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

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ABSTRACT

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