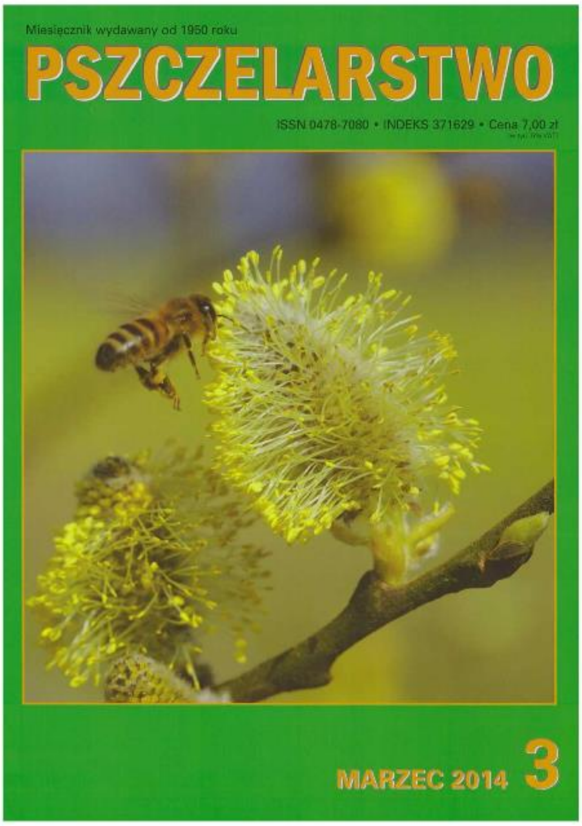

BEEKEEPING, 2014/3

NAUKA PRAKTYCE

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ARTYKUŁ AUTORYZOWANY

Badania aktywności nowego preparatu zabezpieczającego pszczoły przed skutkami skażenia środowiska

Artykuł ten dedykujemy redaktor naczelnej miesięcznika „Pszczelarstwo" – pani Krystynie Wyzner oraz współzałożycielce Stowarzyszenie Pszczelarzy Polskich „Polanka" – pani Elżbiecie Kowalczyk

APPROVED ARTICLE

Protecting bees from neonicotinoides

Contemporary agriculture is struggling with many problems, yet still the largest one of them is insects which damage and malform crops, transmitting pathogens such as viruses, fungi, or bacteria, which results in decreasing harvest yield. Currently, it is estimated that modern agriculture suffers a loss of 10–15% of the total yield due to the pests. And these are truly considerable losses. For this very reason, there will always be a social need for environmentally friendly plant protection products—for products that will always be in balance with the real needs of agriculture itself.

Until the mid-twentieth century, pest eradication in agriculture was largely based on inorganic and natural insecticides which, over time, proved to be insufficient.

They were replaced by chlorinated hydrocarbons, organophosphorus compounds, methyl carbamates, and pyrethroids. However, the effectiveness of these insecticides decreased over time due to the emergence of immunity thereto. This situation triggered the need to create a new type of insecticide. The search for new chemical structures of new plant protection products led to discovering strong new generation insecticides known as neonicotinoids. Neonicotinoids, out of all the plant protection measures, are the most commonly used ones in agriculture, horticulture, and gardening.

The main target of the neonicotinoids is the nervous system and the midgut of insects. Neonicotinoids compounds are structurally and functionally similar to nicotine. Due to their chemical structure, they are also called chloronicotinyls. Their use in agriculture is necessary but, recently, there have appeared many publications concerning their possible adverse impact on *Apis mellifera.*

Numerous laboratory and field tests report on the adverse effect of neonicotinoids on the behaviour and the health of bees. Studies also demonstrate that neonicotinoids impair smell, memory, and learning ability. In addition, they interfere with physiological functions such as breathing or the development of thoracic glands. The fact that insects survive acceptable doses of neonicotinoids does not always indicate their good condition, which was also noticed in the research on bumblebees. Neonicotinoids effectively imitate the effects of nicotine in the central nervous system. In small concentrations, they cause activation of nicotinic type cholinergic receptors and depolarization of neuronal membranes, i.e. the state of arousal, while in higher concentrations they block synaptic conductance. This condition persists because there are no esterases to break down nicotine analogues in the synaptic space. This leads to impaired transmission of nerve impulses, causing the hyperactivity of postsynaptic neurons. Nicotine and its analogues cause damage and atrophy of neurons (Excitotoxicity), which, in turn, leads to the death of insects. In addition to neurological symptoms, neonicotinoids also reduce the immunity of bees to diseases.

It should be noted that the decrease in immunity may be caused by a neonicotinoid alone, but may also be associated with the stress resulting from exposure to neonicotinoid. It is one side of the coin. On the other hand, there are numerous publications informing us about the harmlessness of neonicotinoids on pollinating insects. So how do we reconciliate these contradictory reports? It seems that everything that is new (and neonicotinoids are only a dozen or so years old) is not yet fully understood.

So far, no reports of an effective method or means have been given in the literature that would allow for eliminating the effects of neonicotinoid compounds used in insecticide preparations or protecting bees therefrom. Due to large losses in the bee population, pressure from environmentalists, and taking into account the position of EFSA (the European Food Safety Authority) on this issue, the European Union adopted a two-year memorandum restricting some neonicotinoids. However, there is no certainty whether they will be definitively withdrawn. One should also take into account the fact of registering other preparations. Therefore, one should look for alternative solutions that may result in rational changes in relation to the views on the use of systemic pesticides, the presence of which in modern agriculture seems inevitable.

Great hopes are connected with the research on the new preparation VITAEAPIS developed by BioActive-Tech Sp. z.o.o. from Lublin. This preparation has so far been tested on over 2000 bee colonies throughout Poland. The research conducted on the preparation confirms that a solution has been found that will enable bees to survive, despite the real threat resulting from the need to protect agricultural products from pests using the latest generation preparations. In addition, it was proved that the preparation significantly affects the hygienic behaviour of the bee colony, stimulating the instinct of self-cleaning not only in relation to the hive environment, but also to "itself"—an individual bee.

Tests:

The test used a preparation developed by BioActive-Tech Sp. z.o.o. from Lublin, called VITAEAPIS (its symbol is a verbal and graphic trademark applied for at the Patent Office of the Republic of Poland).

The preparation VITAEAPIS, which is of fully natural origin, is attractive for bees in terms of smell and taste. Due to its taste, bees consume it in a sugar solution willingly and quickly. However, due to its smell, bees undertake effective attempts to rob the families which have been served the preparation in syrup, despite the protection and the presence of benefits in the area.

The neonicotinoids used for the study are ones whose active substances—imidacloprid and thiamethoxam—are present in preparations purchased for testing at retail outlets trading in plant protection products.

Initial research was carried out in the apiary of Mr J. Jasina in Lublin. The effect of the concentration of the active compounds in the sugar syrup on the survival of bees was examined on the sample of 100 bees placed in mating boxes. Carniolan honey bees *(Apis mellifera carnica)* of different strains absorbed a protective preparation dissolved in the standard sugar syrup in three concentrations: respectively, 5, 10 and 20 mg of active ingredient per 1 litre for three days. The concentration of the preparation was tested in three independent hives. After three days of consuming the protective preparation, the bees received imidacloprid at a concentration of 375 µl/l in a volume of 5 ml of syrup (similar doses are used to eradicate insects in vegetable farming). Three hives, 100 bees each, which were not fed with a protective preparation constituted the control group. The samples of bees receiving the preparation in sugar syrup with three concentrations of the preparation and the samples from the control group were tested in three consecutive series. The test results confirmed the effectiveness of the preparation.

However, the discussed research should be considered preliminary because, at the time of its performance, the conditions significantly differed from natural ones. This research technique is repeatedly used by many researchers as a typical model system.

100 specimens of bees in a mating box, which, after three days of absorbing the preparation, received imidacloprid at a concentration of 375 µl/l (microlitres per litre) in a volume of 5 ml of syrup. Three concentrations of VITAEAPIS were applied in three consecutive series.

However, in conditions deviating from the natural environment, a handful of bees, the absence of brood and mother (no typical odours of the bee colony) and other food and concentration of CO_2 may significantly interfere with the results of experiments carried out on model systems. Therefore, similar studies to those discussed were made at the beginning of July 2012 in three families containing about 40 000 bees. The control group consisted of the bees from the apiary which were fed only with syrup. Honeycombs with bees (see the set of photos) were sprayed with imidacloprid dissolved in water at a concentration of 125 µl/litre (microlitres per litre). It is half the concentration of imidacloprid used in the field cultivation of vegetables. The aim of the study was to assess low doses of imidacloprid, to which mainly the bees collecting nectar and pollen during agrotechnological treatments and immediately after them were exposed. It should be mentioned here that many farmers apply agrotechnological treatments during the bee flight, which is reprehensible.

The research was carried out on two groups. The bees in the control hives did not receive protection in the form of VITAEAPIS. The experimental groups were bee families which were not given a protective preparation, but imidacloprid (1 hive), two bee families which received VITAEAPIS preparation through the bee feeder, 10 mg/l (milligrams per litre) in syrup for one week and were then sprayed with imidacloprid at a concentration of 125 µl/l (set of photos).

The aim of the study was to verify the previously adopted hypothesis, according to which VITAEAPIS protects bees from the toxic effects of the studied neonicotinoids which enter the hive together with nectar and pollen.

The results, illustrated in the form of a set of photographs documenting the studies, show the behaviour of bees 2 hours, one day and 13 days after application, in the control group (without the protective preparation) and experimental group (with protective preparation), respectively. Based on this research, it can be concluded that in the case of bees which did not receive the VITAEAPIS cover, imidacloprid significantly contributed to their mortality. It can be noticed that two hours after spraying, almost all bees were smitten (Photo A in the set of photos). On the first day after spraying, the bees that survived abandoned caring for the brood and became a large group similar to the one from the winter period, called a "cluster" in beekeeping. In addition, the bees were very weakened and did not set up guards before the hive (Photo B in the set of photos). About half of the population died. Bees threw part of the brood outside. Even after two weeks from the treatment, the bees were still clustering, abandoning their care over the brood. The general condition indicated their considerable weakness, which resulted in the lack of guards and bees dwelling in the hive without flying out for food (carrying pollen by bees was not observed).

13 days after application of imidacloprid, the bees continued to gather in clusters (Photo C in the set of photos), they did not fly out of the hive and did not produce honey. They required feeding, because otherwise they died.

Bees which took VITAEAPIS behaved in a different way (Photos D, E, F in the set of photos). As already mentioned, the syrup containing the protective preparation was more attractive for them and during the first day the bees absorbed it in the quantity of 2.5 litres. As in the control group, the use of imidacloprid did not bring about such drastic effects. Some of the bees died (about 10%), but the remaining population set up guards the following day and did not leave the brood without attention. They carried pollen, which means they did not lose their memory. The general appearance of the bee colony demonstrated the high effectiveness of VITAEAPIS. Partial mortality of bees can be explained by the application of the preparation to the honeycombs, on which imidacloprid also impacted young bees, which, due to the hierarchy prevailing in the beehive (division of labour), may not have consumed a sufficient amount of the preparation.

Fig A. The interior of the control hive *2* for hours after spraying. As you can see, almost all bees have been affected

Fig D. The interior of the beehive in which the bees received VITAEAPIS protective preparation, two hours after the application of imidacloprid. A similar image was observed in the case of the second hive used, in which a protective preparation was applied. Dead specimens can be seen, but the picture is significantly different compared with the control hive

Fig B. Honeycombs from the control hive (without protective preparation) on the first day after spraying. It can be noticed that the bees have abandoned caring for the brood, clustering into a large group similar to one of the winter period. In addition, the bees were very weakened and did not set up guards in front of the hive

Fig E. Honeycombs from the beehive in which bees received VITAEAPIS protective preparation, one day after the application of imidacloprid. The bees did not abandon the brood and set up guards. The overall picture is similar to that of healthy bees

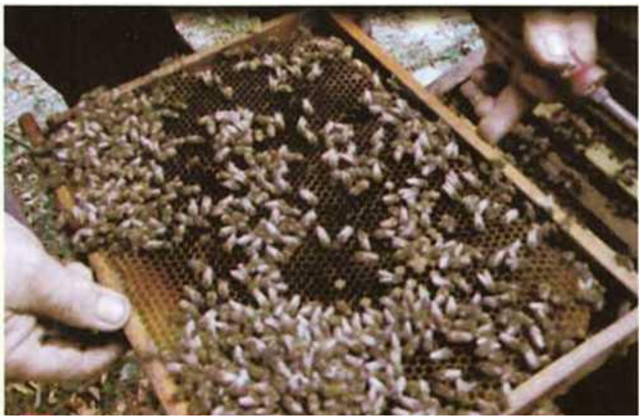

Fig C. The honeycomb from the control hive (without protective preparation) 13 days after spraying with imidacloprid. Highly weakened bees; they do not carry pollen or set up guards in front of the hive

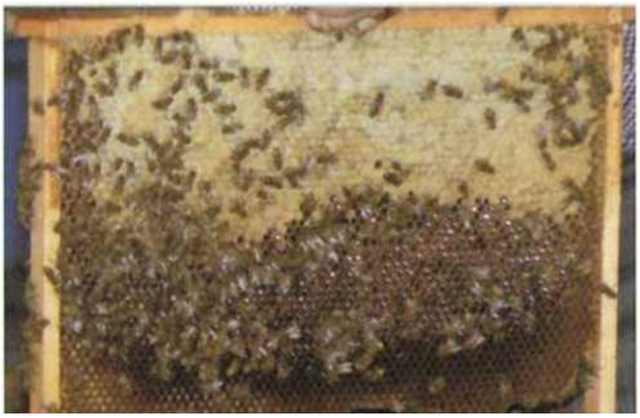

Fig F. The honeycomb from the hive in which the bees received the protective preparation VITAEAPIS, 13 days after spraying with imidacloprid. The bees did not abandon the brood and set up guards; they are carrying pollen and nectar (the visible capping consists of

Set of photos. The image of the frames with bees which had been sprayed with the above-mentioned concentration of imidacloprid. Photo A, B and C—the family without VITAEAPIS protection. Photo D, E and F—the family protected with VITAEAPIS

During the consecutive days following the poisoning, the bees flew out to the feeding grounds (goldenrod flowers) and brought food back pollen. After two weeks from the application of imidacloprid, the bees from both hives treated with the protective preparation showed no difference in behaviour in comparison with the hives containing healthy bees.

Assuming that the most reliable assessment of the research results is based on the data collected in field conditions, further research was carried out, this time on full bee colonies.

The research began on 31 May 2013. It lasted until the end of the season. The experiment was carried out in an apiary consisting of 25 bee colonies, settled in Dadant type hives (frame 435 x 300 mm). Styrofoam hives were equipped with removable screened bottom boards, which were additionally equipped with a diagnostic tray. The brood box of the hives consisted of ten frames. A group of 25 bee colonies was divided into six groups. Each group consisted of four hives.

- Group I—the control one, which consisted of bee families which had not undergone any treatment.
- Group II—bee families poisoned with thiamethoxam, but not protected with VITAEAPIS.
- Group III—bee families treated only with the protective preparation VITAEAPIS in the form of spray.
- Group IV—bee families treated only with the protective preparation VITAEAPIS in the form of spray and poisoned with food-borne thiamethoxam.
- Group V—bee families treated only with the protective preparation VITAEAPIS in the form of spray and poisoned with thiamethoxam by contact.
- Group VI—the remaining five bee families on which experiments were carried out using the minimum and maximum doses of VITAEAPIS in combination with poisoning with thiamethoxam by contact.

Observations and conclusions derived from the research:

1. In group I, no abnormalities in the behaviour of bees were observed. These were standard, healthy bee colonies.
2. In group II, in which the protective preparation had not been applied and the bees were poisoned with thiamethoxam at the concentration of the active ingredient of 0.1 g/l, all colonies died after several minutes with typical symptoms of poisoning.

3. In group III, in which thiamethoxam had not been applied, and where bees had been sprayed with the protective preparation VITAEAPIS at the concentration of the active substance of 40 mg/l, it was observed that the preparation improved the behaviour of the bee colony and significantly influenced the hygienic instinct of bees. After securing the families with the preparation, a much larger amount of impurities was observed in the diagnostic tray, compared with the control families.
4. In Group IV, in which the bees were poisoned with thiamethoxam at a concentration of 0.1 g/l (through the oral route), 35% mortality was observed 24 hours after applying the protective preparation VITAEAPIS with active substance concentration of 40 mg/l (dead bees lying on the hive bottom were weighed). The families poisoned through the oral route which survived, resumed flights to fetch pollen after 24 hours. They did not lose their memory. All the bees protected with VITAEAPIS and then subjected to poisoning survived the waiting period of the preparation. On the day that the waiting period expired, all of the poisoned but previously protected families carried pollen and showed increased traffic at the hive exit.

5. In group V, in which the bee colonies had been poisoned by contact, 24 hours after applying the protective preparation VITAEAPIS with the active substance concentration of 40 mg/l, 10% mortality was observed. The families poisoned by contact, which survived, resumed flights to fetch pollen after 24 hours. All the bees protected with the preparation and then poisoned survived the waiting period of the agent. On the day that the waiting period expired, all of the poisoned but previously secured colonies carried pollen and showed increased traffic at the hive exit.

6. In the case of using the protective preparation VITAEAPIS at a concentration of 0.08 mg/l (0.01 mg per family)—group VI—

and the application of poisoning with thiamethoxam in the amount of 0.05 g/l 24 hours after applying protection, it was observed that about 60% of the bee colony did not survive the treatment. After applying VITAEAPIS in the amount of 0.01 mg per family, no symptoms of increased vigour in the bees, as described in group III, were observed. The bees that survived did not resume flights for pollen either after 24 hours or after 48 hours. It was, however, observed that the bees were gathering in clusters. The behaviour of the bees which survived is similar to the behaviour exhibited after using imidacloprid (the poisoned control bees gathered in clusters and did not fly out of the hive). In order to strengthen the vigour of the surviving 40% of the bee colony population, VITAEAPIS at a concentration of 1.6 mg/l was sprayed. This family received a dose of 2 mg. After 48 hours, the bees resumed flights around the hive. After one week after being spraying with VITAEAPIS, the behaviour of the bee colony returned to normal condition, that is, the bees carried pollen. After two weeks from poisoning, the bees were no different from the control ones. This means that VITAEAPIS can also be used in cases of already existing poisoning.

- In the case of using VITAEAPIS with the active substance concentration of 1.6 mg/l (0.2 mg per family)—in group VI—about 40% of the bee family did not survive the treatment. However, after 48 hours, the surviving bees resumed flights to fetch pollen.

- When VITAEAPIS with the active substance concentration of 16 mg/l (2 mg per swarm) was applied—group VI—almost all the bees survived the treatment and there were few deaths. The bees that survived, resumed flights to fetch pollen after 24 hours. Seven days after nobbling, it was found that bees protected with VITAEAPIS preparation which survived did not show any symptoms of poisoning.

In the case of applying VITAEAPIS preparation with the active ingredient concentration of 8 g l (1g per family)—group VI—the bees poisoned with the pesticide, and protected with the preparation 24 hours earlier, 90% survived and only 10% mortality was recorded. The inspection of the beehive performed 24 hours after the application of VITAEAPIS preparation and before the pesticide poisoning was carried out, showed that a dose of one gram per beehive, which exceeds 500 times the beneficial dose, did not cause any toxic effects. There was not a single dead bee on the bottom board of the hive. After seven days, the bees did not show any symptoms of poisoning.

- In the case of applying VITAEAPIS preparation with the active substance concentration of 16 mg/l (2 mg per family) in groups IV-V, queen bees mortality was discovered in group V after 24 hours, and in group IV after 72 hours. After 24 hours, black, hairless, glistening bees, that showed no sign of intoxication, were observed in all cases of poisoning. These bees made flights to fetch pollen and nectar, and were let into the hive by the guards without any resistance. Such a state lasted until the physiological replacement of generations.
- Black, hairless bees (the colour change caused by thiamethoxam) protected with VITAEAPIS and the behaviour of guard bees (admission of black, hairless bees to the hive) is of particular importance in differential diagnosis. Similar looking individuals are also observed in other disease conditions, including viral diseases, nosemosis, mycosis and food poisoning. Black bees in such conditions, however, are not allowed into the hive.
- After the use of VITAEAPIS, in all examined concentrations of the active substance, intensified bee movement at the exit ensued and the defence of the beehive increased. In addition, there was intensified activity and noise in the beehive and an increased number of bee flights compared to flights from the test hives. In the first half of August in the beehives protected with VITAEAPIS, a significant increase in queen bee reproduction was observed in comparison with the test group. This means a significant increase in the strength of the bee family before winter, which considerably impacts its survival.
- Moreover, the research established the possibility of reintroducing bees protected with the preparation to areas contaminated with pesticides, where, until now, it has been impossible. The beehive, in which the bee family without the protection of the preparation died, was populated with a new, also unprotected family, which also died in a short time. The beehive contaminated with the pesticide and its metabolites is poisonous for bees even after a few weeks, whereas, after settling such a hive with a family protected with VITAEAPIS, the bees were still alive and showed no signs of poisoning.
- The molecular mechanism of the operation of VITAEAPIS has not been revealed so far.
- **The authors of the article invite the scientific community and beekeepers to pursue further research and discussions, the results of which may become an inspiration for further activities which will aid the honeybee in the future. Thanks to it, as it was predicted by Albert Einstein, we can have a vision of the future and dreams in our lifetime.**

More information: Prof. dr hab. Mariusz Gagoś – inventor of the idea and the formula of the preparation.
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