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The factors influencing the pollinators visitation of the oilseed rape cultivars

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Abstract: Currently, the hybrid cultivars are predominant in the cultivation of winter oilseed rape in Europe. Cultivation of hybrid cultivars instead of the traditional line can affect the visitation of pollinators. Beekeepers and farmers claim that hybrid cultivars are not visited by pollinators as much as the line. Ten yellow and one white flowering oilseed rape cultivars were used to compare the visitation rates of pollinators (*Apis mellifera* L. and *Bombus* sp.) during flowering in the years 2015–2017. At the same time, the visitation of hybrid and line cultivars by pollinators was evaluated. Visitation of pollinators on each cultivar was calculated from observed visitations to flowering oilseed rape plants in an area 2.1 m^2 from the edge of single plots for 20 s. The results from this study clearly show that the individual cultivars, whether hybrids or lines, did not have a major influence on the pollinators' visitation, either by honey bees or bumble bees. It is thus proved that hybrid cultivars do not affect the pollinator visitation and there is no need to worry about the prevalence of these cultivars in the Czech fields. However, a more significant effect for both pollinator groups appears to have been the color of the flower.

Keywords: attractiveness; crop pollination; oilseed rape hybrid; agroecology

Currently, the hybrid cultivars are predominant in the cultivation of winter, oilseed rape in Europe. The availability of such a large number of hybrid cultivars strongly affects the proportion of the total crop area grown. Estimates suggest European countries plant approximately 90% hybrid cultivars (Kleffmann Group 2018).

There have been suggestions that the cultivation of hybrid cultivars instead of traditional line cultivars could affect the visitation by honey bees and other pollinators. Sláma (2019) stated (oral statement) that winter rape stands are less frequented by bees due to the cultivation of hybrid cultivars. He also reports lower yields of honey from these stands.

However, oilseed rape is still considered by most growers to be a very attractive crop for insect pollinators due to the deep yellow color of its flowers, its special aromas, and its sugar content in nectar (Masierowska and Pietka 2014). Abrol (2011) also noted that oilseed rape provides about 25 to 91 million flowers per hectare per day, making it one of the most important and safest sources of bee pasture. Veselý et al. (2009) found that older line cultivars with a high content of erucic acid and glucosinolate had similar flowering biology to the currently grown hybrids.

Stanley et al. (2013) report that a wide variety of insects, including bees (*Apis mellifera* L.), bumble bees (*Bombus* sp.), other bees and flower flies (*Eristalis*

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hoverflies L.), are visiting winter oilseed rape but most insect pollination in oilseed rape is affected by honey bees, which is the predominant pollinator of *B. napus* L. (Pordel et al. 2007, Blochtein et al. 2014).

Pollination is also important for oilseed rape growers. Oilseed rape is considered a predominantly self-pollinating crop, but as described by Chambó et al. (2014), insects can increase their yield by pollination visits. Veselý et al. (2009) even indicate that after adding 3 to 4 colonies per 1 ha of cultivated oilseed rape, the yield may increase by up to 35%. The main reason for the higher yield is the higher number of seed pods after pollination. Conversely, the elimination of pollinators resulted in a 27% reduction in seed production and a 30% reduction in weight, with comparable control values from the oilseed rape field attended by pollinators (Stanley et al. 2013).

Ouvrard and Jacquemar (2019) point out that there are many factors influencing pollinators in their selection. Pollinator preference may depend on sowing time (winter or springform), type of cultivar (hybrid or line), and other parameters, such as the size of the area under observation or nectar offer of plants.

It has been shown that honey bees do not respond to all substances in flowers, but only to some biologically active compounds, which limits the number of traits that breeders should consider when choosing bee-attractive crops. Higher amounts of some bioactive components may alter the response of bees and cause potential repellency (e.g., decanal) (Pichersky et al. 2006). The proportion of these biologically active substances was found to be 30% due to the type of cultivar (Mas et al. 2018).

Ouvrard and Jacquemar (2019) report that only a few cultivars of oilseed rape have been evaluated about pollinators in scientific studies due to the rapid variation of cultivars. In cultivated sunflower hybrids where a single cultivar has predominated, many attempts have been made to determine hybrid/line effects on pollinator visitation. Shein et al. (1980), for example, confirmed that honey bee visitation was influenced not only by the genotype but also by several factors. Hybrid cultivars appear to be one of the important ones. Luzaić et al. (2008) conducted an experiment in Poland with six different sunflower hybrids (H1, H2, H3, H4, H5, H6). They observed that H2 had the lowest pollinator visit, while H4 had the highest visit. However, the differences between other hybrids were not statistically significant. Similarly, Stejskalová et al. (2018) confirmed statistically significant differences in the attractiveness of sunflower cultivars for bumble bees.

The study reported here was aimed at comparing the visitation rate of pollinators on cultivated cultivars of winter oilseed rape in 2015–2017. In addition to the visitation of hybrid versus line cultivars, we compared visitation on yellow-flowering cultivars with a white-flowering cultivar.

MATERIAL AND METHODS

In 2015–2017, the small-plot experiments were conducted for the purpose of examining the influence of oilseed rape on the visitation of two groups of pollinators: bumble bees (*Bombus terrestris* L. and *Bombus lapidaries* L.) and honey bee *(Apis mellifera* L.).

Description of location. The experiments were carried out at a demonstration field belonging to the Czech University of Life Science Prague (50°12'99.7"N, 14°37'37"E).

Experiment with oilseed rape cultivars. In the first year of the experiment, pollinator visitation was observed on winter oilseed rape cultivars: Artoga, DK Explicit, Sidney, Sherpa, and Witt. During the second year, the experiment was extended to include the cultivars: Andromeda, Arabella, Dozzen, DK Exception, DK Exprit and Mentor (Table 1). Witt is the only white-flowering cultivar available on the market in the Czech Republic. The experiment was established in small-plots in a randomized, complete block design (RCBD) with 3 replicates. The size of each plot was 10 m^2. In these trials, no pesticide protection was used. The cultivars were sown in mid-August each year (18 August 2014, 14 August 2015, 20 August 2016). All pollinators evaluated were naturally occurring.

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

$$VR = \frac{\frac{\sum_t^t n_1}{\sum_t^t n_1 + n_2 + n_x}}{\frac{\sum_{t+y}^t n_1}{\sum_{t+y}^t n_1 + n_2 + n_x}} \times 100$$

Where: t – number of observations; n_x – number of observed pollinators on cultivar; y – number of cultivars in each trail year.

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Table 1. The characteristics of oilseed rape cultivars used in experiment

Cultivar	Type	Colour of bloom	Distributor	Trail year
Andromeda	hybrid	yellow	SELGEN a.s., Prague	–, 2016, 2017
Arabella	line	yellow	Limagrain Central Europe S.E.,	–, 2016, 2017
Artoga	hybrid	yellow	Limagrain Central Europe S.E.,	2015, 2016, 2017
Dozzen	hybrid	yellow	VP AGRO spol. s.r.o.	–, 2016, 2017
DK Exception	hybrid	yellow	MONSANTO ČR s.r.o.	–, 2016, 2017
DK Explicit	hybrid	yellow	MONSANTO ČR s.r.o.	2015, 2016, 2017
DK Exprit	hybrid	yellow	MONSANTO ČR s.r.o.	–, 2016, 2017
Mentor	hybrid	yellow	NPZ H.-G. Lembke, Germany	–, 2016, 2017
Sidney	line	yellow	SAATBAU LINZ ČR spol. s.r.o.,	2015, 2016, 2017
Sherpa	hybrid	yellow	NPZ H.-G. Lembke, Germany	2015, 2016, 2017
Witt	line	white	Soufflet Agro a.s.	2015, 2016, 2017

Monitoring of pollinator visitation. In cultivar experiment, pollinator visitation assessments were conducted according to the same methodology as used by Stejskalová et al. (2018), during days with the following conditions for pollinators flight: air temperature higher than 20°C; very light or no wind; and, no precipitation. Pollinator visitations were counted and recorded from an area of 2.1 m^2 from the edge of single plots of flowering oilseed rape for 20 s.

Pollinator visitation observations for cultivar experiments started with the beginning of the flowering of the first cultivar until the end of the flowering of the last cultivar. Visitation data were collected from 24 April to 20 May 2015 (27 observations), from 20 April to 20 May 2016 (55 observations) and from 4 to 26 May 2017 (77 observations).

Statistical analysis. The results of the pollinator visitation on cultivars of oilseed rape were statistically evaluated by the variance analysis method. A more detailed evaluation was performed with some non-parametric tests at the level of significance $\alpha \leq 0.05$ (tests are deeply described in results). The analysis was conducted in Statistica 12 CZ software (Palo Alto, USA).

RESULTS AND DISCUSSION

In small-plot field experiments with winter oilseed rape, the differences in the numbers of honey bees and bumble bees were observed between types of varieties and cultivars with different flower colors. The differences in the number of visits by honey bees and bumble bees are shown in Table 2. The total number of pollinator visits recorded in this experiment was 6864; of these, 68.3% were from honey bees (n = 4691) and 31.7% from bumble bees (n = 2173). Thus, in the oilseed rape, the honey bee appears to be the main pollinator. However, Stanley et al. (2013) refute this statement, saying that bumble bees were the main oilseed rape pollinator in Ireland in the field experiments. But compared to the Czech Republic, Ireland is one of the countries with the lowest number of colonies per km^2.

Factors affecting the visitation of oilseed rape by pollinators. The differences in pollinator visitation in observed years are mainly caused by the date of oilseed rape and fruit tree blooming. When flowering at the same time, fruit trees are more attractive to honey bees, or the dandelions may also be competitive, as Free (1968) states. The pollinator traffic in oilseed rape is also influenced by the course of weather, especially temperature, precipitation, and wind strength. Thus, the number of days optimal for pollinator flight varies each year (Farkas 2008). This is because bumble bees can fly at lower temperatures and in more adverse weather conditions than honey bees (Tuell and Isaacs 2010). Farkas (2008) also mentions nectarodity as an important factor, which makes the cultivars more attractive to honey bees. The author studied the production of nectar and sugar composition of four cultivars of oilseed rape (Baldur, Bekalb, Catonic, and Colombo). It was found that weather conditions had a significant effect on nectar production. Bees visited plants only in sunny and dry weather, either because the concentration of sugar in nectar was more attractive to them, or because the temperature conditions were more favorable to honey bee activity. Blažytė-Čereškienė et al. (2010) state that honey bees can discriminate among rape genetic lines that give more or less reward and adapt

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Table 2. Number of honey and bumble bees and visitation rate (VR, %) of oilseed rape cultivars

Cultivar	Honey bee						Bumble bee					
	2015		2016		2017		2015		2016		2017	
	n	VR	*n*	VR	*n*	VR	*n*	VR	*n*	VR	*n*	VR
Andromeda			133	99.1^{ab}	127	55.5^{a}			2	48^{bc}	160	99.3^{a}
Arabella			90	67.1^{b}	242	105.6^{a}			2	48^{bc}	136	85.0^{a}
Artoga	145	104.0^{a}	161	119.9^{ab}	172	75.0^{a}	55	77.5^{ab}	17	375^{a}	169	105.5^{a}
Dozzen			181	134.7^{a}	252	110.0^{a}			3	60^{bc}	162	100.3^{a}
DK Exception			118	88.0^{ab}	263	115.0^{a}			2	48^{bc}	229	143.2^{a}
DK Explicit	198	142.1^{a}	116	86.4^{ab}	257	112.3^{a}	93	131.4^{b}	3	60^{bc}	125	77.3^{a}
DK Exprit			97	72.1^{bc}	250	109.3^{a}			5	109^{ac}	178	110.8^{a}
Mentor			150	111.8^{ab}	278	121.4^{a}			7	145^{ac}	141	87.4^{a}
Sidney	164	118.0^{a}	135	100.3^{ab}	196	85.8^{a}	86	121.8^{b}	1	24^{bc}	203	126.5^{a}
Sherpa	160	114.9^{a}	134	99.9^{ab}	303	132.5^{a}	103	145.5^{b}	1	24^{bc}	118	73.0^{a}
Witt	29	21.0^{b}	162	120.8^{ab}	178	77.7^{a}	17	23.7^{a}	7	157^{bc}	148	91.7^{a}
Total	696	100	1477	100	2518	100	354	100	50	100	1769	100
	2015: H (4, n = 135) = 18.95463; P = 0.0008						2015: H (4, n = 135) = 31.55058; P = 0.0000					
	2016: H (10, n = 605) = 24.34431; P = 0.0067						2016: H (10, n = 605) = 35.25189; P = 0.0001					
	2017: H (10, n = 847) = 10.49621; P = 0.3981						2017: H (10, n = 847) = 12.48561; P = 0.2539					

The statistic evaluation for visitation rate of oilseed rape cultivars is shown by letters in columns. Data in columns followed by the same letter do not differ significantly as based on the Kruskal-Wallis test at confidence level 95%

the number of their visits to the nectar secretion rate. In their study, the oilseed rape cultivars (SW Savann, Ural) were not similar in attractiveness or access to nectar to honey bees; therefore, insects visited cv. SW Savann more often than cv. Ural. Veselý et al. (2009) add that the production of nectar varies from year to year. Nectar production is most influenced by the physiological state of the plant (supply of water, nutrients, etc.). Also, according to beekeepers' observations, oilseed rape cultivars produce more nectar in areas where soils are well supplied with moisture and at temperatures around 22°C. Danka et al. (2006) report that the total sugar content of oilseed rape nectar varies during the day and flowering. The authors measured higher values in the afternoon compared to the morning. Pernal and Currie (1998) also measured higher values in the first two weeks of oilseed rape blooming, compared to the last two weeks.

Cultivar as a monitored factor. In 2015, DK Explicit was the most visited cultivar by honey bees. Bee visitation rate on this cultivar reached VR 142%. It was found that among the yellow-flowering cultivars, the lowest attendance rate was on cv. Artoga (VR 104%). However, the lowest statistically significant differences in visitation rate were on the white flowering cultivar, Witt (VR 20%). In 2016 and 2017 the larger number of cultivars was evaluated. The most visited cultivar in 2016 was the Dozzen cultivar (VR 134%). The lowest visitation of honey bees was found in cv. Arabela (VR 67%) and cv. DK Exprit (VR 72%). Statistically significant differences were found in 2016 only between the cultivars with the highest attendance, Dozzen, and the lowest attendance cultivars, Arabella and DK Exprit. In 2017, the most visited cultivar was Sherpa (VR 132%). The lowest honey bee visitation was found in the Andromeda (VR 55%), Artoga (VR 75%), and Witt (VR 77%) cultivars. In 2017, there was no statistically significant difference in bee attendance among any tested cultivars. The effect of oilseed rape cultivars on honey bee attendance is therefore low. Ouvrard and Jacquemar (2019) report that rapeseed cultivars are not stigmatized in relation to pollinators due to the rapid variation of the cultivar. However, Blažytė-Čereškienė et al. (2010) examined the relationship between honey bee density and flower density on 2 rape cultivars and found that honey bee density in the investigated oilseed rape cultivars was positively correlated with flower density. Thus, an increase in flowering intensity was followed by an increase in honey bee density in both rape cultivars and, as well, conversely.

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In 2015, the most visited oilseed rape cultivar by bumble bees was cv. Sherpa (VR 146%). The absolutely least attractive cultivar was the white flowering cv. Witt (VR 23%). Statistically significant differences in bumble bee attendance were found only between the cv. Witt and the cvs. DK Explicit, Sherpa, and Sidney. In 2016 and 2017, the same as in the case of honey bees, the larger number of cultivars was evaluated. The visitation of bumble bees on the experimental plot in 2016 was very low. The most visited cultivar was cv. Artoga (VR 375%). The lowest number of visitors was found in the cvs. Sidney and Sherpa (both VR 24%). Statistically significant differences in bumble bee attendance were found in 2016 between the most visited cv. Artoga and other cultivars except cvs. DK Exprit and Mentor. In 2017, the most visited cultivar by bumble bees was cv. DK Exception (VR 143%). The lowest bumble bee attendance was found in the cv. Sherpa (73% of the average attendance) and cv. DK Explicit (77.3%). In 2017 there was no statistically significant difference in the visitation of bumble bees among tested cultivars. The influence of oilseed rape cultivars on bumble bees visitation is low.

Type of cultivar as a monitored factor. In our study, the influence of the cultivation of hybrid and line cultivars on pollinator visitation was also studied (Figure 1a,b). Ouvrard and Jacquemar (2019) point out that the type of cultivar is one of the factors influencing pollinators in their selection. In 2015, hybrid cultivars were visited by honey bees by 77% more than line cultivars, and by 63% in case of bumble bees. This difference was statistically significant in both groups. A large difference in visitation was mainly caused by the white-flowering cv. Witt, which bloomed in pure white that year and showed a low visitation of pollinator. In the following years 2016 and 2017, the visitation of hybrid and line cultivars was almost balanced, and there was no statistically significant difference in either of the studied pollinator groups.

Bloom color as a monitored factor. In all monitored years, the number of pollinators in oilseed rape

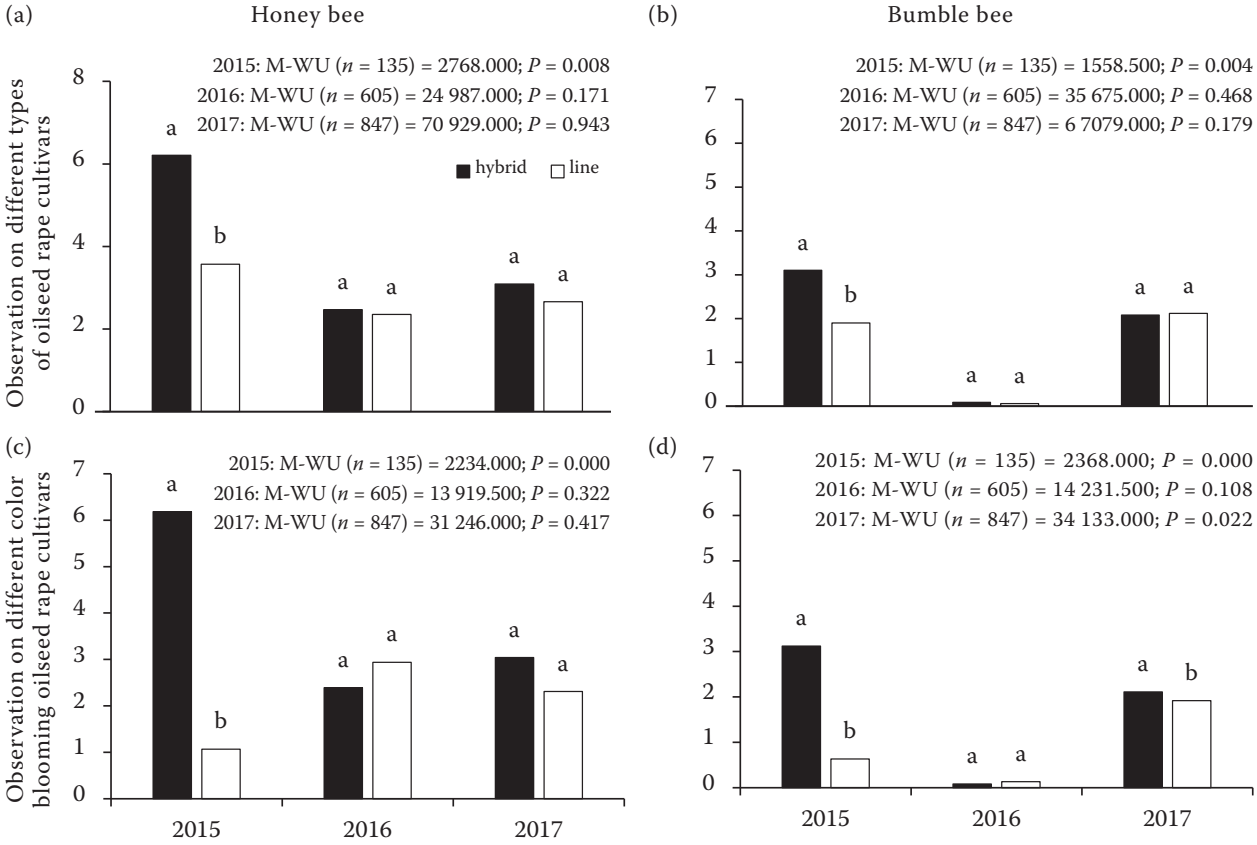

Figure 1. Average number of honey and bumble bees per observation on different types of oilseed rape cultivars and color blooming oilseed rape cultivars. The statistic evaluation of this is shown by letters above columns for each year separately. Columns with the same letter do not differ in distribution significantly as based on the Mann-Whitney U-Test at significance level 0.05

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cultivars was compared according to the color of the flower (Figure 1c,d). When testing the difference in pollinator attendance on the pure white flowering cv. Witt compared to the yellow flowering cultivars in 2015; it was found that yellow flowering cultivars were visited more than the white flowering cultivar on average by 476% in the case of honey bees and 395% in case of bumble bees. This difference was statistically significant in both groups. The low attractiveness of this white-flowering cultivar for honey bees, but also insect pests, was also observed in the experiments of authors (Giamoustaris and Mithen 1996).

In 2016, the second-highest visitation was observed in both groups of pollinator on the cv. Witt, which blossomed that year noticeably yellowish. The visitation of yellow cultivars was 81.2% compared to cv. Witt by honey bees and 61.5% by bumble bees. Again, in 2017, and similar to 2015, the Witt oilseed rape cultivar bloomed more whitish, the yellow flowering cultivars were again higher by 131.6% compared to the cv. Witt in honey bees and 110% in bumble bees. The experiments showed a fluctuation in visitation according to the saturation of the white color of the cv. Witt. Producer of cv. Witt explains the annual changes in the flower color of this cultivar by the fact that the whiteness of the flowers depends on the amount of sunshine during flowering. The fact that the cultivation of cultivars of different colors could have an effect on pollinator visitation is also evidenced by experiments established by Chittka and Waser (1997), who cultivated red flowering oilseed rape and subsequently evaluated its influence on pollinator visitation. The attractiveness for honey bees was not comparable to the yellow-flowering cultivar, probably due to poor visibility of the red color by bees. However, Cook et al. (2005) believe that the olfactory signals from pollen and nectar remain, which should be so attractive to bees that sufficient pollination is ensured.

Among the individual cultivars, there were no significant differences found in pollinator visitation in the observed years. Individual cultivars do not have a major impact on pollinator visitation. The more significant result is that the difference in visitation by honey bees and bumble bees of line and hybrid cultivars is statistically inconclusive (2016 and 2017), or the visitation of line cultivars prevails (the year 2015). It is thus proved that hybrid cultivars do not affect the pollinator visitation and there is no need to worry about the prevalence of this type of cultivars in the Czech fields. However, a more serious effect on visitation for both pollinator groups is the color of the flower. The white flowering cv. Witt has a different VR each year, which fluctuates strongly in relation to the change in the hue of the flower. This knowledge should draw attention to the fact that any other cultivars with changed color of the flower should always be checked for the perception of this change by pollinators.

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