Determination of Terbacil in Blueberries

A method for the analysis of terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) in blueberries, utilizing electron-capture gas chromatographic detection, has been developed and used to determine terbacil residues in both highbush and lowbush blueberries treated with terbacil alone and terbacil in combination with other herbicides. The maximum terbacil residue found was 2.0 ppb. The limit of detection was 1.0 ppb based on a 25-g sample and recoveries were in the order of 90%.

Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) is registered in Canada for selective weed control in strawberries, apples, peaches, and pears. It has also been registered in the United States for use on alfalfa, apples, blueberries, peaches, mint, and sugarcane. In Canada, terbacil has shown potential for use in both highbush blueberries (*Vaccinium corymbosom* L.) (Hughes and Cruickshank, 1974; Hughes and Horvath, 1975) in British Columbia and lowbush blueberries (*Vaccinium angustifolium* Ait.) (Jackson, 1971, 1975) in Nova Scotia and Prince Edward Island. However, analytical procedures for the determination of terbacil rersidues in treated blueberries have not yet been reported. The present paper describes a highly sensitive method of analysis for determining terbacil residues in blueberries using electron-capture gas chromatography, based on an earlier method of Pease (1968) who determined terbacil residues in a variety of other fruits. The method was used to determine terbacil residues in both highbush and lowbush blueberries which had been treated with terbacil as well as with terbacil in combination with other herbicides.

MATERIALS AND METHODS

Herbicide Treatments. Two varieties of highbush blueberries were treated at Richmond, British Columbia. Triplicate 9.2-m^2 plots of highbush blueberries (variety, Ivanhoe) were sprayed on May 16, 1974 with the following herbicide treatments: terbacil (2.24 kg/ha; 3.36 kg/ha); terbacil + diuron (1.12 kg/ha + 1.12 kg/ha; 1.68 kg/ha + 1.68 kg/ha); terbacil + paraquat (3.36 kg/ha + 1.12 kg/ha); and terbacil + glyphosate (3.36 kg/ha + 1.12 kg/ha). The various herbicide treatments were applied as directed basal sprays using 468 L/ha of water to the established crop (120–180 cm high). The blueberries were harvested on Aug 5, 1975.

The following herbicide treatments were also applied to triplicate 7.4-m^2 plots of established (120–180 cm high) highbush blueberries (variety, Jersey) on July 3, 1975 as directed basal sprays using 848 L/ha of water: terbacil (3.36 kg/ha); terbacil + diuron (1.68 kg/ha + 1.68 kg/ha); terbacil + paraquat (3.36 kg/ha + 1.12 kg/ha); and terbacil + glyphosate (3.36 kg/ha + 1.12 kg/ha). The blueberries were harvested on Sept 22, 1975.

Two fields of lowbush blueberries were treated at the Agriculture Canada Project Farm at Fenwick, Nova Scotia. The A field, consisting of nursery grown plants set out in the spring of 1971, was treated with terbacil (1.12 kg/ha) plus atrazine (2.24 kg/ha) on July 25, 1972 and again on July 29, 1973. The herbicide treatments were applied with 187.2 L/ha of water by a boom sprayer operated at 210 kPa. A sawdust mulch was applied to the ground surface in the fall of 1972, 3 months after the first application. The plants were burned in a normal pruning program in the spring of 1974 and fruit produced in 1975. The blueberries were harvested on Aug 12, 1975. The B field, consisting of nursery grown plants set out in the spring of 1974, was treated with terbacil (1.12 kg/ha) plus atrazine (2.24 kg/ha). The herbicide treatment was applied with 187.2 L/ha of water by a boom sprayer operated at 210 kPa on July 15, 1974. A sawdust mulch was applied to the ground surface 3 months after this treatment. The blueberries were harvested on July 29, 1975.

Lowbush blueberries were also treated at a breeding nursery at Kentville, Nova Scotia. In 1974, simazine was applied as a granular application with a fertilizer spreader at 2.24 kg/ha. In May 1975, terbacil (0.84 kg/ha) was applied with 749 L/ha of water by a boom sprayer operated at 210 kPa. The blueberries were harvested from Aug 5 to 10, 1975.

Sampling. Samples (0.5 kg) of lowbush blueberries, from the Project Farm at Fenwick, Nova Scotia, were collected from random sites (approximately 20 m^2) in both the A and B fields. The lowbush blueberries from the breeding nursery at Kentville, Nova Scotia, were collected from several sites within the treated areas of the nursery and were pooled to form a composite sample. Samples (0.5 to 1.0 kg) of highbush blueberries were collected from the plots at Richmond, British Columbia.

The blueberries were frozen in sealed polyethylene bags immediately after harvest, shipped to Regina in dry ice, and, upon arrival, stored in a freezer at −10 °C until extraction.

Chemicals. All solvents were pesticide grade and used as received. The analytical grade terbacil was supplied by E. I. du Pont de Nemours & Co., Wilmington, Del.

Sample Extraction. Twenty-five grams of blueberries was blended (Virtis 45 Hi-Speed homogenizer) in 80 mL of 1% NaOH for 3 min at medium speed, transferred to a 250-mL centrifuge bottle, and centrifuged at 3000 rpm for 10 min. The liquid was decanted through a glass wool plug into a 250-mL separatory funnel and the solids transferred from the centrifuge bottle to the homogenizer flask with 80 mL of 1% NaOH, blended again at medium speed for 3 min, and then centrifuged as before. The liquid was decanted through the same glass wool plug into the 250-mL separatory funnel and the combined decantates were acidified with 6 mL of 18 N H_2SO_4 and extracted 3 times with 50 mL of $CHCl_3$. Each $CHCl_3$ extraction resulted in the formation of an emulsion which was effectively broken by slowly draining each $CHCl_3$ layer from the separatory funnel into a second separatory funnel which contained 150 mL of $CHCl_3$. The combined $CHCl_3$ extracts were separated from the aqueous layer carried over from the emulsions and evaporated just to dryness under reduced pressure using a rotary evaporator.

Florisil Cleanup. Florisil (10 g; heated at 400 °C for 4 h and then deactivated by the addition of 9% water) was placed in a 17 mm i.d. × 300 mm column, 10 mL of Na_2SO_4 (heated at 600 °C for 48 h) was added, and the column was prewashed with 25 mL of hexane. The blueberry extract residue was taken up with 2 mL of $CHCl_3$ and washed onto the Florisil column with ca. 3 mL of 5% acetone in hexane. The column was washed with 25 mL of hexane and then eluted with 110 mL of 5% acetone in hexane. The last 90

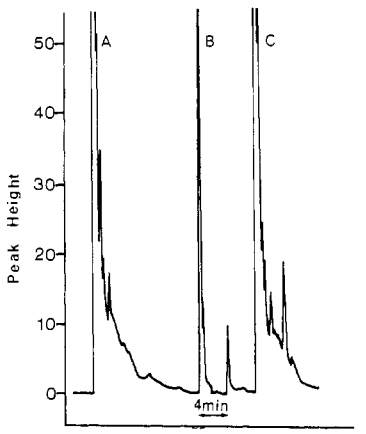

Figure 1. Chromatograms A, B, and C; attenuator ×8, chart speed 0.1 in./min: (A) 4-μL extract from blueberry check; (B) 0.04 ng of terbacil in acetone (4 μL, 0.01 ppm); (C) 0.04 ng of terbacil plus 4 μL of extract from blueberry check.

mL of eluate was concentrated to ca. 5 mL using a rotary evaporator and the volume adjusted to 10 mL prior to gas chromatographic analysis.

Fortification. Recoveries of terbacil were determined by extraction of blueberries fortified at both 1.0 and 0.1 ppm of terbacil on a fresh weight basis. The blueberries were fortified with terbacil as follows: 25 g of frozen blueberries was allowed to thaw in a 150-mL beaker. The berries were crushed and 5.0 mL of 5.0 or 0.5 ppm of terbacil in methanol was added. The crushed blueberries and terbacil solution were thoroughly mixed and left in darkness at room temperature in the open beaker for 48 h before extraction and analysis.

Gas Chromatography. A Hewlett-Packard Model 5713A gas chromatograph, equipped with a ^{63}Ni detector, was used with a Honeywell Electronik 194 1-mV recorder. The 1.2 m × 4 mm i.d. coiled glass column was packed with 3% QF-1/2% DC-200 on 60/80 mesh Gas-Chrom Q. The retention time for terbacil was 4.0 min with the following operating conditions: 95% argon–methane (carrier gas), 40 mL/min; injector and column, 170 °C; detector, 300 °C. Under these conditions, with the attenuator set at ×8, 0.4 ng of terbacil gave a full-scale deflection. A linear response was observed over the range of 0.04 to 4.0 ng of terbacil.

RESULTS AND DISCUSSION

Recoveries of terbacil from lowbush blueberries fortified at both 0.1- and 1.0-ppm levels were determined from a standard calibration curve constructed by plotting nanograms of terbacil against peak height. Five replicates were analyzed at each fortification level, the recoveries being 98.5 ± 3.4% at the 1.0-ppm level and 90.7 ± 13.3% at the 0.1-ppm level. The recoveries of terbacil from highbush blueberries fortified at the same levels were not significantly different.

In the original method of Pease (1968), the residue from the $CHCl_3$ extract was taken up in 1 N NaOH, washed twice with hexane, and partitioned twice with ethyl acetate and the ethyl acetate extract was concentrated prior to Florisil column cleanup. Even though these cleanup steps were eliminated in the present method, chromatograms of the blueberry extracts indicated that coextracted plant substituents did not significantly interfere with the detection of terbacil. A typical chromatogram from a cleaned up blueberry check extract is shown in Figure 1, chromatogram A. Unknown plant substituents resulted in a tailing of the solvent peak and, consequently, the terbacil peak was imposed on a somewhat sloping baseline. The gas chromatograph was operated at a sensitivity which permitted a peak height of 9 units for 0.04 ng of terbacil (Figure 1, chromatograms B and C). Using a 4-μL injection size, this was equivalent to 4.0 ppb of terbacil. At this sensitivity, the limit of detection was 1.0 ppb.

Terbacil residues, which were not corrected for recoveries, in both the Jersey and Ivanhoe varieties of highbush blueberries did not exceed 2.0 ppb. The Ivanhoe highbush blueberries were harvested 1 year after the herbicide treatments, whereas the Jersey highbush blueberries were harvested the same year as the herbicide treatments. Four herbicide treatments were carried out in both years: terbacil (3.36 kg/ha); terbacil + diuron (1.68 kg/ha + 1.68 kg/ha); terbacil + paraquat (3.36 kg/ha + 1.12 kg/ha); and terbacil + glyphosate (3.36 kg/ha + 1.12 kg/ha). No significant differences in terbacil residues were observed in highbush blueberries harvested the year after herbicide treatment and the year of herbicide treatment nor in highbush blueberries treated with terbacil alone or with terbacil in combination with the other herbicides. Thus, paraquat, diuron, and glyphosate did not have an effect on the terbacil residues found in highbush blueberries at the rates applied.

Except for one sample, none of the lowbush blueberries from Nova Scotia gave terbacil residues. The lowbush blueberries from the nursery at Kentville, Nova Scotia were harvested in the same year as they were treated with herbicide. The lowbush blueberries from the B field at the Project Farm at Fenwick, Nova Scotia were harvested 1 year after treatment, whereas those from the A field were harvested 2 years after treatment.

All samples were analyzed in duplicate, except the composite sample of lowbush blueberries from Kentville, Nova Scotia, from which four analyses were done.

The registration of terbacil for selective weed control in apples and strawberries in Canada was based on terbacil residues in these fruits of less than 40 ppb (Huston, 1976). Thus, the terbacil residues determined in lowbush and highbush blueberries resulting from the herbicide treatments used in this study would not appear to be a problem.

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