975) concluded from a 2-year feeding study that ETU was not biologically deleterious to the rat at concentrations of 5 and 25 ppm.

One of the important crops requiring protection from fungal attack is tomatoes. Diseases such as early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), and anthracnose (caused by *Colletotrichum phomoides*) are common on tomatoes, and broad spectrum fungicides such as the EBDC provide effective control. Although other fungicides are recommended (Publication 363, 1977) for the control of these diseases, they may not always be viable because of cost, the possibility of skin irritation, or the lack of season-long control.

Residues of EBDC and ETU in tomatoes have been reported (Engst et al., 1968; Newsome et al., 1975; Newsome, 1976; Pease and Holt, 1977), but because of the range of results and the need for additional data (Federal

Provincial Pesticide Residue Testing Laboratory, Ontario Ministry of Agriculture and Food, c/o University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

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