# DISAPPEARANCE OF AZOXYSTROBIN, CYPRODINIL, AND FLUDIOXONIL RESIDUES ON TOMATO LEAVES IN A GREENHOUSE

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**Abstract:** The objective of this work was to estimate the disappearance parameters of azoxystrobin, cyprodinil and fludioxonil initial deposits, active ingredients of Amistar 250 SC, and Switch 62,5 WG, at present, commonly used for the protection of fruits and vegetables against diseases of fungal origin. The tests were carried out on tomato plants grown in commercial greenhouses sprayed with homogenous 0.1% aqueous solution of these plant protection products. It was found that on tomato leaves azoxystrobin, cyprodinil, and fludioxonil residues dropped by half on average within 13, 9, and 31 days after treatments, respectively. Therefore, in conditions of high infection pressure, there is a need to repeat the fungicide application but not earlier than 10 days after previous application of Amistar 250 SC and Switch 62,5 WG.

**Key words:** azoxystrobin, fludioxonil, cyprodinil, disappearance trends, tomato leaves

## INTRODUCTION

In a greenhouse tomatoes develop during all of summer and parts of spring and autumn. The fruits ripen gradually and are usually harvested weekly (sometimes two times a week). Therefore, to protect tomatoes from gray mould (*Botrytis cinerea*), pesticides with a short preharvest interval of 1 week or less are required.

In Poland, there are numerous fungicides used against gray mould (*Botrytis cinerea* Pers.) such as: chlorothalonil, cymoxanil, buconazole, pyrimethanil, iprodione and thiophanate methyl. Recently, some new fungicides, such as azoxystrobin, cyprodinil, and fludioxonil, were introduced on the market. Studies on the behaviour of those fungicides after treatments on grapes and on tomato fruits are reported in literature while no studies have been carried out on tomato leaves.

The objectives of this work were to determine disappearance parameters of azoxystrobin, cyprodinil and fludioxonil deposits on tomato plants in commercial greenhouses to optimize Switch 62,5 WG and Amistar 250 SC application together with other fungicides of the same mode of action, and to avoid an accumulation effect of fungicide residues due to repeated applications of the same or different active ingredients in periods of high infection pressure.

## MATERIALS AND METHODS

The experiments were carried out in commercial greenhouses located in south-eastern Poland, 17 km from Rzeszów, air warmed and equipped with a drop irrigation system. Tomato plants of the Cunero variety, receiving routine horticultural practices, were sprayed in the evening with Amistar 250 SC (active ingredient: 250 g of azoxystrobin per 1 l of the plant protection product) and Switch 62,5 WG (a.s.: 375 g of cyprodinil and 250 g of fludioxonil per 1 kg of the plant protection product) in the form of homogeneous aqueous slurry in concentrations recommended by The Institute of Plant Protection – National Research Institute. 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).

**Extraction procedure**

Samplings were started the next days (about 12 hr) after treatments and each sample consisted of eight tomato leaves. Thirty two discs were cut out from the sampled leaves using a leaf punch sampler (of inner diameter 1.1 cm) placed in a blender jar of Waring apparatus containing 100 ml of distilled water and homogenised for 2 minutes with 150 ml of acetone. An aliquot of filtrate, equivalent of one fifth of the analytical portion, was taken and placed in a separatory funnel. Fungicide residues were extracted by the method published in the scientific journal of the Plant Protection Institute (Sadło 1998), based on the solvent system proposed by Luke *et al*. (1975) with its subsequent modification (Ambrus *et al*. 1981), and combined extracts were evaporated to dry-

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ness with a Rotavapor-R of Büchi below 40°C. Residues were transferred quantitatively with petroleum ether to a 10-ml flask and then cleaned up using florisil mini columns (Cabras *et al.* 1997).

**Apparatus and chromatography**

Gas chromatographs, Agilent 6890 and Hewlett Packard 5890, equipped with electron capture detector (ECD) connected to ChemStation (used for determination of azoxystrobin residues) and nitrogen-phosphorus detector (determination of cyprodinil and fludioxonil residues) were employed. The HP-5MS narrowbore (5%-Phenyl)-methylpolysiloxane capillary column (30-m length, 0.25-mm i.d., and 0.25-μm film thickness) was used. The injector and the detector were operated at 240 and 250°C, respectively. Sample extracts (1–2 μl) were injected splitless and the oven temperature was programmed as follows: 100°C for 1 min, raised to 260°C (10°C/min), and held for 4 min. Good linearity was achieved in the range of 0–0.5 ng. Under these conditions, the detection limits for azoxystrobin, cyprodinil and fludioxonil were 0.02 μg/cm$^2$. Fungicide residues on leaves were expressed in μg/cm$^2$, and then their average levels and standard deviations (SD) were calculated.

**Statistical analysis**

The degradation kinetics of fungicide residues were determined by plotting residue concentration against time and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves. For all the samples studied, exponential relations were found to apply, corresponding to first order rate equation:

$$R_t = R_0 e^{-kt}$$

where $R_t$ represents the concentration of the fungicide residue at time $t$, $R_0$ represents the initial concentration and $k$ is the rate constant in days$^{-1}$. The half lives ($t_{1/2}$= ln(2)/$k$) and one tenth ($t_{1/10}$=ln(10)/$k$) of the initial deposits were determined from $k$ value for each experiment.

Disappearance trends of the average fungicide initial deposits were also described by regression linear equations:

$$R_t = R_0 + mt$$

and then half-life periods of the fungicides tested were calculated.

**Chemicals**

Acetone, dichloromethane and petroleum ether were of analytical grade. Azoxystrobin, cyprodinil, and fludioxonil were purchased from Ehrenstorfer (Germany) and their stock standard solutions (10 μg/ml) were prepared in acetone and stored at 4°C. Working standard solutions (0.2 μg/ml) were obtained by diluting the stock solution with petroleum ether.

## RESULTS AND DISCUSSION

**Disappearance of azoxystrobin residues on tomato leaves**

Azoxystrobin, the IUPAC name methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, is a beige substance practically insoluble in water (6.0 mg/l), belonging to a new class of pesticidial compounds called β-methoxyacrylates which are derived from the naturally-occurring strobilurins. Its biochemical mode of action is the inhibition of electron transport.

Azoxystrobin is of low chronic toxicity to humans for which Acceptable Daily Intake (ADI) was established at the level of 0.1 mg/kg of body weight/day. In the form of concentrates it is used as a preventive and curative systemic fungicide with activity against several diseases on many edible crops and ornamental plants. In Poland, it is registered for 10 years under trade name Amistar 250 SC product of Syngenta Limited, though the behaviour of its active ingredient after treatment was poorly recognized and popularized.

Laboratory studies show that azoxystrobin is moderately persistent in soil in the absence of light and moderately mobile in soil profile. In field trials azoxystrobin on grapes disappeared quite slow ($t_{1/2}$ = 15.2 days) (Cabras *et al.* 1997; Cabras and Angioni 2000). This fungicide was also stable on tomato fruits and the absence of any decrease in greenhouse conditions was due to the systemic activity of azoxystrobin (Garau *et al.* 2002). However there is no information about the behaviour of azoxystrobin on tomato leaves.

Applications of Amistar 250 SC were performed on September 24 (2002), April 11 (2003) and May 26 (2004). Sampling was performed by collecting randomly eight leaves from each plot. Samples were taken the next day after application in order to allow enough time for the solution to dry. The collected leaves were put in polyethylene bags and transferred to the laboratory. The residue data determined by the multiresidue method and expressed in μg/cm$^2$ were presented in tables 1, 2, and 3.

Table 1. Average residues of azoxystrobin [μg/cm$^2$±SD] on tomato leaves, Test 1

| Day after treatment | Residue | % of initial level |
|---|---|---|
| 1 | 0.88±0.39 | 100 |
| 3 | 1.05±0.31 | 119 |
| 6 | 0.98±0.37 | 111 |
| 10 | 0.64±0.44 | 73 |
| 14 | 0.49±0.37 | 56 |
| 17 | 0.38±0.46 | 44 |

Table 2. Average residues of azoxystrobin [μg/cm$^2$±SD] on tomato leaves, Test 2

| Day after treatment | Residue | % of initial level |
|---|---|---|
| 1 | 1.10±0.27 | 100 |
| 4 | 1.36±0.50 | 124 |
| 7 | 0.91±0.24 | 83 |
| 11 | 0.87±0.08 | 79 |
| 13 | 0.56±0.39 | 51 |

Table 3. Average residues of azoxystrobin [μg/cm²±SD] on tomato leaves, Test 3

| Day after treatment | Residue | % of initial level |
|---|---|---|
| 1 | 1.15±0.23 | 100 |
| 5 | 0.81±0.19 | 70 |
| 12 | 0.58±0.66 | 51 |
| 16 | 0.62±0.27 | 54 |

As shown from these tables, the initial deposits of azoxystrobin residues on tomato leaves in successive field tests were 0.88, 1.10, and 1.15 μg/cm² (on average 1.04 μg/cm²). Between days 3 and 5 following the application of Amistar 250 SC, azoxystrobin residues remained relatively stable and generally declined slowly with time. In relation to initial deposits the percentage dissipation of azoxystrobin residues two weeks after the applications was 56%, 51% and 52%.

Disappearance trends of azoxystrobin deposits expressed by first order rate equation and linear regression were presented in table 6 and on Figure 1. Both exponential and linear equation indicate that the initial deposits of azoxystrobin applied in the form of Amistar 250 SC in the recommended application doses dropped by half not earlier than 12 days (on average within two weeks) after treatments. Therefore the effect of repeated applications, at 5-day intervals should lead to an increase of residue levels on leaves of tomato plants. The same increase may be expected in the case of tomato fruits because of the lack of significant dilution effect due to their growth.

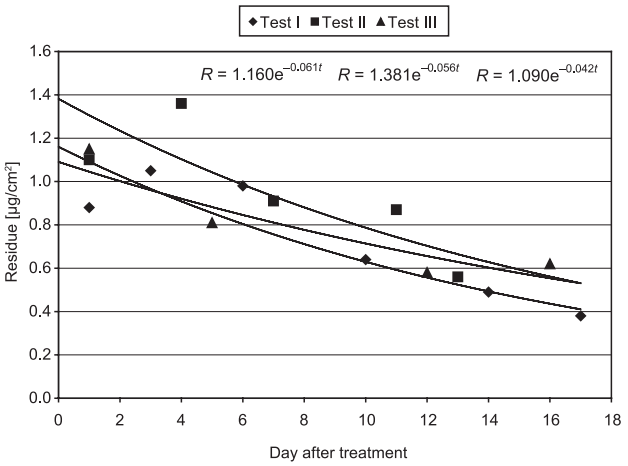


Fig. 1. Disappearance trends of azoxystrobin initial deposits

**Disappearance of Cyprodin il and Fludioxonil residues on tomato leaves**

Cyprodinil, the IUPAC name 4-cyclopropyl-6-methyl-*N*-phenylpyrimidin-2-amine, belonging to pyrimidine fungicides and fludioxonil, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile, belonging to pyrrole fungicides are the active ingredients of Switch 62,5 WG which is used as a contact (and systemic) fungicide with protective and curative properties. In Poland, Switch 62,5 WG was registered for use two years ago, though the behaviour of its active ingredients after treatment was poorly recognized and popularized.

These compounds show a high level of activity against *B. cinerea* strains, resistant to commonly used fungicides. Cyprodinil is anilinopyrimidine whose mechanism of action consists of the inhibition of methionine biosynthesis (Mastner *et al.* 1994). Fludioxonil, a phenylpyrrole, is a nonsystemic fungicide, which affects the transportation processes in the plasmatic membrane (Jespers *et al.* 1993).

Cabras *et al.* (1997) has studied the persistence of cyprodinil and fludioxonil in grapes. It was found that these fungicides show different decay rates after treatment with first-order kinetics and half-lives 12 and 24 days for cyprodinil and fludioxonil, respectively.

**Field test 1**

Cyprodinil residues on tomato leaves averaged at 2.88 μg/cm², with Relative Standard Deviation (Variation Coefficient) amounting to 50% (Table 4), and decreased rapidly according to first order rate equation $R$=2.719e$^{-0.096t}$. Initial cyprodinil residues dropped by half ($t_{1/2}$) and reached the tenth percent level ($t_{1/10}$) in 7 and 24 days after treatment, respectively.

Table 4. Average residues of cyprodinil and fludioxonil [μg/cm²±SD] on tomato leaves, Test 4

| Day after treatment | Cyprodinil | | Fludioxonil | |
|---|---|---|---|---|
| | residue | % of initial level | residue | % of initial level |
| 1 | 2.88±1.46 | 100 | 2.44±0.86 | 100 |
| 4 | 1.93±0.39 | 67 | 1.65±0.48 | 68 |
| 8 | 1.30±0.25 | 45 | 1.43±0.40 | 59 |
| 11 | 0.47±0.47 | 16 | 1.03±0.95 | 42 |
| 15 | 1.04±0.46 | 36 | 1.56±0.48 | 64 |

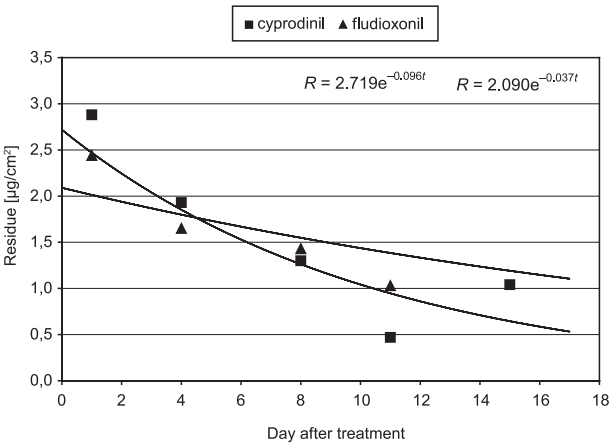


Fig. 2. Disappearance trends of cyprodinil and fludioxonil initial deposits, test 1

On the other hand, fludioxonil residues on the same tomato leaves taken the next day after treatments were 2.44 μg/cm² on average with Variation Coefficient amounting to 35% (Table 4), and decreased slower than cyprodinil according to equation $R$=2.090e$^{-0.037t}$. Thus, initial fludioxonil residues dropped by half and reached the tenth level in 18 and 61 days after treatment, respectively (Table 6).

**Field test 2**

Slightly different disappearance trends were observed during the second field test. Cyprodinil residues on tomato leaves averaged at 3.28 μg/cm², with Variation Coefficient amounting to 18% (Table 5), and decreased according to equation $R$=3.272e$^{-0.066t}$. Its initial deposits dropped by half ($t_{1/2}$) and reached the level $t_{1/10}$ in 11 and 35 days after treatment, respectively.

Fludioxonil residues on tomato leaves taken the next day after treatments were, on average 2.84 μg/g, with Variation Coefficient amounting to 26% (Table 5), and decreased very slowly according to equation $R$=3.043e$^{-0.015t}$. Initial fludioxonil residues dropped by half ($t_{1/2}$) and reached the level $t_{1/10}$ in 45 and 152 days after treatment, respectively (Table 6).

Table 5. Average residues of cyprodinil and fludioxonil [μg/cm²±SD] on tomato leaves, Test 5

| Day after treatment | Cyprodinil | | Fludioxonil | |
|---|---|---|---|---|
| | residue | % of initial level | residue | % of initial level |
| 1 | 3.28±0.58 | 100 | 2.84±0.74 | 100 |
| 4 | 2.22±0.25 | 68 | 2.86±0.53 | 101 |
| 7 | 1.86±0.37 | 57 | 2.95±0.69 | 104 |
| 11 | 1.48±0.50 | 45 | 2.48±0.63 | 87 |
| 17 | 1.11±0.72 | 34 | 2.29±1.40 | 81 |

Table 6. Statistical parameters corresponding to the disappearance trends of azoxystrobin, cyprodinil and fludioxonil residues on greenhouse tomato leaves

| Test | Compound | Equation | $R_0$ | r | $R^2$ | $t_{1/2}$ | $t_{1/10}$ |
|---|---|---|---|---|---|---|---|
| 1 | azoxystrobin | $R$=1.078$^{-0.040t}$ | 1.078 | –0.922 | 0.851 | 13 | 24 |
| | | $R$=1.160e$^{-0.061t}$ | 1.160 | –0.939 | 0.882 | 12 | 41 |
| 2 | | $R$=1.317$^{-0.050t}$ | 1.317 | –0.824 | 0.680 | 13 | 24 |
| | | $R$=1.381e$^{-0.056t}$ | 1.381 | –0.706 | 0.840 | 12 | 41 |
| 3 | | $R$=1.087$^{-0.035t}$ | 1.087 | –0.906 | 0.822 | 16 | 28 |
| | | $R$=1.090e$^{-0.042t}$ | 1.090 | –0.920 | 0.847 | 16 | 54 |
| 1 | cyprodinil | $R$ = 2.646$^{-0.144t}$ | 2.646 | –0.865 | 0.748 | 9 | 17 |
| | | $R$=2.719e$^{-0.096t}$ | 2.719 | –0.671 | 0.450 | 7 | 24 |
| | fludioxonil | $R$ = 2.133$^{-0.066t}$ | 2.133 | –0.705 | 0.497 | 16 | 29 |
| | | $R$=2.090e$^{-0.037t}$ | 2.090 | –0.671 | 0.450 | 18 | 61 |
| 2 | cyprodinil | $R$ =3.095$^{-0.129t}$ | 3.095 | –0.956 | 0.913 | 12 | 22 |
| | | $R$=3.272e$^{-0.066t}$ | 3.272 | –0.984 | 0.992 | 11 | 35 |
| | fludioxonil | $R$ =3.0198$^{-0.039t}$ | 3.020 | –0.850 | 0.723 | 39 | 70 |
| | | $R$=3.043e$^{-0.015t}$ | 3.043 | –0.859 | 0.738 | 45 | 152 |
| Mean | azoxystrobin | – | 1.21 | – | – | 13 | 45 |
| | cyprodinil | – | 3.00 | – | – | 9 | 29 |
| | fludioxonil | – | 2.57 | – | – | 31 | 106 |

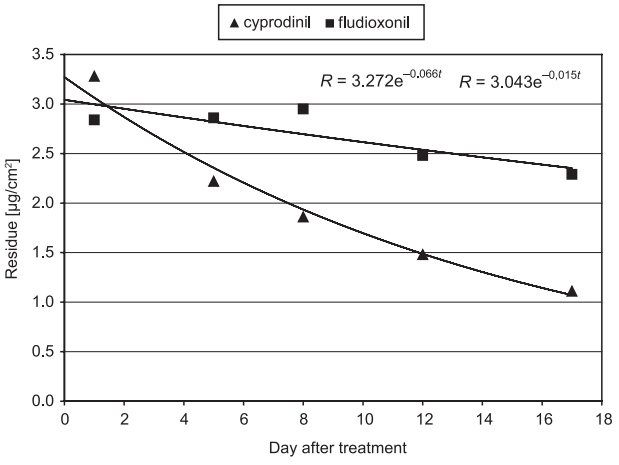


Fig. 3. Disappearance trends of cyprodinil and fludioxonil initial deposits, test 2

Disappearance trends of cyprodinil and fludioxonil initial deposits on tomato leaves suggest that Switch 62,5 WG (active ingredients: 375 g of cyprodinil and 250 g of fludioxonil per 1 kg of the plant protection product) should protect tomato crops within 10-day period of time. In order to assure effective protection the next treatment should be carried out within 10 days.

## CONCLUSIONS

Reported research showed that residues of azoxystrobin, cyprodinil and fludioxonil on tomato leaves in commercial greenhouses became reduced by half within 13, 9 and 31 days after treatments, respectively. In case of a subsequent treatment, performed on the seventh day after former application of Switch 62,5 WG or Amistar 250 SC, the ripe tomato fruits shall still contain over 50% of their initial deposits. Therefore, since tomatoes are harvested twice a week (every 3 or 4 days) and chemical treatments are performed immediately after harvest,

it should be said that in conditions of high infection pressure, significant summation of residues in ripening tomato fruits may take place.

On the other hand, within 7 days after the treatment, the azoxystrobin, cyprodinil and fludioxonil deposits on leaves became reduced no more than by half and should ensure effective protection for tomato crops. Therefore, in conditions of high infection pressure when there is a need to repeat the fungicide application, the next spraying of tomato plants should be carried out as early as after 10 days following the previous application of Switch 62,5 WG or Amistar 250 SC.

The studies of fungicide behaviour on crops constitute an important step towards rationalization of crop protection against diseases of fungal origin and their results shall be included in instructions of use (or labels) of specific crop protection agents.

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## POLISH SUMMARY

### ZANIKANIE AZOKSYSTROBINY, CYPRODINILU I FLUDIOXONILU NA LIŚCIACH POMIDORA SZKLARNIOWEGO

Celem pracy było ustalenie zanikania azoksystrobiny, cyprodinilu i fludioksonilu, substancji aktywnych preparatów Amistar 250 SC i Switch 62,5 WG, aktualnie powszechnie stosowanych do ochrony owoców i warzyw przed chorobami pochodzenia grzybowego. Doświadczenia polowe wykonano na pomidorach szklarniowych rosnących w komercyjnych szklarniach opryskiwanych 0,1% roztworem wodnym wymienionych wyżej preparatów chemicznych. Stwierdzono, że depozyty początkowe azoksystrobiny, cyprodinilu i fludioksonilu obniżyły się o połowę po 13, 9 i 31 po zabiegu. W warunkach dużej presji czynnika chorobotwórczego, gdy istnieje potrzeba wykonania kolejnego zabiegu, zabieg ten należy wykonać nie wcześniej niż po 10 dniach po aplikacji preparatów Amistar 250 SC i Switch 62,5 WG.