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Disappearance of Pyrimethanil Residues on Tomato Plants

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The objective of this work was to estimate the disappearance of pyrimethanil, the active ingredient of Mythos 300 SC, at present, commonly used for the protection of greenhouse vegetables against diseases of fungal origin. Disappearance trends of the chemical deposits were studied on tomato plants grown in commercial greenhouses sprayed with a homogeneous 0.15% aqueous solution of the plant protection product. It was found that, on average, pyrimethanil residues on ripening fruits dropped by half and reached detection limit level in 5.7 and 13.7 days after Mythos 300 SC application, respectively. Pyrimethanil residues on tomato leaves dropped by half within 4.0 days and reached detection limit level in the first 10.5 days. Therefore, in conditions of high infection pressure, there is a need to repeat the fungicide application as early as after 3 or 4 days after previous application of Mythos 300 SC.

KEYWORDS: Disappearance; pyrimethanil residues; tomato; leaves and fruits

INTRODUCTION

Pyrimethanil [IUPAC name *N*-(4,6-dimethylpyrimidin-2-yl)-aniline] is a colorless crystalline substance practically insoluble in water (121 mg/L), belonging to the anilinopyrimidine class, for which the Acceptable Daily Intake (ADI) was established at the level of 2 mg/kg of body weight/day (*1*). In the form of concentrates (tradenames of Mythos, Clarinet, and Scala) it is used as a contact fungicide with protective and curative properties. In Poland, Mythos 300 SC was registered for use six years ago, although the disappearance process of its active ingredient after treatment has not been described so far.

Pyrimethanil inhibits the secretion of fungal enzymes relevant for pathogenicity. It controls gray mold (*Botrytis cinerea* Pers.) on fruits, vegetables, and ornamentals as well leaf scab (*Venturia inaequalis* or *pirinal*) on pome fruit (*1*). Compounds of the anilinopyrimidine class were not utilized so far. This means, therefore, that growers have a new agrochemical of original mode of action.

The objective of this work was to determine disappearance parameters of pyrimethanil deposits on tomato plants grown in commercial greenhouses after Mythos 300 SC application against *Botrytis cinerea* Pers. and *Alternaria solani* Sorauer. It is important to know these parameters, because Poland's annual production of greenhouse tomatoes now exceeds 250000 tons.

EXPERIMENTAL PROCEDURES

Materials and Methods. Four experiments were carried out in 10-ha commercial greenhouses, air warmed and equipped with a drip irrigation system. Tomato plants (cv. Cunero F_1), receiving routine horticultural practices, were sprayed in the evening with Mythos 300 SC (active ingredient: 300 g of pyrimethanil per liter of the plant protection product) in the form of a homogeneous aqueous slurry in a concentration of 0.15%. A completely randomized plot scheme was used with four replications. Each single plot consisted of two double rows and contained 140 plants (2.5 plants per square meter).

Table 1. Pyrimethanil Recoveries and Detection Limits for Tomato Plants

sample	spiking level	av (%) ± RSD	detection limit
fruits	0.14 μg/g	99 ± 6.7	0.01 μg/g
leaves	1.83 μg/cm^2	91 ± 4.5	0.02 μg/cm^2

Sampling was started the next day (~12 h) after treatments and repeated in 1, 8, 15, and 22 days (experiment 1), 1, 3, 5, 7, and 10 days (experiments 2 and 4), and 1, 4, 6, 8, and 11 days (experiment 3) after treatment. Each analytical sample consisted of eight tomato fruits and leaves.

Extraction Procedure. One hundred grams of each fruit sample was weighed into the blender jar of a Waring apparatus and homogenized for 2 min with 150 mL of acetone. Homogenate was filtered through a Büchner funnel, and an aliquot of the filtrate, equivalent to 20 g of the analytical portion, was placed in a separatory funnel. Pyrimethanil residues were extracted according to the method published in the scientific journal of the Plant Protection Institute (*2*), based on the solvent system proposed by Luke at al. (*3*) with its subsequent modification (*4*), and combined extracts were evaporated to dryness with a Rotavapor-R of Büchi below 40 °C. Residues were transferred quantitatively with *n*-hexane to a 25-mL flask.

Thirty-two disks were cut from the sampled leaves using a leaf punch sampler (inner diameter of 0.5 cm), placed in the blender jar of a Waring apparatus containing 100 mL of distilled water, and homogenized for 2 min with 150 mL of acetone. An aliquot of filtrate, equivalent to one-fifth of the analytical portion, was taken, and further analysis followed as described above.

Recovery Assays of Pyrimethanil Residues. Samples of untreated tomato fruits and leaves were spiked with 1 mL of a 10 μg/mL acetone standard solution and taken through the extraction procedure four times. Recoveries obtained with this method were satisfactory (**Table 1**).

Apparatus and Chromatography. A gas chromatograph, Hewlett-Packard 5890, was employed, equipped with a nitrogen−phosphorus detector (NPD) connected to an HP 3396 series II integrator. The HP

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Table 2. Average Residues of Pyrimethanil on Tomato Plants (Experiment 1)

	fruits			leaves	
days after treatment	wt (g)	residue (μg/g·cm^2)	% of initial level	residue (μg/g·cm^2)	% of initial level
1	119 ± 5	0.33 ± 0.15	100	1.00 ± 0.45	100
8	125 ± 2	0.05 ± 0.03	16	0.02 ± 0.00	2
15	115 ± 7	0.02 ± 0.01	5	<0.02	0
22	121 ± 9	<0.01	0	<0.02	0

Table 3. Average Residues of Pyrimethanil on Tomato Plants (Experiment 2)

	fruits			leaves	
days after treatment	wt (g)	residue (μg/g·cm^2)	% of initial level	residue (μg/g·cm^2)	% of initial level
1	130 ± 15	0.16 ± 0.07	100	0.78 ± 0.48	100
3	131 ± 4	0.20 ± 0.08	124	0.80 ± 0.22	103
5	134 ± 9	0.07 ± 0.03	43	0.64 ± 0.09	82
7	128 ± 4	0.05 ± 0.03	34	0.45 ± 0.09	58
10	127 ± 3	0.06 ± 0.06	38	0.22 ± 0.05	28

Table 4. Average Residues of Pyrimethanil on Tomato Plants (Experiment 3)

	fruits			leaves	
days after treatment	wt (g)	residue (μg/g·cm^2)	% of initial level	residue (μg/g·cm^2)	% of initial level
1	142 ± 4	0.30 ± 0.14	100	1.40 ± 0.11	100
4	115 ± 8	0.28 ± 0.13	95	0.31 ± 0.09	22
6	123 ± 1	0.21 ± 0.15	70	0.15 ± 0.05	11
8	117 ±14	0.12 ± 0.07	41	<0.02	0
11	120 ±19	0.09 ± 0.05	30	<0.02	0

17 wide-bore fused silica capillary column (10-m length, 0.53-mm i.d., and 2.0-μm film thickness) was used. The injector and the detector were operated at 240 and 260 °C, respectively. Sample extracts (2 μL) were injected splitless, and the oven temperature was programmed as follows: 150 °C for 1 min, raised to 260 °C (10 °C/min), and held for 6 min. Good linearity was achieved in the range of 0.00−0.50 ng. Under these conditions, the detection limits for pyrimethanil were 0.01 μg/g (fruits) and 0.02 μg/cm^2 (leaves), respectively. Pyrimethanil residues on fruits and leaves were expressed in micrograms per gram and micrograms per square centimeter, respectively, and then their average levels and coefficients of variation (relative standard deviations; RSD) were calculated.

Chemicals. Acetone, dichloromethane, and petroleum ether were of analytical grade. Pyrimethanil was purchased from Ehrenstorfer, and its stock standard solution (10 μg/mL) was prepared in acetone and stored at 4 °C. Working standard solution (0.2 μg/mL) was obtained by diluting the stock solution with petroleum ether.

RESULTS AND DISCUSSION

The average recoveries of pyrimethanil from spiked tomato fruit and leaves are given in **Table 1**. In all cases, their levels were >80% with coefficients of variation not exceeding 7%. These levels are generally considered to be satisfactory for residue determinations and are comparable to those obtained for the other compounds insoluble in water (*2*).

The residue data obtained in the disappearance studies of pyrimethanil deposits on tomato plants after spraying carried out with Mythos 300 SC are summarized in **Tables 2**−**5**. These data were subjected to mathematical analysis using the Excel program, and the results obtained are summarized in **Table 6**. The degradation trend of pyrimethanil deposits was well

Table 5. Average Residues of Pyrimethanil on Tomato Plants (Experiment 4)

	fruits			leaves	
days after treatment	wt (g)	residue (μg/g·cm^2)	% of initial level	residue (μg/g·cm^2)	% of initial level
1	127 ± 7	0.34 ± 0.19	100	2.22 ± 0.91	100
3	121 ± 4	0.27 ± 0.06	79	0.90 ± 0.15	41
5	121 ± 6	0.23 ± 0.20	69	0.56 ± 0.08	25
7	121 ± 9	0.05 ± 0.04	15	0.25 ± 0.09	11
10	131 ± 9	0.05 ± 0.06	15	0.00 ± 0.00	<0.02

Table 6. Statistical Parameters Corresponding to the Disappearance Trends of Pyrimethanil Residues on Greenhouse Tomato Plants (Experiments 1–4)[a]

expt		eq	R_0	r	R^2	$t_{1/2}$	$t_{R=0}$
1	fruits	$R = 0.3178 - 0.1106$ Ln(t)	0.32	−0.9848	0.9699	4.2	17.7
	leaves	$R = 0.9700 - 0.3948$ Ln(t)	0.97	−0.9788	0.9581	3.4	11.7
2	fruits	$R = 0.1877 - 0.0153t$	0.19	−0.7920	0.6272	6.1	12.2
	leaves	$R = 0.9294 - 0.0676t$	0.93	−0.9667	0.9346	6.9	13.8
3	fruits	$R = 0.3417 - 0.0236t$	0.34	−0.9616	0.9246	7.2	14.5
	leaves	$R = 1.3665 - 0.6859$ Ln(t)	1.37	−0.9934	0.9868	2.7	7.3
4	fruits	$R = 0.3732 - 0.0356t$	0.34	−0.9425	0.8883	5.3	10.4
	leaves	$R = 2.1271 - 0.9639$ Ln(t)	2.22	−0.9924	0.9849	3.1	9.1
mean	fruits		0.28			5.7	13.7
	leaves		1.35			4.0	10.5

[a] Equation = the best approximations; R_0 = initial residue/deposit; r = correlation coefficient; R^2 = coefficient of determination; $t_{1/2}$ = half-life period.

described by linear ($Y = mX + b$) or logarithmic ($Y = c$ Ln(X) + b) equations, where Y is the concentration of pyrimethanil and X is the time elapsed between application and sampling. In the case of the disappearance study expressed by linear regression, the dependent variable Y represents residue level (R) at a given moment (t) after treatment, whereas b and m values express the initial residue of pesticide (R_0) and rate of its decrease with time. Finally, linear regression takes the form of $R = R_0 + mt$, whereas the logarithmic line $R = R_0 + c$ Ln(t).

Disappearance of Pyrimethanil Residues on Tomato Plants (Experiment 1). The first laboratory samples were taken the next day (∼12 h) after treatment. Pyrimethanil residues in tomato fruits averaged 0.33 μg/g, with a coefficient of variation amounting to 46% (**Table 2**), and decreased rapidly according to the logarithmic line $R = 0.3178 - 0.1106$ Ln(t). The course of the line indicated that initial residues, $R_0 = 0.32$ μg/g, dropped by half ($t_{1/2}$) and reached the detection limit level ($t_{R=0}$) 4.2 and 17.7 days after treatment, respectively. About 12 h after treatment, the average initial deposit of pyrimethanil on tomato leaves was at the level of 1.0 μg/cm^2 and dropped by half within 3.4 days, reaching the detection limit level after 11.7 days. The results obtained indicated that pyrimethanil deposits disappeared rapidly. Thus, proper sampling procedure changes were introduced in methodology before the next three experiments.

Disappearance of Pyrimethanil Residues on Tomato Plants (Experiments 2—4). Pyrimethanil residues in ripe tomato fruits taken the next day after treatments were, on average, 0.16, 0.30, and 0.34 μg/g and were in good agreement with the application rates (*5*). The average levels dropped by half within 5.3−7.2 days after treatment by the route of real disappearance (lack of dilution effect caused by fruit growth) (**Table 6**). Pyrimetanil residues reached the detection limit value within 2 weeks. ADI, established for pyrimethanil at the level of 0.2 mg/kg of body weight/day (*1*), shows that a 60-kg person could eat even 40 kg/day of ripe tomato fruits harvested immediately after treatment containing the average residue of 0.30 mg/kg.

The average initial pyrimethanil deposits on fully developed tomato leaves were, on average, 0.78, 1.41, and 2.22 $\mu g/cm^2$ and then dropped by half within 2.7−6.9 days after treatments (**Table 6**). The fast disappearance rate of pyrimethanil deposits on leaves strongly suggests that Mythos 300 SC (active ingredient: 300 g of pyrimethanil per liter of the crop protection product) protects tomato crops only within a 4-day period of time. To ensure effective protection, the next treatment should be carried out.

Conclusions. Reported research showed that residues of pyrimethanil in tomato fruits became reduced by half within 6 days after spraying of tomato plants and reached detection limit level after 2 weeks. Because tomatoes are harvested twice a week (every 3 or 4 days) and chemical treatments are performed immediately after harvest, it should be said that any significant summation of residues in ripening tomatoes may take place only in conditions of high infection pressure. In case of a subsequent treatment, performed on the seventh day after the former application of Mythos, the ripe tomatoes shall contain only the residues of the last applied plant protection product.

Within 4 days after the treatment, the pyrimethanil deposits on leaves become reduced by half and might not ensure effective protection for tomato crops. Therefore, in conditions of high infection pressure, there is a need to repeat the fungicide application as early as after 3 or 4 days after the previous application of Mythos 300 SC.

Studies on the disappearance rates of fungicide residues on crops constitute an important step toward rationalization of crop protection against diseases of fungal origin, and their results shall be included in instructions of use (or label) of specific crop protection agents.

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