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Food Control 19 (2008) 247–256
FOOD CONTROL
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# Changes of pesticide residues in apples during cold storage

Jana Ticha [a], Jana Hajslova [a,*], Martin Jech [a], Jiri Honzicek [a], Ondrej Lacina [a], Jana Kohoutkova [a], Vladimir Kocourek [a], Miroslav Lansky [b], Jana Kloutvorova [b], Vladan Falta [b]

[a] *Institute of Chemical Technology (ICT Prague), Department of Food Chemistry and Analysis, Technická 5, 166 28 Praha 6, Czech Republic*
[b] *Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy 1, 508 01 Horice, Czech Republic*

Received 18 December 2006; received in revised form 15 March 2007; accepted 20 March 2007

**Abstract**

The dynamics of incurred pesticide residues in apples, variety Melrose, was monitored during their cold storage at 1–3 °C for 5 months. Of 21 active ingredients contained in pesticide preparations applied within four experimental pre-harvest regimes, only six fungicides (captan, cyprodinyl, dodine, pyrimethanil, tebuconazole, tolyfluanid) and one insecticide (phosalone) were detected at the time of harvest. The other active ingredients – acetamiprid, chlorpyrifos-methyl, difenoconazole, diflubenzuron, dithianon, EBDCs (represented by mancozeb and thiram in this study), fenoxycarb, kresoxim-methyl, teflubenzuron, thiacloprid, triazamate, trifloxystrobin and triflumuron did not exceed detection limit of LC–MS/MS or GC–MS methods used for sample analysis. Successive decrease of residues occurred during storage period, after 5 months only fungicide dodin and insecticide phosalone were detected.


*Keywords:* Apples; Storage; Field experiments; Pesticide residues; LC–MS/MS; GC–MS

## 1. Introduction

Various pesticide preparations are used for crops protection worldwide to increase their quality and yield as well as to extend storage lifetime. Although Maximum Residue Limits (MRLs) have been exceeded in fruit/vegetable available at EU market in only a few cases in recent years (Pesticide Residues in Europe, 2006), consumers are very much concerned on health risks associated with occurrence of detectable pesticide residues in their food supply. On this account, attention has to be paid to selection of treatment regimes enabling not only effective control of pests, but also leaving minimal residues of active ingredients used for respective plants protection (Hamilton & Crossley, 2004).

Apples are the major fruit crop grown in temperate geographical zone. Among apple varieties, large differences exist not only in eating attributes and storage potential, but also in agronomic traits such as yield, fruit size distribution and tolerance to various disorders (Pennel, 2006). Pest and disease pressure varies considerably from year to year and this, consequently, affects requirements for apple trees protection. Besides a variety of insects such as codling moth (*Cydia pomonella*), sawfly insects (*Hoplocampa testudinea*), tortricid (*Tortricidae*), aphids (*Dysaphis plantaginea*) and fruit tree red spider mite (*Panonychus ulmi*) controlled within the pre-harvest time by organophosphates, carbamates and other insecticides, development of fungal diseases, namely apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and apple canker (*Nectria galligena*) has to be prevented during vegetation period by suitable fungicide preparations such as Hattrick, Merpan, Euparen Multi, Delan etc. It should be noted that apples decay may also occur during the post-harvest period (Athanasopoulos, Kyriakidis, & Stavropulos, 2004; Watkins, Nock, Weis, Jayanty, & Beaundry, 2004). Sometimes incipient infection is too small to be seen prior to fruit storage but may develop under favourable conditions (high humidity) as a result of sporulation from older lesions. Key apple storage

* Corresponding author. Tel./fax: +420 220443185.
*E-mail address:* jana.hajslova@vscht.cz (J. Hajslova).

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doi:10.1016/j.foodcont.2007.03.011

diseases are apple scab (*V. inaequalis*) and rots caused by a variety of species (*Botrytis cinerea*, *Monilia fructigena*, *Gloeosporium* spp., *Penicillium expansum* etc.) (Pennel, 2006).

While relatively rapid decline of pesticide residues takes place within the pre-harvest period (Holland, Hamilton, Ohlin, & Skidmore, 1994; Rasmussen, Poulsen, & Hansen, 2003) due to various environmental factors, their drop in post-harvest time might be slower, depending on the storage conditions (Athanasopoulos et al., 2004; Cano & De LaPlaza, 1987; Johnson, 1997; Mahajan & Chopra, 1992; Panagiotis & Pappas, 2000).

The aim of presented study was to assess four treatment regimes realized on apple trees in terms of pesticide residues left in fruit not only at the time of harvest, but also with regard to changes of residues during post-harvest period under cold storage conditions.

## 2. Materials and methods

### 2.1. Field work

Melrose apples examined in this study were obtained from our project partner Research and Breeding Institute of Pomology, Holovousy, Czech Republic. The field experiments were performed at the orchard Holovousy Kamenec, Czech Republic. Melrose apple trees (17 years old) were planted in spacing $4 \times 2.5$ m; experimental area was 0.20 ha. The overview of pesticide preparations, commercial names and some relevant characteristics of active ingredients are listed in Table 1. Pesticides were applied with a tractor mounted sprayer Tifone Vanguard 1070 equipped with the Albuz ATR nozzles; the operating volume and pressure were 400 L per ha and 1.3 MPa, respectively.

The experimental apple orchard was divided into four plots, and obtained specific pesticide treatment (FT 1–FT 4) shown in Table 2a in the first phase. In the second phase, each plot was subdivided into three sections (ST /1–ST /3). In sections ST /1, no pesticides were used, while in ST /2 and ST /3, fungicides against storage pests were applied according to the scheme in Table 2b.

The first set of samples was taken for analysis at the time of harvest (October 21, 2004), the next two after 2 and 5 months of cold storage (December 9, 2004 and March 4, 2005, respectively). Sampling in orchard was performed by hand-picking of apples (approximately 1 kg of fruit, at

Table 1
Pesticide preparations and physico-chemical properties of active ingredient

| Pesticide preparation | Active ingredient | Mode of action | Content in preparation | MRL (mg/kg) | Safety period (days) | Physico-chemical properties of active ingredient | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Molecular weight | log $K_{OW}$ | Water solubility (mg/l) |
| Kuprikol WP 50 | Copper oxychloride | Fungicides | 0.05–0.1% | – | – | 427.1 | – | <10-5 mg/l (pH 7, 20 °C) |
| Discus | Kresoxim-methyl | | 50% | 0.2 | 35 | 313.4 | 3.40 (pH 7, 25 °C) | 2 (20 °C) |
| Delan 700 WG | Dithianon | | 0.07% | 0.1 | 21 | 296.3 | 3.2 | 0.14 (pH 7, 20 °C) |
| Zato 50 WG | Trifloxystrobin | | 500 g/kg | 0.5 | 14 | 408.4 | 4.50 (25 °C) | 610 (25 °C) |
| Mythos 30 SC | Pyrimethanil | | 0.75–1 l/ha | 1 | 28 | 199.3 | 2.84 (25 °C) | 121 (pH 6.1, 25 °C) |
| Syllit 65 WP | Dodine | | 0.075–0.1% | 1 | 21 | 287.4 | – | 630 (25 °C) |
| Chorus 75 WG | Cyprodinil | | 750 g/kg | 1 | 28 | 225.3 | 4.00 (25 °C) | 20 (pH 5.0, 25 °C) |
| Merpan 80 WG | Captan | | 80% | 3 | 35 | 300.6 | 2.80 (25 °C) | 3.3 (25 °C) |
| Foligreen | Agricultural micronutrient | | | | | | | |
| Score 250 EC | Difenoconazole | | 0.2 l/ha | 0.02 | 49 | 406.3 | 4.20 (25 °C) | 15 (25 °C) |
| Kumulus WG | Sulphur | | 1000 l/ha | – | 3 | 32.1 | | Practically insoluble |
| Thiram Gran. | Thiram | | 80% | | 14 | 240.4 | 1.73 | 18 (room temperature) |
| Euparen Multi | Tolylflunid | | 50% | 1 | 7 | 347.3 | 3.90 (20 °C) | 0.9 (20 °C) |
| Dithane M 45 | Mancozeb | | 0.2–0.45% | 3 | 21 | 265.3 | | 6.2 (pH 7.5, 25 °C) |
| Hattrick | Tebuconazole | | 10% | 0.5 | 28 | 307.8 | 3.70 (20 °C) | 36 (pH 5–9, 20 °C) |
| | Tolylfluanid | | 40% | 1 | 28 | | | |
| Oleoekol ME | Chlorpyrifos | Insecticides | 30 g/l | 0.5 | – | 350.6 | 4.7 | 1.4 (25 °C) |
| | Coleseed oil | | 75% | – | – | | | |
| Mospilan 20 SP | Acetamiprid | | 20% | 0.05 | 28 | 222.7 | 0.80 (25 °C) | 4250 (25 °C) |
| Insegar 25 WP | Fenoxycarb | | 25% | 0.05 | 60 | 301.3 | 4.10 (25 °C) | 7.9 (pH 7.55–7.84, 25 °C) |
| Calypso 480 SC | Thiacloprid | | 480 g/l | 0.3 | 14 | 252.7 | – | 185 (20 °C) |
| Aztec140 EW | Triazamate | | 140 g/l | 0.1 | 7 | 314.4 | 2.10 (25 °C) | 399 (pH 7, 25 °C) |
| Dimilin 48 SC | Diflubenzuron | | 0.25 l/ha | 1 | 28 | 310.7 | 3.89 | 0.08 (pH 7, 25 °C) |
| Zolone 35 EC | Phosalone | | 0.20% | 2 | 21 | 367.8 | 4.01 (20 °C) | 3.05 (25 °C) |
| Nomolt 15 SC | Teflubenzuron | | 150 g/l | 0.5 | 28 | 381.1 | 4.30 (20 °C) | 0.019 (23 °C) |
| Reldan 40 EC | Chlorpyrifos-methyl | | 400 g/l | 0.5 | 28 | 322.5 | 4.24 | 2.6 (20 °C) |
| Alsystin 480 SC | Triflumuron | | 480 g/l | 1 | 28 | 358.7 | 4.91 (20 °C) | 0.025 (20 °C) |

Table 2a
Application schedule and treatment rates of pesticides used in the first phase of pre-harvest field treatment

| Application dates year 2004 | Active ingredients (see Table 1 for names and relevant characteristics of pesticide preparations) used in the first phase pre-harvest period | | | |
|---|---|---|---|---|
| | FT 1 | FT 2 | FT 3 | FT 4 |
| April 15 | Copper oxychloride (5.0 kg)[a] | Copper oxychloride (5.0 kg) | Copper oxychloride (5.0 kg) | Copper oxychloride (5.0 kg) |
| April 21 | Chlorpyrifos (10 l) | Chlorpyrifos (10 l) | Chlorpyrifos (10 l) | Chlorpyrifos (10 l) |
| April 21 | Kresoxim-methyl (0.2 kg) Dithianon (0.3 kg) | Trifloxystrobin (0,10 kg) Captan (1.0 kg) | Kresoxim-methyl (0.2 kg) Dithianon (0.3 kg) | Trifloxystrobin(0.10 kg) Captan (1.0 kg) |
| May 3 | Trifloxystrobin (0.15 kg) | Trifloxystrobin (0.15 kg) | Trifloxystrobin (0.15 kg) | Kresoxim-methyl (0.2 kg) |
| May 12 | Pyrimethanil (1.0 l) Acetamiprid (0.25 l) | Thiram (3.0 kg) Thiacloprid (0.25 l) | Tolylflunid (2.0 kg) Phosalone (3.0 l) | Thiram (3.0) Thiacloprid (0.25 l) |
| May 25 | Dodine (1.0 kg) Fenoxycarb (0.3 kg) | Dodine (1.5 kg) Diflubenzuron (0.25 l) | Dithianon (1.0 kg) | Kresoxim-methyl (0.2 kg) Triflumuron (0.25 l) |
| June 6 | Cyprodinil (0.25 kg) | Dithianon (1.0 kg) | Mancozeb (3.0 kg) | Captan (2.0 kg) |
| June 14 | Captan (2.0) Thiacloprid (0.2 l) | Captan (2.0) Phosalone (3.0) | Dodine (1.5 kg) Chlorpyrifos-methyl (1.25 l) | Mancozeb (3.0 kg) Acetamiprid (0.25 l) |
| June 22 | Dithianon (1.0 kg) | Dodine (1.5 kg) | Pyrimethanil (1.0 l) | Captan (2.0 kg) |
| June 28 | Triazamate (0.5 l) Micronutrient (1.0 l) | Triazamate (0.5 l) Micronutrient (1.0 l) | Triazamate (0.5 l) Micronutrient (1.0 l) | Triazamate (0.5 l) Micronutrient (1.0 l) |
| June 30 | Difenoconazole (0.2 l) | Captan (2.0 kg) | Tebuconazole, Tolylfluanid (1.125 kg) | Mancozeb (3.0 kg) |
| July 7 | Sulphur (7.0 kg) | Dithianon (1.0 kg) | Captan (2.0 kg) | Captan (2.0 kg) |

[a] Per hectare dosage of applied pesticide preparation.

Table 2b
Application schedule and treatment rates of pesticides applied in the second phase – protection against storage diseases – of pre-harvest field experiments

| Codes | | Fungicides used in the second phase of pesticide treatment (application rate kg/ha) | Date of treatment | Sampling dates | | |
|---|---|---|---|---|---|---|
| Experiment | Apple origin | | | Harvest | 2 months storage | 5 months storage |
| ST 1/1 | FT 1 | Tolyfluanid (2.0) | – | October 21, 2004 | December 9, 2004 | March 4, 2005 |
| ST 1/2 | | | September 27 | | | |
| ST 1/3 | | | September 17 | | | |
| ST 2/1 | FT 2 | Dodine (2.0) | – | | | |
| ST 2/2 | | | September 16 | | | |
| ST 2/3 | | | August 26 | | | |
| ST 3/1 | FT 3 | Thiram (3.0) | – | | | |
| ST 3/2 | | | September 23 | | | |
| ST 3/3 | | | September 9 | | | |
| ST 4/1 | FT 4 | Dithianon (1.0) | – | | | |
| ST 4/2 | | | September 16 | | | |
| ST 4/3 | | | August 26 | | | |

For names of respective pesticide preparations see Table 1.

least 10 apples per sample) from various places of the experimental fields in accordance with the principles specified in Commission Directive 2002/63/EC. After harvest, apples were stored in the store with regulated temperature (1–3 °C).

### 2.1.1. Weather

Climate conditions (temperature, humidity and precipitations) were monitored by automatic weather station. Between first spraying (April 15) until harvest (October 21), minimum, maximum and average temperatures were 4 °C, 26 °C and 14 °C respectively. The total precipitations from the first pesticide application (April 15) until harvest (October 21) were 218.8 mm. There were two major rainfalls from the last pesticide preparation application until harvest. The average humidity during respective vegetation period was 82%.

## 2.2. Chemicals

Certified standards of active ingredients of pesticide preparations shown in Table 1 (purity of chemicals in the range 92–99%) were obtained from Dr. Ehrenstorfer GmBH (Germany). Stock solutions were prepared by dissolving of neat standards in acetonitrile, for analysis by liquid chromatography coupled with tandem mass spectrometry analysis (LC–MS/MS), and toluene, for analysis employing gas-chromatography mass spectrometry (GC–MS). Working standards

consisting of mixtures of target pesticides (concentration of individual pesticides was 1 μg/ml) were prepared in acetonitrile (for LC–MS/MS analysis) and toluene (for GC–MS analysis). By appropriate dilution of these working standards (2×, 10×, 20×, 100× and 200×, respectively), calibration standards were prepared.

Organic solvents for pesticide residue analysis were the highest purity grade from Sigma–Aldrich, Germany, (acetonitrile), Merck, Germany (cyclohexane, toluene, and methanol) and Scharlau, Spain (ethyl acetate). Anhydrous sodium sulphate obtained from Penta, Czech Republic was dried at 600 °C for 7 h and then stored in a tightly closed glass container prior to use.

### 2.3. Analytical methods

Multiresidue LC–MS/MS and GC–MS methods encompassing whole spectrum of examined pesticides within the presented study were applied. For determination of mancozeb and thiram, compounds representing a group of ethylene bisdithiocarbamates (EBDCs), single residue method consisting of the following steps was used for examination of apple samples: (i) solid phase micro-extraction (SPME) of carbon disulphide (degradation product of EBDCs) from head space of sample digested by hydrochloric acid in the presence of stannous chloride and (ii) GC–MS identification/quantification of analyte thermally desorbed in GC injector port. Since in none of samples residues of EBDCs were detected (LOD of the method was 0.5 μg/kg), no more detailed description is provided here.

#### 2.3.1. LC–MS/MS method

*Sample preparation*. Extraction was carried out as described in our previous study (Tichá et al., 2006). Blended apples (12.5 g) were extracted by homogenization with acetonitrile, the suspension was filtered under vacuum. The residue left after evaporation of crude extract was made-up with methanol and filtered through polytetrafluoroethylene filters (PTFE, 5 μm; National Scientific, USA) prior to LC–MS/MS analysis. The matrix content in examined sample was 0.25 g/ml.

*LC–MS/MS identification/quantification.* HPLC 2695 Alliance module (Waters, UK) coupled to mass spectrometric detector Quattro Premier XE (Waters, UK) was used for determination of polar pesticides in sample extracts. All separations were carried out using a reversed phase Discovery $C_{18}$ column (150 × 3 mm, 5 μm) maintained at 25 °C. The mobile phase was water (A) and methanol (B); flow rate 0.3 ml/min, gradient was employed at starting composition of 50% B, rising linearly to 100% B over 6 min and then held for 11 min at 100% B followed by 10 min re-equilibration to initial mobile phase composition. Injection volume was 20 μl. Identification/quantification of target analytes was performed using tandem quadrupole mass spectrometric analyser operated in a positive electrospray (ES+) ionisation mode. Multiple reaction monitoring (MRM) conditions (collision energy and cone voltage) were optimised for each pesticide during infusion (5 μl/min) of individual pesticide solution (1–5 μg/ml) into the mobile phase flow (A:B 50:50, (v/v)). Following parameters were employed for all experiments: capillary voltage 3.5 kV, extractor voltage 4 V, source temperature 120 °C, desolvation temperature 250 °C, cone gas flow 100 L/h and desolvation gas flow 700 L/h (both gases were nitrogen). Argon was used as a collision gas ($3.3 \times 10^{-3}$ mbar). Tuned and optimised MS/MS transitions, specific cone voltages and collision energies are summarized in Table 3. Analytes were divided into time segments based on their elution characteristics. The MS/MS transitions were monitored in MRM mode at the same dwell time 0.005 s, inter-channel delays, and inter-scan delays of 10 ms for all transitions.

Quantification of pesticide residues in apple extracts was realized by a multilevel matrix-matched calibration curves. 50 μl of working standard solution $S_{1L}$–$S_{6L}$ (in acetonitrile) were added to 950 μl of blank apple extract for following LC–MS/MS analysis.

Generated experimental data were processed using MassLynx software version 4.0 Service Pack 4, Software Change Note #462.

#### 2.3.2. GC–MS method

*Sample preparation.* Isolation and clean-up of GC amenable pesticides was realized as described in our previous study (Tichá et al., 2006). Briefly, 25.0 g of representative apple sample were extracted by homogenization with ethyl acetate and anhydrous sodium sulphate and filtered under vacuum. Concentrated crude extract was after filtration through polytetrafluoroethylene filters (PTFE, 5 μm;

Table 3
MS/MS transitions used for quantification and confirmation in LC–MS/MS method

| Analyte | Transition (*m*/*z*) | Cone (V) | Collision (V) |
|---|---|---|---|
| Acetamiprid | 223 > 126 | 31 | 14 |
| | 223 > 56 | 31 | 14 |
| Diflubenzuron | 311 > 158 | 25 | 10 |
| | 311 > 141 | 25 | 29 |
| Dithianon | 296 > 264 | 30 | 15 |
| | 296 > 267 | 30 | 15 |
| Dodine | 228 > 57 | 45 | 22 |
| | 228 > 186 | 45 | 18 |
| Etofenprox | 394 > 177 | 20 | 14 |
| | 394 > 135 | 20 | 26 |
| Pyrimethanil | 200 > 107 | 54 | 24 |
| | 200 > 82 | 54 | 24 |
| Teflubenzuron | 381 > 158 | 23 | 18 |
| | 381 > 141 | 23 | 13 |
| Thiacloprid | 253 > 126 | 35 | 25 |
| | 253 > 186 | 35 | 13 |
| Triflumuron | 359 > 156 | 29 | 16 |
| | 359 > 139 | 29 | 30 |

National Scientific, USA) purified using automatic high performance gel permeation chromatography (HP GPC, Gilson France) equipped with PL-gel column (600 × 7.5 mm, particle size 10 μm, 50 Å), the mobile phase was ethyl acetate–cyclohexane (1:1, v/v). The eluate containing "pesticide" fraction was after evaporation and concentration to near dryness with a steam of nitrogen redissolved in 1 ml of toluene for following GC–MS analysis.

*GC–MS identification/quantification.* Gas chromatograph 6890N (Agilent Technologies, USA) equipped with a mass-selective detector 5975 Inert XL (Agilent Technologies, USA) and autosampler 7683 Series (Agilent Technologies, USA) was used for GC analysis. Pulsed splitless injection (pressure pulse 60 psi, pulse period 2 min, inlet temperature 250 °C, injection volume 1 μl) was used. All separations were carried out on capillary column DB-5MS (60 m × 0.25 mm × 0.25 μm, J&W Scientific, Agilent Technologies, USA). Oven temperature program started at initial temperature 90 °C (hold 2 min), rising 5 °C/min to 180 °C, then 2 °C/min to 280 °C (hold 5 min). Helium was used as a carrier gas at a constant rate 19 cm/s. MS detector was equipped with quadrupole analyzer operating in electron ionization mode (EI); ion source temperature was 230 °C and MS Quad temperature 150 °C. Identification/quantification of target analytes was performed in selected ion monitoring mode (SIM); see Table 4 for monitored ions ($m/z$).

GC amenable pesticides were quantified by a multilevel matrix-matched calibration curves. Residue after evaporation of solvent from purified blank apple extract was re-dissolved in 1 ml of appropriate standard working solution $S_{1G}$–$S_{6G}$ (in toluene) prior to GC–MS analysis.

All GC–MS chromatographic data were processed using ChemStation® Software (A.04.05, Hewlett-Packard, USA).

### 2.4. Quality control

Recoveries of all pesticides were tested by fortifying blank apple homogenate with pesticide mixture (spikes concentration corresponded to 0.05 mg/kg), which was then processed as described above. Performance characteristics of both employed analytical methods obtained via validation process are summarized in Table 5.

As a part of external Quality Control, laboratory has been successfully participating in available proficiency tests – Food Analysis Performance Assessment Scheme (FAPAS®) and European Commission's Proficiency Testing Program (EU-PT). Both LC–MS/MS and GC–MS methods have been accredited according to ISO/IEC 17025.

Table 4
Monitored ions ($m/z$) of the GC–MS analytical method

| Analyte | quantitation ion ($m/z$) | confirmation ions ($m/z$) |
|---|---|---|
| Captan | 149 | 79, 264 |
| Chlorpyrifos | 314 | 199, 258 |
| Chlorpyrifos-methyl | 286 | 125, 288 |
| Cyprodinil | 224 | 210, 225 |
| Difenoconazole | 323 | 207, 267, 281 |
| Fenoxycarb | 255 | 116, 186 |
| Kresoxim-methyl | 206 | 116, 131 |
| Phosalone | 182 | 121, 367 |
| Tebuconazole | 250 | 125, 163, 252 |
| Tolylfluanid | 137 | 181, 238 |
| Triazamate | 314 | 227, 242, 262 |
| Trifloxystrobin | 131 | 116, 222 |

Table 5
Performance characteristics of the analytical methods employed for apple analysis (mean values of five replicate measurements)

| Analyte | Method | Repeatability (RSD, %) at 0.05 mg/kg | Recovery (%) | LOQ (mg/kg) |
|---|---|---|---|---|
| Captan | GC–MS | 9 | 92 | 0.01 |
| Cyprodinil | (SIM) | 17 | 87 | 0.007 |
| Difenoconazole | | 4 | 95 | 0.008 |
| Fenoxycarb | | 10 | 97 | 0.01 |
| Chlorpyrifos | | 8 | 94 | 0.009 |
| Chlorpyrifos-methyl | | 7 | 93 | 0.011 |
| Kresoxim-methyl | | 9 | 94 | 0.004 |
| Penconazole | | 10 | 110 | 0.006 |
| Phosalone | | 10 | 96 | 0.004 |
| Pyridaben | | 5 | 72 | 0.004 |
| Tebuconazole | | 8 | 89 | 0.006 |
| Tetraconazole | | 5 | 90 | 0.004 |
| Tolylfluanid | | 5 | 100 | 0.004 |
| Triazamate | | 8 | 103 | 0.007 |
| Trifloxystrobin | | 8 | 98 | 0.004 |
| Acetamiprid | LC–MS/MS | 8 | 92 | 0.004 |
| Diflubenzuron | | 11 | 83 | 0.004 |
| Dithianon | | 13 | 83 | 0.01 |
| Dodine | | 12 | 85 | 0.004 |
| Etofenprox | | 8 | 94 | 0.004 |
| Pyrimethanil | | 9 | 87 | 0.004 |
| Teflubenzuron | | 7 | 86 | 0.004 |
| Thiacloprid | | 6 | 94 | 0.004 |
| Triflumuron | | 6 | 89 | 0.004 |

## 3. Results and discussion

Post-harvest diseases can be a limiting factor for the long-term storage of apples. As mentioned in Introduction, orchard practices such as sanitation and fungicide application as well as a strategy of insects control can have a great impact on the types and amount of decay potentially occurring during cold post-harvest storage.

Regarding crop storage lifetime, fungicides applied near harvest time may provide some control of damage-causing pathogens originated both from the latest fungal infection of fruit in the orchard and those developed by fungal infection of wounds (punctures, bruises etc.) caused by harvest and post-harvest handling practices.

However, it should be emphasized that besides of benefits obtained by chemical crop protection, also health

hazards associated with pesticides use have to be taken into consideration. To meet both consumers and toxicologists concerns, residues potentionally occurring in food supply have to be controlled.

While in our previous study (Tichá et al., 2006) the dynamics of pesticide residues in apples within the pre-harvest period was investigated, and treatment regimes leaving minimum residues in fruit intended for direct consumption and/or baby food production were searched, in the current study we focused on the fate of residues during the post-harvest period, under conditions of cold storage. The overview of detected pesticide residues used for orchard treatment in field experiments FT 1, FT 2 and FT 3 in apples at the time of harvest is shown in Fig. 1. (In field experiment FT 4, none of pesticides used for apple trees treatment left detectable residues at the harvest time.)

Of 21 active ingredients of pesticide preparations used for apple trees protection (see Table 1 for detailed information), only six fungicides and one insecticide were found in apples at the beginning of storage period. Residues of acetamiprid, chlorpyrifos-methyl, difenoconazole, diflubenzuron, dithianon, EBDCs (represented by mancozeb and thiram in this study), fenoxycarb, kresoxim-methyl, teflubenzuron, thiacloprid, triazamate, trifloxystrobin and triflumuron dropped below detection limits of analytical methods employed in this study (see Table 5 for overview of *LOQs* of respective analytes). Further decrease of residues occurred during cold storage, see Fig. 2; dodine was only one of those detected fungicides found after 5 months in one of experiments. High persistency was also documented for organophosphorus pesticide phosalone (see Fig. 2). In both cases, pesticide residues were below 0.01 mg/kg that is maximum residue limit required by baby food producers for raw material to be processed.

In the following paragraphs (Figs. 1–6), more detailed information on pesticides we monitored during post-harvest period is provided. Worth to notice, that compounds representing various chemical classes were selected. They

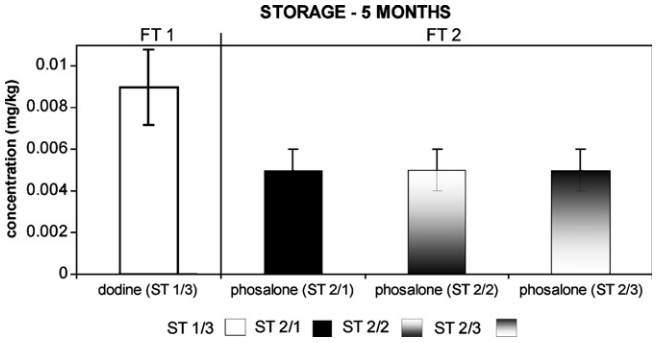


Fig. 2. Overview of pesticide residues in apples stored 5 months; experiments FT 1 and FT 2. For storage treatments (ST) details see Table 2. Error bars express the expanded analytical uncertainty of respective results.

are commonly used in conventional apple orchards and are often detected in matured apples within surveillance programs (Stepán, Tichá, Hajslová, Kovalczuk, & Kocourek, 2005; Tichá et al., 2006).

### 3.1. Captan

Captan belongs to the pesticides with protective and curative action that is used to control a wide range of fungal diseases e.g. apple scab (*V. inaequalis*), storage rots (e.g. *Gleosporium*), sooty blotch (*Gloeodes pomigena*) and fly speck (*Schizothyrium pomi*).

Although applied in each of field treatments FT 1–FT 4, its residues occurred only in the ST 1/2 (one application) and in ST 2/1 (two applications) at concentration levels ranged from 0.01 mg/kg (ST 1/2) to 0.015 mg/kg (ST 2/1) in harvested apples and degraded during storage time. Contrary to expectations, no captan residues were detected in FT 4, where captan was applied repeatedly (four applications) during vegetation period. It might be caused by intensive rainfalls (20 mm on July 8) which occurred after captan application and could possibly remove surface residues of

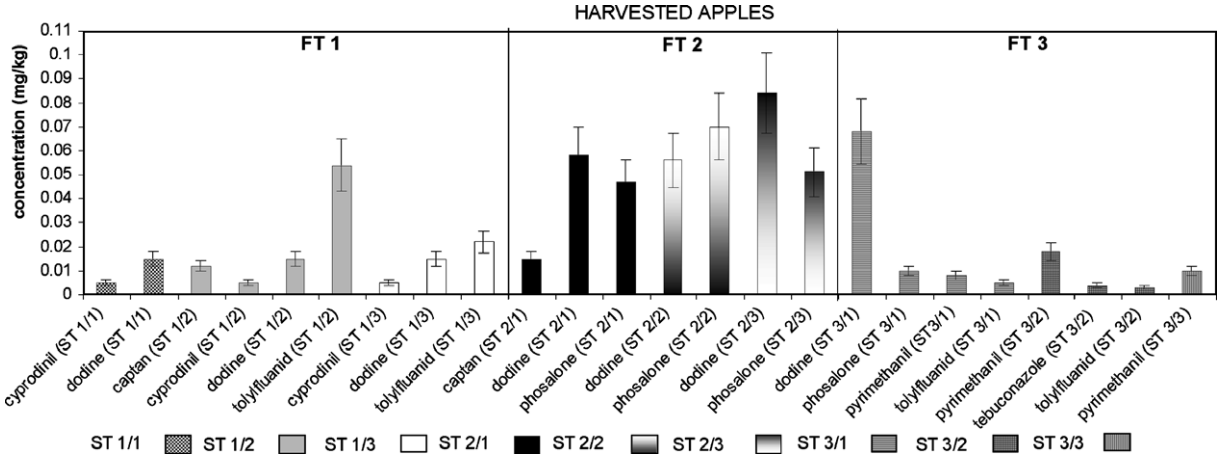


Fig. 1. Overview of pesticide residues in harvested apples; experiments FT 1, FT 2 and FT 3. (For legend of storage treatments (ST) details see Table 2b.) Error bars express the expanded analytical uncertainty of respective results.

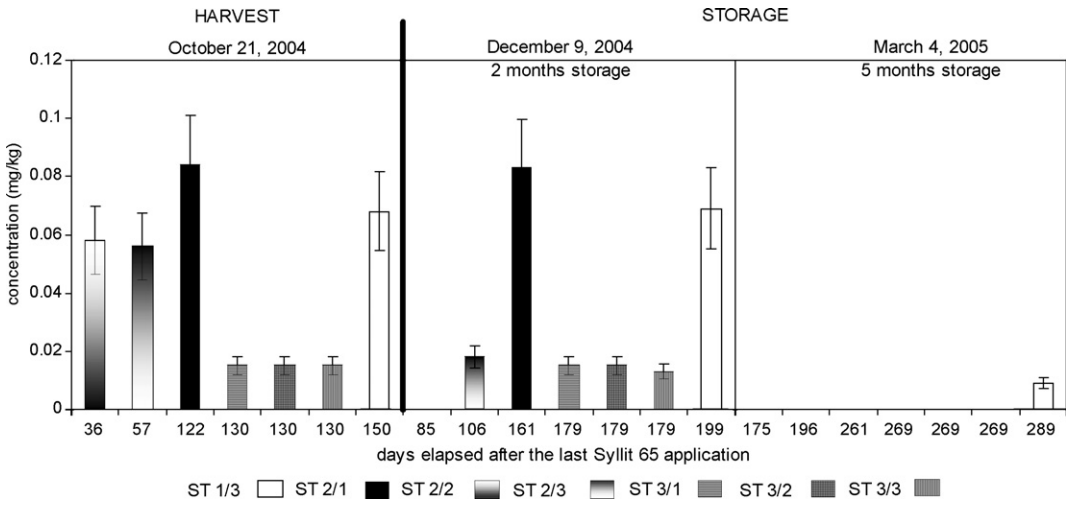


Fig. 3. The dynamics of dodine residues during cold storage in samples from experiments FT 1, FT 2 and FT 3. Error bars express the expanded analytical uncertainty of respective results.

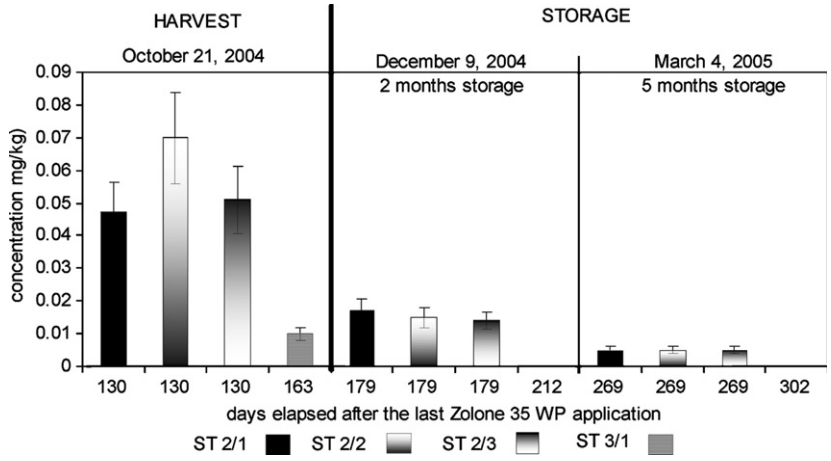


Fig. 4. The dynamics of phosalone residues during cold storage in samples from experiments FT 2 and FT 3. Error bars express the expanded analytical uncertainty of respective results.

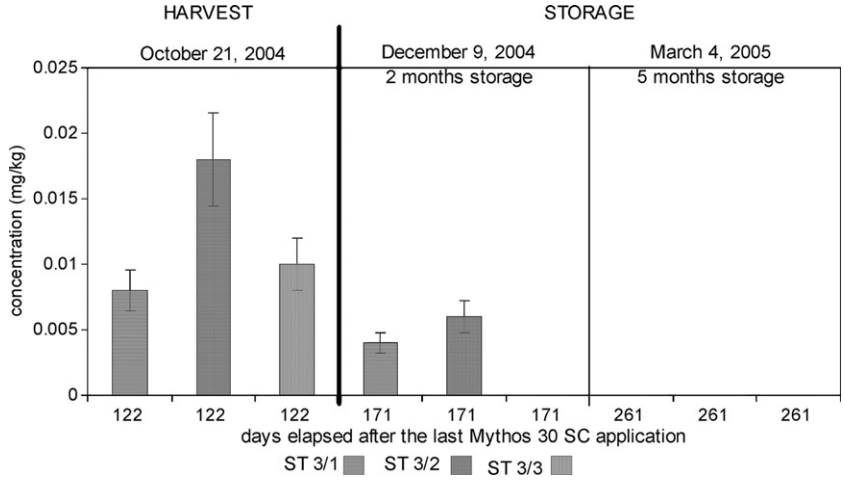


Fig. 5. The dynamics of pyrimethanil residues during cold storage in samples from experiment FT 3. Error bars express the expanded analytical uncertainty of respective results.

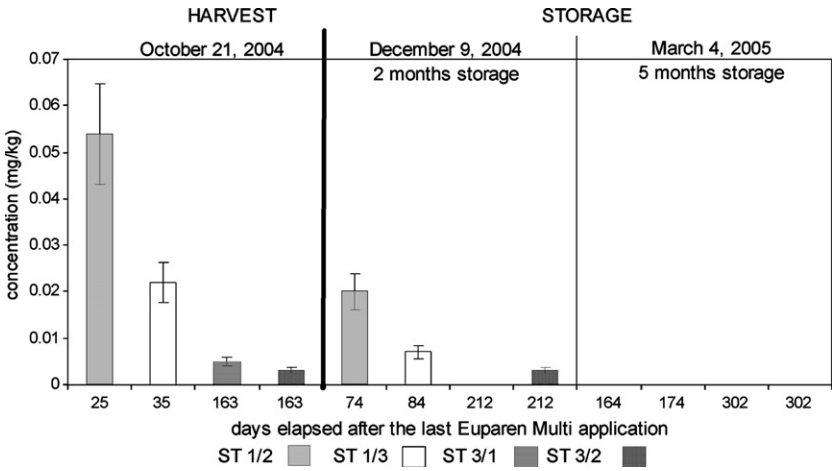


Fig. 6. The dynamics of tolylfluanid residues during cold storage in samples from experiments FT 1 and FT 3. Error bars express the expanded analytical uncertainty of respective results.

captan. However, residues in FT 1 and FT 2 were not fully removed due to their earlier penetration into surface walls.

### 3.2. Cyprodinil

Systemic fungicide cyprodinil is used as a foliar pesticide that controls a wide range of pathogens, such as *Alternaria* spp., *Venturia* spp. and *Monilinia* spp. After foliar application and transport throughout the tissue, it inhibits penetration and mycelial growth both inside and on the leaf surface.

Only traces of cyprodinil were found in the harvested apples obtained from field treatment FT 1 (ST 1/1, ST 1/2, and ST 1/3); residues did not exceed respective LOQ (see Table 5). Its content successively declined during storage and after 5 months of storage no residues were detected.

### 3.3. Dithianon

Dithianon is a foliar fungicide with protective and also curative action used to control a variety of foliar diseases (with the exception of powdery mildews) in apples. Although used for crop protection in all field treatments FT 1, FT 2, FT 3, and FT 4 within the first phase of experiment and in FT 4 in treatment against storage diseases, no dithianon residues were found. Low stability under field conditions (mainly at sunlight) causes relatively fast residues dissipation (The Pesticide Manual, 2002).

### 3.4. Dodine

Dodine represents one of the most important fungicides used to protect apples against storage pests; its main feature is to control the extent of apple scab (*V. inaequalis*). In our experiments, dodine was applied within experiments FT 1, FT 2 and FT 3 in the first phase of our field treatments. Second phase of experiment (protection against storage pests) was realized in FT 2 (experiments ST 2/1, ST 2/2, and ST 2/3).

As illustrated in Fig. 3, relatively high dodine residues were detected in harvested apples in FT 2 (repeated applications) and in FT 1 compared to lower residues found in FT 3. A decline occurred between subsequent samplings, specifically in the case of FT 2 (ST 2/1, ST 2/2, and ST 2/3). In apples stored for 5 months, no dodine residues were detected with the exception of trace concentration in ST 1/3.

### 3.5. Phosalone

Phosalone represents non-systemic insecticide and acaricide showing localised penetration into plant cuticle. It is often used against aphids, fruit tree red spider mite (*P. ulmi*) and *Lepidoptera* (*C. pomonella*) on apple trees. Relatively high persistency of phosalone was earlier documented in our study concerned with monitoring of pesticide residues in fresh apples (Stepán et al., 2005).

In none of examined samples of matured apples phosalone levels exceeded MRL (2 mg/kg) at the time of harvest. Fairly higher content of phosalone residues found in field treatment FT 2 (ST 2/1, ST 2/2, and ST 2/3) was probably due to application of this preparation nearer to harvest as compared to treatment in FT 3. As shown in Fig. 4, successive decline of phosalone residues occurred during storage time, similar results were obtained by experiments on apples realized by Branca, Quagloino, and Navone (1992). After 5 months of cold storage (temperature 1–3 °C), only trace concentrations of phosalone were found in examined fruits.

### 3.6. Pyrimethanil

Pyrimethanil is a fungicide with a protective action against apple scab (*V. inaequalis*). Due to a relatively early pyrimethanil application, its residues were not found in

matured apples originated from field treatment FT 1 while in the samples from later treatment carried out in FT 3 (see Fig. 5), detectable residues were present. Residues of pyrimethanil dropped between samplings; only trace concentrations were detected after 2 months storage, no residues were found in 5 months stored fruits.

### 3.7. Tebuconazole

Tebuconazole is a systemic fungicide with eradicate action that is rapidly absorbed into the vegetative parts of the plant. It helps to protect the crop against a variety of pathogens causing apple trees diseases, e.g. powdery mildew (*P. leucotricha*), apple scab (*Venturia* spp.), white rot (*Botryosphaeria dothidea*) and *Monilinia* spp. The fungicidal preparation applied in our study contained this compound together with tolylfluanid. (Synergic action against target pests is obtained in this way.)

Residues of tebuconazole applied within field treatment FT 3 were found in harvested apples (114 days after the application) only on a very low concentration levels and completely dissipated within 2 months of cold storage.

### 3.8. Tolylfluanid

As described in Experimental (see Tables 2a and 2b), tolylfluanid was applied as Euparen Multi preparation in FT 1 within the second phase of experiments (protection against storage pests) as a fungicide treatment against apple diseases caused by e.g. *Venturia*, *Monilia*, *Gloeosporium*, *Phytophthora*, *Penicillium* and in FT 3 within general field treatment as a Hattrick preparation (together with tebuconazole). In the harvested apples, its residues were fairly below MRL (1 mg/kg) in both field treatments, FT 1 and FT 3.

Successive decline was observed during storage period (see Fig. 6). It should be noted that the relative decrease rate was higher in FT 1 compared to FT 3 in which treatment with Euparen Multi was carried out earlier. Also in FT 1 (experiments ST 1/1, ST 1/2, and ST 1/3) – concentration level in ST 1/2 was higher than in ST 1/3 where tolylfluanid was applied 10 days earlier. In apples analyzed after 5 months of storage, no residues of tolyfluanid were found. These results are in agreement with the study by Rasmussen et al. (2003) who reported significant reduction of tolylfluanid residues during cold storage of apples (variety *Discovery*). It is assumed that the reduction of tolylfluanid during the cold storage might be caused by its relatively low persistence to hydrolysis.

### 3.9. Post-harvest diseases control

Effectiveness of crop protection against storage diseases was evaluated for the occurrence of apple scab. Pesticide applications carried out within field treatment regimes FT 1, FT 2, FT 3 and FT 4, mainly fungicide treatments with Euparen Multi, Syllit, Thiram Granuflo and Delan were shown to be effective. After five months of apple storage, minimal development of storage diseases such as apple scab (*V. inaequalis*) or rot (*B. cinerea*, *M. fructigena*, *P. expansum*) was observed.

## 4. Conclusions

Based on the results obtained in this study, two general conclusions can be drawn:

- All four tested pesticide field treatments (FT 1, FT 2, FT 3 and FT 4) were efficient in protection of apples (variety *Merlose*) against such crops devastating storage diseases as an apple scab and/or rot.
- All field experiments could be classified from chemical safety point of view as reasonable treatment strategies; none of apple samples contained residues exceeding EU MRLs and, moreover almost all residues determined in harvested apples dissipated during storage to non-detectable/only trace levels.

It should be noted; however, that dynamics of residues both before harvest and in the store is dependent on many factors and therefore validation experiments are needed whenever different conditions as compared to those in our study occur.

## Acknowledgements

Financial supports for field experiments and sample analysis provided by the project NAZV 1G46073 for realization of presented study is gratefully acknowledged. The development and validation of multiresidue analytical methods was funded by project MSM 604 613 75 05 (Ministry of Education, Youth and Sports of the Czech Republic).

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