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# The chronic effect of the botanical insecticide Neem EC on the pollen forage of the bumble bee *Bombus terrestris* L.

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**Abstract.** The botanical insecticide Neem EC is allowed for use as a pest control agent in organic farming. Although the preparation is considered safe for honey bees, its effect on bumble bees has been less studied. The aim of this study was to investigate the effect of sublethal chronic doses of the botanical insecticide Neem EC (1% azadirachtin) on the pollen forage of the bumble bee *Bombus terrestris* L. Four pairs of colonies (one pair consisting of a test and a control colony) were placed at 0, 400, 800 and 1200 m from leguminous fields. Prior to taking the colonies to the field the test colonies were fed with a sublethal dose of Neem EC (0.01 ppm azadirachtin in the food) for a three-week period, whereas the control colonies were fed untreated food. Pollen loads of homing bees were removed and analysed. Our results show that sublethal doses of Neem EC may affect the pollen forage of bumble bees**.**

**Key words**: bumble bee *B. terrestris* L., Neem EC, pollen forage, legumes

## INTRODUCTION

The majority of food resources for humans and animals are dependent on pollinators, making them a very important insect group (Williams, 1996). Also the seed yield of leguminous crops depends largely on pollination. The flower morphology of many leguminous plant species causes incapability for self-pollination or wind pollination (Free, 1993). All kinds of bees, including bumble bees, belong to the important pollinators of these crops (Williams, 1996).

During the last decades a decrease in the number of bumble bees and other wild bees has been noted in agricultural landscapes in many European countries (Goulson, 2003). Intensive use of pesticides has been partly held responsible for this. Once contaminated by a certain insecticide, the bee might be killed by a lethal dose, or carry a sublethal dose to the nest with contaminated nectar or pollen. Inside nests, contaminated food fed to larvae can cause various malformations in pupae and emerging adults (Thompson, 2003). Bumble bees are often more vulnerable than honey bees owing to the long period in spring when only queens are collecting food. Moreover, their colonies are smaller, which makes them more vulnerable to loss of members in the colony (Alford, 1975). Restrictions on application of insecticides often target the foraging time of honey bees, e.g. spraying is allowed early in the morning or late in the evening, which is exactly the time when bumble bees are most active (Thompson, 2003). The need for more environmentally friendly pest management is continuously increasing. The botanical insecticide Neem EC is recommended for use also in organic farms. Tests have shown that only extremely high dosages can affect the larvae of honey bees through feeding of contaminated nectar (Natural Research Council, 1992; Naumann & Isman, 1996). Neem preparations (active ingredient: azadirachtin) are reported to be safe for bees owing to their fast degradation time and the high concentration that bees can be exposed to. Studies about the effect of Neem EC on bees as non-target species have mostly focused on honey bees. Neem preparations are safe for adult honey bees at dosages likely to be encountered in the field and bees are not repelled by the insecticide (Naumann et al., 1994). However, very few studies have addressed bumble bees, although they constitute an important pollinator group; for many plants, especially legumes (e.g. lucerne, red clover), bumble bees are the sole effective pollinators as they are heavy enough to be able to open the flowers (Watanabe, 1994). The aim of the study was to test the effect of chronic doses of Neem EC on the pollen forage of bumble bee *Bombus terrestris* L. workers that had previously been fed with a sublethal dose of the insecticide.

## MATERIALS AND METHODS

The study was carried out during the flowering period of leguminous crops in 2003, 2005 and 2006 at the Jõgeva Plant Breeding Institute, Jõgeva County, Estonia. In all years bumble bees (*Bombus terrestris* L.) had access to three abundant leguminous flower resources: hybrid lucerne *Medicago x varia* Mart., red clover *Trifolium pratense* L. and white clover *Trifolium repens* L. The experimental area was situated on intensely cultivated arable land, with adjacent legume fields next in an area of 1.5 ha.

Commercially produced bumble bee colonies were bought from Koppert Biological Systems (Koppert B.V., Postbus 155, 2650 AD Berkel en Rodenrijs, Netherlands). Bumble bee hives NATUPOL contained colonies with the queen, workers, brood and larvae of bumble bee *B. terrestris*. In 2003 four pairs of colonies were placed at 0, 400, 800 and 1200 m from leguminous fields to establish the general pollen forage of bumble bees. The area was surrounded by cereal crops. In 2005 and 2006 the design of the field experiment was the same, however, prior to taking the colonies onto the field the test colonies were fed with a sublethal dose of Neem EC for a three-week period. The original food of all commercially produced colonies was replaced with water and a mixture of fresh pollen and sugar solution (30%). Nothing else was added to the food of the control colonies. In the case of the test colonies (one of each pair), a sublethal dose (0.01 ppm azadirachtin in the food) of Neem EC, obtained from India (M/S RYM Exports – The Indian Neem Tree Company), was added to the food. The preparation of Neem EC (1% azadirachtin) was diluted with distilled water.

In 2003 and 2005 the experiment was conducted in the intensive blooming period and in 2006 at the end of the blooming season of leguminous crops. Pollen loads from both hind legs of bees were removed from 30 homing bumble bees per colony during three consecutive days. The pollen loads were dried and later analyzed in the laboratory. In 2006 we measured the whole amount of pollen collected by the test and control colonies. The mean weight of the bumble bee pollen load per four control colonies was 34.35±1.09 mg and per four test colonies 34.74±1.1 mg. However, we didn't find any statistically significant differences between the given mean weights (*t*=0.25, *df* =478, *P*=0.79) and for that reason we didn't carry out any further analyses concerning the weight of the pollen loads.

Acetolysis (Kearns & Inouye, 1993), which removes protoplasm and other organic debris around the exine, was used to separate the pollen grains. A total of 200 pollen grains out of each sample were counted and identified by light microscopy. The *Chi-square* test (STATISTICA 7.0) was used to test for statistical differences in the proportions of leguminous pollen in the samples.

## RESULTS AND DISCUSSION

In 2003 bumble bees from colonies of same locations collected similar proportions of pollen from three mass-flowering crops (hybrid lucerne, red clover and white clover). There were no statistically significant differences between the colonies in all pairs (Table 1). Insect flight is one of the most energy consuming activity performed in the animal kingdom. The task of the foraging bee is to collect as much nectar and pollen as possible at the shortest time and energy costs. Therefore, bees rely on their memory to a high extent: they remember the colour (White et al., 2001) and smell of profitable flowers (Dobson, 1987) and learn how to handle these in the most efficient way (Møller, Eriksson, 1995, White et al., 2001).

Another reason why paired colonies use similar food resources is that bumble bees have relatively restricted flying distances compared, for instance, with honey bees (Walther-Hellwig & Frankl, 2000, Goulson, 2003). They do not have the same recruitment methods as honey bees but they have indirect ways of pronouncing about profitable food sources and the proportions of pollen from legume crops. They remember the pollen and nectar odours and colours that they were fed with when being larvae or indoor workers (Dobson, 1987). Thus all individual foragers have the same task: to find an optimal foraging strategy and this makes them behave similarly.

In 2005 there were statistically significant differences between the test and the control colonies (Fig. 1). When the hives were close to the food source, the test bumble bees foraged significantly more leguminous pollen (0m: $\chi^2 = 4.6$, $df = 2$, $P = 0.03$). When the colonies were further away, the test bees gathered significantly less pollen compared to the control colonies (400m: $\chi^2 = 435$, $df = 2$, $P < 0.001$; 800 m: $\chi^2 = 1093$, $df=2$, $P < 0.001$; 1200m: $\chi^2 = 477$, $df = 2$, $P < 0.001$). In the case of longer distances, the bees from the test colonies foraged less on abundant leguminous plants and visited more other (wild) plants.

The results of the experiment in 2006 show that out of all colony-pairs the ones treated with Neem EC gathered significantly more leguminous pollen even when the legume fields were as far as 1200 m from the hive (0 m: $\chi^2 = 282$, $df = 2$, $P = 0.0001$; 400 m: $\chi^2 = 119$, $df = 2$, $P = 0.0001$; 800 m: $\chi^2 = 52$, $df = 2$, $P = 0.0001$; 1200m: $\chi^2 = 1001$, $df = 2$, $P = 0.0001$) (Fig. 2). These results are contradictory to the results of the similar experiment conducted in 2005, where more leguminous pollen was foraged by bumble bees from the control colonies, when the legume fields were at least 400 m away.

**Table 1.** The proportions of leguminous pollen in samples ($n$ = 30) collected by bumble bee colonies at four distances from the leguminous fields in 2003.

| Distance (m) | Colony (No) | Leguminous pollen grains (%) | *Chi-square* test, $df$=2 | $P$ |
|---|---|---|---|---|
| 0 | 1 | 82.0 | 3.5 | 0.1 |
| | 2 | 87.1 | | |
| 400 | 1 | 72.5 | 0.3 | 0.6 |
| | 2 | 74.8 | | |
| 800 | 1 | 61.4 | 3.4 | 0.1 |
| | 2 | 61.9 | | |
| 1200 | 1 | 52.5 | 1.9 | 0.2 |
| | 2 | 54.4 | | |

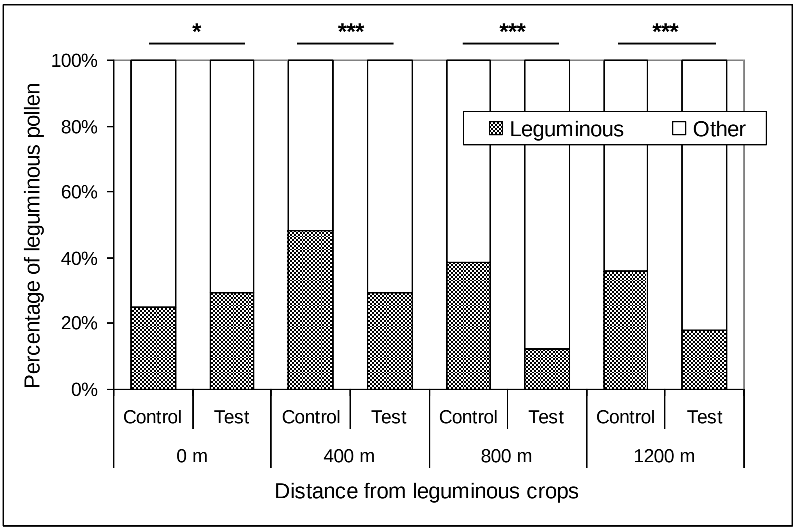


**Fig. 1.** In 2005 the proportions of leguminous pollen in the samples ($n$ = 30) collected by bumble bee colonies at four distances from leguminous fields. Control – colonies fed on sucrose solution; Test – colonies fed on sucrose solution containing Neem EC (0.01 ppm azadirachtin in the food); * - $P$ < 0.05; *** - $P$ < 0.001

In 2005 the experiment was carried out at the beginning of the blooming season when flowers are very attractive to bumble bees, whereas the study of 2006 was conducted at the end of the blooming season when there is less pollen in the flowers, making them less attractive to bees. Bumble bee foragers decrease their degree of flower constancy when the blooming period is ending and change their increasingly sparse main food plant species to other species that are more productive (Teräs, 1985). Our results do not enable to show exactly how big the influence of the different stage of flowering was, therefore further research on this topic is needed.

In 2005 and 2006 we found significant differences between the pollen loads of the test and the control colonies, however, the differences can even be greater. This is due to the behaviour of drifter workers: a small number of bumble bees from the adjacent colonies, motivated by the opportunity to lay eggs, can carry their pollen loads not to their own colony, but to the colony next to it. It means that some pollen foraged by the test bees could have ended up in the control colony hives and vice versa (Lopez-Vaamonde et al., 2004).

Neem preparations are said to be safe for honey bees (National Research Council, 1992), but their effect on bumble bees has been less studied. In the experiments of Melathopoulos and coworkers (2000) honey bee adults were not affected by different dosages of Neem preparations. Honey bee larvae were susceptible to the compounds and in the case of sublethal doses different malformations occurred when young bees emerged from the cocoons. The malformations appeared only in the case of high dosages. Naumann and coworkers (1994) have found the deterring effect of Neem preparation (0.1 ppm azadirachtin) for honey bees. In our experiments of 2005 and 2006 we used ten times lower sublethal doses (0.01 ppm azadirachtin), nevertheless, we found that the Neem EC had a statistically significant effect on the pollen forage of bumble bees**.** This is a pilot study and further research is needed in order to gain full knowledge of the effects of Neem EC on the pollen forage of bumblebees. Since between-year comparisons can be confounded with seasonal differences, future experiments should be run over the whole flowering season and also take different climatic conditions of different years into account.

Biopesticides appear safer for pollinators and their use could retard disappearance of natural pollinator species. Still, when sprayed on flowering crops contaminants can be carried into nests by bees and be fed to the larvae. Even very small changes in the pollen forage may affect the survival rate of colonies, especially in intensely managed agricultural areas where distances between food sources are larger.

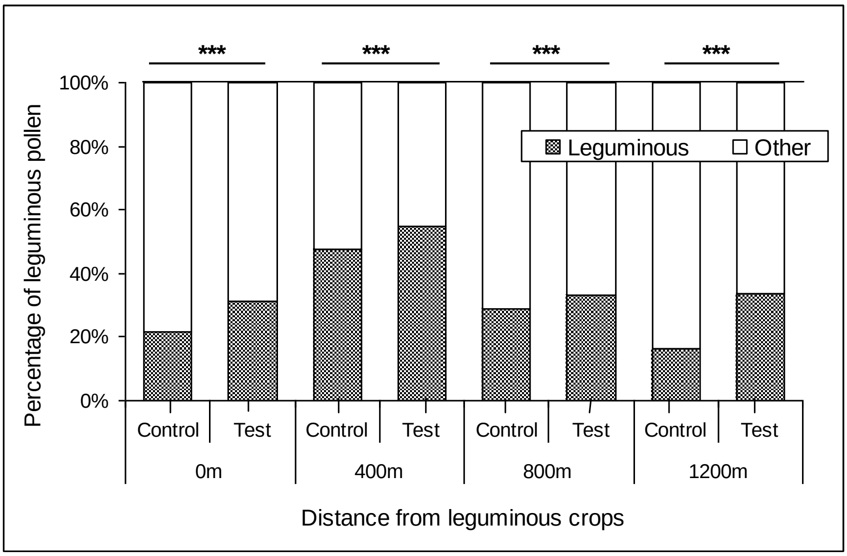


**Fig. 2.** In 2006 the proportions of leguminous pollen in the samples ($n$ = 30) collected by bumble bee colonies at four distances from leguminous fields. Control – colonies fed on sucrose solution; Test – colonies fed on sucrose solution containing Neem EC (0.01 ppm azadirachtin in the food); *** - $P$ < 0.001

## CONCLUSIONS

Although Neem EC is considered to be safe for bees (Melathopoulos et al., 2000), our results show that chronic treatment may affect the pollen forage of bumble bees. In the experiments of 2005 and 2006 chronic dosages of Neem EC were ten times lower than the recommended field rates. Despite this, feeding with a chronic sublethal dose of Neem EC caused some differences in the pollen forage of bumble bees, which in turn may affect the survival rate of colonies, especially in intensely managed agricultural areas where distances to the food sources are larger.

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