4.13 Synergistic effects between variety of insecticides and an EBI fungicide combinations on bumble bees (*Bombus terrestris* L.)

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Abstract

In recent year´s severe decline in honey bees as well as in bumble bee populations have been observed all over the world. Pesticides have been proposed as one of the main cause of pollinators decline. Several studies show that variety of pesticides co-exist in environment and also in bee products at the same time and might therefore synergise.

Fipronil, cypermethrin, thiamethoxam and imidacloprid are agriculturally well known and used insecticides as well as fungicide imazalil. EBI fungicides like imazalil are functioning as detoxification inhibitor tools in insects. Thereby, the fungicide and insecticide co-occurrence might lead to synergy in bees. The cocktail-effects between insecticides and fungicides are still little studied. Aim of this study was to assess the impact of previously mentioned pesticides and their mixtures impact on bumble bee longevity and feeding rate. The bumblebee (*Bombus terrestris* L.) were fed with syrup containing different single pesticides and their combinations. Bees mortality and feeding rate was daily monitored.

Here we show that 3 of these insecticides are synergising with fungicide and due that causing significant decrease in bumble bees longevity and feeding rate. The results from this experiment allows us to suppose that EBI fungicide imazalil inhibits the detoxification processes in bees and due that toxicity of insecticides increases.

Although fungicides are considered as quite safe to bees when used appropriately and alone but in combination with insecticides might lead to faster individual death. Several studies have demonstrated impacts of single pesticides on bees, but yet there is a lack of data of synergistic effects. Future research should focus on synergistic effects of environmentally relevant doses of EBI fungicides and insecticides on pollinators longevity and physiology.

Reference

Published full text article can be found from journal Pest Management Science.
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4.14 Developing methods for field experiments using commercially reared bumblebee colonies – initial colony strength and experimental duration as influential factors

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Summary

Semi-field and field experiments with commercially used bumblebees (e.g. *Bombus terrestris*) gain more and more importance for both ecological studies and trials on potential side effects of plant protection products. However, standardized, replicable experimental methods are lacking so far and need further development. For example, initial strength of bumblebee colonies may vary across experiments but may be a key factor in successful colony development under field conditions. Trial duration and termination may impact results on total reproductive output (e.g. number of newly produced queens). In this study commercially reared bumblebee colonies of different initial strengths (number of worker bees) were placed along the field margin of each of six field sites. Each site was nested within one of two seasons and planted with one of two arable crops (*Brassica napus* and *Phacelia tanacetifolia*). Each colony was spaced approx. 50 m apart from the next colony, and its development was monitored once a week. While the development of half of the colonies was terminated at the first sighting of newly emerging queens within the nesting area, the other half of the colonies was left to develop further until the end of their natural colony cycle. Newly emerging queens were kept within the colonies using queen excluders. Colonies of different initial strengths showed very similar developmental patterns with medium and large colonies peaking slightly earlier than small colonies. Results may help to develop optimal parameters for standardized field tests.

Introduction

Semi-field and field experiments with commercially used bumblebees gain more and more importance for both ecological studies and trials on potential side effects of plant protection products. However, standardized, replicable experimental methods are lacking so far and need further investigation. In this study we evaluated two different factors which can be standardized: (1) Initial colony strength at the beginning of experiments may be a key factor in successful colony development; (2) Trial duration and termination may impact results on total reproductive output. Here we present data on colony weight.

Materials and Methods

Commercially reared buff-tailed bumblebee colonies (*Bombus terrestris*) of different initial strengths were used in two arable crops: oilseed rape (*Brassica napus*; hereafter referred to as "OSR") and phacelia (*Phacelia tanacetifolia*; hereafter "Ph"). According to their initial strenght (number of worker bees) colonies were categorized as small, medium, and large ("S", "M", "L", respectively). The initial strength (average ±standard error) of small colonies was 58 ±1.7 bees, of medium colonies 79 ±2.2 bees, and of large colonies 110 ±3.9 bees in the OSR setup. Colonies in the phacelia setup contained 40 ±3.7 bees (S), 53 ±1.9 bees (M), and 72 ±2.1 bees (L) respectively. Four colonies of each strength class were placed along the field margin of each of three OSR fields in spring 2015, and four new colonies along the field margin of each of three phacelia fields in summer 2015 resulting in a total of six field sites and 72 colonies. Sites were located in and around Brunswick, Germany. Each colony was spaced approx. 50 m apart from the next colony, and its development (colony weight and number of workers) was monitored once a week. Colony exits were closed in the morning of sampling before start of bee flight. Nest boxes were transfered to a portable container which kept out light, contained a scale and held a camera. Nest boxes were weighed, and bees were allowed to settle down in the dark before photos of the nest with the bees sitting on it were taken. After crop withering colonies were moved to a common area. At the first sighting of newly emerging queens (switching point of the colonies), development of half of the colonies was terminated by freezing, while the other half of the colonies was left to develop further until the end of their natural colony cycle. Newly emerging queens were kept within the colonies using queen excluders. All measured parameters were compared between colonies of different initial strength using LMM and paired contrasts. Statistical analysis was performed in *R* (R Core Team, 2014).

Results

In both arable crops colonies switched from worker to queen production after four weeks, while the end of the natural colony cycle was detected after seven and nine weeks in phacelia and OSR, respectively.

<u>Population development in oilseed rape</u>

In OSR, colonies of different initial strengths showed very similar developmental patterns with medium and large colonies peaking slightly earlier than small colonies (Fig. 1). Colonies of different initial strength revealed significantly different weights depending on the week of observation (LMM on log-transformed data, Initial strength x Week, $F_{12,144}=2.74$, $p=0.002$). Small colonies weighted significantly less than large colonies in week two (Z-ratio=-3.5, p=0.002), three (Z-ratio=-3.6, p=0.001) and four (Z-ratio=-2.8, p=0.017). Weight of small colonies was signifiantly lower than weight of medium colonies in week two (Z-ratio=-2.4, p=0.04). Weight of large colonies did not significantly differ from weight of medium colonies in any of the sampling weeks.

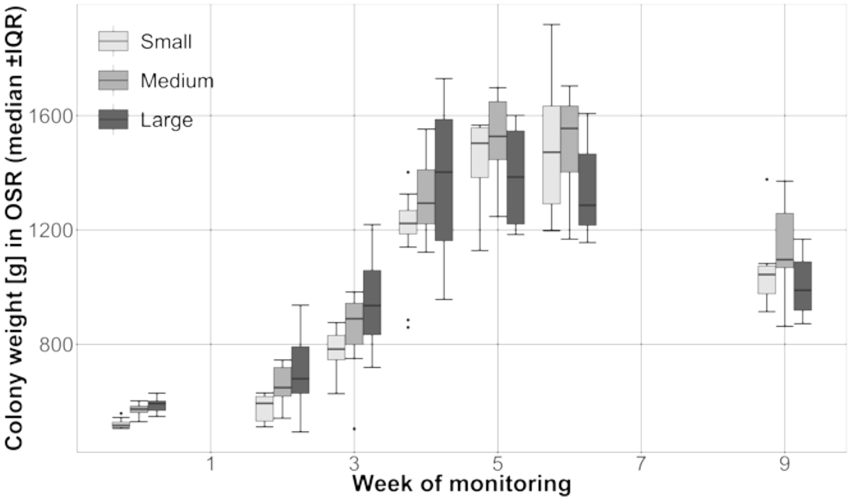

Fig. 1 Median weight, interquartile ranges (IQR) ±1.5*IQR whiskers of bumblebee colonies of different initial strenght (small, medium, large) in oilseed rape.

Population development in phacelia

In phacelia, we did not reveal a clear peak in colony development (Fig. 2). Colony weight decreased in a similar manner within each group of the same initial strength over the period of the trial (LMM, Initial strength x Week, $F_{8,114}=0.63$, p=0.75). Colony weight differed significantly between the three strength groups (LMM, Initial strength, $F_{2,33}=6.0$, p=0.006); while small colonies weighed always less than large colonies (Z-ratio=-3.34, p=0.002), weights of medium colonies did neither significantly differ from weights of small colonies (Z-ratio=1.97, p=0.12) nor large colonies (Z-ratio=-1.37, p=0.36). Except for one medium colony with three young queens, colonies did not produce queens.

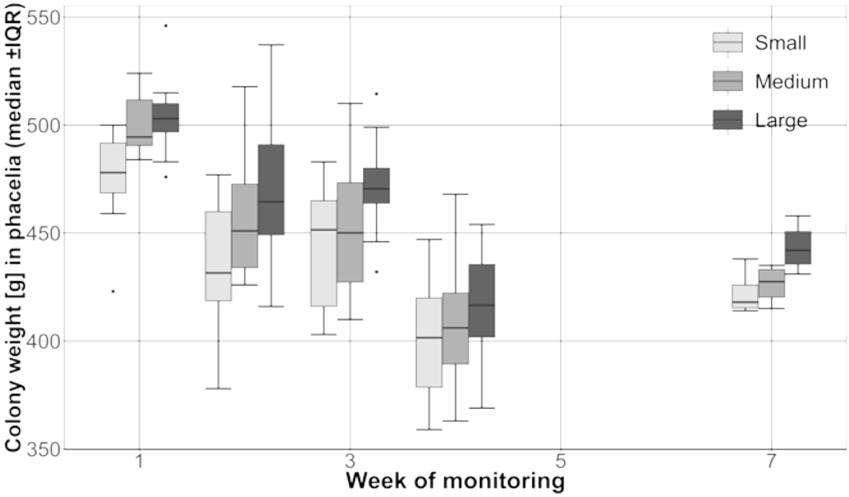

Fig. 2 Median weight, interquartile ranges (IQR) ±1.5*IQR whiskers of bumblebee colonies of different initial sizes (small, medium, large) in phacelia.

Summary and conclusion

In OSR, buff-tailed bumblebee colonies´ development showed very similar patterns for colonies of different initial strength. Colonies grew heavier in all strength classes with a peak in growth around week four to six. While small colonies needed longer to grow heavier they eventually reached similar weight like medium and large colonies. In phacelia, colony weight did not show the same pattern. Large colonies stayed heavier than small colonies although old founder queens did not die earlier in small or medium compared to large colonies. We could not reaveal a clear peak in colony development in summer colonies; on the contrary weight decreased in all strength classes from the beginning. This discrepancy between seasons may be a result of bee biology rather than foraging constrains. While oilseed rape and phacelia may not be directly comparable as a food resource they are known to be both nectar- and pollen-rich and an attractive foraging crop for bumblebees (Westphal et al. 2006, Stanley et al. 2013). Differences in colony development between spring and summer colonies over the course of each trial are therefore unlikely to have been caused by a shortage of food.

For field experiments with commercial reared bumblebees in spring, medium-strength colonies may be most favorable showing even variation in development across weeks and less variation between colonies than large colonies. They can also be handled easily. For summer experiments, large colonies may be a better suit; however, reproductive success may in general not be adequately testable later in the season. The duration of experiments in spring may have to last longer (nine weeks) than in summer (seven weeks) to cover the full development cycle of the buff-tailed bumblebee. However, spring experiments may be more suited to show experimental effects due to their natural developmental progession including a growth peak and switching point.

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