Persistence of Chlorothalonil on Grapes and Its Effect on Disease Control and Fruit Quality

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The fungicidal efficacy of chlorothalonil against *Plasmopara viticola* (downy mildew) and *Uncinula necator* (powdery mildew) of grapevines in Ontario, Canada, was determined. Applications of 1.5–1.9 kg of AI/ha at 10–14-day intervals prevented foliar infections of both diseases. Harvest residues 46 days after completion of both three- and six-spray programs were 0.6–0.8 μg/g for wettable powder (WP) and 1.0–3.3 μg/g for flowable (F) formulations. For determination of the dissipation rate, a seventh application of chlorothalonil (WP) was made August 30, thereby increasing berry residues from 1.9 to 7.5 μg/g, which degraded slowly to 4.9, 3.1, 1.6, 1.6, and 2.5 μg/g after 7, 14, 21, 27, and 36 days. The time for the initial residue to decline to one-half was calculated from first-order kinetics and from asymptotic regression to be 10–15 days. Flowable formulations were phytotoxic to De Chaunac berries. Concentrations of soluble solids and titratable acid and the pH of juice were not appreciably affected by the several chlorothalonil programs.

Mancozeb [zinc ion and manganese ethylenebis(dithiocarbamate)] has been extensively used for the control of downy mildew [*Plasmopara viticola* (Berk & Curt) Berl. & de Toni] of grapes in Ontario. However, toxicological concern over the conversion of mancozeb and other ethylenebis(dithiocarbamate) fungicides into ethylenethiourea (ETU) might restrict its future use and create a need for an alternative fungicide (Ripley et al., 1978). Chlorothalonil (tetrachloroisophthalonitrile) has proved effective in Australia (Maitland, 1978) against downy mildew and powdery mildew [*Uncinula necator* (Schw.) Burr.] of grapes and in the states of California (Maitland, 1978) and New York (Braun, 1964) against grape powdery mildew. The present research examined its efficacy under the climatic conditions of southern Ontario. Determinations were made of the late-season dissipation of chlorothalonil and the effect of its formulation upon foliar disease control, fruit quality, and residues on berries and in expressed juice.

EXPERIMENTAL SECTION

Plots consisted of either 5 or 10 mature vines, surrounded by unsprayed vines, planted 2.9 m between vines and 3.4 m between rows and pruned to a four-cane kniffen system. Plots were randomized and replicated in three blocks. Experiment 1, conducted on Agawam grapes in 1976, determined the efficacy of Bravo 7.2 F (7.2 lb of chlorothalonil/imperial gal) at four rates, compared to the rates of Phaltan [50% folpet [*N*-(trichloromethylthio)phthalimide]] and Dikar [72% mancozeb and 4.4% dinocap (mixture of 2,4-dinitro-6-octylphenyl crotonate and 2,6-dinitro-4-octylphenyl crotonate)] recommended by the Ontario Ministry of Agriculture and Food (1976). Four applications were made: June 17 (prebloom), July 9 (postbloom), July 20 (first cover), and August 11 (second cover). Experiment 2 was conducted on De Chaunac (Seibel 9549) vines in 1978 and compared three formulations of Bravo: the 75% wettable powder (WP), the 7.2 flowable (F), and the 500 flowable (F) (500 g of chlorothalonil/L), used in concentrations equivalent to 68 g of chlorothalonil/100 L [1.5 kg of chlorothalonil/(ha application)]. Materials were applied as both a full-season program of six applications [June 15 (prebloom), June 29 (postbloom), July 11 (first cover), July 21 (second cover), July 31 (third cover), and August 11 (fourth cover)] and a late-season program of three sprays (July 11, July 31, and August 11).

Application Method. Fungicides were applied by a hydraulic sprayer at 2100 kPa using two five-nozzle booms mounted vertically on a vehicle driven at 2.5 km/h. The spray volumes used in experiment 1 were 1700 L/ha prebloom, 2270 L/ha postbloom, and 2840 L/ha in the first and second cover sprays. In Experiment 2 applications were made by using 2270 L/ha for each spray. An application of Pirimor [pirimicarb [2-(dimethylamino)-5,6-dimethyl-4-pyrimidyl dimethylcarbamate]] on July 20, 1976, was the only insecticide used in these experiments.

Disease Assessment. Downy mildew was assessed September 7–9, 1976, by examining the lower (abaxial) surfaces of mature mid-season-developed leaves. Sampling was by means of a metal grid consisting of 20 square apertures, each 20 × 20 cm, placed against the vine. One leaf was sampled through each aperture. In experiment 1, 50 leaves were sampled from each of the east and west sides of each plot. No evaluation was made in experiment 2 because of low disease incidence in the check.

The leaves sampled for downy mildew in experiment 1 were also evaluated for severity of powdery mildew on the upper (adaxial) surface by using a rating system of six categories described previously (Chiba et al., 1973). In experiment 2, 20 leaves on each of the east and west sides of each plot were rated for powdery mildew severity August 22–23, 1978, and expressed as a percentage of leaf area infected.

Dissipation Study. A further application of Bravo 75 WP was applied on August 30, 1978, to half of each De Chaunac plot in experiment 2 that had previously received six applications of the same formulation. Bunches were collected from the high and low branches close to the trunk from each of the three replicate plots. Bunches were cut into thirds and placed at random into three plastic bags to give three composite samples of 1 kg for each sample date, of which one was analyzed. Samples were taken prespray and at intervals to 36 days postspray and frozen at −10 °C. Prior to residue analysis, the berries were separated from stalks, weighed, and counted; only berries were analyzed.

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Berry Residues at Harvest. Composite samples (1 kg) were taken September 26 from each of the seven main treatments in experiment 2 and frozen at −10 °C. Residue analyses were subsequently performed on the berries only.

Berry and Juice Residues. On September 28, bunch samples were collected separately from each plot and held in plastic bags at 1 °C. Berries were separated from stalks 15 days later, and a representative 750-g subsample was refrigerated while a further 1.25 kg of berries was juiced. The volume of extracted juice was measured, and its soluble solids were determined with a hand refractometer (American Optical) and expressed as degrees Brix (°B) (Balling). The acidity and the quantity of titratable acid were determined potentiometrically, and the latter is expressed as g of tartaric acid/100 mL of juice. These data were statistically analyzed for treatment differences by analysis of variance and Duncan's Multiple Range Test.

The three replicate samples from each treatment were combined, giving three composite samples of 750 g of berries and 350 mL of juice which were frozen on October 13 for subsequent residue analysis.

Analytical Methods. Chlorothalonil and its metabolite 4-hydroxy-Daconil (DAC-3701; 4-hydroxy-2,5,6-trichloroisophthalonitrile) were determined by electron-capture gas–liquid chromatography using modifications of previously published methods (Pesticide Analytical Manual, 1970; Ballee et al., 1976). Fifty grams of grapes was blended for 5 min with 250 mL of acetone. Five milliliters of 50% H_2SO_4 was added, and the sample was reblended for an additional 5 min. The sample was filtered under vacuum and rinsed with 2 × 25 mL of acetone. The filtrate was concentrated until no acetone remained; concentration continued for 15 min after cessation of the boiling action of acetone. Saturated NaCl (5 mL) was added, and the residue was transferred to a 250-mL separatory funnel with 2 × 40 mL rinses of water followed by 2 × 20 mL rinses of ethyl ether.

For juice samples (150 mL), 5 mL of 50% H_2SO_4 was added and the samples were allowed to stand on the bench for 30 min; 40 mL of ethyl ether and 5 mL of saturated NaCl were then added. The samples were gently shaken and allowed to stand for 15 min. The aqueous phase was transferred to a second separatory funnel and repartitioned with another 40 mL of ethyl ether. The ether extracts were combined, 50 mL of saturated NaCl and 10 mL of H_2O were added, and the solution was shaken for 4 min and allowed to stand for 20 min. The aqueous phase was discarded, and the ether was evaporated just to dryness.

A chromatographic column (18 mm i.d.) containing 15 g of 2% deactivated Florisil was prepared and prewashed with 30 mL of CH_2Cl_2. The crop extract was dissolved in 2 × 10 mL of 5% acetone in CH_2Cl_2 (eluant A) and placed on the column. The column was eluted with an additional 100 mL of eluant A; the receiver was changed, and the column was eluted with 100 mL of 50% acetone in CH_2Cl_2 (eluant B). Each eluate was evaporated just to dryness. The eluate A residue (chlorothalonil) was redissolved in 5 mL of hexane for GLC analysis.

The eluate B residue (4-hydroxy-Daconil) was dissolved with 10 mL of H_2O and 1 mL of 50% H_2SO_4 and transferred to a 125-mL separatory funnel with rinses of 10 mL of H_2O and 30 mL of ethyl ether. The solution was shaken for 4 min and allowed to stand for 10 min, and the aqueous phase was discarded; the solution was allowed to stand for a further 5-min period, and any remaining water was discarded. The ether was evaporated just to dryness, 2 mL of cold diazomethane solution was added, and the mixture was allowed to stand for 5 min; if the yellow color did not persist, an additional 2 mL of diazomethane was added. This solution was evaporated to dryness with a gentle flow of nitrogen and redissolved in 5 mL of benzene.

Table I. Comparison of the Efficacies of Bravo, Dikar, and Phaltan against Late-Season Downy Mildew and Powdery Mildew Infection of Agawam Grapevines

fungicide and formulation	concn of active ingredient, g/100 L	powdery mildew: leaf area infected, %	downy mildew: leaves infected, %
Bravo 7.2 F	180	1.4 A[a]	0.7 A
Bravo 7.2 F	135	1.6 A	1.8 AB
Bravo 7.2 F	90	1.7 A	2.4 AB
Bravo 7.2 F	68	2.4 AB	5.3 B
Dikar 80 W	160	4.1 AB	4.1 AB
Phaltan 50 WP	100	7.8 B	0.8 AB
water check		47.5 C	91.0 C

[a] Means followed by different letters are significantly different ($P = 0.05$) by Duncan's Multiple Range Test.

Table II. Effects of Six- and Three-Spray Programs of Three Formulations of Bravo[a] on the Control of Powdery Mildew of De Chaunac Grapevines and on the Residues of Chlorothalonil and 4-OH-Daconil on Fruit at Harvest[b]

fungicide and program	leaf area with mildew, %	residues in grapes, μg/g: chlorothalonil	residues in grapes, μg/g: 4-OH-Daconil
full season:			
6 applications			
Bravo 75 WP	0 A[c]	0.8	0.006
Bravo 7.2 F	0 A	1.0	0.014
Bravo 500 F	tr[d] A	3.3	0.017
late season:			
3 applications			
Bravo 75 WP	tr A	0.6	0.002
Bravo 7.2 F	tr A	2.5	0.008
Bravo 500 F	0 A	1.7	0.008
unsprayed check	30 B	ND[d] (<0.002)	ND (<0.002)

[a] Bravo formulations were applied at 1.5 kg of AI/(ha application). [b] Interval between final application (August 11) and harvest (September 26) was 46 days. [c] Means followed by different letters are significantly different ($P = 0.05$) by Duncan's Multiple Range Test. [d] tr = trace; ND = not detectable.

The extracts were analyzed on a Hewlett-Packard 5713 gas chromatograph equipped with a constant-current electron-capture detector and a 6 ft × 4 mm i.d. glass column packed with 3.6% OV-101 and 5.5% OV-210 on 80–100 mesh Chromosorb W, AW, DMCS treated. Chromatographic conditions were the following: oven, 200 °C; detector, 300 °C; 5% argon–methane carrier gas, 60 mL/min. Under these conditions chlorothalonil eluted in 7.1 min and the methylated 4-hydroxy-Daconil eluted in 8.6 min. Mean recoveries in grapes and juice were 88% for chlorothalonil and 85% for 4-hydroxy-Daconil. No interferences were encountered with these substrates.

RESULTS AND DISCUSSION

Disease Prevention. In experiment 1, even the lowest concentration of Bravo 7.2 F (68 g of chlorothalonil/100 L) was as effective as the two standard fungicides Dikar and Phaltan for the prevention of powdery mildew and late-season downy mildew on Agawam foliage (Table I).

Three formulations of Bravo used at 68 g of chlorothalonil/100 L [1.5 kg of AI/(ha application)] were equally effective against powdery mildew on De Chaunac grapevines (Table II) and showed good residual protection

Table III. Decline in Residues of Chlorothalonil and 4-OH-Daconil on Grape Berries Relative to Postspray Interval, Cumulative Rainfall, and Berry Weight after a Seventh Application of Bravo WP[a]

days after application	cumulative rainfall post-spray, mm	residues in grapes, μg/g: chlorothalonil	residues in grapes, μg/g: 4-OH-Daconil	mean berry wt, g
0 unsprayed	0	ND (<0.02)	ND (<0.002)	1.40
0 prespray[b]	0	1.9	0.018	1.39
0 postspray	0	7.5	0.058	1.40
1	0	8.7	0.090	1.45
3	0	6.1	0.034	1.96
7	7.0	4.9	0.024	1.54
11	18.0	5.4	0.017	1.77
14	57.0	3.1	0.014	1.85
21	137.2	1.6	0.016	1.76
27	137.4	1.6	0.013	1.90
36	191.7	2.5	0.017	1.90

[a] Bravo formulation was applied at 1.5 kg of AI/(ha application). [b] Residues remaining from six previous applications of Bravo 75 WP.

until harvest, a 46-day postspray interval (August 11–September 26), despite severe infection on adjacent unsprayed vines. The three-spray late-season program, commenced immediately after the first appearance of powdery mildew, was most effective, and there was no advantage in the application of earlier sprays for the prevention of powdery mildew on this cultivar.

Chlorothalonil (DAC 2787, 75 WP) was effective in New York State against powdery mildew infection of grapevine foliage but inadequate for protection of bunches at rates equivalent to 1.1–1.3 kg of AI/ha (Braun, 1964). The rates currently recommended in Australia (Maitland, 1978) are equivalent to 1.3–1.7 kg of AI/ha. In the present research, under Ontario conditions, the low rate of 68 g of AI/100 L was equivalent to 1.9 kg of AI/ha in the first and second cover sprays of experiment 1, but in Experiment 2, where only 2270 L of spray/ha was used per application, the rate was equivalent to 1.5 kg of AI/ha.

Phytotoxicity. The 7.2 F and 500 F formulations caused a superficial epidermal roughness and scaling of berries, as well as a bleaching of the early blotchy coloration of De Chaunac berries examined 14 days after the final application. The injury was most evident on bunches on the upper branches, exposed to direct sunlight. By contrast, the wettable powder formulation caused negligible injury to the fruits. By harvest, September 26, the development of dark purple pigment in De Chaunac berries and a heavy wax bloom almost entirely masked the injury caused by the flowable formulations. The sizes of berries, bunches, and leaves appeared normal.

The extent of varietal susceptibility to Bravo phytotoxicity and its commercial consequence has not been established, although berry injury has been observed in Australia and California (Maitland, 1978). A certain amount of injury could be tolerated for wine grapes but not for dessert varieties.

Harvest Residues. The harvest residues of berries, 46 days after the final application August 11, were similar for the three- and six-spray programs except that the residues tended to be higher from the flowable formulations (1.0–3.3 μg/g) than those from the wettable powder (0.6–0.8 μg/g) (Table II). Residues were not detectable (ND) in the unsprayed check. The residues of 4-OH-Daconil, the chlorothalonil metabolite, were also lower in the fruit treated with the wettable powder than that treated with the flowable formulations (Table II).

Chlorothalonil Dissipation Study. The seventh application of Bravo WP increased chlorothalonil residues on fruits from 1.9 to 7.5 μg/g (Table III). Residues decreased to 4.9 and 3.1 μg/g after 7 and 14 days, respectively, and thereafter declined slowly to 1.6–2.5 μg/g, during the subsequent 22-day period. The 4-OH-Daconil residues (Table III) were low in all cases and varied between 0.3 and 1.0% of the chlorothalonil residues. This was similar to both the range of 0.2–2.0% of the chlorothalonil residues in grape juice (Table IV) and a range of 0.5–1.9% on onion foliage (Dzikowski and Ripley, 1979).

The decline of chlorothalonil residue (Table III) showed a high correlation with the length of the postseventh spray interval ($r = -0.86$) and also with cumulative rainfall ($r = -0.84$) (Table III). The relation between residue present and time after application was further examined by first-order kinetics and asymptotic regression, and due to the scattering of the data both models adequately described the dissipation of chlorothalonil. The erratic data for the 14–36-day period left questions concerning the nature and concentration of the terminal residue. From these equations, the time taken for the residue to dissipate by half was of the order of 10–15 days, which was substantially longer than the 3-day interval on onion foliage (Dzikowski and Ripley, 1979) and an approximately 5-day interval on apple foliage (Gilbert, 1976).

The effect of rain on residue dissipation was not clear from this study (Table III). However, the bunches were moderately protected by the foliage from rain water and direct sunlight.

The average maximum and minimum temperatures during the dissipation study on grapes were 21 and 11 °C, respectively, for the 36-day period, although a 4-day rain-free period with an average maximum of 27.4 °C, which occurred between 3 and 7 days postspray, could have contributed to the early residue decline. Chlorothalonil applied to the leaves of 12 tree species showed a persistent fungitoxicity to *Monilinia fructicola* (Wint.) Honey for an average of 20 days, but climate conditions were not obviously correlated with its persistence (Neely, 1970). On De Chaunac grapes chlorothalonil gave excellent residual

Table IV. Effect of Six- and Three-Spray Programs of Three Formulations of Bravo on Residues of Chlorothalonil and 4-OH-Daconil (μg/g) in Refrigerated De Chaunac Grapes and Juice

fungicide and program	grape berries: chlorothalonil	grape berries: 4-OH-Daconil	grape juice: chlorothalonil	grape juice: 4-OH-Daconil
full season: 6 applications				
Bravo 75 WP	0.3	ND (<0.002)	0.3	ND (<0.0005)
Bravo 7.2 F	0.8	0.006	0.7	0.002
Bravo 500 F	1.6	0.008	0.9	0.002
late season: 3 applications				
Bravo 75 WP	0.2	ND (<0.002)	0.3	ND (<0.0005)
Bravo 7.2 F	0.7	0.011	0.8	ND (<0.0005)
Bravo 500 F	0.6	0.006	0.2	0.004
unsprayed check	ND (<0.002)	ND (<0.002)	ND (<0.001)	ND (<0.0005)

Table V. Characteristics of De Chaunac Grape Juice in Relation to Three Formulations of Bravo Sprayed in Six- and Three-Application Programs

fungicide and program	juice/ berries, mL/kg	sol solids, °B	acidity, pH	tartaric acid equiv, g/100 mL
full season:				
6 applications				
Bravo 75 WP	555 A[a]	15.9 A	3.1 A	1.08 A
Bravo 7.2 F	529 A	15.8 A	3.1 A	1.15 ABC
Bravo 500 F	552 A	15.3 AB	3.0 AB	1.16 ABC
late season:				
3 applications				
Bravo 75 WP	542 A	14.8 AB	3.1 AB	1.17 ABC
Bravo 7.2 F	531 A	14.8 AB	3.0 B	1.19 BC
Bravo 500 F	551 A	15.2 AB	3.0 AB	1.23 C
unsprayed check	542 A	14.1 B	3.1 AB	1.09 AB

[a] Means in the same column followed by different letters are significantly different ($P = 0.05$) by Duncan's Multiple Range Test.

protection against powdery mildew infection for 37 days after the completion of a five-spray program (Northover and Neufeld, 1980).

The decline in chlorothalonil residues from 7.5 to 2.5 μg/g over 36 days represented a reduction of 67%, but the growth of berries from 1.40 g to 1.90 g during this period accounted for a dilution of 26%, leaving only 41% reduction due to loss of residues.

Berry and Juice Residues. Grapes that were refrigerated at 1 °C for 15 days before being frozen showed appreciably lower residues (Table IV) than those associated with grapes frozen immediately after harvest (Table II). This may have been due to the different storage conditions and partially to the dissipative effect of 6 mm of rain between the two harvest dates. The chlorothalonil and 4-OH-Daconil residues from the wettable powder formulation were lower than those from the flowable formulations, confirming the trend in the harvest samples. The chlorothalonil residues in the cold-expressed juice were similar to those in the refrigerated berries, but the very low levels of 4-OH-Daconil appeared lower in the juice than in the berries (Table IV).

Compared with the unsprayed check, the several Bravo programs had no effect on juice yield/kg of grapes (Table V). Soluble solids were higher in the six-spray program of Bravo WP and Bravo 7.2 F than in the check, but the differences in pH and titratable acid were minor. The soluble solids and acidity were lower, and titratable acid was slightly higher than average levels for Ontario-produced De Chaunac (S9549) juice: respectively, 16.6 °B, pH 3.27, and 1.00 g of tartaric acid/100 mL (Zubeckis, 1963).

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Search for Linuron Residues in Tributaries of the Chesapeake Bay

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It has been suggested that herbicides which are used on corn and soybean fields bordering the Chesapeake Bay and its tributaries may be a contributing factor to reported declines in the abundance of grasses in the Bay. As part of the program to determine the contribution, if any, of linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] to the problem, samples of mud and water have been taken from areas likely to show linuron if it is being transferred from fields into adjacent bodies of water. Samples from drainage basins receiving up to 45000 kg of linuron annually showed no linuron residue, i.e., less than 10 ng/g (10 ppbw) in the mud and less than 0.2 μg/L (0.2 ppb, w/v) in the water. These samples were analyzed by using an extraction procedure followed by measurement with liquid chromatography. From these data, it is concluded that linuron is not accumulating in the Chesapeake Bay.

Declines in the abundance of aquatic grasses in the Chesapeake Bay have been reported recently, and this situation is of concern because of the role which these grasses play in the propagation of waterfowl, shellfish, and finfish. Similar declines have been noted in the past, and the reasons for these cycles are not understood to any significant degree.

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