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**Original article**

# Residues of captan (contact) and difenoconazole (systemic) fungicides in bee products from an apple orchard

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**Abstract** – Ten bee colonies were placed in the middle of a ten-hectare-apple orchard at about 10% in bloom. The orchard was sprayed with a mixture of a contact fungicide, Captan 50WP (active ingredient captan) and a systemic fungicide, Score 250 EC (active ingredient difenoconazole). The residues of fungicides in honey, pollen, and bee bread were then measured by gas chromatography. Honey had very low contamination – 0.0006 mg·kg$^{-1}$ of difenoconazole and 0.009 mg·kg$^{-1}$ of captan. Contamination of pollen was much higher – about 0.043 and 2.99 mg·kg$^{-1}$ of difenoconazole and captan, respectively. The most contaminated was bee bread, 0.27 and 6.39 mg·kg$^{-1}$ of difenoconazole and captan, respectively. This finding may be due to some chemical reactions between difenoconazole and some plant metabolites taking place in pollen and bee bread. Difenoconazole, a systemic fungicide, penetrates about 1.66 and 1.16 times more efficiently into honey and bee bread, respectively, than the contact fungicide captan. But in pollen pellets from apple, the penetration coefficient was lower than 1. This observation corroborates the suggestion that in fresh pollen some fungicides may be fixed by sugars, aminoacids, or even proteins.

**honey / pollen / contamination / systemic fungicide / contact fungicide**

## 1. INTRODUCTION

Contemporary horticultural production requires heavy chemical protection against a multitude of diseases, among them fungal ones. Several crops (strawberry, raspberry, cherry, apple and others) require protection during blooming. Since most

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fungicides are relatively non-toxic for bees, short prevention periods were established [11]. This carries a risk that bees foraging on sprayed plant could collect contaminated nectar and pollen and thus contaminate bee products.

In our previous papers we have found that contact fungicides contaminate mostly pollen, whereas honey remains much less contaminated [5, 6]. It was noted that systemic insecticides may be poisonous to bees, even when the spray was done several days before the bloom [3, 4, 9, 10]. Thus it is interesting to determine if systemic fungicides are transported in significant quantities to nectaries and anthers and contaminate collected honey and pollen.

In our earlier experiments on blackcurrants [7] and cherries [8] we have found that systemic fungicides contaminate honey and pollen more than contact ones. But our results obtained so far are incomplete. Blackcurrants turned out to be completely unattractive for bees as a source of pollen. Cherries offered nectar and pollen for bees, but iprodione and vinclozolin – fungicides regarded as contact ones-behave as a systemic. Therefore, it was necessary to find a crop that would be a good source of both nectar and pollen and a pair of fungicides differing distinctly in their mode of action. Apple pollen and nectar are willingly collected by bees. So captan and difenoconazole serve well as models of contact and systemic fungicides. The aim of this work was to investigate contamination of apple honey, pollen and bee bread with the systemic and contact fungicides, and to check if the systemic fungicide penetrates easier into bee products.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of bees and orchard

Ten bee colonies of equal strength (8–9 frames of Dadant type, fully covered by bees) were transferred into hives containing only empty combs and foundations. The queens were confined on a single comb. The hives were placed in the middle of a 10 hectare apple (*Malus domestica* Mill. cv. Cortland, Golden Delicious, Gloster, and Idared) orchard on May 12th at about 10% in bloom, and on the same day in the afternoon the orchard was sprayed with a mixture of Captan 50WP (2 kg·ha$^{-1}$) and Score 250 EC (0.2 l·ha$^{-1}$, active ingredient difenoconazole), using 2000–2500 l of spraying liquid per hectare.

### 2.2. Collecting samples

The hives were equipped with pollen traps and pollen was collected daily. During the experiment, weather conditions were harsh, and amounts of harvested pollen were small. Thus, the pollen pellets collected daily in all 10 hives were combined and treated as one sample. At the end of the experiment (May 26th – after moving out the bees) the honey was extracted and bee bread was carefully scraped out from each cell. Because of the small amounts of honey and bee bread (stored pollen with small amounts of nectar and glandular secretion added by bees) collected, the samples collected from two hives were combined. All collected bee products were stored in a freezer at –20 °C until analysis.

Samples of apples were collected on June 5 and September 10 and consisted of harvesting about 2 kg of fruit from randomly selected trees. The samples were stored until analysis in a freezer at –20 °C.

### 2.3. Extraction of captan and difenoconazole from honey

25 g of honey was dissolved in 50 ml of distilled water saturated with n-hexane. Then 25 ml of n-hexane was layered on top of the water phase and the bilayer was carefully stirred for 1.5 h with a magnetic stirrer at low speed (not more than 20 rpm) to avoid

emulsion formation. The upper, hexane layer was removed, new solvent was added, and extraction was repeated. Both hexane extracts were collected, dried over anhydrous $Na_2SO_4$, evaporated under reduced pressure, and the dry residue was dissolved in 2 ml of n-hexane.

### 2.4. Extraction of captan and difenoconazole from pollen and bee bread

Twenty-five milliliters of ethyl acetate was added to 2 g of pollen or bee bread and vigorously shaken for 1 h. Then the liquid phase was filtered off and the filtrate extracted again with a new portion of ethyl acetate. The extracts were combined, dried over anhydrous $Na_2SO_4$, evaporated under reduced pressure and the dry residue was dissolved in 5 ml of n-hexane.

### 2.5. Extraction of captan and difenoconazole from apples

Two kilograms of apples were chopped to small pieces and one subsample of about 0.5 kg each were taken and homogenised in a laboratory blender. 25 g of the homogenate was mixed with 100 ml of ethyl acetate in a 250 ml Erlenmeyer flask and shaken vigorously for 5 min. The mixture was then filtered through a Buchner funnel and the filtrate was washed twice with 30 ml of ethyl acetate. The extracts were combined, dried over anhydrous $Na_2SO_4$, and made up to 200 ml with ethyl acetate. Fifty ml of this solution was evaporated under reduced pressure and the dry residue was dissolved with 5 ml of n-hexane.

### 2.6. Purification of n-hexane extracts of captan and difenoconazole

N-hexane extracts obtained as described above were passed slowly through a Bakerbond SPE-phenyl disposable extraction columns (Baker) preconditioned by washing with 5 ml of hexane. The columns were then rinsed with 4 ml of n-hexane and the sample was eluted with 10 ml of a mixture of n-hexane: ethyl acetate (1:1, v/v). The eluate was evaporated under reduced pressure and the dry residue was dissolved in 2 ml of methanol. Methanol solution was mixed with 20 ml of distilled water and passed slowly through a Bakerbond SPE-phenyl disposable extraction column previously preconditioned by washing with methanol and water. The column was then rinsed with 6 ml of water, dried with a stream of air for two hours, and the adsorbed fungicides were eluted with 10 ml of n-hexane: ethyl acetate mixture (1:1, v/v). The solution was evaporated and the dry residue was dissolved in a proper volume of toluene (usually in 1 ml or less in the case of honey samples or higher volumes for samples of pollen or bee bread). 1 μl samples of this solution were taken for chromatographic analysis.

### 2.7. Chromatographic analysis of captan and difenoconazole

Analysis of captan and difenoconazole were performed on a HP 5890 gas chromatograph (Hewlett Packard) equipped with a capillary column HP-5, 30 m × 0.32 mm, film thickness 0.25 μm, and ECD detector. The carrier gas was helium, 3 ml·min$^{-1}$ and the make up gas was nitrogen, 60 ml·min$^{-1}$. Temperature of the injector was 260 °C, and of the detector, 300 °C. Initial temperature of the oven was maintained at 100 °C for 2 min, then increased at a rate of 20 °C·min$^{-1}$ to 250 °C, and then to 280 °C at a rate of 30 °C·min$^{-1}$. Time of analysis was 20 min. Retention time for captan in such conditions was 10.5 min and for difenoconazole 17.9 min. Each sample of honey, pollen and bee bread was analysed in three to five replications and chromatographic analysis was repeated at least two times. Data presented in the tables represent arithmetical means ± standard deviations of all replications, to

characterise the repeatability of the procedure. In the last line of Table II are arithmetical means ± standard deviations of residues of all bee colonies which characterise variation among individual bee hives. The last line in Table III gives the weighted averages for which standard deviations were not calculated.

### 2.8. Fortification of honey, apples, pollen, and bee bread

The standards of captan (99.0%, Promochem) and difenoconazole (99.0%, Ciba-Geigy) were used. Two levels of fortification were checked: I – 10 μg of captan and1 μg of difenoconazole; II – 1 μg of captan and 0.1 μg of difenoconazole.

#### *2.8.1. Fortification of honey and apples*

The standards dissolved in toluene were put into an empty Erlenmeyer flask. Toluene was evaporated under vacuum, water solution of honey, or pulp of apples was added, and purification procedures were performed as is in steps 2.3 and 2.5.

#### *2.8.2. Fortification of pollen and bee bread*

The standards dissolved in toluene were added to a suspension of 2 g of either pesticide free pollen or bee bread in 100 ml of toluene. The mixture was then shaken for 30 min, the solvent was evaporated under reduced pressure and dry pollen or bee bread, evenly surface-coated with both fungicides, was used for all steps of purification and analysis as described above. The detection limit of captan and difenoconazole standards on gas chromatograph was 0.01 ng. The recovery of captan and difenoconazole from honey was 98.0% and 71.0%, respectively. The recovery from pollen and bee bread were 93.5% for captan and 97.1% for difenoconazole, the recovery for apples was 86% for captan and 91.5 for difenoconazole. The detection limits of difenoconazole and captan residues in honey, bee bread, pollen and apples were $4 \times 10^{-4}$ and $5 \times 10^{-3}$ mg·kg$^{-1}$, respectively when the volume of final solution before analysis on GC was 1.0 ml.

### 2.9. Pollen analysis

#### *2.9.1. Identification of pellets*

Pollen pellets were manually segregated according to their shape and colour in white and UV lights (with Wood's filter for identification of fluorescing pellets). In most cases, only pellets from apple were separated, but sometimes samples of pollen pellets were divided into subsamples for all identified species.

#### *2.9.2. Identification of pollen in individual pellets and bee bread*

One 4 g sample of pellets or bee bread was suspended in 10 ml of distilled water alkalinised with diluted NaOH solution to pH 9.0. After several minutes swollen pollen grains were examined under a microscope with 500 × magnification and compared with examples from the literature.

#### *2.9.3. Identification of pollen in honey*

One 10 g sample of honey was diluted with 20 ml of warm (50 °C) distilled water and centrifuged at 3000 rpm (1407.1 *g*) for 5 min. The pellet was collected, carefully mixed with a small amount of water, and examined under microscope.

## 3. RESULTS AND DISCUSSION

The blooming period of apples in central Poland began after May 10th. On May 12th, when the bees were moved into the experimental orchard, there were plenty of other plants blooming on which the bees could forage. Pollen analysis of bee

products revealed that honey and bee bread collected during the period of the experiment originated from several plants (Tab. I). It was found that the amount of pollen in honey varied for different species [1]. Thus, pollen composition in multiflower honey does not reflect the relative share of various nectars, but it can be concluded that for a given species, content of its pollen in a honey should be proportional to the relative content of nectar collected from its flowers.

Sometimes, the composition of pollen taken from pollen traps and composition of bee bread differed conspicuously. Pollen from pollen traps consisted on average of 60.8% apple pollen (Tab. III), whereas bee bread contained only 22.3% apple pollen grains (Tab. I). It is likely that pellets of apple pollen were larger than pollen pellets of another species and they could be more easily broken apart in pollen traps. A similar phenomenon has been observed with strawberry pollen [6].

If one assumes that all nectar collected from apple flowers was contaminated to the same extent, there should have been a positive correlation between a sample of apple nectar and contamination of honey. But there was no such correlation in analysed samples (Tabs. I and III). This discrepancy can be explained if some of the foragers collected nectar from unsprayed apple orchards located within foraging distance from the nest.

Average contamination of honey with difenoconazole was very low – only 0.0006 $mg \cdot kg^{-1}$. However, the amount of this compound recommended for spraying (only 50 $g \cdot ha^{-1}$ of pure substance) also was small. Residues of captan were much higher, and the amount of active substance used in a spray also was much higher – 1000 $g \cdot ha^{-1}$. To compare the ability of both these substances to contaminate bee products, the same molar quantities should be used for spraying (which would be unacceptable for fruit production), or the results should be recalculated as if the same molar quantities of pure active substance were used. Thus we used a recalculated contamination or recalculated residue, expressed as $(mg \cdot kg^{-1})_M$. For example, 1 $kg \cdot ha^{-1}$ of captan was used in a spray, which was equivalent to 3.33 Mol, whereas for difenoconazole 50 g was equivalent to only 0.12 Mol. To compare these preparates for contamination, the experimental value for captan in honey = 0.009 $mg \cdot kg^{-1}$ was divided by 3.3 to give 0.0027 $(mg \cdot kg^{-1})_M$. A similar calculation for difenoconazole gives 0.0006 / 0.12 = 0.005 $(mg \cdot kg^{-1})_M$ (Tab. II). The penetration coefficient, a ratio of recalculated residues of systemic and contact preparates serves as an indicator of the ability of the given fungicide to penetrate. For example, for honey this coefficient was $\eta = 1.66$ (Tab. II), which means that difenoconazole entered honey 1.66 times more readily than captan. Identically calculated values for pollen and bee bread were $\eta = 0.35$ and $\eta = 1.16$, respectively (Tabs. II and III). Thus, the systemic fungicide, difenoconazole more effectively contaminated honey and bee bread than pollen. These results are partly consistent with results obtained earlier on cherries [8], in which the penetration coefficient of pollen pellets also was $\eta = 0.36$. There were no substantial differences between the recalculated arithmetical or weighted average for apple pollen (Tab. III), meaning that the quantity of difenoconazole detected in pollen was very small compared to bee bread.

In our previous experiments on cherries, a lag period was observed between the spray time and maximum contamination of pollen with all systemic and contact pesticides used. The maximal contamination of pollen was observed 3–5 days after application of a fungicide [8]. A similar lag period was observed in contamination of apple pollen. In general, the dynamics of pollen contamination was the same for systemic (difenoconazole) and contact (captan) fungicides (Fig. 1). The maximum contamination of pollen with both fungicides occurred three days after spraying (Fig. 1). However, those

**Table I.** Composition of honey and bee bread collected during the blooming period of apple.

| Colony no. Plant | Share of pollen (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 + 2 | | 3 + 4 | | 5–6 | | 7–8 | | 9–10 | | **Average** | |
| | honey | bee bread | honey | bee bread | honey | bee bread | honey | bee bread | honey | bee bread | **honey** | **bee bread** |
| *Malus* | 6.65 | 35.78 | 11.47 | 23.28 | 14.32 | 34.48 | 7.12 | 10.76 | 12.24 | 7.21 | **10.34 ± 3.42** | **22.31 ± 12.21** |
| Brassicaceae | 84.1 | 29.92 | 77.10 | 36.23 | 79.95 | 8.90 | 79.06 | 64.31 | 75.59 | 76.94 | **78.55 ± 2.22** | **40.98 ± 25.06** |
| *Acer* | 5.77 | 12.03 | 15.01 | 18.39 | 11.23 | 22.26 | 3.98 | 14.70 | 7.36 | 6.13 | **8.62 ± 4.40** | **15.08 ± 35.52** |
| *Rubus* | 0.59 | 10.27 | 0.57 | 3.47 | – | 13.96 | – | 5.73 | 2.65 | 4.53 | **1.26 ± 1.19** | **8.12 ± 3.45** |
| *Taraxacum* | 0.58 | 1.16 | 0.29 | 3.17 | 0.28 | 0.26 | 0.26 | 2.45 | 0.87 | 4.45 | **0.44 ± 0.24** | **2.40 ± 1.73** |

**Table II.** Residues of difenoconazole and captan in honey and bee bread.

| Bee product | Honey | | | | Bee bread | | | |
|---|---|---|---|---|---|---|---|---|
| Colony No. | Apple pollen (%) | Difenoconazole ($mg \cdot kg^{-1}$) | Captan ($mg \cdot kg^{-1}$) | η dif/capt | Apple pollen (%) | Difenoconazole ($mg \cdot kg^{-1}$) | Captan ($mg \cdot kg^{-1}$) | η dif/capt |
| 1 + 2 | 6.65 | 0.0003 ± 0.0001 | 0.007 ± 0.0001 | | 35.8 | 0.411 ± 0.075 | 4.74 ± 1.01 | |
| 3 + 4 | 11.47 | 0.0005 ± 0.0001 | 0.004 ± 0.0003 | | 23.3 | 0.180 ± 0.061 | 5.03 ± 0.58 | |
| 5 + 6 | 14.32 | 0.0006 ± 0.0001 | 0.009 ± 0.0001 | | 34.5 | 0.405 ± 0.096 | 6.78 ± 0.40 | |
| 7 + 8 | 7.12 | 0.0005 ± 0.0001 | 0.019 ± 0.0013 | | 10.8 | 0.157 ± 0.067 | 8.09 ± 0.79 | |
| 9 + 10 | 12.24 | 0.0009 ± 0.0001 | 0.006 ±0 .0003 | | 7.2 | 0.198 ± 0.011 | 7.30 ± 1.11 | |
| **Average** | **10.34** | **0.0006 ± 0.0001** | **0.009 ± 0.0001** | **1.66** | **22.3** | **0,271 ± 0.126** | **6.39 ± 1.45** | **1.16** |

Recalculated residues of difenoconazole and captan in honey are 0.005 $(g \cdot kg^{-1})_M$ and 0.003 $(mg \cdot kg^{-1})_M$, respectively.
Recalculated residues of difenoconazole and captan in bee bread are 2.251 $(mg \cdot kg^{-1})_M$ and 1.93 $(mg \cdot kg^{-1})_M$.

**Table III.** Residue of difenaconazole and captan in separated apple pollen pellets.

| Days | Apple pollen (%) | Difenoconazole* ($mg \cdot kg^{-1}$) | Captan* ($mg \cdot kg^{-1}$) | η (dif/capt) |
|---|---|---|---|---|
| 12 V | 89.94 | 0.017 ± 0.001 | 0.04 ± 0.004 | |
| 15 V | 34.15 | 0.166 ± 0.003 | 18.97 ± 2.40 | |
| 16 V | 70.95 | 0.191 ± 0.003 | 14.66 ± 2.80 | |
| 17 V | 59.21 | 0.020 ± 0.001 | 5.54 ± 0.20 | |
| 18 V | 67.54 | 0.029 ± 0.001 | 0.29 ± 0.07 | |
| 22 V | 77.48 | 0.019 ± 0.002 | 0.20 ± 0.03 | |
| 23 V | 54.87 | 0.033 ± 0.002 | 0.16 ± 0.03 | |
| 24 V | 43.36 | 0.023 ± 0.008 | 0.15 ± 0.03 | |
| 25 V | 50.04 | 0.043 ± 0.009 | 0.04 ± 0.004 | |
| **Average** | **60.83** | **0.043** | **2.99** | **0.35** |

* Weighted average.
Recalculated residues of difenoconazole and captan in pollen pellets were 0.352 $(mg \cdot kg^{-1})_M$ and 0.996 $(mg \cdot kg^{-1})_M$ respectively.
Penetration coefficient (0.352/0.996 = 0.35).

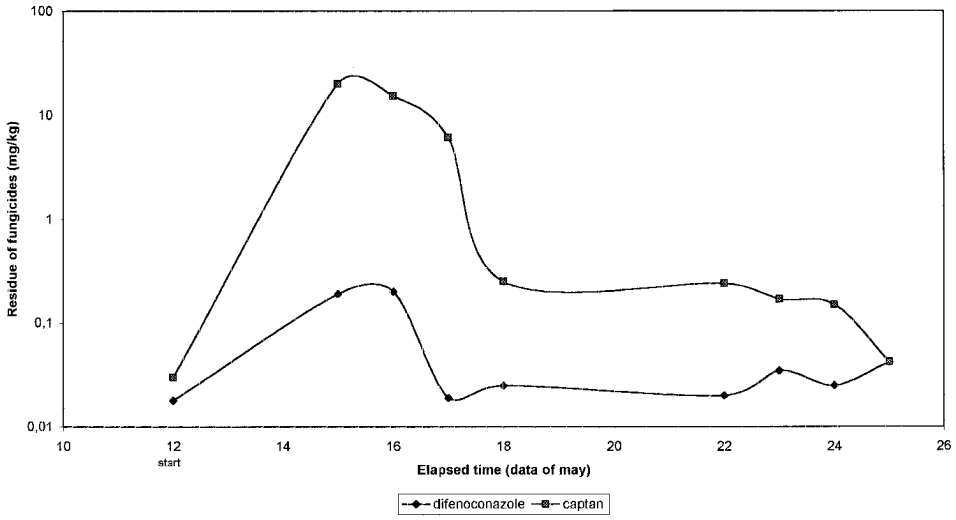


**Figure 1.** Contamination of apple pollen collected from an apple orchard sprayed with a mixture of Captan 50 WP, and Score 250 EC. ——- ▩ ——- residue of captan, ——- ◆ ——- residue of difenoconazole. Spraying of the orchard a with mixture of captan and difenoconazole was performed on May 12th in the evening.

three days were cold and drizzly and unsuitable for bees to forage, so data for two days was missing. Therefore, the possibility can not be excluded that in good weather the maximum of contamination would be observed on the next day after spraying without any lag period. There was only one clear difference between contamination with

contact (captan) and systemic (difenoconazole) fungicides. After several days, contamination with captan dropped to 1%, whereas for difenoconazole it dropped to only 15–25% of the maximal value, meaning that the systemic fungicide persisted in the plant and was effectively transported to young, developing pollen grains. Despite high contamination of pollen, the residue level of both fungicides in fruits at harvest was below detection limit. Some residues were detectable only at fruitlet stage (Tab. IV).

Contamination of bee bread was 0.27 mg·kg$^{-1}$ for difenoconazole and 6.39 mg·kg$^{-1}$ for captan. If one considers that bee bread consists only of 22% of apple pollen, the contamination of apple pollen in bee bread was actually 1.227 and 29.04 mg·kg$^{-1}$ for difenoconazole and captan respectively, instead of 0.043 and 2.99 mg·kg$^{-1}$ as was presented in Table III.

Apple pollen pellets were extracted in the same way as the bee bread. Extraction of difenoconazole from freshly harvested pollen with toluene, as well as with ethyl acetate was completed within 15 min [2]. Further prolongation of the extraction time did not increase the amount of fungicide. The sample of apple pollen was collected on May 24th, so it was unlikely that pollen grains were surface contaminated. Thus, this result can shed light on the extraction of fungicides present inside of the pollen grains.

It is clear that extraction of fungicide from pollen pellets of apple was less effective than from bee bread. The reason for this phenomenon was not due to the incomplete extraction of difenoconazole from the outer surface of pollen grains, because it was shown that recovery of standard difenoconazole applied on the surface of pollen grains was about 97.1%. Although some of the differences in extracted amounts may have been a result of differences with water and honey content in both the products, it is not likely to explain the observable differences. The most probable explanation of this phenomenon, as we proposed previously for vinclozolin and iprodione [8], is the formation of a chemical conjugate between difenoconazole and some plant metabolites. Such a compound could not be extracted from pollen grains with the solvents used or, if extracted, would not be detected as difenoconazole. During storage of bee bread such a compound could be broken down and a free pesticide released.

A partial explanation for the greater amount of contact fungicide in bee bread than in pollen pellets is that apple pollen may not have been the only source of fungicides in bee bread. Dandelion growing in abundance in the grass under apple trees may be have been another source of fungicides. But the average content of dandelion pollen in bee bread was only about 2.4% (Tab. I). Thus, despite its relatively high contamination (at maximum on May 16th up to 4.9 mg·kg$^{-1}$ of captan and 0.018 mg·kg$^{-1}$ of difenoconazole), contamination of dandelion pollen can not fully explain the phenomenon.

**Table IV.** Contamination of apples with difenoconazole and captan.

| Time of samples collection | Difenoconazole | | Captan | |
|---|---|---|---|---|
| | (mg·kg$^{-1}$) | SD | (mg·kg$^{-1}$) | SD |
| 5.06 | 0.0249 | 0.001 | 0.0591 | 0.002 |
| 10.09 | NOP | – | NOP | – |

A practical conclusion from our results is that special attention should be paid while collecting pollen because contamination of this product with systemic pesticides could be higher than permissable residue levels, thus such pollen should not be recommended for use as a source for human food, medicines and cosmetics.

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**Résumé – Résidus de deux fongicides, l'un de contact (captane) et l'autre systémique (difénoconazole), dans les produits de la ruche provenant d'un verger de pommiers.** Plusieurs cultures (fraises, framboises, cerises, pommes et certains légumes) nécessitent durant la floraison des traitements chimiques contre les maladies, entre autres contre les maladies fongiques. Les fongicides utilisés sont généralement non toxiques pour les abeilles si bien que les périodes de prévention sont courtes, une à deux heures. Le but de ce travail est de vérifier si les fongicides systémiques contaminent plus les produits de la ruche que les fongicides de contact.

Dix colonies d'abeilles domestiques (*Apis mellifera* L.) ont été transvasées dans des ruches ne contenant que des rayons vides et des feuilles de cire gaufrée et placées au milieu d'un verger de pommiers (*Malus domestica* Mill) de 10 hectares, le 12 mai en début de floraison. L'après-midi du même jour, le verger a reçu une pulvérisation d'un mélange de Captan 50 WP (2 kg·ha$^{-1}$, matière active captane) et de Score EC (0,2 l·ha$^{-1}$ ; matière active: difénoconazole). Les ruches possédaient une trappe à pollen et les pelotes de pollen ont été récoltées chaque jour. Le miel a été extrait et le pain d'abeilles prélevé dans les rayons à la fin de l'expérience.

Dans tous les produits de la ruche une analyse pollinique a été effectuée et les résidus des fongicides ont été déterminés par chromatographie en phase gazeuse.

La contamination du miel était généralement faible : de 0,0006 mg·kg$^{-1}$ pour le difénoconazole à 0,009 mg·kg$^{-1}$ pour la captane (Tab. II). Le pollen de pommier dans les pelotes était en moyenne beaucoup plus contaminé : 0,043 mg·kg$^{-1}$ pour le difénocozanole à 2,99 mg·kg$^{-1}$ pour le captane (Tab. III). Le pain d'abeilles était le produit le plus contaminé : 0,271 mg·kg$^{-1}$ pour le difénocozanole à 6,39 mg·kg$^{-1}$ pour le captane (Tab. II). Mais si le pain d'abeilles ne contenait que du pollen de pommier, sa contamination serait de 1 227 mg·kg$^{-1}$ pour le difénocozanole et de 29,04 mg·kg$^{-1}$ pour le captane, c'est-à-dire respectivement 34,2 fois et 10,9 fois celle des pelotes de pollen de pommier récoltées dans la trappe. À la récolte les fruits des vergers traités étaient indemnes de résidus des pesticides utilisés.

L'explication la plus probable de cette forte contamination du pain d'abeilles est que le difénoconazole est fortement adsorbé sur la structure de la paroi cellulaire à l'intérieur des grains de pollen ou qu'il est chimiquement lié à quelque(s) métabolite(s) de la plante et ne peut donc être extrait et détecté par les méthodes utilisées. Seuls 3 % du fongicide présent dans les grains de pollen peuvent être extraits et détectés. Au cours de la fermentation du pain d'abeilles la liaison est cassée et le fongicide libéré. Le captane, fongicide de contact, ne pénètre pas profondément dans le tissu de la plante et sa liaison, si elle existe, est donc moins intense.

Pour déterminer la capacité d'un fongicide donné à contaminer les produits de la ruche, les mêmes quantités molaires doivent être utilisées pour les pulvérisations et les résidus de fongicides détectés doivent être recalculés comme si les mêmes quantités molaires avaient été utilisées pour chaque matière active (résidu recalculé). Si le rapport résidu recalculé pour le systémique/résidu recalculé pour le fongicide de contact (coeffi-

cient de pénétration η) est supérieur à 1, cela signifie que le fongicide systémique pénètre plus facilement dans un produit de la ruche que le fongicide de contact. Nos résultats montrent que le difénoconazole pénètre respectivement 1,66 et 1,16 fois plus facilement dans le miel et le pain d'abeilles (Tab. II). Mais pour le pollen frais, $\eta = 0{,}35$, c'est-à-dire que la quantité de fongicide détectée est plus faible que dans le pain d'abeilles. Les méthodes de détermination des pesticides dans les pelotes de pollen devraient donc êtres spécialement adaptées afin d'éviter des erreurs suite à l'utilisation des méthodes habituelles d'extraction.

**miel / pollen / contamination / fongicide de contact / fongicide systémique / résidu**

**Zusammenfassung – Rückstände der Fungizide Captan (kontakt) und Difenoconazolen (systemisch) in Bienenprodukten aus Apfelplantagen.** Viele Nutzpflanzen (Erdbeeren, Himbeeren, Kirschen, Äpfel und einige Gemüsesorten) müssen während der Blütezeit chemisch gegen Pilzbefall geschützt werden. Die eingesetzten Fungizide sind für Bienen nicht giftig. Deshalb sind nur 1 oder 2 Stunden als Wartezeit vorgeschrieben. Bienen fliegen häufig in die Anpflanzungen, um Nektar und Pollen zu sammeln, die beide mit groβer Wahrscheinlichkeit kontaminiert sind. Ziel dieser Versuche war die Überprüfung der Hypothese, dass systemische Fungizide die Bienenprodukte stärker kontaminieren als Kontaktfungizide.

Zehn Bienenvölker (*Apis mellifera)* wurden in zweizargige Beuten mit Mittelwänden umgesetzt und zu Beginn der Blüte (12.Mai) mitten in eine 10 Hektar groβe Apfelplantage gebracht (*Malus domestica* Mill. cv. Cortland, Golden Delicious, Gloster und Idared). Am gleichen Nachmittag wurde die Plantage mit einer Mischung aus Captan 50WP (2 kg·ha$^{-1}$, Wirkstoff Captan) und Score 250 EC (0,2 l·ha$^{-1}$, Wirkstoff Difenoconazolen) gesprüht.

Die Beuten waren mit Pollenfallen versehen und die Pollenhöschen wurden täglich gesammelt. Am Ende des Experiments wurde Honig geschleudert und Bienenbrot aus der oberen Zarge entnommen. Von allen Bienenprodukten wurden Pollenanalysen durchgeführt und die Rückstände der angewendeten Fungizide mit Gaschromatographie bestimmt.

Die Kontamination von Honig war insgesamt gering – von 0,0006 mg·kg$^{-1}$ Difenoconazolen bis zu 0,009 mg·kg$^{-1}$ Captan (Tab. II). Reiner Apfelpollen war viel stärker kontaminiert – von 0,043 mg·kg$^{-1}$ Difenoconazolen bis zu 2,99 mg·kg$^{-1}$ Captan (Tab. III). Aber das Bienenbrot war am stärksten belastet. Bei einer Berechnung unter der Annahme, dass es sich nur um Apfelpollen gehandelt hätte, wäre es 34,2 mal höher mit Difenoconazolen- (1,227 mg·kg$^{-1}$) und 10,9 mal höher mit Captan (29,04 mg.kg$^{-1}$) belastet als Apfelpollen von Höschen aus den Pollenfallen. Bei der Ernte konnten bei den Äpfeln aus den gesprühten Plantagen keine Rückstände der Fungizide nachgewiesen werden (Tab. IV).

Die wahrscheinlichste Erklärung für dieses Phänomen ist die Annahme, dass Difenoconazolen stark an die Zellwandstrukturen im Pollenkorn gebunden wird oder sich chemisch mit pflanzlichen Zwischenprodukten des Stoffwechsels verbindet und daher nicht mit der hier angewendeten Methode extrahiert und nachgewiesen werden kann. Nur 3 % der in den Pollenkörnern vorhandenen Fungizide konnte extrahiert und nachgewiesen werden. Während der Fermentation des Bienenbrots bricht die Bindung auseinander und die Fungizide liegen wieder in freier Form vor. Captan als Kontaktfungizid dringt wahrscheinlich nicht so tief in das Pflanzengewebe ein, sodass seine Bindung nicht so fest ist oder gar nicht erfolgt.

Um die Unterschiede in der Kontamination von Bienenprodukten einzelner Fungizide zu bestimmen, müssten die gleichen molaren Mengen gesprüht werden. Eine andere Möglichkeit liegt in einer Neuberechnung

unter der Annahme, dass gleiche molare Mengen der aktiven Substanzen eingesetzt worden wären (recalculated residue). Wenn das entsprechend neu berechnete Verhältnis der Rückstände von systemischen zu Kontaktfungiziden (penetration coefficient η) größer ist als 1, dringt das systemische Fungizid stärker in ein bestimmtes Bienenprodukt ein als das Kontaktfungizid. Unsere Ergebnisse zeigen, dass Difenoconazol 1,66 mal stärker den Honig und 1,16 mal stärker das Bienenbrot kontaminiert als Captan (Tab. II). Allerdings betrug bei frischem Pollen der Koeffizient $\eta = 0{,}35$. In diesem Fall war die mit dieser Methode nachweisbaren Menge des Fungizids kleiner als im Bienenbrot.

Für die Praxis würden die Ergebnisse folgendes bedeuten: Das übliche Verfahren der Bestimmung von Pestiziden in Pollenhöschen müsste speziell für diesen Zweck verändert werden, denn es können große Fehler auftreten, wenn man Routinemethoden der Extraktion benutzt.

**Honig / Pollen / Kontamination / systemische Fungizide / Kontaktfungizide**

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