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Is pollinator visitation of *Helianthus annuus* (sunflower) influenced by cultivar or pesticide treatment?

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A R T I C L E I N F O

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A B S T R A C T

Beneficial insects, such as *Apis mellifera* (honey bees) and *Bombus* sp. (bumble bees), play important roles in the environment as pollinators, and they are directly affected by agronomical practices, such as pesticide spraying or the selection of crop cultivar. Sunflower is an important resource for these pollinators, and its yield is highly dependent on pollination. Six sunflower hybrid cultivars (ES Biba, Gonzalo, Drake, Vellox, NK Neoma, and P63LE10) were used to compare the visitation rates (VR) of pollinators during flowering in three years (2015, 2016, and 2017). From this set, cultivar P63LE10 was selected to evaluate how pesticide treatments (mixture of two insecticide products containing acetamiprid and pirimicarb, respectively, and two fungicide products containing propiconazole plus prochloraz and dimoxystrobin plus boscalid, respectively) affect pollinator visitation. The amount of pesticide residue in sunflower inflorescences was analyzed to determine the content over the duration of the experiments. The sunflower cultivar P63LE10 was significantly the most attractive to both groups of pollinators in 2017. This cultivar was visited by the highest number of honey bees also in the other years. For bumble bees, cultivar P63LE10, along with cultivars NK Neoma and Vellox, had the highest visitation rates in 2015 and 2016. In 2017, plants treated by the fungicide dimoxystrobin plus boscalid had the highest visitation rates for both groups of pollinators. For bumble bees plants treated with this fungicide were significantly more visited (VR 23.1 ± 2.1%) than these treated with insecticide with pirimicarb (16.4 ± 2%) and the untreated control (17.9 ± 1.7%), and for honey bees plants treated with dimoxystrobin plus boscalid were significantly more visited (VR 24 ± 4.5%) than all other treatments except with acetamiprid (20.5 ± 3.5%). The residues of tested pesticides were found in blooms for the whole time of the experiment (range of values for 2016 and 2017 [ng/g]: acetamiprid 144–6, 3360–56; pirimicarb 177–12, 223–10; prochloraz 380–6, 1970–912; propiconazole 324–17, 1024–1018; boscalid 216–154, 9600–1319; dimoxystrobin 452–137, 6560–911). The influence of cultivar was more important than pesticide treatment in the selection of a preferred food source by pollinators.

1. Introduction

Helianthus annuus L., sunflower, is one of most important oilseed crops worldwide (Cantamutto and Poverene, 2007; Skoric et al., 2007) both in developed and developing countries. Sunflower cultivation is important economically and as an alternative rotation crop, especially with cereals, and provides intercropping and the succession of crops in seed-producing regions (Porto et al., 2007). The forecast for sunflower production in Europe for the 2017/18 season is approximately 4.22 million hectares (ha), with a production forecast of 8.5 million tons (USDA, 2017).

Wind is generally accepted as the main pollinator of flowering plants; however, wind is not sufficient for the pollination of sunflower because it is not able to provide homogeneous pollination and is not able to carry heavy pollen grains (Parker, 1981a). Therefore, sunflower is highly dependent on animal pollinators (Klein et al., 2007) to supplement wind pollination. Sunflower is predominantly allogamous and is highly entomophilous (Putt, 1940). *Apis mellifera* L. - honey bee pollination is crucial for achene production (Parker, 1981a; Oz et al., 2009). Research by Shein et al. (1980) indicates that honey bee visitation to sunflower is affected by its genotype, whereas the pollen was not found to be an important attractant.

Although Maurizio and Schaper (1994) state that nectar and pollen production are generally at high levels, the nutritional value of sunflower pollen for honey bees is considered to be low (Odoux et al., 2004). Chamer et al. (2015) state that sunflower yield may be limited

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by the quality of pollination processes.

Field trials conducted in Kenya to identify the effects of a diversity of pollinators on pollination and the resulting influence on the seed yield of sunflower found that the honey bee was the most common pollinator and had the highest pollination efficiency index. On average, plots that insects could access had a 53% higher seed yield compared to plots where insects were excluded (Nderitu et al., 2008). Charrière et al. (2010) found that pollination was not interrupted when sunflower was cultivated in areas with alternative food sources, even during blooming or during the winter months, even though they confirmed a lower interest of honey bees in sunflower nectar.

Pisanty et al. (2014) conducted an experiment in Israel to compare the pollination of sunflower by honey bees to that by two species of wild bees (genera *Lasioglossum*). They found that honey bees strongly outperformed the wild bees. The sunflower was solely dependent upon pollination by honey bees because wild bees did not effectively pollinate sunflowers in their studied system.

Degrandi-Hoffman and Chambers (2006) used ten self-fertile sunflower cultivars to evaluate seed set in the presence and absence of exposure to honey bees (open and bagged). In the first experiment, the number of bees was lower compared to the second experiment, and the seed set did not significantly differ between variants with bagged and open inflorescences. In the second experiment, the cultivars had greater seed set when the inflorescences were exposed compared to when they were not, and the weight of the seeds from open inflorescences was greater than that from those that were bagged. Degrandi-Hoffman and Chambers (2006) add that environmental conditions influence seed set, as evidenced by differences in seed set among bagged inflorescences. They also add that, at higher temperatures, some open-pollinated cultivars produced four times more seeds compared to bagged cultivars.

In general, pollinator visitation is highly affected by treatment of the sunflower crop with pesticides. In several European countries, bee keepers have reported the weakening of honey bee colonies located near blooming sunflower fields that were identified as being seed treated with insecticides (imidacloprid) (Laurent and Rathahao, 2003). Colony collapses of honey bees and bumble bees due to the exposure of certain pesticides have been reported across all of Europe (Henry et al., 2012; Whitehorn et al., 2012). In Switzerland (and France), neonicotinoids are forbidden for use in sunflower, but a weakening of colonies was still reported in locations close to sunflower fields (Charrière et al., 2010).

Many lethal and sublethal effects of neonicotinoid insecticides on bees have been described in laboratory studies (Shi et al., 2017). No effects were observed in field study of Blacquière et al. (2012) using field-realistic dosages. Although Tosi et al., 2017 described that the neonicotinoid thiamethoxam in chronic exposure impaired honey bee flight ability.

Bonmatin et al. (2005) and Chauzat et al. (2006) found low ppb levels of imidacloprid in a high percentage of pollen samples collected from maize, sunflower and canola, and when pesticide residues from all matrices were pooled together, analyses did not show a significant relationship between the presence of pesticide residues and the abundance of brood and adults, and no statistical relationship was found between colony mortality and pesticide residues (Chauzat et al., 2009).

No residues of imidacloprid were detected in any of the components of beehives that were placed in the center of seed-treated sunflower fields, and no side effects were observed in the short- or long-term analysis of the colony growth parameters (Stadler et al., 2003).

Variables other than pesticide treatment also affect pollinator visitation of sunflower. The type of cultivar also seems to be important. Lužaić et al. (2008) conducted an experiment in Poland using six different sunflower hybrids (H1, H2, H3, H4, H5, H6). They observed that H2 experienced the lowest visitation by pollinators, while H4 showed the highest. The differences among the other hybrids had no statistical significance. During flowering, the main pollinator was honey bees (99.53%), with bumble bees (0.32%) and flies (0.15%) being the other pollinators.

In 2011, Cerrutti and Pontet (2016) began a 3-year study addressing the differential attractiveness of sunflower cultivars to honey bees. They used 13 current sunflower cultivars at two sites. Sunflower genetics was a major factor influencing honey bee attendance to plots, and the discrepancy between the most- and least-visited cultivars reached a factor of 3. While none of the tested cultivars were totally neglected, the highest numbers of visits by pollinators were observed for cultivars 12, 10 and 6, with cultivar 11 having the highest number of visits in site B 2013 and site B 2012. Results regarding the consistency of attendance between years and locations suggest that honey bees show a stable preference for cultivars, even if genotype × environment interactions are to be expected.

A trial conducted by Parker (1981b) designed to compare the attractiveness of cultivars consisted of six male-sterile cultivars (SW 504, 506, 509, 514, 517, 526) and a single male fertile cultivar (RW 637). He found that cultivars 504 and 509 were more highly preferred by bees compared to the others.

We hypothesized that the pesticides sprayed on sunflowers can attract or repel pollinators. A certain level of repellency to bees is desirable to prevent any sublethal effects of the pesticides (Colin et al., 2004). Clearly, proper pollination is necessary for the good production of achenes and thus a greater yield (Parker, 1981a; Oz et al., 2009; Degrandi-Hoffman and Chambers, 2006). Therefore, in our study, we wanted to examine which factor most influences pollinator visitation to sunflower: pesticide treatment or sunflower cultivar. We chose six sunflower hybrid cultivars to compare the visitation rate of pollinators during flowering. From this set, we selected one cultivar to evaluate how certain pesticide treatments affect pollinator visitation. We also analyzed the amount of pesticide residue that occurs in sunflower inflorescences after spraying at the beginning of our observations and at the end of flowering, which was also the end of the experiment.

2. Materials and methods

In 2015–2017, small-plot experiments were conducted to examine the influences of sunflower cultivar and pesticide treatment on visitation by two groups of pollinators: *Bombus terrestris* L. and *Bombus lapidaries* L. (bumble bees) and *Apis mellifera* L. (honey bees).

2.1. Description of location

The experiments were carried out in a demonstration field belonging to the Czech University of Life Science in Prague (50°12′99.7″N 14°37′37″E).

2.2. Experiment with sunflower cultivars

Pollinator visitation was observed on the sunflower hybrid cultivars ES Biba, Gonzalo, Drake, Vellox, NK Neoma, and P63LE10 (Table 1) across three consecutive years. The experiment was established using small plots in a randomized complete block design (RCBD) with 4 replicates. The size of the plot was 10 m^2, and the flowers were spaced at 75 × 25 cm. In these trials, no pesticide protection was used. The

Table 1
The characteristics of the sunflower hybrid cultivars used in the cultivar experiment.

No.	Cultivar	Earliness of flowering	Distributor
1	Gonzalo	Very early	Strube
2	Drake	Very early	SAATBAU ČR s. r. o.
3	Vellox	Early	VP AGRO, s. r.o.
4	ES Biba	Early	Euralis Semences
5	NK Neoma	Mid-early	Syngenta Czech s.r.o.
6	P63LE10	Very early	DuPont Pioneer

cultivars were sown in mid-April every year (14 April 2015, 18 April 2016, 19 April 2017). All pollinators were naturally occurring.

To quantify honey bee attendance on each cultivar during the blooming period, a visitation rate (VR) index was calculated. Expressed as a percentage, it represents the total number of pollinator visits to the cultivar during the blooming period relative to the total number of visits during the whole trial. This index is calculated as follows:

$$\mathrm{VR} = \frac{\sum_{t+6}^{t} n_1}{\sum_{t+6}^{t} n_1 + n_2 + n_x} x\ 100$$

t = number of observations; n_x = number of bees on the cultivar.

2.3. Experiment with pesticide treatments

To examine the effect of pesticide treatments on pollinator VR, we selected one sunflower cultivar (P63LE10) used in the previous experiment. This experiment was carried out using small plots in a Randomized Complete Block Design (RCBD) with 3 replicates, also for three consecutive years. The size of each plot was also set to 10 m^2, and flowers were spaced at a distance of 75 × 25 cm. The plots were sown in mid-spring (16 April 2015, 20 April 2016, 17 May 2017). All pollinators were naturally occurring. In 2017, five honey bee colonies were placed 400 m away from the experiment by a private beekeeper.

The pesticide treatments consisted of 2 insecticides: Mospilan (acetamiprid) and Pirimor (pirimicarb) and 2 fungicides: Bumper Super (propiconazole plus prochloraz) and Pictor (dimoxystrobin plus boscalid), all commonly used in commercial sunflower production, and an untreated control (Table 2). The plots were treated one time each year (10 July 2015, 12 July 2016 and 21 July 2017) (see Table 3).

2.4. Monitoring of pollinator visitation

In both the cultivar and pesticide experiments, pollinator visitation was recorded during days with the following conditions for pollinator flight: an air temperature higher than 20 °C, no or only light wind, and no precipitation. Pollinator visitation to 20 sunflower inflorescences in single plots was observed and recorded for 20 s.

Pollinator visitation observations for the cultivar experiment started with the beginning of flowering of the first variety until the end of flowering of the last variety. Visitation data were collected from 7 to 22 July 2015 (36 observations), from 13 to 27 July 2016 (48 observations), and from 21 July to 8 August 2017 (205 observations).

Pollinator visitation observations for the pesticide experiment began immediately after pesticide application and finished with the senescence of sunflower inflorescences. Visitation data were collected from 16 to 22 July 2015 (49 observations), from 13 to 27 July 2016 (54 observations), and from 21 July to 8 August 2017 (122 observations).

2.5. The analysis of pesticide residues in/on treated plants

The samples for the analysis of pesticide residue quantification were collected from treated plants. Samples were taken from each pesticide treatment 2 days (2016) and 4 days (2017) after application and again at the end of the flowering period in both years. Pesticide residue quantification was not conducted in 2015. Three sunflower inflorescences from each treatment were sampled. From these samples, only disk and ray flowers were removed and analyzed.

Table 2
The characteristics of the pesticides used in the pesticide treatment experiment.

Pesticide	Active ingredient	Registration dose	Producer
Mospilan 20 SP	Acetamiprid	0.1 kg/ha	Nisso Chemical Europe GmbH
Pirimor 50 WG	Pirimicarb	0.5 kg/ha	Syngenta Limited
Bumper Super	Prochloraz, Propiconazole	1 L/ha	Makhteshim-Agan Ltd.
Pictor	Dimoxystrobin, Boscalid	0.5 L/ha	BASF AG, Agricultural Products

2.5.1. Chemical analyses

The flower samples were analyzed for pesticide residues at the Department of Food and Nutrition Analysis at the University of Chemistry and Technology in Prague.

2.5.2. Analytical procedure

A multi-residue quantification method based on a modified version of the QuEChERS extraction approach (Anastassiades and Lehotay, 2003) and coupled to liquid chromatography – triple quadrupole tandem mass spectrometry (LC-QqQ-MS/MS) was fully validated and applied. Recoveries of the entire analytical procedure ranged from 70 to 120%, with repeatabilities of up to 20%. Limits of quantification (LOQs) were 4 μg kg^{-1}.

The targeted pesticides were extracted from a portion of homogenized sample using acetonitrile after matrix hydration with water. After the separation of the water and acetonitrile layers (performed by mixing with anhydrous $MgSO_4$ and NaCl), an aliquot of the upper layer was transferred to a vial for LC-MS/MS determination. The U-HPLC analyses of pesticides were performed using a 1290 Infinity II LC system (Agilent Technologies, Santa Clara, USA). Analytes were separated using an Acquity UPLC HSS T3 analytical column (Waters) maintained at 40 °C using water and methanol (with the addition of ammonium formate and formic acid) as a mobile phase. The U-HPLC system was coupled to a Triple Quadrupole G6495 (Agilent Technologies) mass spectrometer with electrospray ionization in positive ion mode (ESI+). The instrument was operated in dynamic multiple reaction monitoring (dMRM) mode.

3. Results

3.1. Experiment with sunflower cultivars

The total number of pollinator visits recorded in this experiment was 78,522; of these, 7.1% were from honey bees (n = 5572) and 92.9% from bumble bees (n = 72,950).

For both groups of pollinators, the VR of P63LE10 was significantly different from that of all others in 2017 and it was the most attractive. In 2015, 2016, the P63LE10 cultivar had the highest VR, along with cultivars NK Neoma and Vellox for bumble bees. P63LE10 started blooming and retained a high number of pollinators over the whole period of blooming (Fig. 1). The lowest attendance of bumble bees was on the cultivars Gonzalo, Drake and ES Biba in 2015. In 2016, the lowest attendance was on cultivar Gonzalo, and the lowest in 2017 was on cultivar Drake.

The overall abundance of honey bees was lower than abundance of bumble bees in all experimental seasons (years) (Table 4). The P63LE10 cultivar was visited by the highest number of honey bees in all experimental seasons (Table 4).

Cultivar P63LE10 started blooming early and maintained its higher attractiveness to honey bees even at the end of the observation period (Fig. 1, inset). Cultivar NK Neoma began to bloom later and was not very attractive to honey bees during the whole period of blooming. The cultivars Velox and ES Biba were the latest blooming cultivars in the experiment. Cultivar ES Biba was attractive mainly in the second part of the observation period.

3.2. Experiment with pesticide treatments

The total number of visitations of pollinators recorded in the experiment with pesticide treatments was 48,990, of which 30% were from honey bees (n = 14,732) and 70% from bumble bees

Table 3
Number of bumble bees and visitation rate (VR) of sunflower hybrid cultivars. The statistical evaluation of the VR of sunflower hybrid cultivars is shown by the letters in columns. Data in columns followed by the same letter do not significantly differ based on the Kruskal-Wallis test at a confidence level of 95% (2015: H (5, N = 864) = 85.21846, p = 0.0000; 2016: H (5, N = 1152) = 148.6993, p = 0.000; 2017: H (5, N = 4920) = 310.9707, p = 0.000).

Cultivar	Year					
	2015		2016		2017	
	n	VR (%) ± SD	n	VR (%) ± SD	n	VR (%) ± SD
Gonzalo	2449	14.1 ± 7a	900	9.3 ± 3.5a	5691	12.4 ± 4.7a
Drake	2419	13.9 ± 5.3a	1456	15.0 ± 4.7b	5765	12.6 ± 4.3a
Vellox	3066	17.6 ± 7.6b	1606	16.6 ± 4.2bcf	6967	15.2 ± 4.7b
ES Biba	2416	13.9 ± 4a	1410	14.6 ± 4bd	6906	15 ± 4.6bc
NK Neoma	3358	19.3 ± 4bc	2256	23.3 ± 6.1e	8630	18.8 ± 6.5d
P63LE10	3667	21.1 ± 14bd	2056	21.2 ± 7.9ef	11932	26 ± 9.8e
Total	17375	100	9684	100	45891	100

(n = 34,258).

Treatment with the fungicide Pictor (a. i. dimoxystrobin and boscalid) resulted in the highest abundance of bumble bees in all experimental seasons (Table 5). The VR of plants treated with this fungicide was significantly different from that of those treated with the insecticide Pirimor (a. i. pirimicarb) and untreated controls in the year 2017 (see Table 6).

The plots treated with the insecticide Pirimor showed the highest abundance of honey bees in the year 2015. In 2017, the plots treated with the fungicide Pictor exhibited the significantly highest VR, followed by those treated with Mospilan (a. i. acetamiprid).

Analyses of the active compounds (Table 7) were carried out to verify whether the treated sunflower inflorescences contained detectable levels of the active ingredients of the used pesticides over the whole period of pollinator observation.

3.3. Visitation rate comparison of sunflower cultivars and pesticide-treated plots

The comparison of the VR from the experiments with sunflower cultivars and pesticide-treated plots show that pollinators were more strongly influenced by cultivar than pesticide treatment (Fig. 2). In particular, cultivars P63LE10 and NK Neoma influenced the pollinator VR more than any pesticide treatment. Honey bees were more strongly

Table 4
- Number of honey bees and visitation rate (VR) of sunflower hybrid cultivars. The statistical evaluation of the VR of sunflower hybrid cultivars is shown by the letters in columns. Data in columns followed by the same letter do not significantly differ based on the Kruskal-Wallis test at a confidence level of 95% (2015: H (5, N = 864) = 15.78022, p = 0.0075; 2016: H (5, N = 1152) = 5.851756, p = 0.3209; 2017: H (5, N = 4920) = 844.5763, p = 0.000).

Cultivar	Year					
	2015		2016		2017	
	n	VR (%) ± SD	n	VR (%) ± SD	n	VR (%) ± SD
Gonzalo	41	12.3 ± 0.3a	63	14.8 ± 0.7a	534	11.1 ± 0.7a
Drake	52	15.6 ± 0.3a	47	11 ± 0.5a	474	9.9 ± 0.5a
Vellox	60	18 ± 0.3a	54	12.6 ± 0.5a	463	9.6 ± 0.4a
ES Biba	50	15 ± 0.3a	64	15 ± 0.7a	456	9.5 ± 0.4a
NK Neoma	50	15 ± 0.3a	42	9.8 ± 0.5a	364	7.6 ± 0.4a
P63LE10	81	24.3 ± 0.3a	157	36.8 ± 2a	2520	52.4 ± 2.4b
Total	334	100	427	100	4811	100

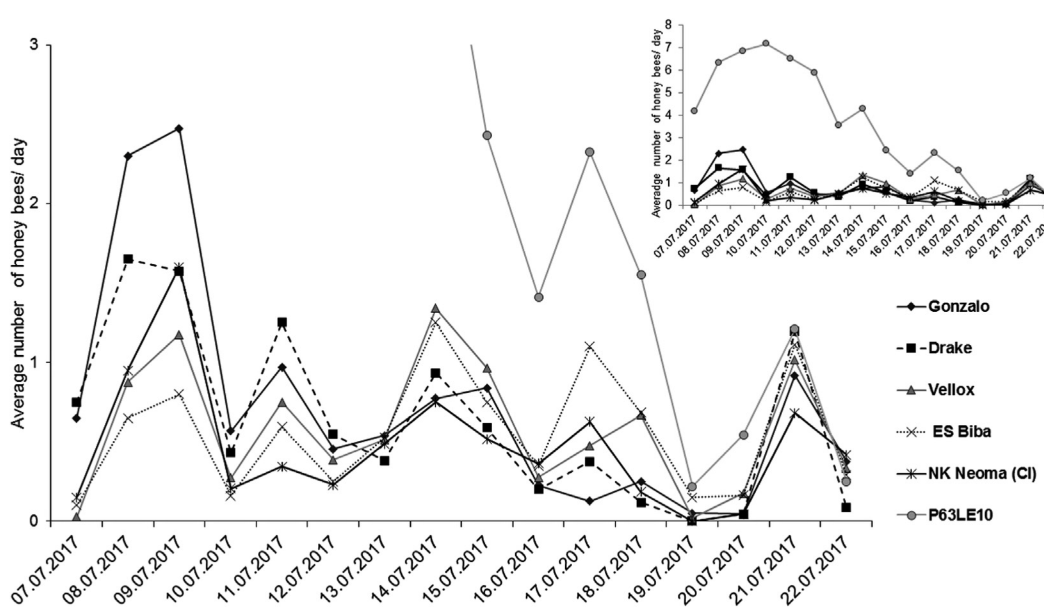

Fig. 1. The full graph is shown in the inset. The average abundance of honey bees on sunflower cultivars over one day during the observation period in 2017. The x-axis begins at the time in which even the latest flowering cultivar was in BBCH 63.

Table 5
Number of bumble bees and visitation rate (VR) in the experiment with different pesticide treatments. The statistical evaluation of VR for the different pesticide treatments is shown by the letters in columns. Data in columns followed by the same letter do not significantly differ based on the Kruskal-Wallis test at a confidence level of 95% (2015: H (4, N = 735) = 5.721197, p = 0.2210; 2016: H (4, N = 810) = 12.96935, p = 0.0114; 2017: H (4, N = 1830) = 31.20927, p = 0.0000).

Pesticide	Year					
	2015		2016		2017	
	n	VR (%) ± SD	n	VR (%) ± SD	n	VR (%) ± SD
Control	3973	20 ± 11.7a	1784	19.8 ± 7.4ae	961	17.9 ± 1.7ac
Mospilan 20 SP (acetamiprid)	3608	18.2 ± 9.7a	1653	18.3 ± 7.2bcde	1148	21.3 ± 1.9bcf
Pirimor 50 WG (pirimicarb)	4063	20.5 ± 12.1 a	1682	18.7 ± 6ad	885	16.4 ± 2a
Bumper Super (prochloraz, propiconazole)	4031	20.3 ± 11.8a	1941	21.5 ± 7.8a	1146	21.3 ± 2.1ce
Pictor (dimoxystrobin, boscalid)	4184	21.1 ± 13.8a	1958	21.7 ± 5.6ac	1241	23.1 ± 2.1def
Total	19859	100	9018	100	5381	100

Table 6
Number of honey bees and visitation rate (VR) in the experiment with different pesticide treatments. The statistical evaluation of VR for pesticide treatment is shown by the letters in columns. Data in columns followed by the same letter do not significantly differ based on the Kruskal-Wallis test at a confidence level of 95% (2015: H (4, N = 543) = 12.85695, p = 0.0120; 2016: H (4, N = 810) = 10.45979, p = 0.0334; 2017: H (4, N = 1830) = 50,23612, p = 0.0000).

Pesticide	Year					
	2015		2016		2017	
	n	VR (%) ± SD	n	VR (%) ± SD	n	VR (%) ± SD
Control	53	16.2 ± 0.5a	21	12.6 ± 0.2a	2855	20.1 ± 3.2bcd
Mospilan 20 SP (acetamiprid)	52	15.9 ± 0.4a	45	26.9 ± 0.2a	2925	20.5 ± 3.5ad
Pirimor 50 WG (pirimicarb)	84	25.6 ± 0.6a	24	14.4 ± 0.1a	2145	15.1 ± 3.1c
Bumper Super (prochloraz, propiconazole)	77	23.5 ± 0.6a	35	21 ± 0.3a	2898	20.4 ± 4.6d
Pictor (dimoxystrobin, boscalid)	62	18.9 ± 0.5a	42	25.1 ± 0.2a	3414	24 ± 4.5a
Total	328	100	167	100	14237	100

Table 7
Amounts of active ingredients in the inflorescences during the period of pollinator monitoring [ng/g]. Control indicates the untreated plots. In 2016, the first sampling occurred two days after the pesticide treatment. In 2017, the first sampling occurred four days after treatment.

Active ingredient	2016		2017	
	July 14	July 30	July 25	August 4
Control	0	0	0	0
Acetamiprid	144	6	3360	56
Pirimicarb	177	12	223	10
Prochloraz	380	6	1970	912
Propiconazole	324	17	1024	1018
Boscalid	216	154	9600[a]	1319
Dimoxystrobin	452	137	6560[a]	911

[a] A detection failure occurred in the sample from 25 July 2017; these sample data were replaced with the data from 28 July 2017.

influenced by cultivars than bumble bees.

4. Discussion

The total number of bumble bees observed in the sunflower experiment was 72,950 (93%), and it was 5572 (7%) for honey bees. Bumble bees were more attracted by the sunflower cultivars than honey bees, and we can assume that they are more important to sunflower pollination. This is in accordance with the findings of Aslan and Yavuksuz (2010) from Turkey, who confirmed in their research that bumble bees had a stronger effect on sunflower pollination than honey bees. On the other hand, Lužaić et al. (2008) monitored the pollinators attending sunflower hybrid cultivars in Croatia, and the visitation rate of bumble bees was just 0.32%, while that of honey bees was 99.53% (and 0.15% for *Volucella zonaria*).

The characteristics of each sunflower cultivar were an important factor influencing the pollinator visitation rates. Of these factors, the earliness of flowering influenced the attendance of pollinators in all experimental seasons. The P63LE10 cultivar flowers very early, which caused the high visitation rates of bumble bees and honey bees during all experimental seasons. This cultivar showed, from the beginning of the observation periods, a very high average number of attending bees (4 bees/day) compared to other cultivars, and it flowered for the duration of the experiments; therefore, it retained the highest number of pollinators for the whole time (Fig. 1). The Drake cultivar also flowered very early, but this cultivar did not attract as many pollinators as P63LE10. Therefore, we assume that other characteristics, such as the amount of nectar and pollen or some unidentified genotypic characteristic, also influenced the cultivar's attractiveness to pollinators. This effect was also observed by Chambó et al. (2011), who studied the influences of nectar and pollen production levels and genotypes. They confirmed that the density of nectar-collecting pollinators was higher than that of pollen-collecting species and that the number of bees visiting some hybrid cultivars differed from that observed visiting other genotypes.

The comparison of visitation rates among sunflower cultivars and pesticide treatments (Fig. 2) formed our conclusion that the type of cultivar had a stronger influence on visitation rate than the type of pesticide.

The proportions of bumble bees in the pesticide treatment experiment were 70 and 30% that of honey bees, which is in agreement with the results from the cultivar experiment. In the year 2017, the plots treated with the fungicide Pictor (a. i. dimoxystrobin and boscalid) showed the significantly highest VR, followed by the Mospilan (a. i. acetamiprid) treatment. The consumption of boscalid (one of the active ingredients in Pictor) in pollen increased honey bee mortality in a cage study (Degrandi-Hoffman et al., 2015).

The visitation rates of bumble bees/honey bees in plots treated with the insecticide Pirimor were not significantly different from those observed for other treatments and, on average, the visitation rate was low

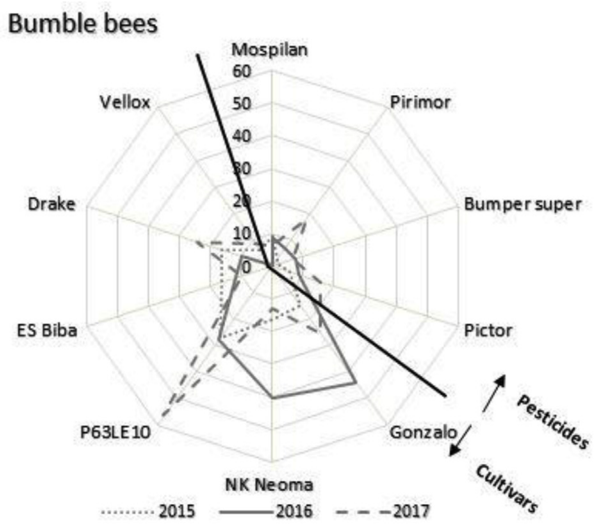

Fig. 2. Comparison of pollinator visitation in experiments with sunflower cultivars and pesticide treatments. Graphs show differences in the theoretical share of the visitation rate (VR %). The theoretical share of pollinator VR was 16.6% in the cultivar experiment and 20% in the pesticide treatment experiment. In the 'Honey bees' graph, the values for P63LE10 extend past the graph boundaries because of high differences - 161.7% in 2016 and 215.7% in 2017.

during all experimental seasons for both groups of pollinators. The observed low visitation rate may have occurred because pirimicarb, the a.i. of Pirimor, is toxic to honey bees. This insecticide, when applied at the rate of 0.28 kg active ingredient/ha to fully blooming oilseed rape in the morning before bees had commenced flying, caused bee mortality for 4 days after spraying. Therefore, this compound cannot be recommended for application to flowering crops (Clinch and Palmer-Jones, 1974).

The active ingredient - pirimicarb have been also found in honey (Irungu et al., 2016; Sanchez-Bayo and Goka, 2014; Blasco et al., 2003). Worldwide screening of neonicotinoids residues revealed also acatamiprid - a. i. of Mospilan in large number of honey samples from Europe and in Asia and South America (Mitchell et al., 2017).

The fungicide Bumper Super, with the active ingredients propiconazole and prochloraz, showed average pollinator visitation in all seasons of the experiment. Propiconazole belongs to the group of demethylation inhibitors (DMIs), also referred to as ergosterol biosynthesis inhibitors (EBIs), and can be a plant growth regulator (Iwasa et al., 2004). Certain compounds of low toxicity can increase the susceptibility of an organism to toxic substances. This is known as potentiation or synergic toxicity (Manning et al., 2017). DMI fungicides can potentiate insecticide toxicity by breaking the production of detoxification enzymes in bees (Manning et al., 2017). A fungicide containing propiconazole potentiated the toxicity of the insecticide acetamiprid, and its field rate caused 100% mortality in honey bees. Fungicides containing propiconazole also increased the honey bee toxicity of acetamiprid 6.0-, 244- and 105-fold in a laboratory experiment conducted by Iwasa et al. (2004). The second a.i. of Bumper Super is prochloraz, which is a sterol biosynthesis inhibitor (SBI) that functions through the inhibition of the fungal cytochrome P450-monooxygenase (P450)-mediated synthesis of ergosterols. Prochloraz has been shown to inhibit normal P450 detoxification activity in honey bees, as well, particularly in relation to the detoxification of pyrethroid insecticides (Pilling et al., 1995). In experiments with the SBI fungicide prochloraz, an almost 2000-fold increase in the toxicity of tau-fluvalinate was observed; tau-fluvalinate is also used as an in-hive treatment against *Varroa destructor* Anderson and Trueman (Johnson et al., 2013). The SBI family of fungicides is known to be present in bee-collected pollen, wax and in the bees themselves (Chauzat and Faucon, 2007).

In the Czech Republic and elsewhere in Europe, it is very common to apply insecticides together with fungicides, growth regulators or fertilizers in a "tank mix" during a single field application. Therefore, even if an insecticide possesses high repellency to pollinators, the fungicide applied with it may mitigate this beneficial feature (trait), potentially attracting pollinators, and they could therefore receive a toxic dose of an insecticide. To prevent this unintended consequence, there should be an appeal to farmers to separate the fungicide and insecticide applications into two separate applications to avoid the potential for pollinator intoxication. Another option may be botanical pesticides, e. g. NeemAzal T/S with a. i. 1% Azadirachtin A obtained from tree *Azadirachta indica* A. Juss. (Pavela et al., 2009). Azadirachtin was tested in the experiment with sunflower treatment and it was proven to have no residual toxicity such as chemical insecticides (Pashte and Patil, 2017a), and it is not repellent and does not affect pollinators foraging activity after treatment (Pashte and Patil, 2017b).

In conclusions the sunflower cultivar P63LE10 was shown to be the most attractive to honey bees in 2017. For bumble bees, cultivar P63LE10 had also a high VR, along with cultivars NK Neoma and Vellox, in 2015 and 2016. Therefore, cultivar P63LE10 could be recommended for agronomical practice for his attractivity to pollinators. In the year 2017, plants treated with the fungicide Pictor (dimoxystrobin plus boscalid) had the highest VR for both groups of pollinators. For bumble bees, Pictor was significantly more attractive than the insecticide Pirimor (pirimicarb) and the untreated control, and for honey bees, Pictor was significantly more attractive than all other treatments except Mospilan (acetamiprid). When comparing the two main factors – cultivar and pesticide treatment – pollinator preferences based on VR indicate that cultivar is the most important agent in food source preferences by pollinators. For future research we suggest to investigate the repellency effect of "tank mixes" as the toxicity of these mixtures for pollinators is usually stated higher than in a divided product application and the repellency effect of "tank mixes" is unknown.

Declarations of interest

None.

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