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Preliminary semi-field study on the effect of the neonicotinoid containing seed dressings to honeybees as pollinator in sunflower

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Sándor, A., Csikász, T. Farkas, S. Rácz, T. & Sárospataki, M.: *Preliminary semi-field study on the effect of the neonicotinoid containing seed dressings to honeybees as pollinator in sunflower.*
Abstract: The key of the success of the neonicotinoid-type insecticides is that they have the greatest affinity to the nicotinic acetylcholine receptors of the insect central nervous system. They block them irreversibly, eventually, they cause the destruction of pests insects. Ultimately, they affect the healthy functioning of ecosystems: their effect also can be extended to plant visitor pollinators – bees, butterflies, hoverflies, etc. –, causing poisoning-, health- and behaviour-changing symptoms.
In 2013, we investigated the effect of the residues of a neonicotinoid seed treatment of sunflower's among semi-field conditions More than 19 000 died individuals were collected during the measurements. On the basis of the results it can be stated that the literature known symptoms required about two weeks to appear on bees. The result have underlined the relevancy of our examinations to interrelation of beekeeping and modern crop systems.

Keywords: neonicotinoid, nAChR, bee destruction, insecticide

Introduction

Honey as nutrient is an integral part of a complete eating of modern man. It contains a lot of important and useful minerals, not to mention the fact that its use as sweetener is much healthier than refined sugars. However, in addition to the production of honey, bees are carried another, much larger in volume benefit by the pollination of crop plants. As prey they have an important part in the food repertoire of different species, for exam-

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ple some mammals, birds, reptiles and amphibians as well. Consequently that any insecticide, which drastically reduces the number of pollinators have a huge impact on the agricultural sector and ultimately affect the healthy functioning of ecosystems (The Wildlife Trusts Position Statement, 2012).

Farmers can act more effectively against the pests and pathogens of crops owing to the development of crop protection. The range of the chemicals used – pesticides –, fungicides, insecticides, herbicides are increasing day by day. The active components of pesticides is checked by the European Commission, but the application- and usage control carried out by the individual Member States (Responsibility for Pesticides, 2009). All the hazardous materials to the bees can only be used under strict control (MAINI et al. 2010, MARZARO et al. 2011).

Sown seed dressing is one of the starter, chemical steps in the arable plant protection, which is a defending device against soil pests and designed to protect seedlings. According to some views the so-called neonicotinoid containing seed dressings can be held responsible for the formation of the so-called Colony Collapse Disorder (CCD) (WENNER and BUSHING, 1996). CCD is a new, widely spread disorder in the Northern Hemisphere, in which bees suddenly disappear from one day to the next, leaving all the food, as well as the broods in the hive (SCHACKER 2008). This problem also makes mysterious that often they are found any dead individuals, although it is often a lot of pathogen are present in the colonies, they did not show the symptoms of diseases, pests, or parasitism. There are many theories to explain this phenomenon. A long list of biological, chemical and environmental stress factors have been associated with CCD, including Varroa mites (COX-FOSTER et al. 2007, BLANCHARD et al. 2008, DE MIRANDA et al. 2010), Nosema ceranae (HIGES et al. 2008) and the regular contact with neonicotinoid insecticides (GIROLAMI et al. 2009, MAINI et al. 2010). The enterprise-wide beekeeping with migratory practices, where are often moed the hives to new pollinating location and long distances can also as well as inadequate and monoculture nutrition be made responsible for the formation CCD (SPIVAK et al. 2011).

The sudden appearance of the phenomenon in the United States in 2006-2007 and later in other countries made it probable, that it is a worldwide problem. In some European countries, the growing concern about the link between neonicotinoids and CCD led to the partial or full ban on some neonicotinoid (CHANG et al. 2013, Commission Implementing Regulation 2013).

Seed dressings containing neonicotinoid active ingredients protect against a number of species of ground dwelling, piercing-sucking and chewing pests (wireworm, larva of each bollworms, corn rootworm, aphids and cicadas) (ALTMANN 2003, MARZARO et al. 2011, ELBERT et al. 1998, NAUEN et al. 2003). Their spectrum of acting is very wide. By stone fruits, apple types, citrus types, grapes, horticulture and industrial crops and ornamentals are those alike regularly use. By seed dressing neonicotinoids may reach the stem, the leaf tips and later the flowers – the nectar and the pollen as well (BLACQUIÈRE et al. 2012) – too. Those insects which eat this plant, are destroyed; however, bees, bumble bees, hoverflies, butterflies collecting contaminated pollen and nectar, may be poisoned too (MASON et. al. 2013).

The acting mechanism of neonicotinoids is based on their irreversible block in the nicotinic acetylcholine receptors of insects as agonist in the postsynaptic side (I50 values: 1 nM), stopped this impulses, causing death of the insect (NAUEN et al. 2003; TOMIZAWA and CASIDA 2005, MACCAGNANI et al. 2008, MUCCINELLI 2008, LAURINO et al. 2010).

A reduced dose can acting presumably by bees which does not cause mortality, but the bees will be more susceptible to certain diseases of bees (e.g. varroosis, nosemosis),

since the agents weaken their immune system. The plant-protection products are primarily exposed to the worker bees (SANFORD. 2003). Visual memorization of the landmarks is essential for spatial orientation of the pollinators. Bees use landmarks to find a source of food and to be able to provide information to the other members of the colony which direction and how far can it reach (VON FRISH 1967). After feeding in a pesticide-treated fields can inaccurately determine the location of the food source (6 ppb enough to mess them up (COLIN et al. 2004), possibly they integrate the landmark and getting lost. Pesticides may affect the accuracy of the hidden information of the hive returning bee's dance.

The direct intoxication symptoms are:

Uncoordinated - and uncontrolled movements (BRUNNER et al. 2001, SINGH et al. 2004.), trembling, shaking, tumbling, flexion of the abdomen and / or rotation of the abdomen and cleaning, while the legs in the back rubs (SUCHAIL et al. 2001), inability to maintain the correct posture and rotation in lying position (LAURINO et al. 2010). Sensory disturbance are developed secondary (KIRSCHNER et al. 1998, SALERNO et al. 2002).

Our study served the preliminary mapping of the above mentioned phenomenon. Its aim was the detection of the residues the most commonly used neonicotinoid seed treatment of sunflower's from beekeeping aspect, in semi-field conditions.

Material and methods

The measurements were carried out in 2013 in the Forage Crops Research Institute of the Kaposvár University on isolator net tent grown sunflower pollinated by honey bees (*Apis mellifera* L.) in Iregszemcse. For the experiment we used fungicide (Apron XL 350 FS) and insecticide (Cruiser 350 FS) treated seeds. Sunflower seeds were dressed with Niklas type dressing machine by the permitted quantity of seed treatment, then sown it. MTZ 82 and Accord Optima pneumatic planter was used by sowing. The row distance we used was 71 cm and the plant-to-plant distance 24.9 cm. Dressed seeds were planted to 6*45 meter isolator tents. The experiment was designed two sowing time (tent No. 1-22, tent No. 23-33). The net-isolated tents were set approx. 2 weeks before the flowering time. These are able to protect against the weather and the bees' migration as well. To ensure the fabric of the tents we used rigging and grounding by the edges of net. Bees came in traditional Zander-type hives approx. at the 25% flowering time (when 25% of the flowers are open) into the tents. Dead bees were counted individually five times (07.12; 07.19; 07.25; 08.02.; 08.09) with surgical tweezers into screw-top jar. Received data were summarized tent-wise. It was recorded the fertilization and the seed binding too, which showed how „liked" bees that seed treatment, compared with previous results of literary studies. Analytical samples have not taken place during the examination. For technical reasons, we were able to collect samples only the tents, where the hives were situated to the south side (odd numbered tents). The statistical analysis of the resulting data sets (Shapiro-Wilk normality test, Anova, Anova with Tukey Contrasts, Regression and Pearson's product-moment correlation) was carried out with Microsoft Excel and R. The results were analysed separately according sowing times (tent No. 1-21; tent No. 23-33). During the analysis we were looking for a difference between the sowing dates and the genotypes used (tent pairs) as well. We calculated with the averages of the data series. In the second sowing time (tent No. 23-33.) it was used bees treated against Nosema. The testing of the dead bees for the parasites was done by the Institute of Apiculture and Bee Biology in Gödöllő.

Results

The results obtained during the recording period and their aggregates are presented in Table 1. During the measurements we are collected altogether 19 657 individuals from the 17 isolated net tents.

Table 1: The number of bees collected from the tents at different dates and by different genotypes

	Tent No.	07.12	07.19	07.25	08.02	08.09	Σ
Genotype 1.	1	387	842	1564			2793
	3	313	351	1416			2080
Genotype 2.	5	227	591	1048			1866
	7	207	494	1071			1772
Genotype 3.	9	154	284	181			619
	11	340	444	554			1338
Genotype 4.	13	208	172	500			880
	15	113	134	678			925
Genotype 5.	17	269	321	1334			1924
	19	281	406	1121			1808
	21	306	175	799			1280
Genotype 6.	23				57	185	242
	25				128	291	419
Genotype 7.	27				101	301	402
	29				119	412	531
Genotype 8.	31				97	239	336
Genotype 9.	33				78	364	442
	Σ	2805	4214	10266	580	1792	19657

We can find significant difference between the dates tent-wise irrespective of genotype – $p_{1\text{-}21}$=8.11E-5***; $p_{23\text{-}33}$=9.72E-4***. Between the dates of the first 11 isolated net tents – 07. 12 and 07. 25; 07. 19. and 07. 25. – we found a significant difference ($p_{1,3}$ < 0.001 ***; $p_{2,3}$=0.00542 **).

Thereafter we examined the differences between genotypes. Table 2 shows that the genotypes have detectable effect. Our statistical test confirmed this (p=0.018). Female 1. (Tent No. 33) resulted 336 dead bees, while paired with restorers 3638 pieces of individuals (Restorer 1. – Tents No. 5-7) and 1805 pcs (Restorer 2. – Tents No. 13-15). Female 2. with Restorer 1. led to 1957 corpses (Tents No. 9-11), while this in itself was only 442 pcs (Tent No. 33). Finally, in the case when Female 3. paired with Restorer 2., we found 5012 pcs of bees (Tents No. 17-21), while in itself only 933 pieces (Tents No. 27-29.). Similarly, Restorer 1. with the Female 1. (Tents No. 5-7) resulted 3638 pieces, with the Female 2. (Tents No. 9-11) 1957, while Restorer 2. with the Female 1. (Tents No. 13-15) 1805 pieces and with Female 3. (Tents No. 17-21) 5012 pieces of dead bees. Based on these we can see that bee mortality depend on the maternal as well as the restorer line.

Table 2: The tent-wise acting of mother and restorer lines to the bee mortality

	Female 1.	Restorer 1.	Female 2.	Restorer 2.	Female 3.	Σ (/genotyp)
Tent No. 5.	1866	1866				3638
Tent No. 7.	1772	1772				
Tent No. 9.		619	619			1957
Tent No. 11.		1338	1338			
Tent No. 13.	880			880		1805
Tent No. 15.	925			925		
Tent No. 17.				1924	1924	5012
Tent No. 19.				1808	1808	
Tent No. 21.				1280	1280	
Tent No. 27.					402	933
Tent No. 29.					531	
Tent No. 31.	336					336
Tent No. 33.			442			442

Analysing the interaction between dead bees and sunflower lines we found moderately strong correlation among the number of ripe seeds (~ number of flowers) and the sum of dead bees (tents No. 1-21) (r=0.51**). Our model of regression at this sowing time gave the value R^2=0.6 (p=0.11), which is also confirms the previous results (Figure 1). However, no significant effect was observed in the case of tents No. 23-33. (r = -0.75; Fig. 2). The model of regression confirm this (R^2 = 0.141; p=0.464).

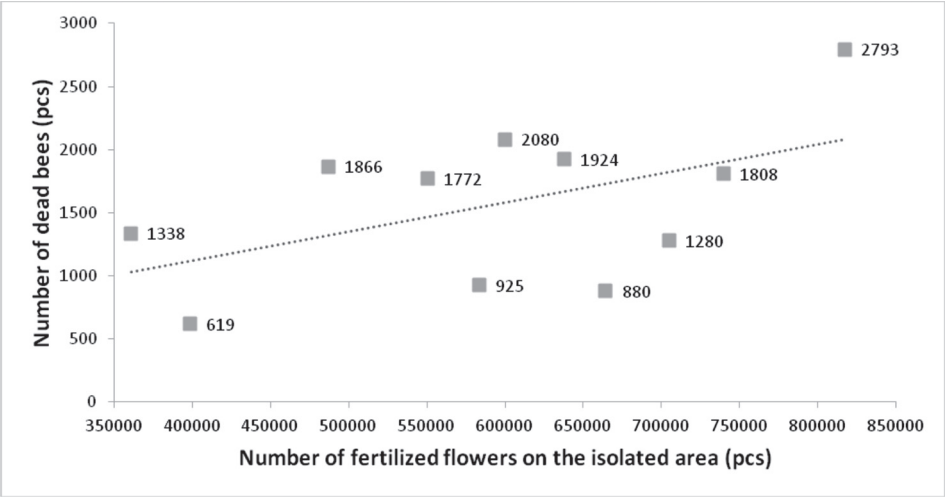

Fig. 1: The correlation between the number of fertilized flowers and the sum of dead bees in tents No. 1-21 (R^2=0.26)

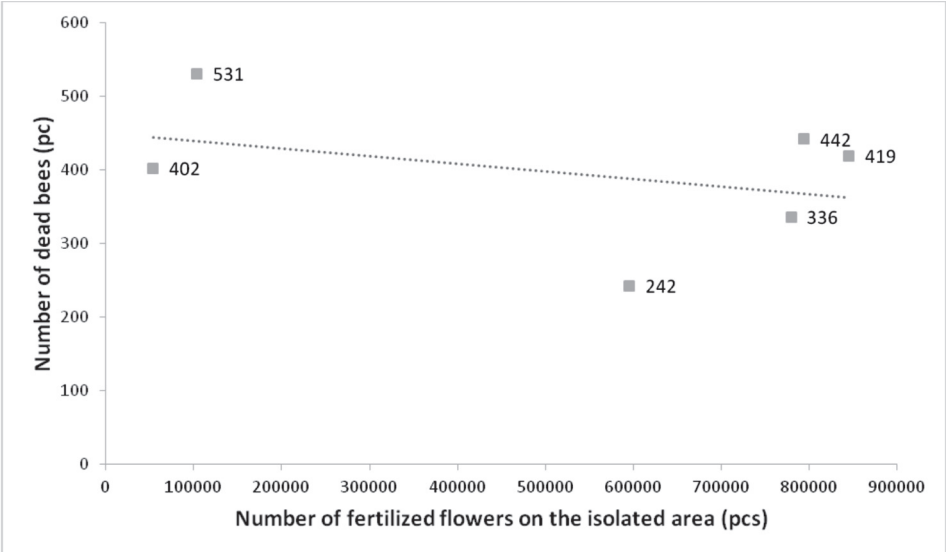

Fig. 2: The correlation between the number of fertilized flowers and the sum of dead bees in tents No. 23-33 (R^2=0.141)

Discussion

The extremely high number of individuals collected during the measurements (100 bees/day is a normal die-off rate (WILSON et al. 1980)) – 19.657 adults – also confirmed our hypothesis that in addition to the natural effects in the test-tents there is prevailed other negative impacts too. The detection of strong Nosema infection of the sample collected by the 1st showing time (tent No. 1-21) given a potential explanation for the observed phenomenon, which could be one inherent of the poisoning of neonicotinoid seed treatment (HIGES et al. 2008). The difference between the numbers of 07.12., 07.19. and 07.25 are attributable to the gradual drug-accumulation beside the reserve nutrient in the hive. The bees have taken up by gathering progressively the ingredients from the flowers, which have weaken their immune system made them more susceptible to the Nosema infection. On the basis of the results it can be stated that the symptoms, known from the literature, required about two weeks to appear in semi-field conditions (KIRSCHNER et al. 1998, BRUNNER et al. 2001, SUCHAIL et al. 2001, SALERNO et al. 2002, SINGH et al. 2004, LAURINO et al. 2010).

Based on Table 2 we can conclude that for the quantity of bee mortality in the case of sunflowers dressed neonicotinoid treated seed dressing among others it may be held responsible the genotype of the sown sunflower, both the maternal and the restorer line. Bees visited some types intensively (they "liked their taste" more) than others, although only parental couples were different. As shown well, the mortality of the more bee-preferred types were higher than those where for example the maternal line was different. Based upon results of statistical tests found that the more nectar and pollen were consumed the bees, the higher mortality appeared, at least in the case of the tents of the first sowing time (Fig. 1). In case of the tent No. 27–33. it is not seen this kind of context therefor because they have been treated against Nosema and contained only maternal line. The experienced rate of mortality is still exceeds the expected number (2372 indi-

viduals collected ↔ ~1400 pieces in theory), which can be attributed to the physiological effects of neonicotinoids.

This issue needs beside the ecological- and agricultural damage it caused and due to the increased national- and international attention further examinations.

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