# MEASUREMENT OF VINCLOZOLIN RESIDUES IN CHERRY POLLEN GRAINS COLLECTED BY BEES FROM PLANTATION SPRAYED AT BLOOM WITH RONILAN 500 SC

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(Received May 9, 2003/Accepted September 10, 2003)

A B S T R A C T

The method for determining vinclozolin residues in bee products, especially for pollen and bee-bread was elaborated. Vinclozolin is adsorbed on the surface of pollen grains, as well as it penetrates their inside, so only after grinding the pollen in methanol (disintegration of walls and membranes) it is possible to measure total amount of this fungicide residues.

**Key words:** vinclozolin, residues, pollen

## INTRODUCTION

Plant protection against fungal diseases requires the spraying of pomological plant plantations with fungicides also during blooming period (Pomological Plant Protection Programme, 1998). Currently used fungicides are not harmful for bees (preventive time – required for the safe access of bees to the sprayed plantation – is about 2 hours for vinclozolin) (Pohorecka, 2002).

Although bees do not suffer, because of spraying flowers, some contamination of nectar, pollen and bee-bread takes place (Pohorecka, 2002). Kubik et al. (1998, 1999, 2000a) found that systemic fungicides contaminated honey and pollen more than the contact agents. Systemic chemicals exhibit abilities for transportation with assimilates to other parts of plants. The contact fungicides do not have this ability (Różański, 1996a).

Vinclozolin is an active substance of Ronilan 500 SC. It is regarded as preventive, used against: *Alternaria* spp., *Botrytis allii, Botrytis cinerea* (Różański, 1996b).

Kubik et al. (1999) compared the decay of methyl thiophanate and vinclozolin in honey collected by bees from cherry orchards protected during blooming period. There was an untypical course of changes of vinclozolin concentration, which during the first few months after harvest, at room temperature, greatly increased. At the same time, contamination with methyl thiophanate decreased in the normal way.

The concentration of vinclozolin was also high in bee-bread. This phenomenon can be caused by the reversible conversion of this chemical into the forms undetectable by the conventional analytical methods of vinclozolin determination (Kubik et al., 2000b).

Vinclozolin, because of its chemical structure, after spraying, may be adsorbed on the pollen grain surface, and a part of it penetrates inside the cells, where it remains in an unchanged or bound form. The bound residues are the part of the pesticide in plant proteins, ingredients of the cell wall or some cytoplasmatic compounds, and this chemically fixed form cannot be extracted and identified (Dec, 1983).

Pollen, because of its great content of nutritional and biologically active substances, is a valuable nutriment, recommended for children and elderly people, and during convalescence – it makes faster bone healing, reduces inflammations and reveals antisclerosis characteristics; it is used as an antidotum and improves immunity (Skowronek, 2001).

On the market it is possible to get dry pollen pellets, and many another preparations, where pollen is a component – for example: honey rich in pollen, a granulate with composition similar to that of bee-bread, tablets of pollen and bee-milk – "Apivit - P" (Prabucki et al., 1998). Pollen used for nutritional and medicinal purposes must be contamination free.

The aim of this work was to elaborate a reliable and repeatable method of vinclozolin determination in pollen.

## MATERIAL AND METHODS

### 1. Sampling and sample preparation

Material for determination of vinclozolin contamination of cherry pollen was taken from a 4.5 ha cherry orchard ('Łutówka') at the Dąbrowice station. On April 27, 1999, five specially prepared bee colonies were brought. They were transferred onto empty combs and foundations, with the queen isolated on one comb, to confine brood rearing. All hives were dowered with pollen traps; pollen was collected each day in the evening, to combine all day crop in one sample. When 10% of flowers were in blossom, the orchard was sprayed with Ronilan 500 SC (1.5 l/ha), according to the Pomological Plant Protection Pro-

gramme for 1998. After one week the spraying was repeated.

Each daily collected sample of pollen grains was manually sorted to separate those of cherries. For further determination only cherry pollen grains were used.

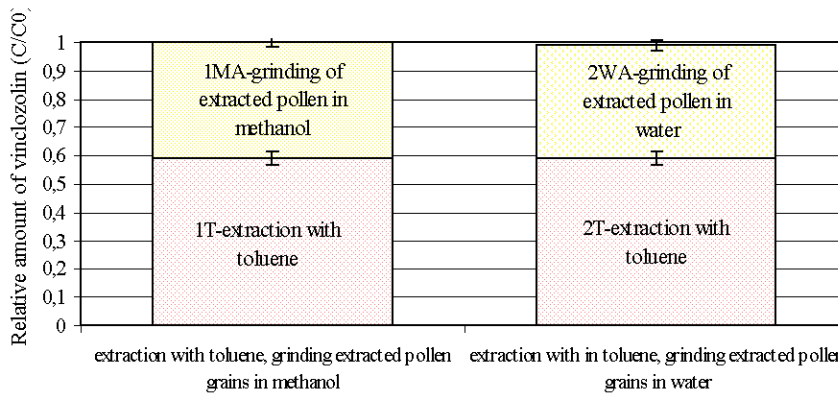
**2. Extraction of vinclozolin from whole and ground pollen grains**

Two 0.5 g samples of pollen were extracted twice with 20 ml of toluene. Those extracts were combined and filtrated and the solvent evaporated under reduced pressure; the residue was dissolved in 5 ml toluene (**1T** and **2T**, Fig. 1). Solid deposit was air dried and that of the first sample was ground in a glass Poter homogenizer with 2 ml of methanol, then extracted twice with 20 ml of acetone. Then the mixture was filtered. The combined solutions were evaporated to dryness under reduced pressure. Dry residue was dissolved in 5 ml toluene (**1MA**, Fig. 1).

Dry deposit of the second sample was ground in a glass Poter homogenizer with 2 ml of water, then extracted twice with 20 ml of acetone. Then the mixture was filtered, 10 ml of water added, and acetone was evaporated under low pressure until its smell vanished. Sample was purified using columns (SPE 500, Baker). The columns were prepared by washing with: 5 ml of hexane, 5 ml of methanol and 10 ml of water. After 15 ml of water was added to the sample, it was loaded on the column. The column was dried and eluted with 5 ml of toluene (**2WA**, Fig. 1).

**3. Extraction of vinclozolin from cherry pollen with different solvents**

A 0.5 g sample of cherry pollen was extracted with 20 ml of toluene for 30 min. Toluene solution was decanted and extraction repeated. Filtred solutions were combined, solvent evaporated under reduced




C - amount of extracted vinclozolin fractions in the sample by the given methods
C0 - total amount of vinclozolin in the sample

**Figure 1.** Comparison of two solvents used for grinding pollen grains during vinclozolin determination

pressure and the residue was dissolved in 5 ml toluene (T, Fig. 2). Two other 0.5 g samples were prepared in the same way, using for extraction methanol (M, Fig. 2) and acetone (A, Fig. 2) instead of toluene.

To a 0.5 g cherry pollen sample 4 ml of methanol was added; after 20 min the sample was extracted twice with 20 ml of toluene, the extracts were combined, filtrated and after the solvent evaporated under reduced pressure, dissolved in 5 ml of toluene (TM, Fig. 2).

Another 0.5 g sample of pollen grains was prepared in the same way, using acetone for extraction (AM, Fig. 2).

**4. Extraction of vinclozolin from ground cherry pollen grains with different solvents**

Sample (0.5 g) of cherry pollen was ground in methanol, then 20 ml of methanol was added, and shaken for 30 min. Methanol solution was decanted, and extraction was repeated. Filtered solutions were combined, the solvent evaporated under reduced pressure, and the remaining residue was dissolved in 5 ml of toluene (MU, Fig. 2).

Another sample of cherry pollen (0.5 g) was ground in 4 ml of methanol in a Poter homogenizer, and extraction was performed during 30 min with acetone (2 x 20 ml), the solution was filtered and evaporated. The residue was dissolved in 5 ml of toluene (AMU, Fig. 2).

Another 0.5 g sample of pollen was ground in 4 ml of methanol in a Poter homogenizer, and further solution was extracted twice with 20 ml of toluene. Then combined solutions were filtered, evaporated to dryness under reduced pressure. Dry residue

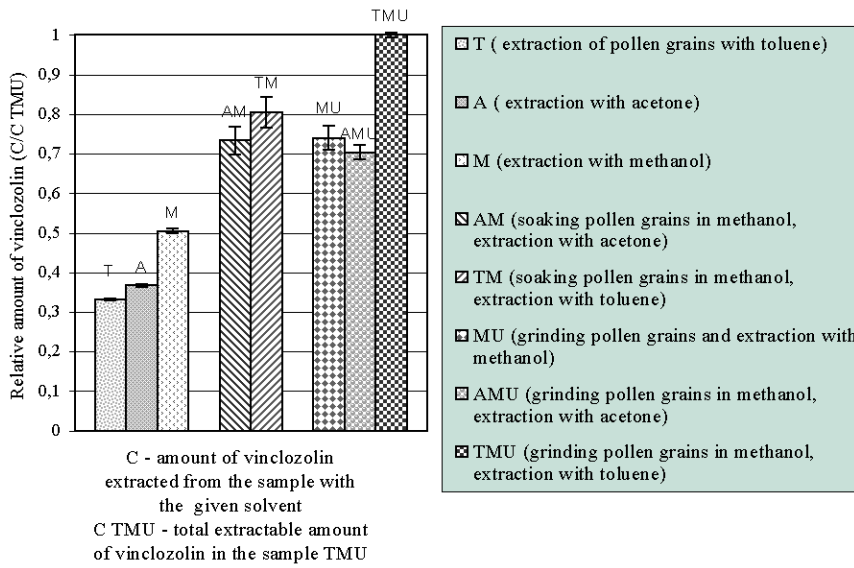



**Figure 2.** Extraction of vinclozolin from pollen grains with different solvents

was dissolved in 5 ml of toluene (TMU, Fig. 2).

**5. Chromatographic analysis**

Vinclozolin was determined by the gas chromatograph HP 5890 (Hewlett Packard, Series II) equipped with a HP-5 capillary column (30 m x 0.32 mm, film thickness 0.25 μm) and ECD detector. Tempetature of injector port 240°C, temp. of detector 300°C, oven programme: initial temperature 90°C, rate 15°C/min up to 180°C, then after 7 min an increase of temperature up to 260°C at the rate of 40°C/min; hold time 1 min.

Carrier gas helium (3 ml/min) and, as a "make up gas" – nitrogen (60 ml/min) were used. 1 μl of toluene sample was injected automatically using the autosampler Agilent (6890 Series Injector).

Identification of vinclozolin was conducted by comparison of the retention time with vinclozolin standard. All samples were prepared and determined in three replicates. Standard deviation was calculated and marked in the figures.

## RESULTS AND DISCUSSION

Figure 1 presents the influence of grinding the pollen sample twice extracted with toluene on vinclozolin recovery. Double extraction with toluene released just more than half of the whole amount of this compound. Pollen grains of many plants have very hard walls, awkward to penetrate (Penny et al., 1985). It is possible, that arrested inside the pollen grains, free particles of vinclozolin are hard to extract with organic solvents. Only after grinding pollen grains in a Poter homogenizer and destroying their walls as well as biological membranes, it is possible to extract the rest of vinclozolin.

The grinding of pollen grains should to be performed in hydrophilic solvents (water, methanol). Water is the chippest, but because of poor mixing with most of organic solvents, the samples need to be completely dry after grinding, before extraction with toluene, or SPE 500 columns should be used, but it slows down this stage of work. The use of methanol as a solvent seems to be the most fortunate, because it shortens the time and lowers the costs.

Other methods of destroying the walls of pollen grains (freezing at – 42°C, grinding them in liquid nitrogen, treatment of pollen grains suspension with microwaves) were also tested and found ineffective as under a microscope the whole pollen grains were still seen.

Essential for the efficient extraction of vinclozolin from pollen is the solvent used (Fig. 2). Toluene alone (T) is able to extract from the sample 0.33 of the total vinclozolin present in pollen grains, methanol alone (M) – 0.50 (it makes together 0.83), but toluene mixed with methanol extracted only 0.80 of this compound. Different results were obtained for other pairs of solvents. Methanol mixed with acetone released from pollen 0.73 of the total amount of vinclozolin whereas methanol alone (M) – 0.50

and acetone alone (A) – 0.37, what makes together – 0.87. The most effective in the extraction of vinclozolin was the mixture of methanol and toluene after the previous grinding of pollen grains in a Poter homogenizer (TMU).

## CONCLUSIONS

1. Obtained result showed that vinclozolin in pollen grains appears in two distinctly separated pools in similar amounts. One of them is relatively easy to wash out or extract with aromatic solvents as toluene and another one needs to be extracted with a polar solvent (acetone, methanol)
2. For the maximum release of vinclozolin from pollen, it is indispensable, to grind the pollen to be analysed in order to destroy the cell wall structure.
3. The best results – the maximum amount of vinclozolin extracted, were obtained when pollen grains were ground in methanol in a Poter homogenizer and extracted with toluene.

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# OZNACZANIE POZOSTAŁOŚCI WINKLOZOLINY W PYŁKU WIŚNI ZEBRANYM PRZEZ PSZCZOŁY Z PLANTACJI OPRYSKIWANEJ PREPARATEM RONILAN 500 SC PODCZAS KWITNIENIA

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## STRESZCZENIE

Metody stosowane do oznaczania pozostałości winklozoliny w owocach nie dają się bezpośrednio przenieść na produkty pszczele. Winklozolina ze względu na swą budowę chemiczną jest głównie adsorbowana na powierzchni ziaren pyłku, ale i częściowo wnika do ich wnętrza.

Opracowano metodę oznaczania pozostałości winklozoliny w pyłku. Największe ilości tego fungicydu uzyskano ucierając obnóża w metanolu w szklanym homogenizatorze Potera i wykonując ekstrakcję toluenem.

**Słowa kluczowe:** winklozolina, pozostałości, pyłek