Table III. Recovery of Residues of 2,4-D from Urine and Hand-Rinse Samples after Regeneration and Reuse of Sep-PAK Cartridge

no. of times Sep-PAK used	% recovery	
	urine (0.5 μg/mL)[a]	hand rinses (2.0 μg/mL)[b]
1	88 ± 8	94 ± 2
2	93 ± 4	86 ± 8
3	94 ± 9	80 ± 5
4	90 ± 9	95 ± 4
5	90 ± 13	100 ± 0

[a] These results are the average of four analyses ± standard deviation. [b] These results are the average of three analyses ± standard deviation.

This work demonstrates a procedure for the efficient determination of residues of 2,4-D and its esters and dimethylamine salt in dermal exposure pads, hand-rinse samples, urine, and perspiration samples based on direct methylation of these compounds with BF_3 and the rapid cleanup of samples with a commercially available cartridge of reverse-phase liquid chromatographic support (Sep-PAK). At present, this procedure is being successfully applied to the analysis of exposure samples collected from monitoring studies of agricultural workers involved in the application of these compounds. The results of these studies will be published later.

ACKNOWLEDGMENT

We thank Wilbur Aller of this laboratory for laboratory assistance in this work.

Registry No. 2,4-D, 94-75-7; 2,4-D isooctyl ester, 25168-26-7; 2,4-D butoxyethanol ester, 1929-73-3; 2,4-D dimethylamine salt, 2008-39-1.

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Received for review July 12, 1982. Accepted February 7, 1983. This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under FIFRA as amended. Also, mention of a commercial product in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture.

Wild Oat Herbicide Studies. 3. Physiological and Biochemical Bases for Interaction of Barban and Growth Regulator Herbicides in Wild Oat

Paul N. P. Chow* and H. Frank Taylor

The instability of barban (4-chloro-2-butynyl *m*-chlorocarbanilate) in tank mixtures with growth regulator herbicides was not a factor contributing to the antagonism between barban and growth regulator herbicides. The antagonism occurred within the plant. Of five growth regulator herbicides, the antagonism was greatest in the mixture of barban/2,4-D[(2,4-dichlorophenoxy)acetic acid] isopropyl ester and 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] triethylamine/barban. Penetration and translocation of [^{14}C]barban in the leaves and to the meristematic tissue of the growing point were inhibited by 2,4-D ester. The meristematic tissue was stimulated to produce [^{3}H]DNA from a precursor, [^{3}H]thymidine, by 2,4-D ester, but inhibited by barban and the barban/2,4-D mixture. It may be concluded that the reduction of barban activity in wild oat (*Avena fatua* L.) by the growth regulator herbicides appeared to be associated with two factors, (1) physiologically reduced penetration and translocation of barban in the leaves to the meristematic tissue of the growing point and (2) biochemically stimulated DNA synthesis by 2,4-D to counteract the inhibitory effect of barban in meristematic tissue.

An ideal herbicide spray effectively controls grass and broadleaf weeds simultaneously. This may be achieved by using a herbicide mixture because most herbicides developed as grass killers control wild oat only. Considerable interest has been shown in package mixtures in recent years. However, herbicides such as barban in mixture with a growth regulator herbicide often reduces wild oat control (Holroyd, 1960; Pfeiffer et al., 1960). The interaction which reduces weed control in herbicide mixtures is defined as antagonism (Colby, 1967).

The metabolism of barban (Shimabukuro et al., 1976; Still and Mansager, 1972) and selectivity of barban between oat or wild oat and cereal crops have been studied (Jacobsohn and Andersen, 1972; Kobayashi and Ishizuka, 1974; Shimabukuro et al., 1976). Barban inhibited synthesis of proteins and nucleic acids (Chow, 1982; Kobayashi

Agriculture Canada Research Station, Brandon, Manitoba, R7A 5Z7 Canada (P.N.P.C), and Agricultural Research Council, Weed Research Organization, Oxford, OX5 1PF U.K. (H.F.T.).

0021-8561/83/1431-0575$01.50/0 © 1983 American Chemical Society

and Ishizuka, 1974), and barban and other carbamate herbicides also inhibited mitosis (Hilton et al., 1963).

Barban has been used for wild oat control for two decades, but little is known about the nature of the interaction of barban mixtures in wild oat. The objectives of this study were to locate the site of the interaction of barban/growth regulator herbicide mixtures in wild oat and to examine the effect of some growth regulator herbicides on penetration and translocation of barban and on DNA synthesis in meristematic tissue. All these factors may be associated with antagonism.

MATERIALS AND METHODS

Measurement of Stability of Barban Mixtures. To study the stability of barban in mixtures, a commercial formulation of barban (12.5% emulsified concentrate with red dye) was used. Barban alone or in mixtures was made equivalent to a 0.35 kg/ha rate and diluted with distilled water in which [*phenyl*-^{14}C]barban (10 μCi/mg) as a monitoring agent was included. After growth regulator herbicides were added at a 0.35 kg/ha rate, the solutions were agitated by shaking vigorously. Samples of the solutions were periodically withdrawn and the radioactivity in the solutions was counted by the standard liquid scintillation counting technique.

Measurement of Effect of Barban Mixtures on Wild Oat. A series of 20 pot experiments was conducted in the greenhouse at the Weed Research Organization in England. Wild oat seeds were sown in pots containing 400 g of sandy loam (10% clay, 10% silt, 60% coarse sand, 20% fine sand, 2.5% organic matter, and pH 7.2). After germination, seedlings were thinned to four plants per pot. In all experiments, the herbicide solutions were applied as a postemergence treatment at the 1.5–2-leaf growth stage. In some specific studies the soil surface of pots was covered with vermiculite to avoid herbicide contamination of the soil during the application. When two herbicide solutions were applied to different leaves, one leaf was covered with aluminum foil. When two herbicide solutions were individually applied to a single leaf, 20–30 min elapsed between the applications to avoid over wetting the leaf surface. A commerical formulation of barban (12.5% a.i.) was used. Barban alone or mixtures diluted in 200 L/ha of demineralized water was applied at 310-kPa pressure by using a Teejet 8001 nozzle, while other herbicide solutions in 350 L/ha demineralized water were applied at 207-kPa pressure with a Teejet 8002 nozzle. The differential volumes and pressures used were based on recommendations for barban and growth regulator herbicide applications. Three weeks after application, foliage fresh weight was measured to assess antagonism. The antagonism was determined by using the method of Colby (1967). Each experiment was arranged in a randomized complete block with four to seven replications depending on the experiment and repeated at least once.

Measurement of Penetration and Translocation of [^{14}C]Barban. When wild oat seedlings were at the 2-leaf growth stage, 2,4-D isopropyl ester (70% in emulsified concentrate) was applied as previously mentioned. Thirty minutes later, 10 spots of [*phenyl*-^{14}C]barban at 5 μL (10 spots each having 0.5 μL) were placed on the middle portion of the first leaf or 5 spots were placed on each leaf depending on the experiment. A microsyringe mounted on a Burkard applicator was used to place the spotting. At different time intervals after application, the leaves were sectioned in several portions designated as A (apical tip above the treated portion), B ([^{14}C]barban-treated portion), C (lower portion below the treated portion), D (meristematic tissue of the growing point), and E (new leaf of the tiller produced after treatment). After the leaves were sectioned, the ^{14}C-treated portion was washed with 70% ethanol for 1 min to collect the unabsorbed [^{14}C]-barban. Each portion of the tissue was placed in a liquid scintillation vial and digested in diluted solubilizer (Soluene/toluene at 1/1 by volume) for 2 days at room temperatures, followed by bleaching with saturated benzoyl peroxide in toluene for 2–3 days. Samples were mixed with 10 mL of acidified toluene scintillation solution–Triton X-100 at 2/1 by volume, and the radioactivity was counted.

Measurement of [^{3}H]Thymidine Incorporated into [^{3}H]DNA. Each of barban, 2,4-D ester, and the barban/2,4-D ester mixture was applied at 0.35 kg/ha to wild oat at the 2.5-leaf stage. Seven days after application, eight sections of meristematic tissue including untreated control were excised and incubated at 21 °C for 2 h in 1 mL (pH 6.5) of 0.005 M Tricine buffer containing 2% sucrose and 0.42 μCi of [^{3}H]thymidine (52 Ci/mmol), a precursor of DNA. The sections were washed 3 times with buffer, ground in a mortar with cold Cl_3AcOH (5%), and allowed to precipitate overnight in the refrigerator at 4 °C. The homogenate samples were then filtered through a Whatman GFA glass fiber filter and washed 3 times with 5% Cl_3AcOH and with a final wash with 80% ethanol to remove lipids, sucrose, and amino acids. The [^{3}H]DNA samples on the filter disks were dried and the radioactivity was counted by the standard liquid scintillation counting technique.

Table I. Reduction of [^{14}C]Barban Radioactivity in Barban and Barban Mixtures When Solutions Were Allowed to Settle

	radioactivity of [^{14}C]barban, relative value			
time, h	barban	barban + 2,4-D	barban + dicamba	barban + MCPA K
0	100	100	100	100
0.5	84	81	87	59
1.0	72	70	55	47
1.5	61	51	51	35
2.0	43	49	42	30
2.5[a]	94	101	100	105

[a] The solutions were reagitated vigorously for 2 min.

Table II. Effect of Growth Regulator Herbicides in Mixtures with Barban on Wild Oat Control

herbicide, kg/ha	shoot fr wt, g/pot	growth inhibition, %
control (unsprayed)	5.0	0
barban, 0.35	1.8	64
barban, 0.35, + 2,4-D diethanolamine, 0.35	2.7	46
barban, 0.35, + 2,4-D isopropyl ester, 0.35	3.7	26
barban, 0.35, + MCPA K, 0.35	1.9	62
barban, 0.35, + MCPA isooctyl ester, 0.35	2.2	56
barban, 0.35, + 2,4,5-T triethylamine, 0.35	3.5	30
LSD (0.05)	1.1	

RESULTS AND DISCUSSION

Stability of Barban Mixtures. The radioactivity of [^{14}C]barban in all four solutions decreased with time (Table I). However, the radioactivity in barban mixtures was recovered when the remaining solutions were reagitated after a 2.5-h period. A parallel experiment, where readings were measured every 5 min, revealed that the reduction of activity of [^{14}C]barban in barban alone or in the mixtures started as early as the first 5-min period after

Table III. Determination of Interaction between Barban and 2,4-D Isopropyl Ester on Wild Oat Control

herbicide, kg/ha	shoot fr wt, g/pot	growth inhibition, %	differential response,[a] %	interaction[a]
barban, 0	7.1	0		
barban, 0.25	2.7	62		
barban, 0.4	1.9	73		
barban, 0.55	1.8	75		
barban, 0, + 2,4-D, 0.4	5.7	20		
barban, 0.25, + 2,4-D, 0.4	5.1	28	−40.3	antagonism
barban, 0.4, + 2,4-D, 0.4	4.8	32	−46.4	antagonism
barban, 0.55, + 2,4-D, 0.4	3.6	49	−30.4	antagonism
barban, 0, + 2,4-D, 0.6	4.7	34		
barban, 0.25, + 2,4-D, 0.6	5.4	24	−50.6	antagonism
barban, 0.4, + 2,4-D, 0.6	4.5	37	−45.8	antagonism
barban, 0.55, + 2,4-D, 0.6	3.7	48	−34.4	antagonism
barban, 0, + 2,4-D, 0.8	4.3	39		
barban, 0.25, + 2,4-D, 0.8	4.5	37	−40.0	antagonism
barban, 0.4, + 2,4-D, 0.8	4.1	42	−42.2	antagonism
barban, 0.55, + 2,4-D, 0.8	4.0	44	−40.2	antagonism

[a] The calculated difference between observed response and expected response, based on Colby's formula (Colby, 1967). The values preceded by a minus sign indicate antagonism between two herbicides.

Table IV. Effect of 2,4-D Isopropyl Ester (0.3 kg/ha) on Wild Oat Control of Barban (0.35 kg/ha) at Different Times of Application

treatment	plant ht, cm	fr wt, g/pot
control (unsprayed)	38.3	0.84
barban alone	22.2	0.32
barban, 20 min, followed by 2,4-D	35.4	0.70
barban, 1 day, followed by 2,4-D	31.4	0.57
barban, 2 days, followed by 2,4-D	23.1	0.36
barban, 3 days, followed by 2,4-D	21.1	0.32
barban, 5 days, followed by 2,4-D	21.9	0.33
barban, 7 days, followed by 2,4-D	22.0	0.29
LSD (0.05)	4.6	0.16

Table V. Effect of 2,4-D Isopropyl Ester (0.7 kg/ha) on Wild Oat Control of Barban (0.35 kg/ha) Applied to Different Leaves at Different Times

treatment				
herbicide applied at				
1st leaf	2nd leaf	time of application followed by 2,4-D, day	plant ht, cm	fr wt, g/pot
control (unsprayed)			46.3	1.15
barban			18.6	0.21
barban	2,4-D	0[a]	34.8	0.70
barban	2,4-D	1	35.0	0.57
barban	2,4-D	2	25.7	0.47
barban	2,4-D	3	22.3	0.41
barban	2,4-D	4	19.9	0.31
LSD (0.05)			7.1	0.18

[a] 30 min followed 2,4-D application.

preparation of the solutions (data not presented). Apparently the barban solutions should be agitated continually during application in the field. However, in our pot experiments, the time that elapsed between filling the spraying jar and the application was not more than 1 min, so the decrease in barban activity in the spray was minimal.

Antagonism on Wild Oat Control. All five growth regulator herbicides (0.35 kg/ha) in mixture with barban (0.35 kg/ha) reduced the wild oat properties of barban (Table II). The greatest effect occurred with 2,4-D isopropyl ester and 2,4,5-T triethylamine at a significant level. The reduced wild oat control of barban with 2,4-D diethanolamine, MCPA [[(4-chloro-*o*-tolyl)oxy]acetic acid] isooctyl ester, and MCPA K herbicides was not significant. In comparison, 2,4-D amine had a lesser effect on activity of barban than 2,4-D ester. This would be expected because the ester form has stronger activity on plants than the amine form.

On the basis of Colby's formula (Colby, 1967), the interaction between barban and 2,4-D isopropyl ester was identified as antagonistic (Table III), confirming the results of early workers (Holyroyd, 1960; Pfeiffer et al., 1960). When the dose rate of barban in mixtures increased from 0.25 kg/ha to 0.55 kg/ha, the antagonistic effect of 2,4-D (0.4–0.8 kg/ha) on barban was slightly reduced but was not completely overcome.

It is necessary to determine whether antagoniam occurs in the plant. The whole plant of wild oat at the 2-leaf stage was first treated with barban (0.35 kg/ha). The treated plants then received an application of 2,4-D isopropyl ester (0.35 kg/ha) at designated periods of 20 min, 1 day, 2 days, 3 days, 5 days, and 7 days. The antagonistic effect of 2,4-D ester on barban was noticed after 20 min and 1 day and then diminished after 2 days (Table IV). In a parallel experiment, barban at 0.3 kg/ha was applied on the first leaf of wild oat, while 2,4-D was applied on the second leaf at various time intervals. The antagonism of 2,4-D on barban occurred as noted at 30 min and 1 day following the application of 2,4-D and then gradually decreased until 4 days later (Table V). The decreased 2,4-D effect on barban in this experiment appeared later than in the previous one, probably due to the time required for the movement of 2,4-D in the plant to the site of barban action. Nevertheless, the results of both experiments clearly indicated that the antagonism between the two herbicides occurred in the plant.

Effect of Penetration and Translocation of [^{14}C]-Barban. The results indicated that the penetration of [^{14}C]barban at the treated portion (B) was inhibited by 23% by 2,4-D application in comparison with the control (Table VI). The translocation to apical leaf tip (A) and down to meristematic tissue (D) was inhibited by 18% and 35%, respectively. However, the translocation of [^{14}C]-barban in the leaf was very small, the greatest movement being toward the tip of the leaf (Foy, 1961). About 95% of the applied [^{14}C]barban was still in the treated portion.

In a separate experiment, 2,4-D inhibited translocation of [^{14}C]barban (Table VII). The 2,4-D had a greater effect on translocation of [^{14}C]barban from the central portion to the meristematic portion (D), which agreed with the previous experiment. The 2,4-D reduced the radioactivity in the meristem tissue by 31% at 4.5 days after application

Table VI. Effect of 2,4-D Isopropyl Ester on Penetration and Translocation of [^{14}C]Barban in the First Leaf of Wild Oat

treatment	radioactivity in leaf portion and meristem tissue of growing point, dpm/g fr wt				B/(A + B + C + D), %
	A[a]	B	C	D	
distilled water applied on 2nd leaf [^{14}C]barban on 1st leaf 2 days later (control)	31 240	748 494	2274	3679	95.0
2,4-D applied on 2nd leaf, [^{14}C]barban on 1st leaf 2 days later	25 558 (−18)[b]	579 975 (−23)	2309 (+2)	2381 (−35)	95.3

[a] A: Apical leaf tip above the [^{14}C]barban-treated area. B: [^{14}C]Barban-treated area in the middle part of the leaf. C: Lower leaf portion below the treated area. D: Meristematic section of the growing point. [b] Relative values in parentheses represent changes against the control in percent.

Table VII. Effect of 2,4-D Isopropyl Ester on Pentration and Translocation of [^{14}C]Barban in Whole Plant of Wild Oat

treatment	radioactivity in plant tissues, dpm/g fr wt					B/(A + B + C + D + E), %
	A[a]	B	C	D	E	
measured at 4.5 days after treatment						
distilled water followed by [^{14}C]barban (control)	42 632	488 085	4856	6272	4542	89.3
2,4-D followed by [^{14}C]barban	44 003 (+3)[b]	461 631 (−5)	4910 (+1)	4358 (−31)	4315 (−5)	88.9
measured at 6.5 days after treatment						
distilled water followed by [^{14}C]barban (control)	52 220	531 480	7599	7678	8727	87.5
2,4-D followed by [^{14}C]barban	45 669 (−13)[b]	506 415 (−5)	6551 (−14)	4337 (−44)	7801 (−11)	88.7

[a] A: Apical leaf tip above the [^{14}C]barban-treated area. B: [^{14}C]Barban-treated area in the middel part of the leaf. C: Lower leaf portion below the treated area. D: Meristematic section of the growing point. E: New leaf or tiller formed after treatment. [b] Relative values in parentheses represent changes against the control in percent.

Table VIII. Effect of Barban and 2,4-D Isopropyl Ester on Incorporation of [^{3}H]Thymidine into [^{3}H]DNA in Meristematic Sections of Wild Oat

herbicide, kg/ha	radioactivity in [^{3}H]DNA	
	dpm/mg fr wt	relative value
control (unsprayed)	5123	0
barban	3498	−32
2,4-D	9803	+91
barban + 2,4-D ester	4249	−17

and 44% at 6.5 days of the application, respectively. The inhibition of [^{14}C]barban accumulation in the meristems by 2,4-D may be associated with antagonism between barban and growth regulator herbicides.

There is not a complete understanding yet as to why growth regulator herbicides inhibit the penetration and translocation of [^{14}C]barban in wild oat. The multiple actions of the growth regulator herbicides in plant cells make interpretation difficult. However, on the basis of radioautographic study, Crafts and Yamaguchi (1964) suggested that there was a plugging of phloem in the treated plant due to cell proliferation caused by 2,4-D. Results in Tables VI and VII indicated that partial plugging of phloem by 2,4-D reducing penetration and translocation of barban in leaves and to meristematic tissues might be involved.

Incorporation of [^{3}H]Thymidine into [^{3}H]DNA in Meristem Tissue. Results obtained by using the [^{3}H]-thymidine incorporation technique showed that the effects of barban and 2,4-D on [^{3}H]thymidine incorporation into [^{3}H]DNA in excised meristematic tissue of wild oat were opposite (Table VIII). The treatment with 2,4-D isopropyl ester stimulated [^{3}H]DNA synthesis from the precursor, [^{3}H]thymidine, with a 91% increase over the control, while barban inhibited the [^{3}H]DNA synthesis by 32%. The unequally opposite effects of the 2,4-D ester/barban mixture on [^{3}H]DNA synthesis resulted in a 17% inhibition of [^{3}H]DNA synthesis.

ACKNOWLEDGMENT

The study was conducted at Weed Research Organization, Oxford, England, during 1979–1980 when the senior author was granted a postdoctorate transfer-of-work project by Agriculture Canada Research Branch. Thanks are extended to Director J. D. Fryer, Dr. K. Holly, Dr. J. Caseley, and W. A. Taylor for the use of facilities. Appreciation is expressed to Dr. D. J. Turner for supplying some growth regulator herbicides and for transportation arrangements to the senior author during the study. Acknowledgment is also due to Fisons Chemical Company, Ltd., for supplying the radioactive and commercial products of barban.

Registry No. Barban, 101-27-9; 2,4-D isopropyl ester, 84603-43-0; 2,4,5-T 2,4,5-triethylamine, 2008-46-0.

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Received for review May 3, 1982. Revised manuscript received December 7, 1982. Accepted December 22, 1982.