Gas Chromatographic Determination of Bromoxynil in Onions

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A sensitive gas chromatographic method is presented to determine in onions (*Allium cepa* L.) residues of the herbicide bromoxynil as its methyl ether. The method was used to monitor the dissipation of bromoxynil residues in onions over a growing season at three sites following three postemergence applications at 100 g ha^{-1} to control broad-leaved weeds. Residues on the day of the third application ranged from 288 to 736 $\mu g\ kg^{-1}$ for the three sites and then decreased to 10–20 $\mu g\ kg^{-1}$ during the subsequent 4–5 weeks. No residues were detected in the mature bulbs. Recoveries of bromoxynil were 94 ± 10% and 117 ± 10% at the 50 and 10 $\mu g\ kg^{-1}$ fortification levels, respectively.

INTRODUCTION

Onions (*Allium cepa* L.), which are used as both a vegetable and a spice, are grown commercially in several areas of Canada. Canada is a net importer of onions (Crete et al., 1981), and there is potential for increased onion production. However, the lack of registered herbicide uses to control broad-leaved weeds is a major limitation to increased production faced by onion growers.

Control of broad-leaved weeds is a necessity in onion production because onions develop very slowly and are poor competitors. With the deregistration in Canada of the use of allidochlor (2-chloro-*N*,*N*-diallylacetamide) to control broad-leaved weeds in onions, there was a need to evaluate other herbicides for this purpose. Although crop tolerance studies have shown that onions were not tolerant to bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) applied at rates recommended for cereals (300–400 g ha^{-1}) (Esau and Rumney, 1986; Valk and Knibbe, 1987), low rates of bromoxynil (70–100 g ha^{-1}) applied at 1–2-week intervals when weeds were in the cotyledon to two-leaf stage gave effective weed control without undue damage to the crop (Esau and Rumney, 1985; Esau, 1987; Goodwin, 1987).

The objective of the present study was to determine the magnitude of bromoxynil residues in onions at selected times after the last of three applications of bromoxynil at 100 g ha^{-1} to control broad-leaved weeds between the cotyledon and two-leaf stages.

EXPERIMENTAL PROCEDURES

Sample Collection. Onion samples were collected from three sites in western Canada: the Alberta Horticultural Research Center, Brooks, Alberta, and Manitoba Department of Agriculture sites near Oakville and Portage-la-Prairie, Manitoba. At each site, the check and herbicide treatments were replicated four times in a randomized complete block. Bromoxynil [formulated as a liquid emulsifiable concentrate (Torch DS; May & Baker Canada Inc.) at 450 g of bromoxynil L^{-1}] at 100 g of active ingredient ha^{-1} was applied when the onions were in the loop stage. The application was repeated when the onions were at the one-leaf stage and again at the two-leaf stage to control subsequent flushes of broad-leaved weeds. Immediately after the third application, a minimum of 200 g of whole onion plants was collected from each plot. Subsequent samplings were conducted 1, 2, 4, 8, and 14 weeks after application at the Alberta site and 1, 5, 10, and 14 weeks after application at the two Manitoba sites. At 14 weeks after application, the onions were mature. Sample size was increased at all three sites in proportion to the growth of the crop. Immediately after collection, the onions were briefly rinsed with tap water to remove adhering soil and then blotted dry with paper toweling. The replicate samples were placed in polyethylene freezer bags and immediately stored at −10 °C. After the last sample collection, the samples were packed with dry ice and shipped to Regina. The 8-, 10-, and 14-week samples were separated into bulb and top components, and then the replicate samples were chopped by using a Hobart food chopper with the addition of powdered dry ice; the chopped tissue was stored at −10 °C until analysis. Replicate samples collected on the other harvest dates were similarly chopped (as whole plants) and stored. Samples were analyzed within 1–4 months of arrival in Regina.

Chemicals. All solvents were distilled-in-glass grade (BDH Chemicals). Florisil (60–100 mesh) was heated at 600 °C for 48 h, cooled, and then deactivated by the addition of 5% water (w/w). Sodium sulfate was heated at 600 °C for 24 h. The matrix extraction solvent was an ethanol/water (80/20 v/v) solution. Bromoxynil methyl ether was prepared as described previously (Cessna, 1980).

Diethyl ether, used for partitioning, was rendered peroxide-free as follows: 250 mL of ether was removed from a 4-L bottle and 250 mL of acidic (1 M H_2SO_4) 1 M ferrous sulfate solution was added and the mixture shaken vigorously. The ferrous sulfate solution was retained in the 4-L bottle until the ether was completely used, at which point the ferrous sulfate solution was discarded. Peroxide-free diethyl ether was dried for diazomethane preparation via distillation using a rotary evaporator.

Sample Extraction. Chopped onion tissue (25 g) was homogenized at high speed in a 250-mL glass homogenizer jar with 70 mL of 80/20 ethanol/water extraction solvent for 1 min. The mixture was filtered under reduced pressure through a Buchner funnel equipped with a glass fiber filter paper. Extraction solvent (70 mL) was used to rinse the homogenizer generator into the homogenizer jar and subsequently to wash the filter cake. The combined filtrates were then taken to volume (200 mL) with extraction solvent. The onion extract (40 mL; equivalent to 5 g of plant tissue) was transferred to a 500-mL round-bottom flask containing 1 mL of 1 N NaOH solution and concentrated to an aqueous residue (approximately 10 mL) by using a rotary evaporator. The aqueous residue was transferred to a 125-mL Erlenmeyer flask followed by three 15-mL 0.1 N NaOH solution rinses of the 250-mL round-bottom flask. The basic extract was then heated at 80 °C (water bath) for 30 min. After the extract was cooled by immersing the flask in an ice–water bath, saturated NaCl solution (25 mL) was added and the pH was lowered to approximately 1 by using 6 N H_2SO_4. The acidified mixture was transferred to a 125-mL separatory funnel and extracted three times with 25-mL portions of peroxide-free diethyl ether. The ether extracts, combined in a 250-mL separatory funnel, were extracted three times with 25-mL portions of 4% $NaHCO_3$ solution. The combined $NaHCO_3$ extracts were acidified to pH 1 by the careful addition of 6 N H_2SO_4 solution and then extracted three times with 25-mL portions of chloroform. Each chloroform extract was passed through 30 mL of anhydrous sodium sulfate (contained in a 9 cm diameter long-stemmed funnel on top of a glass wool plug) into a 250-mL round-bottom flask, with the third extract fol-

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lowed by a 25-mL chloroform wash of the sodium sulfate. The combined chloroform extracts were then concentrated to approximately 25 mL by using a rotary evaporator (water bath at 40 °C), acetone (25 mL) was added, and the mixture was taken just to dryness.

Diazomethane Methylation. Diazomethane in diethyl ether solution was prepared as described previously (Grover et al., 1985), except that dry peroxide-free diethyl ether was used. The extract residue was transferred to a 40-mL graduated centrifuge tube with three 2.5-mL dry peroxide-free diethyl ether rinses of the 250-mL round-bottom flask. The combined ether rinses were concentrated to approximately 0.5 mL by using a gentle stream of nitrogen, and then diazomethane solution (2.5 mL) was added. After 15 min, hexane (2 mL) was added to the centrifuge tube and the reaction mixture was concentrated to approximately 1 mL by using a gentle stream of nitrogen; this procedure was repeated two more times.

Florisil Column Cleanup. Florisil (4 mL) was added to a 10 mm i.d. × 200 mm column containing 10 mL of hexane and then topped with 1 cm of anhydrous sodium sulfate. The hexane was drained to the surface of the sodium sulfate, and the methylated extract in the 40-mL centrifuge tube was transferred to the column, including two 1.5-mL 0.5% acetone in hexane rinses of the centrifuge tube. The column was eluted at 1 mL min^{-1} with 37 mL of 0.5% acetone in hexane, and the first 12 mL of eluate was discarded. For samples collected on the day of spraying, the remainder of the eluate was taken to volume (25 mL) and then appropriately diluted prior to quantitation. For all other samples, the eluate was concentrated by using a rotary evaporator (water bath at 40 °C) and then taken to volume (5 mL) with hexane.

Gas Chromatography. A Hewlett-Packard Model 5890A gas chromatograph, equipped with a ^{63}Ni electron-capture detector and an on-column injector, was used with the Model 7673A autosampler set to inject 2 μL and the Model 5895A data station. A Hewlett-Packard 30 m × 0.53 mm i.d. HP-1 (film thickness of 0.88 μm) fused silica column was used with the following temperature program: 70 °C for 1 min, 10 °C min^{-1} to 270 °C, hold for 1 min at 270 °C. By use of a carrier gas (helium) flow rate of 5 mL min^{-1} and detector makeup gas (nitrogen) flow rate of 70 mL min^{-1}, bromoxynil methyl ether had a retention time of 12.8 min. The detector (350 °C) response for bromoxynil methyl ether was nonlinear, and a piecewise linear calibration was used for quantitation.

Fortification Experiments. Recoveries of bromoxynil were determined by the extraction of chopped untreated onion tissue fortified at 50 and 100 μg kg^{-1} (whole plants) and at 10 and 50 μg kg^{-1} (bulbs only). Bromoxynil (2.5, 1.25, or 0.25 μg) in 2.5 mL of methanol was added to 25 g of chopped onion tissue contained in a 250-mL glass homogenizer jar. The homogenizer jar was placed in a fumehood until the methanol had evaporated and then sealed with parafilm and stored in the dark at −10 °C until extraction. Fortified samples were analyzed as every sixth sample during analysis of the treated and check samples. As a consequence, storage of the fortified samples at −10 °C varied from 24 h to 2 months. Four to six replicates were analyzed for each fortification level of each substrate.

RESULTS AND DISCUSSION

Acidic herbicides, such as mecoprop (Chow et al., 1971), 2,4-D (Feung et al., 1973), dichlorprop (Løkke, 1975), diclofop (Jacobson and Shimabukuro, 1984), picloram (Eliasson and Hallmén, 1972), and benzoylprop (Beynon et al., 1974), are known to form conjugates with plant substituents. Studies by Chow et al. (1971) and Løkke (1975) have shown that acidic herbicides are best released from plant tissue when the extraction includes a hydrolytic step. Two extraction strategies have generally been employed in incorporating the hydrolysis step. One involves subjecting the plant tissue to extended hydrolysis (usually using 0.05–0.5 N sodium hydroxide or potassium hydroxide solution with heating), followed by acidification and partitioning of the "free" acidic herbicide into an organic phase (Bjerke et al., 1967; Cessna, 1980; Bristol et al., 1982; Frank et al., 1983; Galoux et al., 1983; Smith, 1984; Smith et al., 1986; Steinwandter, 1989). The second approach involves extracting the intact conjugates of these compounds directly into an organic phase and then effecting hydrolysis (Hamilton et al., 1971; Buckland et al., 1973; Feung et al., 1973; Beynon et al., 1974; Shimabukuro et al., 1979; Eronen et al., 1979; Scheel and Sandermann, 1981). Aqueous alcohols have been widely used for the extraction of metabolites and intact conjugates for several acidic herbicides (Chang and Vanden Born, 1971; Hamilton et al., 1971; Feung et al., 1973; Hunter and McIntyre, 1974; Robocker and Zamora, 1976; Shimabukuro et al., 1979).

Table I. Percent Recoveries of Bromoxynil from Bulb and Whole Onion Tissue Fortified at 10, 50, and 100 μg kg^{-1}

substrate	level of fortification, μg kg^{-1}		
	10	50	100
whole onion	[a]	94[b]	98
		102	100
		98	99
		106	93
		104	
		102	
onion bulb	120	80	[a]
	110	82	
	120	90	
	100	86	
	120		
	130		
mean ± SD	117 ± 10	94 ± 10	98 ± 3

[a] Recoveries were not determined for this substrate and fortification level. [b] Background interferences in corresponding check samples at the retention time for bromoxynil methyl ether have been subtracted.

In the present study, 80% aqueous ethanol was used to extract bromoxynil and possible conjugates from onion tissue. The ethanol extract was concentrated and then subjected to alkaline hydrolysis (0.1 N NaOH solution at 80 °C). Under these conditions, ester linkages between the bromoxynil hydroxyl group and free carboxyl groups on plant substituents would be readily hydrolyzed (Smith, 1972; Grayson and Stokes, 1979), whereas bromoxynil itself would be stable (Cessna, 1980). The hydrolyzed extract was then acidified and partitioned into diethyl ether. Subsequent partitioning into bicarbonate solution and then, after acidification, into chloroform was necessary to give reproducible background interferences from onion check samples at the retention time for bromoxynil methyl ether.

During initial method development, poor recoveries of bromoxynil from fortified onion tissue led to experiments to determine solvent blank recoveries. Water, fortified at 10, 50, and 100 μg kg^{-1}, was used for this purpose. Maximum recoveries, regardless of fortification level, were in the order of 80%. The low recoveries were found to be due to the presence of diethyl peroxide in the diethyl ether used for extraction purposes as well as for diazomethane derivatization. When different production batches of diethyl ether were studied, the yield of bromoxynil methyl ether was found to be inversely proportional to the amount of peroxide present in the ether. The presence of peroxides in the ether resulted in bromoxynil methyl ether peaks which were broader as well as reduced in area. Use of peroxide-free diethyl ether for both ether extraction and diazomethane derivatization greatly improved recoveries of bromoxynil from fortified onion tissue (Table I).

The presence of diethyl peroxide in the diethyl ether was confirmed by using a Hewlett-Packard Model 5970B mass selective detector (MSD) operated in the scan mode

Table II. Bromoxynil Residues[a] ($\mu g\ kg^{-1}$) Detected in Treated Onions and Background Interferences[a] Detected in Onion Checks at the Retention Time for Bromoxynil Methyl Ether

sampling time, weeks after last application	substrate	response of interfering peak at retention time for bromoxynil, methyl ether, equivalent to $\mu g\ kg^{-1}$ of bromoxynil	experimental site		
			Brooks	Portage-la-Prairie	Oakville
0[b]	whole plants	15 ± 6	288 ± 37[c]	736[d]	551 ± 39
1	whole plants	3 ± 3	19 ± 4	62 ± 18	44 ± 18
2	whole plants	4 ± 2	10 ± 2	*e*	*e*
4	whole plants	3 ± 1	10 ± 2	*e*	*e*
5	whole plants	4 ± 1[f]	*e*	15 ± 10	18 ± 5
8	tops	3 ± 2	5 ± 3	*e*	*e*
	bulbs	2 ± 1	3 ± 1	*e*	*e*
10	tops	5 ± 3[f]	*e*	8 ± 1	9 ± 3
	bulbs	3 ± 3	*e*	2 ± 0	4 ± 3
14	tops	8 ± 1	7 ± 1	*e*	*e*
	bulbs	2 ± 0	2 ± 0	2 ± 1	4 ± 2

[a] Bromoxynil residue and background interference values are the mean of four replicates. [b] Day of last bromoxynil application. [c] Residue values of treated samples do NOT have background interferences subtracted. [d] Two replicate samples were lost. [e] Samples were not collected on this date at this site. [f] Check samples from the Portage-la-Prairie site. All other check samples from the Brooks site.

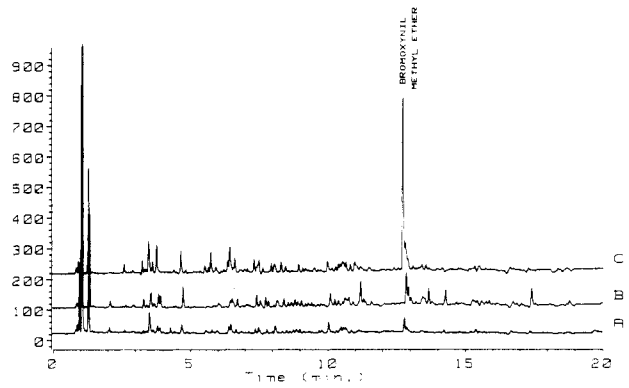

Figure 1. Chromatograms of whole onion samples from the Alberta site 4 weeks after the third bromoxynil treatment: chromatogram A, onion check [area counts (1422) equivalent to 3.9 $\mu g\ kg^{-1}$]; chromatogram B, treated onions [area counts (3325) equivalent to 9.2 $\mu g\ kg^{-1}$]; chromatogram C, onion check fortified with bromoxynil at 50 $\mu g\ kg^{-1}$ [area counts (17 832) equivalent to 54.9 $\mu g\ kg^{-1}$].

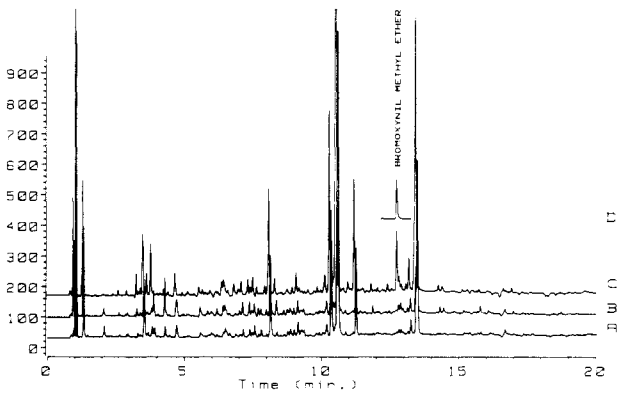

Figure 2. Chromatograms of onion bulbs from the Alberta site 14 weeks after the third bromoxynil treatment: chromatogram A, onion bulb check [area counts (655) equivalent to 1.8 $\mu g\ kg^{-1}$]; chromatogram B, treated onion bulbs [area counts (865) equivalent to 2.4 $\mu g\ kg^{-1}$]; chromatogram C, onion bulb check fortified with bromoxynil at 10 $\mu g\ kg^{-1}$ [area counts (5105) equivalent to 14.5 $\mu g\ kg^{-1}$]; chromatogram D, bromoxynil standard equivalent to 10 $\mu g\ kg^{-1}$ in the bulbs (area counts = 3595).

(50–200 amu). The MSD was interfaced to a Hewlett-Packard Model 5890A gas chromatograph equipped with a 25 m × 0.2 mm i.d. Ultra 1 capillary column (film thickness of 0.11 μm) and a split–splitless injector operated in the splitless mode. The helium carrier gas flow rate was 25 cm s^{-1}, and the column temperature program consisted of 50 °C for 1 min followed by 10 °C min^{-1} to 250 °C. Under these conditions, the retention time for diethyl peroxide was 4.7 min. The mass spectral scan showed a weak parent ion at 91 amu (M + 1), the base peak at 73 amu (M − 17), and a second major ion at 61 amu (M − 29).

Background interferences from whole plant check samples at the retention time for bromoxynil methyl ether were in the order of 4–5 $\mu g\ kg^{-1}$ (Table II; Figure 1, chromatogram A) and readily permitted quantitation at 50 $\mu g\ kg^{-1}$. Whole plant check samples collected from the Alberta site immediately after the third application showed significantly higher background interferences (Table II) due, most likely, to drift during spraying. In those samples separated into top and bulb components, coextractive interferences from the bulbs were in the order of 2–3 $\mu g\ kg^{-1}$ (Table II; Figure 2, chromatogram A) and recoveries at the 10 $\mu g\ kg^{-1}$ fortification level were 117 ± 10% (Table I).

Initial residues of bromoxynil in whole onion plants, immediately following the third application of bromoxynil, ranged from 288 to 736 $\mu g\ kg^{-1}$ (Table II). [Note: residue values for bromoxynil in treated onions (Table II) have not been corrected for background interferences.] These initial residues had decreased by more than an order of magnitude 4–5 weeks after application. Growth dilution would have accounted for most of this decrease; however, additional losses may have occurred due to photochemical decomposition on leaf surfaces and possibly metabolism following uptake (Buckland et al., 1973; Sanders and Pallett, 1987).

Residues of bromoxynil in the 4- and 5-week samples were in the order of 10–20 $\mu g\ kg^{-1}$ (Table II). These residues were confirmed as bromoxynil methyl ether by using the mass selective detector and gas chromatography system described above. When the mass selective detector was operated in the selected ion monitoring mode and the chromatographic conditions described above were used, bromoxynil methyl ether had a retention time of 24.5 min and was confirmed by using ions of 248, 276, and 291 amu and the ion ratio (291/276) of the two most abundant ions. The amount of bromoxynil methyl ether present in a 2-μL injection of a 10 $\mu g\ kg^{-1}$ extract was 20 pg. Amounts less than 10 pg could not be confirmed either because the least abundant ion (248 amu) was not detected or because the 291/276 ratio differed from that for the bromoxynil methyl ether standard by more than 30%.

Residues of bromoxynil were not detected in the bulbs of the 8-, 10-, and 14-week samples. Electron-capture detector responses at the retention time for bromoxynil methyl ether in the chromatograms for these samples were

not significantly different than those for the corresponding check samples (Table II). Because of the small amounts involved in these samples, analysis using the mass selective detector to confirm possible bromoxynil residues was not attempted.

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