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Behavior of Acylanilide and Dicarboximidic Fungicide Residues on Greenhouse Tomatoes

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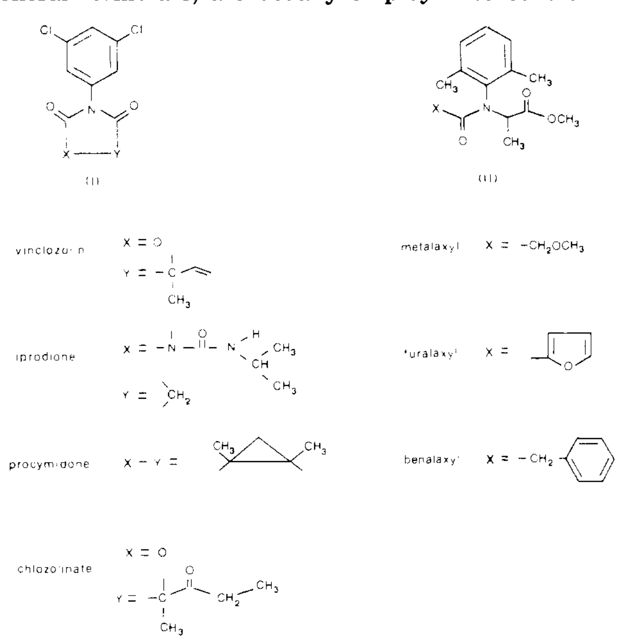
The residual behavior of four dicarboximidic and three acylanilidic fungicides was studied in greenhouse grown tomatoes. In different experiments tomatoes underwent single spraying and five sprayings with an interval of 21 days, at rates of application of 380 and 760 g/ha (acylanilides) and 1500 and 3000 g/ha (dicarboximides). The harvesting was carried out weekly. Residues recovered after repeated sprayings confirmed the well-known trend of the four dicarboximidic fungicides: toxically significant accumulation in fruits several days after recommended preharvest times. Among acylanilides, only furalaxyl and benalaxyl showed the accumulation of residues but always at concentrations lower than legal limits, even before the fixed preharvest times. The possible need is raised to revise preharvest times for both categories of fungicide. When tomatoes were single sprayed, the fungicide disappearance from fruits showed pseudo-first-order rate dependence only for dicarboximides; degradation products of the latter, found in a different matrix (wine), were not detected.

The fungal diseases most commonly present in greenhouse grown tomatoes are those generated by *Botrytis cinerea* and *Phytophthora infestans*. Dicarboximides, of general formula I, are usually employed to control *B.*



cinerea, while acylanilides, of general formula II, are usually employed to control *P. infestans* (Gozzo, 1979).

The behavior of residues of the dicarboximidic fungicides vinclozolin and iprodione has been studied by Vanachter et al. (1979) and Van Wambeke et al. (1980) on greenhouse-grown tomatoes. These authors reported an accumulation effect of fungicide residues, due to repeated applications of the same active ingredient (a.i.). The same effect has been observed on greenhouse-grown lettuce (Meloni et al., 1984; Dejonckheere et al., 1982) and on field-grown grapes and tomatoes (Cabras et al., 1982a,b) as well as with pesticides belonging to different chemical families.

Since this effect could result from different ways of using the fungicides and no data are available on the behavior of the acylanilide residues on greenhouse tomatoes, we decided to compare the behavior of the acylanilides and dicarboximides mentioned above.

The specific objectives were (1) to complete, under standardized conditions, the data of Vanachter and Van Wambeke on the dicarboximidic fungicides vinclozolin and iprodione, also studying the behavior of procymidone and chlozolinate, (2) to ascertain if acylanilide fungicide behavior was similar to that of the dicarboximides, (3) to ascertain if the simultaneous spraying of several different a.i., belonging to the same family, could affect the content of their residues, (4) to ascertain if the same dicarboximide degradation products found in wine (Cabras et al., 1984) could also form in tomatoes, (5) to study the degradation kinetics of all the fungicides used in the experiments, and (6) to check if, in usual agronomic handling (repeated applications, weekly harvest, etc.), the preharvest times produce residues corresponding to toxic risk.

EXPERIMENTAL SECTION

Materials and Methods. The trial was carried out inside a 500-m^2 glasshouse with galvanized iron framework, air warmed and equipped with a drop irrigation system. The tomatoes employed Were Vemone I (SLU GRO) and 81 TI UPM (Clause). Sowing was done on Nov 12, 1982, and transplantation on Dec 10, 1982. A random block

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Table I. Residue (ppm ± SD) of Dicarboximidic Fungicides after Single Spraying[a]

fungicide	sprayed amount, g/ha	days after spraying 0	1	3	6	13	20	28
vinclozolin	1500	1.91 ± 0.18	2.63 ± 0.45	1.63 ± 0.22	1.74 ± 0.54	1.17 ± 0.36	0.69 ± 0.26	0.38 ± 0.17
	3000	5.56 ± 0.50	4.65 ± 0.85	3.82 ± 0.71	3.00 ± 0.62	1.86 ± 0.27	0.91 ± 0.09	0.77 ± 0.10
iprodione	1500	2.60 ± 0.62	2.21 ± 0.56	2.80 ± 1.03	2.61 ± 1.03	1.49 ± 0.51	1.09 ± 0.38	0.46 ± 0.29
	3000	4.74 ± 0.90	5.66 ± 0.76	3.22 ± 0.69	3.08 ± 0.62	2.46 ± 0.62	0.95 ± 0.51	0.92 ± 0.14
procymidone	1000	2.06 ± 0.15	1.80 ± 0.27	1.72 ± 0.49	1.20 ± 0.16	1.01 ± 0.08	0.64 ± 0.21	0.36 ± 0.08
	2000	3.57 ± 0.44	4.12 ± 0.47	2.64 ± 0.49	2.06 ± 0.49	1.78 ± 0.40	1.15 ± 0.38	1.03 ± 0.41
chlozolinate	1500	1.05 ± 0.41	1.41 ± 0.20	1.28 ± 0.55	0.76 ± 0.26	0.42 ± 0.16	0.25 ± 0.09	0.19 ± 0.13

[a] The reported values are the means of duplicate analyses from four replications.

scheme was used with four replications. Each block measured 2.56 m^2 and contained eight plants linearly spaced at 0.4 m with 0.8 m between the rows. Sampling (500 g from each lot) started at the time of commercial ripening. Pesticides were applied with portable mechanical sprayers; solutions were prepared in accordance with the manufacturers' recommendations for producing the following coverage densities: vinclozolin, 1500 g/ha; iprodione, 750 g/ha; procymidone, 500 g/ha; chlozolinate, 750 g/ha; furalaxyl, 380 g/ha; metalaxyl, 350 g/ha; benalaxyl, 380 g/ha. In one experiment, single applications were carried out; in another, the applications were repeated 5 times; in a third, the solutions were sprayed at double strength. In addition, the following single-strength combinations were sprayed: furalaxyl plus metalaxyl; benalaxyl plus metalaxyl; vinclozolin plus iprodione and procymidone plus chlozolinate. Pesticide applications were performed every 21 days, starting Jan 25, 1983. In the single spraying experiments, the harvest was performed on the same day and 1, 3, 6, 13, 20, and 28 days after the application. In the repeated spraying experiments, the harvest was performed 7, 14, 21, 28, and 35 days after the last application. Residue values were corrected for recovery and were submitted to a factorial analysis of variance.

The degradation kinetics of the fungicides were determined following the spraying with the doses suggested by the manufacturers (single-dose experiment (SDE) or with double-strength solutions (double-dose experiment, DDE) and harvesting tomatoes as previously mentioned.

Apparatus and Chromatography. We employed a Varian 5020 solvent delivery system (Palo Alto, CA) equipped with a UV/vis variable-wavelength UV-50 detector, Valco AH 20 injector (loop 50 μL), and a Hewlett-Packard 3390 A reporting integrator.

Merck Hibar RP-8 columns (Darmstadt, West Germany, 250 × 4.0 mm i.d., 10 μm) were employed with a 50:50 mixture of water and acetonitrile as the eluent, at the flow rate of 1.5 mL/min. The detector was set at 200 nm, the best wavelength for the simultaneous detection of the pesticides under study.

The standard curve of each fungicide was constructed by plotting peak areas (internal standard method) vs. concentrations. Very good linearity was achieved in the range 0–5 ppm.

Under these conditions, minimal detectable values (MDV) between 0.02 and 0.04 ppm were achieved; MDV were calcd. as previously reported (Cabras et al., 1982a).

Chemicals. Water was distilled twice and filtered through a Millipore apparatus before use (0.45 μm). Acetonitrile was HPLC-grade solvent while cyclohexane and benzene were pesticide-grade solvents (all from Carlo Erba, Milan, Italy).

Metalaxyl (≥99.9%) and furalaxyl (≥99.9%) were kindly supplied by Ciba Geigy (Saronno, Italy). Benalaxyl (≥99.5%) and chlozolinate (≥99.5%) were supplied by

Table II. Preharvest Times and Legal Limits Fixed by Italian Law for Dicarboximides and Acylanilides on Tomatoes

	legal limit, ppm	preharvest time, days
vinclozolin	1.5	21
iprodione	5.0	40
procymidone	1.5	21
chlozolinate	1.5[a]	21[a]
furalaxyl	1.0	21
metalaxyl	1.0	21
benalaxyl	1.0	21

[a] Proposed values.

Farmoplant S.p.A. (Milan, Italy). Vinclozolin (≥98.5%), procymidone (≥98.5%), and iprodione (≥99.0%) were gifts from BASF Agritalia S.p.A., Shell Italiana, and Rhône-Poulenc (Milan, Italy), respectively.

Extraction Procedure. A total of 0.5 kg of tomatoes, sampled as previously described, was homogenized for 5 min at 3000 rpm with a Waring apparatus (Tecnochimica, Rome, Italy). Ten grams of homogenate was extracted in a screw-capped tube (160 × 1.6 mm i.d.) with 10.0 mL of a cyclohexane–benzene mixture (8:2 v/v).

After thorough blending for 10 min, the sample was centrifuged for 5 min at 3000 rpm.

One milliliter of the organic layer, dried under anhydrous sodium sulfate, was evaporated under reduced pressure ($T < 40$ °C), and the residue was recovered with 1.0 mL of the mobile phase. Recoveries obtained with this method on blank tomatoes, fortified with known amounts of the studied pesticides, ranged between 81.8 and 106.2% (mean SD ±4.5%) at levels of 0.5, 1.0, and 2.0 ppm.

RESULTS AND DISCUSSION

Dicarboximides. *Single Spraying.* From the data reported in Table I it was noted that (1) at the preharvest time fixed by Italian laws (Italian Health Department Act, 1979; Table II) a double residue did not correspond to the double-strength spraying for all the fungicides, as other workers found for vinclozolin and iprodione (Vanachter et al., 1979; Van Wambeke et al., 1980), (2) residue values were always lower than the Italian legal limits at the expiry of preharvest times, whether SDE or DDE, (3) values lower than the legal limits were detected for vinclozolin and procymidone 13 days after application, while for iprodione and chlozolinate only 1–2 h after application, (4) the residue disappearance from tomatoes follows a pseudo-first-order kinetic for all the pesticide; their half-life times are reported in Table III.

Repeated Sprayings. The following was noted from the data reported in Table IV. (1) The simultaneous spraying of two dicarboximides produced neither synergic nor antagonistic effects on their residue levels; in fact, the values were similar to those found after the application of the single pesticides. Only iprodione seemed an exception; its residues were much higher in the first 21 days, when

Table III. Fungicide Half-Life Times of Disappearance from Tomatoes

fungicide	sprayed amount, g/ha	$t_{1/2}$ days
vinclozolin	1500	9.85
	3000	9.78
iprodione	1500	10.18
	3000	10.03
procymidone	1000	11.18
	2000	11.03
chlozolinate	1500	7.45

sprayed together with vinclozolin. (2) The accumulation effect already described in the cited reports of Vanachter et al. (1979) and Van Wembeke et al. (1980) was confirmed for vinclozolin and iprodione and noted for procymidone and chlozolinate. Residues found 7 days after the last of five applications of each pesticide were approximately double those determined 7 days after the single application. (3) Tomatoes contained residue values lower than the legal limits only 28 days after the last application of vinclozolin, procymidone, or chlozolinate. Iprodione residues, although in the same range as those of other dicarboximides, never infringed the law, since the legal limit is much higher (5.0 ppm) than for the other dicarboximides studied (1.5 ppm). Chromatographic analyses of tomato extracts excluded the presence of the dicarboximide degradation products found in wine (Cabras et al., 1984).

Acylanilides. *Single Spraying.* Data of disappearance of these fungicides from tomatoes are reported in Table V. This acylanilide disappearance did not seem to be linear in SDE or DDE; it did not follow a pseudo-first-order kinetic as in the case of dicarboximide disappearance. Furthermore, the data show that furalaxyl, metalaxyl, and benalaxyl had different persistences in tomatoes, even if their preharvest times were the same (21 days, Italian Health Department Act, 1982). The values of furalaxyl residues 13 days after application were lower than the MDV of our analytical procedure in SDD or DDE (see Table V), while those of metalaxyl and benalaxyl were detectable up to 20 days after the last spraying. This different behavior was much more evident when SDE were compared with DDE. Although previous reports (Dejonckheere et al., 1982; Van Wambeke et al., 1980) state that residues arising from tomatoes sprayed with double-strength solutions were always higher, only furalaxyl was always found to show such behavior; metalaxyl and benalaxyl had SDE and DDE residues that tended to equalize 13 and 20 days, respectively, after the last spraying. At the moment, we are not able to formulate any hypothesis to explicate such behavior.

Repeated Sprayings. The study of residue trend following repeated sprayings was carried out applying the fungicide alone or in combination (see Experimental Section). The residues found are reported in Table VI. From these data it is evident that repeated spraying of benalaxyl and furalaxyl produced the accumulation effect already observed on tomatoes with other fungicides and insecticides (Vanachter et al., 1979; Cabras et al., 1982a,b) and on lettuce (Dejonckheere et al., 1982; Foschi et al., 1983; Meloni et al., 1984). Such an effect is well indicated

Table IV. Residues (ppm ± SD) of Dicarboximidic Fungicides after Repeated Spraying[b]

fungicide	sprayed amount, g/ha	mode[a]	days after the last spraying: 7	14	21	28	35
vinclozolin	1500	A	2.67 ± 0.22	2.54 ± 0.41	2.00 ± 0.60	0.80 ± 0.05	0.31 ± 0.05
	1500	B	3.20 ± 0.33	2.67 ± 0.33	1.61 ± 0.33	0.78 ± 0.37	0.35 ± 0.06
iprodione	1500	A	2.58 ± 0.26	3.35 ± 0.66	2.97 ± 0.42	1.88 ± 0.22	1.03 ± 0.19
	1500	B	4.52 ± 0.58	4.53 ± 0.81	3.51 ± 0.55	2.00 ± 0.65	0.74 ± 0.17
procymidone	1000	A	2.45 ± 0.33	2.66 ± 0.52	2.06 ± 0.37	1.12 ± 0.40	0.56 ± 0.04
	1000	B	3.13 ± 0.35	2.96 ± 0.47	2.13 ± 0.23	1.00 ± 0.13	0.53 ± 0.13
chlozolinate	1500	A	2.30 ± 0.56	2.21 ± 0.36	1.75 ± 0.68	0.86 ± 0.26	0.28 ± 0.12
	1500	B	2.29 ± 0.21	2.49 ± 0.50	1.77 ± 0.19	0.60 ± 0.19	0.33 ± 0.09

[a] A = sprayed alone; B = sprayed with the other studied pesticides (see Experimental Section). [b] The reported values are the means of duplicate analyses from four replicates.

Table V. Residues (ppm ± SD) of Acylanilide Fungicides after Single Spraying[a]

fungicide	sprayed amount, g/ha	days after spraying: 0	1	3	6	13	20	28
furalaxyl	380	0.66 ± 0.25	0.56 ± 0.27	0.33 ± 0.09	0.24 ± 0.13	≤0.04	≤0.04	≤0.04
	760	1.49 ± 0.14	1.53 ± 0.16	0.62 ± 0.14	0.51 ± 0.25	≤0.04	≤0.04	≤0.04
metalaxyl	350	0.69 ± 0.10	0.73 ± 0.12	0.51 ± 0.18	0.41 ± 0.04	0.22 ± 0.06	0.18 ± 0.07	≤0.03
	700	1.22 ± 0.12	1.01 ± 0.36	0.60 ± 0.04	0.57 ± 0.19	0.22 ± 0.09	0.16 ± 0.06	≤0.03
benalaxyl	380	0.71 ± 0.24	0.62 ± 0.18	0.50 ± 0.09	0.38 ± 0.14	0.21 ± 0.06	0.17 ± 0.05	≤0.02
	760	0.91 ± 0.20	0.63 ± 0.36	0.78 ± 0.25	0.52 ± 0.27	0.35 ± 0.14	0.21 ± 0.10	≤0.02

[a] The reported values are the means of duplicate analyses from four replicates.

Table VI. Residues (ppm ± SD) of Acylanilide Fungicides after Repeated Sprayings[b]

fungicide	sprayed amount, g/ha	mode[a]	days after last spraying: 7	14	21	28	35
furalaxyl	380	A	0.43 ± 0.09	0.24 ± 0.06	≤0.04	≤0.04	≤0.04
	380	B	0.29 ± 0.12	0.24 ± 0.21	≤0.04	≤0.04	≤0.04
metalaxyl	350	A	0.49 ± 0.15	0.30 ± 0.16	0.14 ± 0.08	≤0.03	≤0.03
	350	B	0.56 ± 0.11	0.35 ± 0.20	0.13 ± 0.06	≤0.03	≤0.03
benalaxyl	380	A	0.84 ± 0.25	0.55 ± 0.08	0.49 ± 0.14	0.20 ± 0.09	0.06 ± 0.01
	380	B	0.66 ± 0.09	0.64 ± 0.06	0.54 ± 0.09	0.18 ± 0.07	≤0.02

[a] A = sprayed alone; B = sprayed with another pesticide (see the text). [b] The repoprted values are the means of duplicate analyses from four replicates.

Table VII. Comparison between Acylanilide and Dicarboximidic Fungicide Residues on Tomatoes after Single (SS) and Repeated Sprayings (RS)[a]

fungicide	sprayed amounts, g/ha	days after spraying: 6th SS	6th RS	6th Δ[b]	14th SS	14th RS	14th Δ[b]	21st SS	21st RS	21st Δ[b]
furalaxyl	380	0.24	0.43	79	≤0.04	0.24				
metalaxyl	350	0.41	0.49		0.25	0.21		0.18	0.14	
benalaxyl	380	0.38	0.84	118	0.21	0.55	125	0.17	0.49	188
vinclozolin	1500	1.74	2.67	54	1.17	2.54	117	0.69	2.00	189
	1000[c]	1.40	3.01	115	0.66	2.59	290			
iprodione	1500	2.61	3.35	27	1.49	2.97	99	1.09	1.88	73
	1000[c]	1.35	4.17	208	1.76	1.91	8			
procymidone	1000	1.20	2.45	100	1.01	2.20	117	0.64	2.06	220
chlozolinate	1500	0.76	2.30	200	0.42	2.12	400	0.25	1.75	600

[a] Residue values (ppm) are the means of four replicated analyses and were statistically treated ($P < 0.05$). [b] Percentage increase of residue level in RS vs. SS. [c] Data from Van Wambeke et al. (1980) at the 6th day after one single spraying and at the 13th day after the last of three sprayings at the rate reported.

by the comparison, reported in Table VII, between the residue values detected 6, 14, and 21 days after the last of five applications and those detected on the same days after the single spraying. Six days following the last of the repeated sprayings, furalaxyl showed residues 79% higher than those 6 days after the single spraying. This difference was not computable at the 13th day. Benalaxyl showed a similr behavior, which appeared to be constant from the 6th to 21st day after application, and its residues were 100% higher after repeated spraying than after single spraying.

Metalaxyl, on the contrary, showed in the same period residual values in the samples with repeated sprayings similar to those with a single spraying.

To date inexplicable, this trend appears to be anomalous if compared to that of the related compounds furalaxyl and benalaxyl and also to the above-mentioned dicarboximides. The percentage increases of the acylanilide residues, arising from tomatoes that received both single and repeated sprayings, were in the same range as that determined for the dicarboximides both in this work and by Vanachter et al. (1979). The absolute values following repeated spraying, however, are lower than those determined for the dicarboximide residues, probably due to the lower acyanilide rate of application with active ingredient per unit area of about $^1/_3$ that of dicarboximides.

Conclusions. All the acylanilides showed residue values lower than their legal limits (1.0 ppm) admitted in Italy and many other European countries (R. Fabbrini, personal communication), several days before the expiration of the preharvest times. Since this happens whether applying the concentrations suggested by the manufacturers or doubling them (Table I) and also after repeated sprayings (Table III), preharvest times of 10–15 days would seem to be adequate, the interval between one spraying and the next remaining unaltered.

Weekly harvesting gives rise to little toxic risk from the acylanilides, while repeated spraying with the same dicarboximide could result in residues toxically significant. Such a problem, which could prejudice the use of these fungicides in greenhouses, might be solved by readjusting their legal limits and preharvest times more in keeping with the toxic characteristics of the specific active ingredient.

The absence of antagonistic or synergic effects on the residue levels in the tomatoes following the use of combined fungicides (Tables IV and VB), allows fungicide alternation in spraying programs to prevent resistance phenomena by the fungi, already reported in the literature (Gullino et al., 1981). The fact that acylanilides show a disappearance rate that does not depend on a kinetic equation of the type $y = Ae^{-kt}$ suggests that their loss from tomatoes is dependent upon more than one factor, such as the formulations' characteristics (Phillips, 1974) and systemic properties of these fungicides (Gozzo, 1979; Worthing, 1979; Farmoplant S.p.A., 1982).

Registry No. Furalaxyl, 57646-30-7; benalaxyl, 71626-11-4; metalaxyl, 57837-19-1; chlozolinate, 72391-46-9; procymidone, 32809-16-8; iprodione, 36734-19-7; vinclozolin, 50471-44-8.

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