Further investigation on the photooxidation of II might elucidate the possible correlation among the formation of VII, VIII, and II.

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An Improved Analytical Procedure for Captafol Residues in Apple Wood, Leaves, and Fruit

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The fungicide captafol is currently used for the control of various crop fungal diseases and has been introduced recently in apple pest management programs for control of scab on both leaf and fruit in a single application technique. We report an improved analytical technique for captafol residues in apple wood, leaves, and fruits. The procedure involves extraction of plant tissue with acetonitrile, partitioning with a mixture of methylene chloride–petroleum ether, cleanup on a Florisil column, and quantitation by gas–liquid chromatography, employing an electron-capture detector. Captafol residues as low as 4 ppb were determined. Average recoveries of captafol from fortified samples were 93.0, 97.1, and 87.1% for apple leaves, wood, and fruit, respectively. Samples from apple trees treated with captafol at two concentrations and at two locations were analyzed periodically to demonstrate the practical application of this method and to determine captafol distribution in the orchards.

Captafol [*N*-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide] is the active ingredient of the commerical formulation of Difolatan (Chevron Chemical Co., Ortho Division, Richmond, CA). It is currently used as a nonsystemic fungicide for the control of many of the major fungal diseases on fruits, ornamentals, vegetables, and turfgrass of economic importance (Chevron Chemical Co., 1965).

Techniques for residue analysis of various crops have been reported in the literature. Both gas and thin-layer chromatography (GC, TLC) methods have been used for the quantitative analysis of captafol residues in plant and animal tissues. Kilgore and White (1967) developed a procedure for the extraction and determination of captafol residues in apricots, cherries, nectarines, peaches, and prunes. The fruits were extracted with benzene (600 mL/300 g sample), analyzed directly, or cleaned up on attaclay–charcoal columns before analysis by GC employing electron-capture detection. Chevron Chemical Co. (1975) described a method using ethyl acetate as the extracting solvent (350 mL/25–50 g sample). Sample cleanup was composed of four steps: (1) ethyl acetate–water partition, (2) acetonitrile–hexane partition, (3) acetonitrile–water–hexane partition, and (4) Florisil column chromatography. Quantitation of the residues were performed by GC using either an electron-capture or flame-photometric detector.

Other techniques using GC for captafol residue determinations have been reported by Crossley (1972), Cooke (1973), Baker and Flaherty (1972), Pomerantz et al. (1970), and Lemperle and Strecker (1971). Chiba and Northover (1977) published a combined TLC and GC analysis of captafol residue in apple leaves and wood. They used tumbling and sonification extraction methods. Samples were purified by TLC prior to GC analysis. Although these methods are satisfactory with regard to recoveries, they generally involve time-consuming extraction and cleanup procedures and large volumes of purified solvents.

We now report the development of an improved analytical procedure for captafol in apple wood, leaves, and fruit and its practical application in the analysis of plant

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tissue from apple trees treated with captafol.

MATERIALS AND METHODS

Reagents. Acetonitrile, benzene, methylene chloride, and petroleum ether (35–60 °C) were distilled prior to use. Florisil (60/100 mesh) was manufactured by Floridin Co., Hancock, WV. It was activated at 140 °C overnight and cooled at room temperature for 45 min prior to use. Captafol (99.34%) was provided by Chevron Chemical Co., Ortho Division, Richmond, CA. Stock solutions of captafol (for recovery trials and preparation of standard curves) were prepared by dissolving 10 mg in 1 mL of acetone and 9 mL of benzene. Two solvents were prepared and stored over anhydrous sodium sulfate prior to use in the column cleanup procedures: (1) 20% methylene chloride in petroleum ether and (2) 50% methylene chloride in 1.5% acetonitrile in petroleum ether. Safe handling of all solvents is advised because of flammability and potential toxicity.

Apparatus. A Tracor Model 222 gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. An electronic digital integrator (Autolab Minigrator, Spectra Physics Model 23000–011) was used to determine areas of peaks. Analyses were performed with a $^1/_4$ in. × 6 ft U-shaped glass column containing a column packing of 3% SP2401 100/120 mesh Supelcoport. The column was conditioned by injecting nanogram quantities of captafol until a constant response was obtained. The temperature of the column, detector, and injection port during the analysis was maintained at 195, 300–350, and 250 °C, respectively. Prepurified nitrogen was used (without further purification) as carrier gas at a flow rate regulated to provide 80 cm^3/min.

Apple Samples. All samples (leaves, wood, and fruit) used in this investigation were provided by the Fruit Research Laboratory, The Pennsylvania State University, Biglerville, PA. Samples were stored under refrigeration (≈–20 °C) prior to analysis.

Spray Application. Samples (wood, leaves, and fruit) used in this investigation were collected from treated and untreated trees in two apple orchards located in south-central Pennsylvania. A single application of captafol (Difolatan 4F, Chevron Chemical Co., Ortho Division, Richmond, CA) was applied to each orchard at the 1.0 cm green bud stage on 1 April 1977 under good to excellent conditions for spray application and drying. Trees in one of the orchards (Longenecker) were mature "York Imperial"/seedling 5.5 m high and spaced 9.0 × 10.5 m. Each captafol plot consisted of a 2.5-ha block of trees planted in ten or more rows. Treatments were applied in this orchard with a John Bean 257 airblast sprayer operated at a speed of 4.0 km/h and delivering 467 L/ha of spray with 80% directed into the upper two-thirds of the trees (50% in the upper one-half). Captafol (Difolatan 4F) at 9.3 and 28.0 L/ha was applied in combination with 12.0 L/ha superior oil (Sunspray 7E, Sun Petroleum Products Co., Philadelphia, PA).

Samples were taken from a second orchard located at The Pennsylvania State University, Fruit Research Laboratory orchard, Biglerville, PA. "Rome Beauty"/M7A trees 3.0 m high and spaced 4.0 × 8.0 m were sprayed to the point of "run-off" (ca. 2.8 kL/ha) with a high-pressure, single-nozzle handgun operated at a pressure of 3100 kPa. Difolatan 4F 0.5 and 1.0 L/100 L was applied in combination with superior oil at 2.0 L/100 L.

Sample Collection. Replicate samples were collected from each orchard for residue analysis from four trees which were representative of the orchard. Wood samples consisted of 25 spurs (blossoming clusters) ca. 5.0 cm long

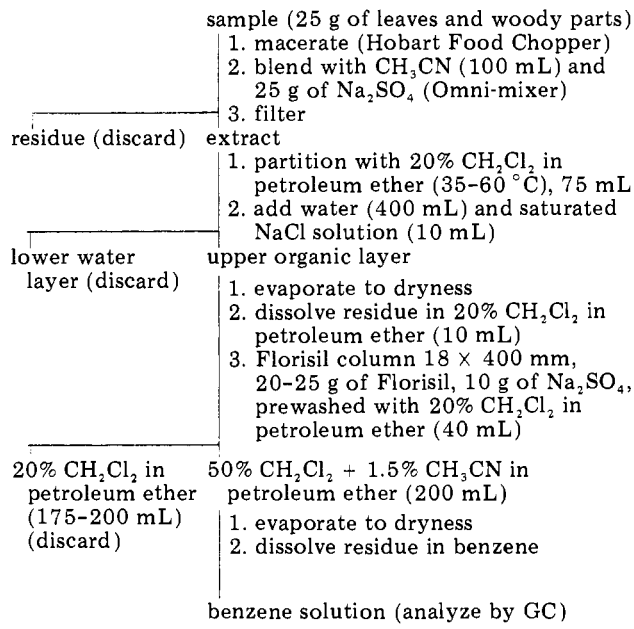

Figure 1. Scheme of developed extraction and cleanup procedure for captafol.

(leaves removed) which were collected randomly at 1.0–2.0 m high around the trees. Leaf samples consisted of the three apical leaves on each of 25 terminal shoots per replicate tree collected from the same area as the wood samples. Fruit samples were collected at harvest on 21 September (Longnecker) and 13 October (PSU Fruit Research Laboratory) by selecting ten fruits at random from around the tree at 2.0–2.5 m high.

Sample Extraction and Solvent Partitioning. Bulk samples were macerated and mixed to provide a representative sample. Apple wood samples were transferred (25 g) to a pint or quart Mason glass jar and 100 mL of acetonitrile and 25 g of sodium sulfate were added. The mixture was blended (Omni-mixer, Ivan Sorvall, Inc.) for 3 min and filtered with suction (Buchner funnel). Apple leaves (25 g) were directly blended in the same fashion as wood samples. Fruit samples were sliced into small pieces including the peel, and an aliquot (150 g) was blended with 150 mL of acetonitrile and 50 g of sodium sulfate in a Waring Blendor for 3–5 min. The resulting mixture was then filtered on a Buchner funnel and the filtrate was directly worked up or stored under refrigeration over sodium sulfate. In this study all sample extracts were analyzed within 48 h. If residue levels were large, a suitable aliquot of the extract was taken for analysis. For recovery tests, the untreated control samples were fortified with a known amount of captafol after placing the macerated material in the Mason jar, but before the addition of the extracting solvent.

The filtrate was transferred to a liter separatory funnel and vigorously shaken with 75 mL of 20% methylene chloride in petroleum ether for 2 min. Layers were allowed to separate, and 10 mL of saturated aqueous sodium chloride and 400 mL of distilled water were added. The resulting mixture was shaken gently, to avoid formation of an emulsion, and then allowed to sit for 15 min. The lower aqueous layer was discarded and the upper organic phase, containing the pesticide residue, was transferred to a 250-mL, round-bottom flask and rotary evaporated to dryness. The residue was then dissolved in 10 mL of 20% methylene chloride in petroleum ether prior to cleanup. For leaf samples richer in plant pigments, the upper organic layer was washed again with a solution of

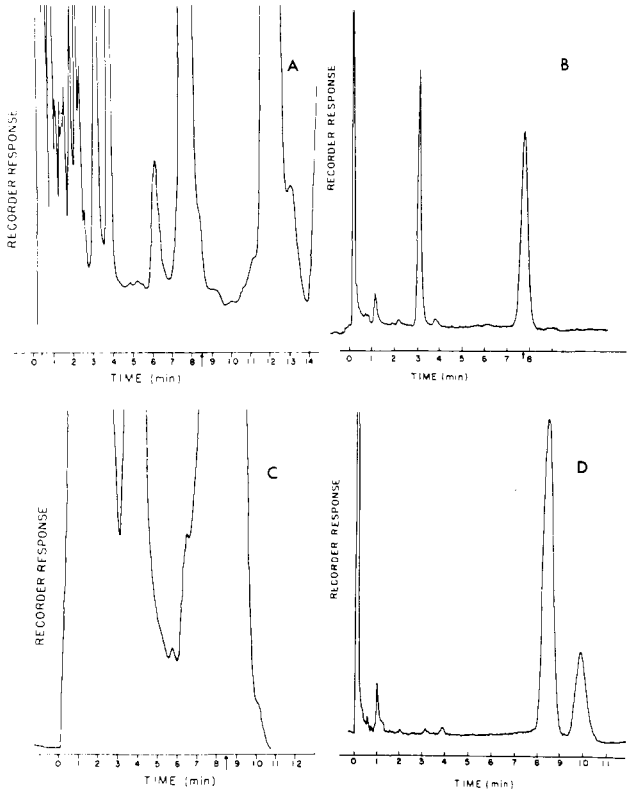

Figure 2. Gas chromatogram of an extract of apple wood fortified with captafol without cleanup (A) and with cleanup (B). Gas chromatogram of an extract of apple leaves fortified with captafol without cleanup (C) and with cleanup (D).

saturated sodium chloride (10 mL) and 100 mL of distilled water.

Florisil Column Chromatography. The Florisil adsorbent was poured into the column (18 × 400 mm) (20 g for fruit and wood samples and 25 g for leaf samples) and topped with 10 g of anhydrous sodium sulfate. The column was then prewashed with 10 mL of 20% methylene chloride in petroleum ether, and the washings were discarded. The plant extract was transferred to the column and the column was then washed with 175–200 mL of 20% methylene chloride in petroleum ether to remove possible interfering materials. The captafol residue was then eluted with 200 mL of 50% methylene chloride plus 1.5% acetonitrile in petroleum ether. The eluate was collected in a 250-mL round-bottom flask and evaporated to dryness with a rotary evaporator at 45 °C. The residue was dissolved in a suitable amount of benzene for quantitative determination by gas–liquid chromatography.

Gas Chromatography Analysis. All samples were analyzed under the operating conditions mentioned previously. Standard straight line curves were prepared for each set of samples by plotting peak area responses vs. nanograms of captafol (dissolved in benzene). A sample linear regression analysis with Minitab II was used to determine the regression line of the standard curve. The amount of residue was quantitatively determined by comparing the average peak area of a replicate series of two chromatograms with the standard curve.

RESULTS AND DISCUSSION

A scheme of the newly developed extraction and cleanup procedure for captafol on apple wood, leaves, and fruit is

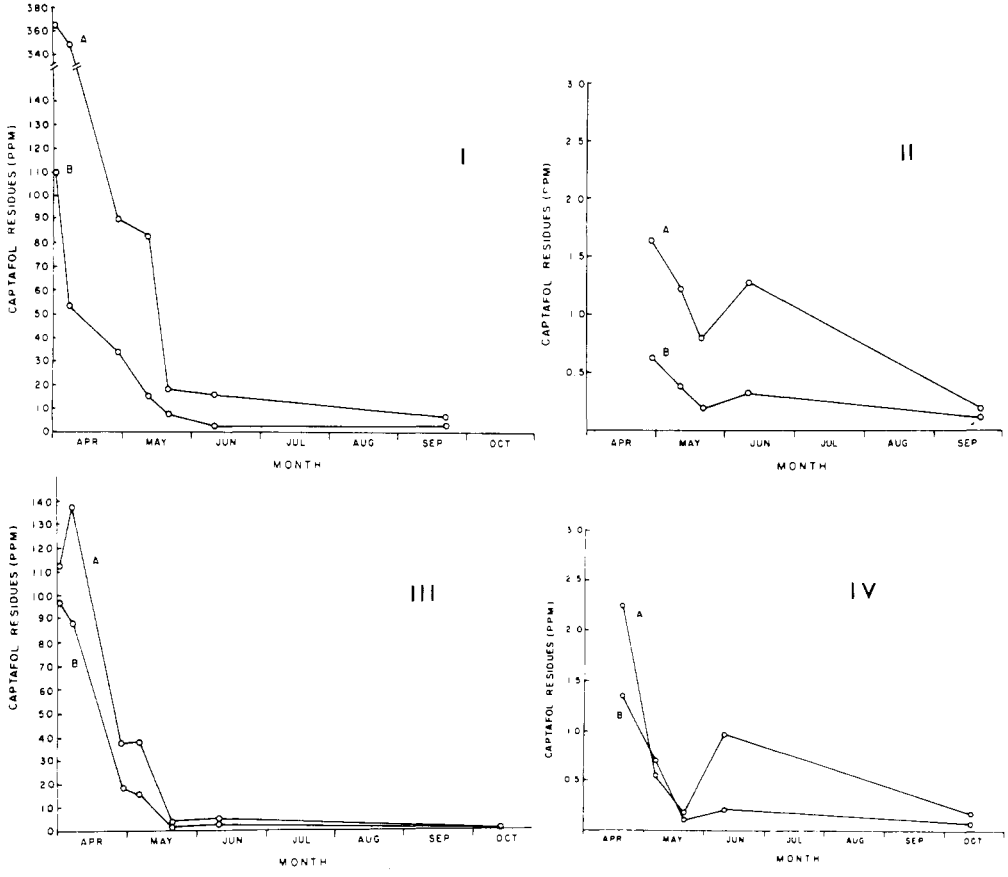

Figure 3. Seasonal changes of captafol residues on apple wood (I) and leaves (II) (Longnecker orchard). Curves A and B represent a treatment of 12 and 4 qt of Difolatan 4F/100 gal of water per acre, respectively. Seasonal changes of captafol residues on apple wood (III) and leaves (IV) (PSU orchard). Curves A and B represent a treatment of 4 and 2 qt of Difolatan 4F/100 gal of water per acre.

Table I. Recovery of Captafol from Fortified Apple Samples

added ppm	av % recov[a]	SD
A. Leaf Samples		
0.10	87.8	2.87
0.50	86.6	3.40
1.00	87.7	2.09
2.00	96.0	4.57
3.00	96.9	3.08
5.00	96.9	2.69
10.00	92.9	4.85
25.00	99.5	3.70
total av recov	93.0	
B. Wood Samples		
1.00	97.5	3.31
2.00	91.0	3.29
5.00	98.3	5.35
25.00	99.5	3.58
40.00	97.1	3.18
100.00	97.3	1.13
500.00	98.6	0.97
total av recov	97.1	
C. Fruit Samples		
0.05	85.1	4.00
0.10	87.5	4.65
0.50	83.5	1.91
1.00	88.3	4.57
2.00	87.8	1.81
5.00	90.3	3.74
total av recov	87.1	

[a] Average of four replicates.

presented in Figure 1. Modifications from previous methods include the use of less and different organic solvents and less partitioning steps resulting in a reduction in time for cleanup. One hundred milliliters of acetonitrile was found sufficient to extract the 25–50-g plant samples. Less acetonitrile would be required with smaller plant samples. Only 75 mL of 20% methylene chloride in petroleum ether and 400 mL of distilled water were used in the partitioning step. Further partitioning steps as used in some other procedures (Pomerantz et al., 1970; Chevron Chemical Co., 1975) were found unnecessary. The partitioned upper-layer organic phase was evaporated to dryness, the residue was redissolved in 20% methylene chloride in petroleum ether and cleaned up on an activated Florisil column. Captafol eluted with 50% methylene chloride plus 1.5% acetonitrile in petroleum ether. Following evaporation to dryness, the residue was dissolved in benzene and analyzed by GC. Since this modified procedure resulted in the use of less organic solvents and partitioning steps it required less time than previous cited methods. Eight samples could be extracted, cleaned up, and analyzed in a 8-h period.

Kilgore and White (1967) reported a procedure applicable to some fruits without a cleanup step. With the apple samples (wood, leaves, fruit) a cleanup procedure was necessary because of interfering substances. Charcoal and attaclay columns did not give satisfactory recoveries (ca. 24%). Moreover the use of benzene for extraction, as suggested by Kilgore and White (1967), Crossley (1972), Iwaida et al. (1974), and Chiba and Northover (1977), is to be avoided because of its potential carcinogenic activity.

Figure 2 shows gas chromatograms of uncleaned and cleanedup samples of apple wood and leaves fortified with captafol. Similar results were obtained with fruit samples. As is evident, the Florisil column used in the cleanup procedure removed significant contaminents. Table I shows the average recovery of captafol from untreated apple wood, leaves, and fruit samples fortified with captafol from 0.05 to 500 ppm. The apple wood samples gave the best recovery (av. 97.1%), while the fruit sample gave the poorest recovery (av. 87.1%). Captafol residues as low as 4 ppb were determined.

To demonstrate the applicability of the developed technique, various samples from field-treated trees were analyzed. Samples were obtained from two different orchards: a privately owned orchard (Longenecker) and The Pennsylvania State University (PSU) fruit research orchard (Biglerville, PA). Figure 3 shows the seasonal change of captafol residues in apple wood and leaves from the day of application to harvest in the two orchards. The increase in captafol residues in June on the leaves represents redistribution of captafol by rainfall. Residue of captafol on fruit average 0.06 ppm.

These data show persistence and redistribution of captafol in the apple trees and provide basic information to make a rational discision for proper, safe, and effective use of this fungicide for disease control. Specifically it examined the suitability of using captafol in a single application technique for control of apple scab for pest management programs.

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