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Residue Analysis of Triadimefon, Triadimenol, and the BAY KWG 1342 Diol and BAY KWG 1323 Hydroxylated Metabolites in Winter Wheat

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Triadimefon is the fully systemic active ingredient 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone in the broad spectrum fungicide Bayleton. This compound shows activity against powdery mildews and rusts (Frohberger 1975; Kaspers et al. 1975; Scheinpflug and Paul 1977) and is used in tree fruits, berry crops, nuts, vegetables, cereals, turf and ornamentals. It provides protective and curative back action preventing disease symptoms after infection has taken place.

The metabolism of triadimefon in beans, barley and soil has been reported (Buchenauer 1976; Vogeler 1976). The metabolite, KWG 0519, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol is also fungicidal and is the active ingredient triadimenol in the seed treatment fungicide Baytan. In a long term experiment with triadimefon, Buchenauer and Rohner (1982) identified two polar metabolites in barley, and under similar conditions Rouchaud et al. (1982) showed the formation of 4-chlorophenol. The major steps in triadimefon metabolism in microorganisms and plants have been reported by Gasztonyi and Josepovits (1984). Triadimenol seems to be more persistent in plants than triadimefon.

Bayleton has been used commercially and Baytan experimentally for control of powdery mildew on winter wheat in Eastern Canada. However, no studies have yet been made for their residues in foliage, straw or grain in this region. This study was undertaken to measure residue levels of Bayleton and Baytan at various periods after fungicide application in the field.

MATERIALS AND METHODS

The first three experiments were carried out at Kentville, Nova Scotia, while the fourth was performed at Charlottetown, Prince Edward Island. Normal management practices were followed. Treatments were applied in 200 L ha^{-1} and replicated four times on 1 x 5 m plots. Crop samples for residue measurement were frozen at -18°C until analysis.

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Shoots and straw samples were ground in a coffee mill (Philips Electronics, Scarborough, ON) and grains were ground in a Cyclone Mill (UDY Corp., Fort Collins, CO). Twenty-five grams shoots or grains or 12.5 g straw were homogenized in 220 mL methanol:water (7:3, v/v) at high speed for 3 min. Contents of the blender cup were transferred into a 500-ml flat-bottomed flask using 60-70 mL methanol:water (7:3 v/v). The flask was fitted with a small magnetic bar and a water cooled condenser and heated at reflux for 90 min, then cooled at room temperature for 30 min. Fifteen grams of Hyflo Super-cel (Johns Manville, washed with 70% methanol, filtered with suction and the cake vacuum dried) were added to each flask. The flasks were swirled and the contents filtered through glass fibre filter paper fitted in a Büchner funnel into a 1-L round-bottomed flask. The filter cake was returned to the original 500 mL flat-bottomed flask, 100-125 ml of methanol were added, the mixture was swirled for 1 min, and filtered through another glass fibre filter paper into the 1-L round-bottomed flask. The filter cake was washed with 50 mL methanol and the combined filtrate evaporated until the aqueous phase was observed using a rotary evaporator at 40°C.

Conjugated residues were released from samples by enzymatic hydrolysis (Mobay Chemical Corp **priv. commun.** 1985). Briefly, an equal volume of sodium acetate buffer (0.2 M) in deionized water, pH adjusted to 5.5 by acetic acid, was added to each round-bottomed flask followed by 150 mg cellulase enzyme (practical grade II, Sigma Chemical Co.) in 2 mL deionized water. The flask was swirled to mix contents, stoppered and sealed with parafilm, then incubated at 37°C in a water bath for 17-18 h. The flask was removed from the water bath, left to cool for about 15 min and then its contents were transferred to a 500-mL separatory funnel using two 125-ml portions of methylene chloride (for grains methylene chloride was substituted by methylene chloride:acetonitrile 2:1, v/v to minimize emulsion formation). The separatory funnel was shaken for 30 sec and the organic phase drained into the original 1-L flask. The aqueous phase in the separatory funnel was reextracted with 250 mL of methylene chloride and the organic phase drained into the 1-L flask. The combined extract was evaporated to near dryness on a rotary evaporator at 40°C and final traces of solvent were removed with a stream of dry nitrogen.

The residue in the 1-L flask above was dissolved in 3 mL chloroform and the resulting solution was transferred to a 13-mL centrifuge tube with a Pasteur pipet. The flask was rinsed with two 3 mL portions of chloroform and the rinsings were transferred to the same tube. The volume was adjusted to 10 mL with chloroform and centrifuged at 1800 rpm for 3 min. The solution was cleaned by gel permeation chromatography using chloroform as eluting solvent collected in a 125-mL round-bottomed flask (discard, collect and wash modes: 24 x 1 min, 120 mL; 12 x 1 min, 60 ml and 10 x 1 min, 50 mL, respectively). The chloroform was evaporated to near dryness and its traces were removed by dry nitrogen. The residue was dissolved in 10 mL petroleum ether-ethyl ether (94:6, v/v) and cleaned up on a 20 x 400 mm Florisil column. A loose plug of glass

wool (pre-washed with methylene chloride) was introduced at the bottom of the column followed by 1-cm fine mesh glass beads to make a level surface bottom for the Florisil. The column was filled with petroleum ether-ethyl ether mixture (94:6, v/v) and allowed to drain until most of the air from the beads and glass wool was removed. Ten grams of water-deactivated (2.5%) Florisil (50-100 mesh) were sprinkled slowly into the column and when settled were topped by 5 g anhydrous sodium sulfate (granular, pre-washed with methylene chloride). The solvent was drained to the top of the sodium sulfate, the 10 mL petroleum ether-ethyl ether solution in the 125 mL flask above was transferred to the Florisil column and the elution rate was adjusted to 2-3 drops/sec. The flask was rinsed with two more 10-mL portions of the solvent mixture and the rinsings were added to the column just as the previous rinse drained into the sodium sulfate. The column was then eluted with 170 mL of solvent mixture and all the eluates were discarded. The 125 mL flask above was rinsed with three 10 ml-portions of hexane-ethyl acetate mixture (60:40, v/v) and each rinsing was transferred to the column just as the last of the previous rinse drained into the sodium sulfate. The column was eluted with an additional 120 mL of the hexane-ethyl acetate mixture at a rate of 2-3 drops/sec and the total eluate (150 mL) was collected in a 250-mL Erlenmeyer flask. This eluate I containing triadimefon, triadimenol and traces of the bound hydroxylated metabolite (KWG 1323) was evaporated on a rotary vacuum evaporator to near dryness. The last traces of solvent mixture were removed with dry nitrogen and the residues were redissolved in 2 mL acetone and analysed directly by gas chromatography with an N/P detector as described below. Ten milliliters of ethyl acetate-methanol mixture (95:5, v/v) were added to the same Florisil column and allowed to percolate through. Then the column was eluted with an additional 140 mL of ethyl acetate-methanol mixture at a rate of 2-3 drops/sec in a 250-mL Erlenmeyer flask. This eluate II which contains the bound diol metabolite (KWG 1342) residue and any remaining KWG 1323 metabolite was vacuum evaporated to near dryness and the last traces of solvents were removed with dry nitrogen. The residues were redissolved in 2 mL methanol and analysed by the same gas chromatographs after derivatization as described below.

The 2 mL methanol above and standards of KWG 1342 and KWG 1323 (1.25 g each) were placed in separate 5-ml Reacti-Vials. The solvent in each vial was evaporated to dryness with dry nitrogen, 50 1 of trifluoroacetic anhydride (Aldrich Chemical Co.) were added, the vials were capped with teflon-lined caps and heated at 45°C for 1 h. When cooled to room temperature, excess trifluoroacetic anhydride and all traces of solvents were removed using dry nitrogen. Residues from foliage samples were dissolved in 2 mL acetone for analysis by the same gas chromatography. Residues from dry straw and grain samples were redissolved in 1 mL benzene and further cleaned up on silica gel before analysis by gas chromatography. A loose plug of glass wool was placed into a Pasteur pipet, 6 cm of 5% water-deactivated silica gel G60 (100-200 mesh, Merck) added and the pipet topped with 0.5 cm sodium sulfate. The column was wetted with 1 mL benzene, then the

trifluoroacetic acid derivatized sample in 1 mL benzene above was transferred to it. When the benzene solvent had just percolated into the sodium sulfate layer the vial was rinsed with 2.5 mL benzene twice, the rinsing added successively to the column. All eluates were discarded and the column was eluted with 10 mL ethyl acetate. The ethyl acetate was evaporated just to dryness using dry nitrogen and residues were dissolved in 2 mL acetone and analyzed by gas chromatography.

Residue analysis employed a Tracor Micro-Tek 220 gas chromatograph equipped with an N/P alkali flame detector. The column (120 cm x 0.32 cm o.d. glass) was packed with 10% DC-200 + 1.5% QF-1 on Chromasorb W (HP), 80-100 mesh. The confirmatory column (120 cm x 0.32 cm o.d. glass) was packed with 20% OV-11 on Chromasorb W (HP), 80-100 mesh. The carrier gas was helium at 60 ml/min and 75 psig. The column, injector and detector temperatures were 220°C, 250°C and 300°C, respectively. Analytical grade standards of triadimefon, 99.4%, triadimenol, 96.4%; Bay KWG 1323, 95%; and Bay KWG 1342, 95% were provided by Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO 64120. A stock solution (1 µg/ µL benzene) of each was prepared and serial dilutions of each standard and composite standards were made in acetone.

Five microliters of each of the acetone solutions (2 mL) in the vials above containing triadimefon, triadimenol, derivatized KWG 1342, or derivatized KWG 1323 were injected onto a 10% DC-200 + 1.5% QF-1 column. The efficiency of the analytical procedures were determined using a series of green foliage, dry straw and grain check samples spiked with the standards and run through the respective procedures. All determinations were performed in duplicate.

RESULTS AND DISCUSSION

Baytan applied as a seed treatment to winter wheat (cv Absolvent) at the rate of 0.6 g a.i./kg seed resulted in no detectable residues in grain at harvest August 31 (<0.02 ppm triadimenol). Similarly, plants sprayed at anthesis June 12 with Bayleton at the rate of 250 g a.i./ha produced grain with no detectable residues of triadimefon (<0.02 ppm). Check samples were also <0.02 ppm.

In the second winter wheat field experiment at Kentville, Lennox winter wheat was sprayed with Bayleton (Table 1). Although residues of triadimefon and its three metabolites were found in green foliage and mature dry straw, little or no residues were found in harvested grain.

In the third Kentville experiment seeds of cv. Lennox were treated with Baytan at the rate of 0.3 g a.i./kg and seeded September 18. Samples of green foliage were taken November 23 and on May 7 and June 14 of the following year and analyzed for triadimenol, KWG 1342, and KWG 1323. Residues of triadimenol at the first sampling date averaged 0.05 ppm and 0.02 ppm at the second and third

sampling dates. No detectable residues (<0.02 ppm) of the metabolites KWG 1342 or KWG 1323 were found in the green foliage at any of the sampling dates. Analysis of grain sampled on August 31 revealed no detectable residues (<0.02 ppm) of any of the three compounds.

Table 1. Residues of triadimefon, triadimenol, KWG 1323 and KWG 1342 in winter wheat sprayed with Bayleton[1].

Wheat Tissues[2]	Residues (ppm)[3]			
	Triadimefon	Triadimenol	KWG 1323	KWG 1342
Green Foliage	1.02	0.15	0.04	0.02
Straw	0.02	1.07	0.35	0.05
Grain	0.02	0.03	0.03	0.02

[1]125 g a.i./ha, June 15.
[2]Green foliage, straw, and grain were sampled June 18, August 20, and August 27, 1982, respectively.
[3]Average of two determinations from each of four plots, expressed on oven-dry basis.

In the fourth experiment at Charlottetown, Lennox winter wheat was treated with Baytan at the rate of 0.3 g a.i./kg seed on August 26 and seeded on September 4. Samples of foliage taken November 16, and on May 6 and June 17 of the following year, as well as grain samples taken on August 19, were analyzed for triadimenol, KWG 1342 and KWG 1323 and no residues were found (< 0.02 ppm) except on November 16 where 0.03 ppm triadimenol was found in the foliage.

Recovery of triadimefon, triadimenol, KWG 1342, and KWG 1323 from fortified foliage, grain, and straw was satisfactory (Table 2).

Table 2. Recovery of triadimefon, triadimenol, KWG 1323 and KWG 1342 from fortified winter wheat.[1]

	Triadimefon		Triadimenol		KWG 1323		KWG 1342	
	F[2]	R[3]	F	R	F	R	F	R
Wheat Sample	(ppm)	(%)	(ppm)	(%)	(ppm)	(%)	(ppm)	(%)
Green Foliage	1.00	91	1.00	103	0.50	99	0.50	90
	0.50	84	0.50	104	0.05	99	0.05	93
Grain	0.10	98	0.10	86	0.10	103	0.10	99
	0.05	90	0.05	101	0.05	107	0.05	102
Straw	0.50	91	0.50	93	0.50	110	0.50	81
	0.10	87	0.10	98	0.10	101	0.10	79

[1]Average of two determinations.
[2]Fortified.
[3]Recovered.

The two metabolites KWG 1323 and KWG 1342 existed in the plant tissues in conjugated forms and their release required a lengthy extraction procedure (17-18 h) with the enzyme cellulase and formation of volatile derivatives suitable for analysis by

gas-liquid chromatography. In the present study residues of these two conjugates were either undetected or slightly exceeded the analytical method detection limit of 0.02 ppm. Should their measurement not be required the analysis of triadimefon and triadimenol can be performed after extraction without enzyme hydrolysis or derivatization.

Triadimenol contains two optically active carbon atoms and accordingly they exist in two diastereomeric forms A and B which cannot be separated on the packed gas chromatograph columns used in this study. Their peak sometimes appeared slightly divided at the top and it is believed that a capillary gas chromatographic column would improve their separation.

Results in this study were confirmed using thin-layer chromatographs on pooled cleaned-up extracts. The R_f values of triadimefon, triadimenol, KWG 1342 and KWG 1323 were 0.75, 0.33, 0.12 and 0.28, respectively, in the solvent system benzene:methanol 9:1 (v/v) and silica gel G with fluorescent binder. Compounds appeared as dark spots against a fluorescent background when viewed under short wavelength UV light.

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