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Attractiveness of oilseed rape cultivars to *Brassicogethes aeneus* and *Ceutorhynchus obstrictus* as a potential control strategy

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Abstract: The abundances of two pests, pollen beetle (*Brassicogethes aeneus* (Fabricius, 1775)) and cabbage seed weevil (*Ceutorhynchus obstrictus* (Marsham, 1802)), were measured before flowering and in the full bloom of oilseed rape cultivars with different phenologies (two yellow-flowering: DK Exssence (the earliest), DK Sensei (the latest) and one white-flowering in time between yellow-flowering cultivars), and the differences in their abundance in the selected cultivars were determined in plot experiments during 2015–2018. No significant differences in pollen beetle and cabbage seed weevil occurrence were observed between the cultivars in the pre-flowering period, but during flowering, the two yellow-flowering cultivars were more attractive than the white-flowering cultivar for both pests. In the white-flowering cultivar, 57% and 69% reductions in the pollen beetle and cabbage seed weevil populations, respectively, were found relative to the two with yellow flowers. Thus, the use of white-flowering cultivar (less attractive, later flowering) as the main crop and the yellow-flowering cultivar (more attractive, earlier flowering) at field edges, with the width of the one-track line, could serve as a strategy to manage oilseed rape pests during flowering. This control strategy which combines more and less attractive oilseed rape cultivars may contribute to a reduction in the use of pesticides and their negative impact on the environment.

Keywords: *Brassica napus* L.; push-pull strategy; pest management; insecticide; trap crops

Agriculture is currently one of the sectors undergoing major changes in terms of crop protection based on synthetic pesticides (Long et al. 2016). It is necessary to adapt cultivation technologies and strategies for pest control with a narrower spectrum of insecticides in crops grown at large scales, such as oilseed rape (*Brassica napus* L.), one of the most important oilseed crops in Europe (Kaasik et al. 2014). The expansion of its cultivation in Europe over the past three decades has created ideal conditions for increasing pest population densities and damages caused by crop pests. The pollen beetle (PB, *Brassicogethes aeneus* (Fabricius, 1775)) and the cabbage seedpod weevil (CSW, *Ceutorhynchus obstrictus* (Marsham, 1802)) are considered members of the major oilseed rape pests in the spring.

Overwintering PB adults colonise fields during the green bud phenological stage of oilseed rape plants (BBCH 51–57). The greatest damage caused by PB adults occurs during the cold weather period in the spring when buds begin to flower (BBCH 57–60). Feeding damage to flower buds caused by adults, or rarely also by larvae, can lead to abscission and subsequent pod-less stalks (Williams and Free 1979,

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Šedivý 1993). The damage caused by adult PBs can be higher on spring-sown oilseed rape cultivars than on autumn-sown rape cultivars. Pollen beetle adults also cause damage to other cruciferous crops grown for seed, such as white mustard and cabbage (Alford et al. 2003).

In Europe, the CSW is a less significant pest of oilseed rape than in North America (Cárcamo et al. 2001). Overwintering adults migrate to oilseed rape during the flowering stages (BBCH 61–65), and warm weather during the flowering period (BBCH 61–65) supports their occurrence. Females lay eggs singly in a hole bitten in the wall of young pods (Lerin 1991), and after hatching, larvae feed on the developing seeds (Williams 2010).

For almost two decades, PB populations in Europe have been effectively controlled by synthetic pyrethroid insecticides, but in 1999, the first report of pyrethroid resistance in PB was documented in France (Derron et al. 2004). Subsequently, pyrethroid-resistant PB populations were detected in 18 European countries (Slater et al. 2011), including Czech Republic (Stará and Kocourek 2018, Hovorka et al. 2021). The risks of increased damage caused by the CSW could be due to the increased resistance of CSW to pyrethroids and neonicotinoids. Cabbage seedpod weevil resistance to acetamiprid was locally documented in Poland in 2009 and 2010 (Zamojska and Węgorek 2014), and resistance to pyrethroids was reported in Germany (Heimbach and Müller 2013). Although there is strong evidence of pyrethroid resistance among populations of the PB (Slater et al. 2011, Spitzer et al. 2020) and CSW (Heimbach and Müller 2013) across Europe, pyrethroids remain the main control agents for these pests. Insecticides are routinely applied to large areas of monocultural crops, frequently without prior screening for the presence of pests (Williams 2010). One of the most recent surveys of insecticide usage in Europe have shown that oilseed rape usually receives between one and four applications; however, it is not unusual for this crop to receive five applications (Menzler-Hokkanen et al. 2006, Richardson 2008). Innovative integrated pest management (IPM) approaches to control these pests is necessary to decrease yield losses and to slow the development of resistance to insecticides (Skellern and Cook 2018). With increasing crop damage caused by the PB and CSW, demand by farmers for new management strategies for these pests also is increasing (Williams 2010).

One of these potential innovative approaches for oilseed rape could be the push-pull control strategy. This control strategy can be defined as a behavioral manipulation method that uses repellent (push) and attractive (pull) stimuli to direct the movement of pests or beneficial insects for pest management (Foster and Harris 1997, Cook et al. 2007). Stimuli that can be used for insect behavioral manipulation include visual and semiochemical cues. These stimuli can be combined with other population-reducing methods, including insecticide application, with a preference for biologically based or selective products (Nalyanya et al. 2000, Zhang et al. 2013). Control strategies against pests, such as the push-pull strategy, have been developed in many areas of pest management. However, despite the problems associated with the development of resistance, chemical control is still preferred in many situations by farmers, because for the time being, there is no other way to protect large scale grown crops like oilseed rape effectively (Cook et al. 2007).

The use of trap crops is an example of a stimulus that attracts (pull) pests. Trap crops divert pest pressure from the main crop because they are more attractive to the pests (Hokkanen 1991, Shelton and Badenes-Perez 2006) and can be plants of a preferred growth stage, cultivar or species. For example, when used as a trap crop, turnip rape (*Brassica rapa* L.) significantly reduced the abundance of the PB in spring-sown oilseed rape compared to that in plots without this trap crop (Cook et al. 2004, Hokkanen and Menzler-Hokkanen 2018). Selective or biological insecticides can be used to reduce pest populations in trap crops. Turnip rape is known to be the preferred host for several oilseed rape pests, as it is for the PB (Barari et al. 2005), and growth stage-related visual and olfactory stimuli are at least partly responsible for the increased preference by pests (Cook et al. 2006). Another example of the push-pull control strategy (in addition to trap crops) is the use of oilseed rape cultivars with different flower (i.e., petal) colours and their different preferences by pests. The high attractiveness of yellow petal colour in oilseed rape for some pests, including the PB and CSW, is well known (Láska et al. 1986, Buechi 1990). However, only a few studies have reported the attractiveness of other petal colours for oilseed rape pests, with their potential to manipulate the location of herbivores or pollinators of oilseed rape and reduce subsequent infestation by pests (Cook et al. 2006, 2013). Cook et al. (2006) reported that blue petals were the least attractive, compared to the yellow, white and red petals, and these results were supported by the use of

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sticky traps, with blue and black traps being the least attractive. Additionally, Cook et al. (2013) reported that red and blue petals of oilseed rape plants were less attractive than yellow and white petals in their experiments with dyed flowers. In both experiments, white petals were the second most attractive for the overwintering PB after yellow.

The aim of our work was to determine in field experiments the preference of PB and CSW by direct counts and sweep netting for white- or yellow-flowering cultivars with different phenologies (two yellow-flowering: DK Exssence (earlier), DK Sensei (latest) and one white-flowering in time between yellow-flowering cultivars). Based on obtained results to propose the potential use of the white-flowering cultivar in a push-pull-based control strategy.

MATERIAL AND METHODS

Field trials. The experiments were carried out in the experimental fields of the Crop Research Institute in Prague (50°05'16.5''N, 14°17'56.0''E, elevation of 345 m a.s.l.) in the spring periods from 2015–2018. The whole region belongs to the temperate climate zone, with an average annual temperature of 7.9 °C and average annual precipitation of 472 mm. The main soil unit is a modal brown soil that is clayey loam and is on a subsoil formed mainly of loess and marlstone.

To simulate the crop rotation system frequently used in Czech Republic, the crop sequence in the experimental fields was as follows: oilseed rape-cereals-oilseed rape-cereals. Thus, oilseed rape was grown in the same field every second year. The experimental fields were located within potatoes-cereals fields and apple orchards (Figure 1). Plots of oilseed rape were separated within a field with 10 m belt of nectar-rich, mainly Leguminosae, plants. No brassicas were planted in the belt of nectar-rich plants.

Winter oilseed rape cultivars were sown on three experimental plots (one plot for each cultivar) at a rate of 400 000 seeds per ha with a Wintersteiger plot seeder (Wintersteiger Sägen GmbH, Arnstadt, Germany). Two yellow-flowering cultivars (DK Exssence and DK Sensei) and one white-flowering cultivar (Witt) were used for the trial (Figure 1).

The two yellow-flowering cultivars of oilseed rape were each sown on a 0.2 ha plot; the white-flowering cultivar was sown on a 0.4 ha plot. Hence, the area

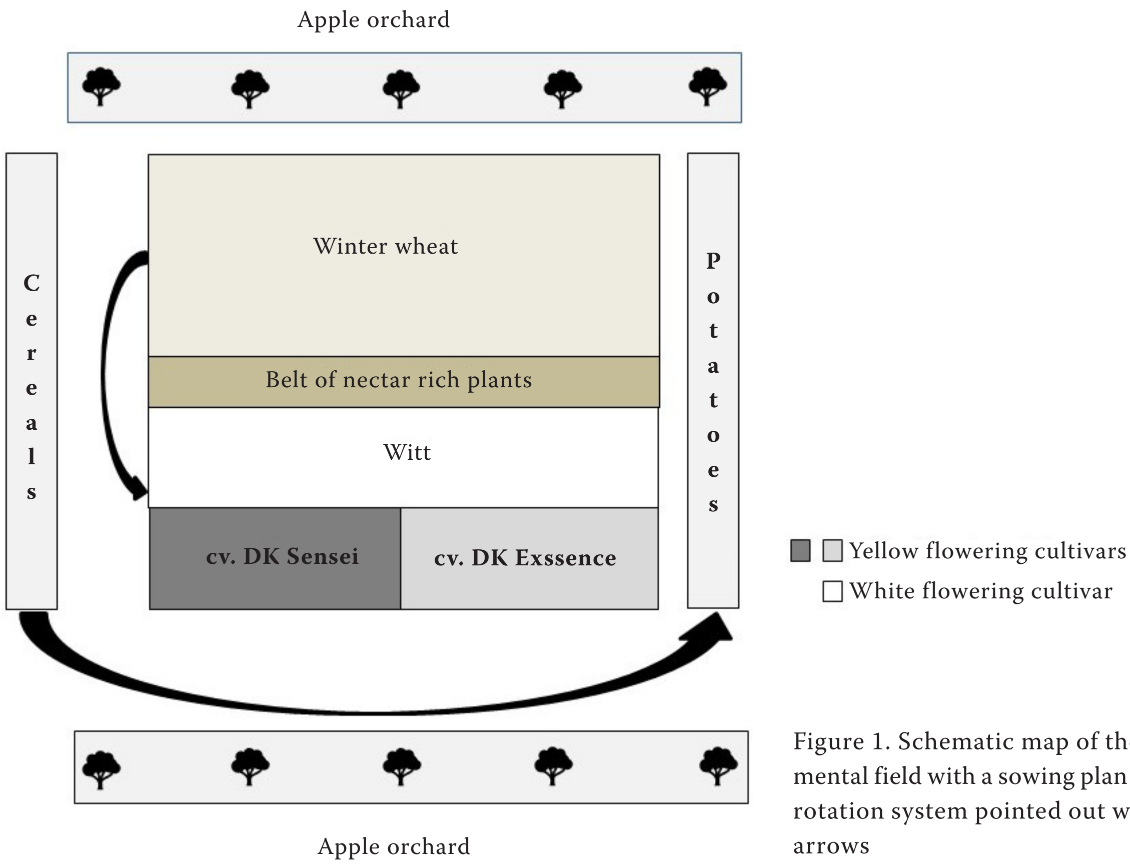

Figure 1. Schematic map of the experimental field with a sowing plan and crop rotation system pointed out with black arrows

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of yellow-flowering cultivars was in total 0.4 ha as the white-flowering cultivar. The plots were left untreated by insecticide, permitting natural infestation by the PB and CSW. Fertilisers (autumn: 40 kg N/ha of UREA$^{stabil®}$, Agra a.s., Střelské Hoštice, Czech Republic; spring: 80 kg N/ha of UREA$^{stabil®}$, Agra a.s., Střelské Hoštice, Czech Republic with StabilureN®, Agra a.s., Střelské Hoštice, Czech Republic) and herbicides (only in autumn: Butisan® Complete (2.25 L/ha), Stratos® Ultra (1.2 L/ha) + Dash® HC (1.2 L/ha), BASF, Prague, Czech Republic and Galera (0.35 L/ha), Dow AgroSciences s.r.o., Prague, Czech Republic) were added as in conventional operating conditions to all cultivars the same way. Two methods of insect sampling, direct counting and sweep netting, were performed in all experimental plots. The samplings of evaluated insects were replicated within a single plot of each cultivar.

Plant material. The yellow-flowering cv. DK Exssence (Monsanto Technology LLC, St. Louis, USA) is a pollen-fertile hybrid with low glucosinolate and erucic acid contents. This cultivar has earlier phenology than the other cultivars (DK Sensei and Witt) used in this experiment, so it flowered first. The other yellow-flowering cultivar, DK Sensei (Monsanto Technology LLC, St. Louis, USA), is a half-dwarf pollen-fertile hybrid with minimal erucic acid and glucosinolate contents. The DK Sensei cultivar is the latest-flowering of the cultivars used in this experiment. The white-flowering cultivar, Witt (Seed Service CZ, Inc.), has plants of an intermediate-range. The phenology of this cultivar is later than that of DK Exssence but earlier than that of DK Sensei. The cv. Witt starts flowering approximately one week after cv. DK Exssence and one week before cv. DK Sensei.

Insect sampling

Direct counts of the pollen beetle. Adult PBs were counted directly from oilseed rape inflorescences in 2016, 2017 and 2018. The beetles from randomly selected plants from each cultivar were beaten down by shaking of the plant from inflorescences onto a sheet of white paper and counted. The total number of beetles from 25 plants of each cultivar was evaluated in the spring during each census. One person collected the samples to ensure these were consistent. The number of censuses varied across years and depended on the duration of the flowering period (2016, 5 censuses; 2017, 7; 2018, 6). The sampling was done at the same time, regardless of the stage development of particular cultivars. The sampling started after the first collection of PB in yellow water traps. The first census began during BBCH 51 and lasted until BBCH 65. The counts were summed and averaged per plant.

Pest sampling by sweep netting. Adult PBs and CSWs were sampled from mid-April until mid-May in 2015, 2016 and 2018 and throughout May in 2017. Five (2015, 2016) and four (2017, 2018) censuses were performed in the period from BBCH 57 to BBCH 65. Insects were sampled on shine or partly cloudy skies at temperatures higher than 10 °C using an entomological sweep net (perimeter 100 cm). Each sample consisted of 75 sweeps in total (25 sweeps with 3 repetitions) on each cultivar. In the yellow flowering cultivars, the first repetition was performed 5 m from the edge of the plot, followed by the second in the middle of the plot and the third repetition 5 m from the edge adjacent to the neighbouring cultivar. Collected samples of insects were then transferred into labelled plastic bags and frozen (–32 °C) until identification. In the laboratory, adult PBs and CSWs were identified and counted using an Olympus SZX7 stereomicroscope (Tokyo, Japan). Pollen beetles were identified according to Kirk-Spriggs (1996), and CSW were identified according to Miller (1956).

Statistical analysis. Since we had only the data from visual counts of PBs on plants averaged per plant and census, we analysed the data using analysis of variance in R version 3.6.1 (R Team 2019), with a log-transformed response variable and cultivar, year (categorical) and their interaction as explanatory terms.

The sweep data were analysed using generalised linear models (GLMs) for the PB and CSW. Based on prior inspection of the residuals, we used a negative binomial error distribution with a log link function (R package MASS), which was superior to the Poisson distribution. The initial model included the cultivar, year (categorical) and their interaction as the explanatory variables and counts of the respective taxonomic group as response variables. The significance of the terms was assessed based on a likelihood ratio χ^2 test (R package car). The percentage difference in the abundance between the cultivars was estimated from a generalised mixed-effect model with a negative binomial error distribution (R package lme4; ref), with cultivar as a fixed effect and a random intercept for the year of sampling. The inclusion of the random effect was justified by a $\Delta AIC > 2$, which differed from the value in the corresponding fixed-effect model.

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RESULTS AND DISCUSSION

Direct counts of pollen beetle. At oilseed rape stages BBCH 51–65, the abundance of PBs per plant evaluated by direct quantification of inflorescences varied across the years ($F_{2,45}$ = 4.697, P = 0.014) but not with the cultivar used ($F_{2,45}$ = 1.027, P = 0.366). Averaged across the cultivars, the abundance per plant increased from 2016 (mean ± standard error; 2.65 ± 0.37) and 2017 (3.57 ± 0.61) to 2018 (10.58 ± 2.33). According to the Czech Central Institute for Supervising and Testing in Agriculture, the threshold number of PB adults on oilseed rape is 3 adults per plant or inflorescence (less than 3 adults/plant: low abundance, 3–10 adults/plant: intermediate abundance, 10 adults/plant and higher: high abundance). Across the study years and cultivars, an average of 5.66 ± 0.94 individuals per plant were reported through visual counts. The abundance of PBs (average number of beetles per plant) on the three cultivars of oilseed rape on various Julian dates over three years (2016, 2017 and 2018) is presented in Figure 2. Our results show that the PB was not able to distinguish the oilseed rape cultivars used in this study before flowering, but from the beginning of flowering, the PB preferred the yellow-flowering cultivars with the highest mean predicted abundance found for cv. Exssence (116.37, 95% CI: 48.42–280.03) over the white-flowering cv. Witt (50.55, 95% CI: 21.08–122.94).

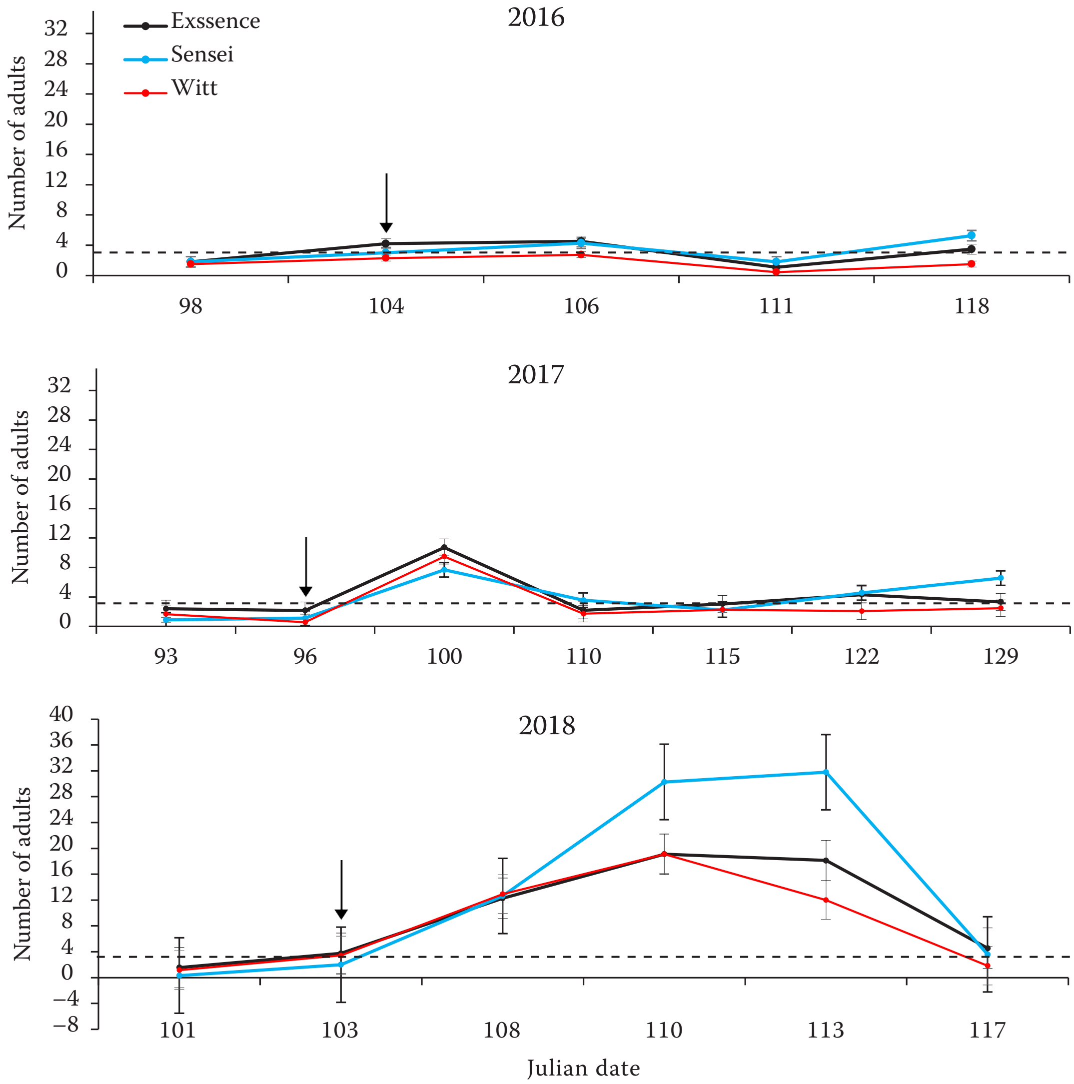

Figure 2. The abundance of *Brassicogethes aeneus* (average number of beetles per plant *via* direct counting = *y*-axis) on three cultivars of oilseed rape over three years (2016–2018) on Julian dates (*x*-axis) associated with BBCH 51–65. The arrows show the optimal treatment dates for the pollen beetle based on the abundance exceeding the threshold (shown by the dashed line))

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Table 1. The effects of oilseed rape cultivar and year of sampling on the abundance of pollen beetle and cabbage seed weevil on flowers (generalised linear model with negative binomial distribution). The significance of the terms was assessed based on a likelihood ratio χ^2 test

Term	Likelihood ratio	*df*	*P*
Pollen beetle			
Cultivar	20.29	2	< 0.001
Year	101.24	3	< 0.001
Cultivar × year	10.78	6	0.095
Cabbage seed weevil			
Cultivar	44.08	2	< 0.001
Year	78.84	3	< 0.001
Cultivar × year	6.56	6	0.363

Sweep netting of the pollen beetle. Just before and during flowering (BBCH 57–65), the abundance of the PB (as evaluated by sweep netting) in all cultivars was low in 2015 (1 623 adults in total), intermediate in 2016 (2 250 adults) and 2017 (2 050 adults) and high in 2018 (9 717 adults). Data from sweep netting demonstrated that adult PBs were able to discriminate between flowering oilseed rape cultivars and that the response to a particular cultivar was constant across the years included in this study (Table 1). The highest mean predicted abundance was found for cv. Exssence (116.37, 95% CI: 48.42–280.03), followed by cv. Sensei (100.40, 95% CI: 41.80–241.69), but the observed decrease in abundance (14%) was not significant ($z = -0.78$, $P = 0.44$). The cv. Witt with white flowers attracted significantly fewer individuals (50.55, 95% CI: 21.08–122.94; 57% reduction compared to cv. Exssence; $z = -4.29$, $P < 0.001$) than the two cultivars with yellow flowers. The overall variation in the abundance among the cultivars and across the years is shown in Figure 3A. The preference of the PB for yellow-flowering oilseed rape cultivars over white-flowering oilseed rape cultivars is also described by Giamoustaris and Mithen (1996), who stated that the abundance of the PB was approximately 50% lower on white-flowering oilseed rape than on yellow-flowering oilseed rape. Cook et al. (2013) found using different methodology (dyed flowers with a water solution of food colouring) that a white-flowering cultivar was as attractive to the PB as the same cultivar with yellow petals, but plants with red and blue petals were significantly less attractive. The high incidence of PBs on white flowers in the study by Cook et al. (2013) was interpreted as a consequence of their high UV reflectance. Döring et al. (2012) found that the number of PBs caught in red, blue, white, grey or black water traps (Petri dishes, diameter 14 cm) in comparison to yellow and green, where was the

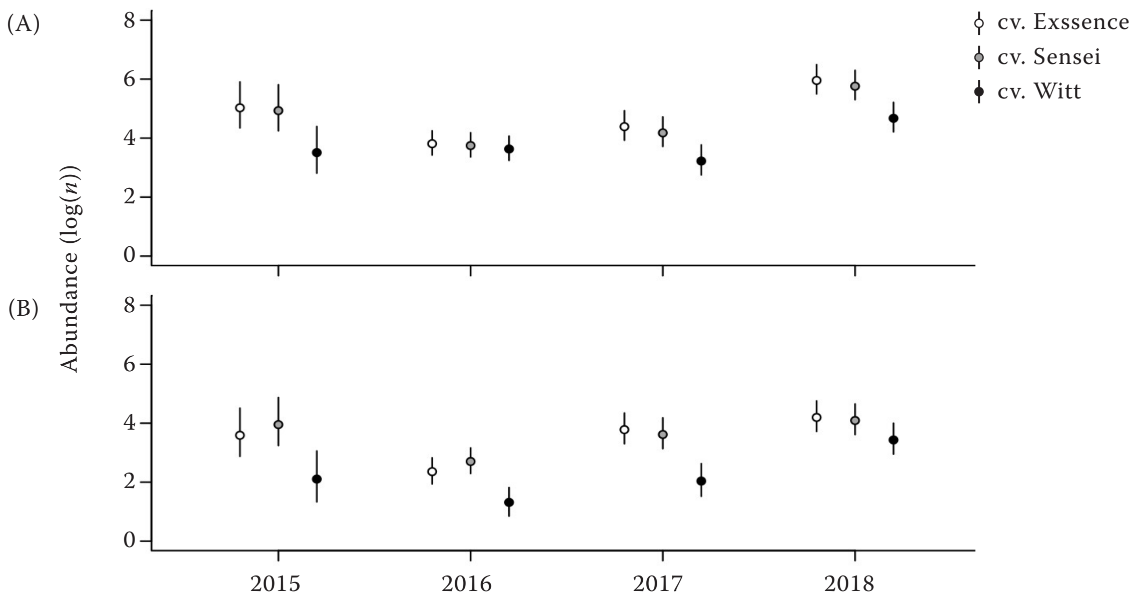

Figure 3. The abundance of (A) *Brassicogethes aeneus*, and (B) *Ceutorhynchus obstrictus* collected on three cultivars of oilseed rape over four years of sampling by sweep netting. The points represent the predicted mean abundance, and the vertical lines represent the 95% confidence intervals as predicted by a GLM (negative binomial distribution) ($n = 5$ for 2015, $n = 18$ for 2016, $n = 12$ for 2017 and 2018)

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highest number of beetles caught, was generally very low. These results support our study and proposed control strategy, which is listed below.

Sweep netting of the cabbage seedpod weevil. In the flowering period of oilseed rape (BBCH 61–65), the response of adult CSWs to the oilseed rape cultivars was very similar to that of the PB (Table 1). The mean density on cv. Exssence was 32.29 individuals (95% CI: 13.81–76.10), and the 10% increase in abundance on cv. Sensei (mean predicted abundance: 35.49, 95% CI: 15.23–83.91) was not significantly different from that on cv. Exssence (z = 0.47, P = 0.64). The CSW was less abundant on cv. Witt (mean predicted abundance: 10.00, 95% CI: 4.25–23.59; 69% reduction compared to cv. Exssence; z = –5.69, P < 0.001) than on the two cultivars with yellow flowers. The overall variation in abundance among the cultivars and across the years is shown in Figure 3B. In Canada, white mustard (*Sinapis alba* L.) was used in canola breeding for resistance to CSW damage (Dosdall and Kott 2006). Some canola genotypes that showed greater resistance to the CSW had different chemical compositions and glucosinolate contents in the pod developing seeds. The effectiveness of using trap crops to control the CSW was investigated in southern Alberta, Canada (Cárcamo et al. 2007). In one study, turnip rape flowered approximately one week earlier than the main crop, oilseed rape, and effectively concentrated CSWs, allowing them to be controlled with pyrethroid insecticide to prevent their movement into the main crops (Cárcamo et al. 2007).

In conclusion, knowledge about the relative attractiveness of oilseed rape cultivars to the PB and CSW can be utilised in pest control strategies adjusted for particular cultivars based on the push-pull strategy (Cook et al. 2007). At the beginning of immigration into oilseed rape fields, the PB prefers plants in the bud formation phase and neglects phenologically delayed plants or cultivars. Hence, it is recommended to sow cultivars with the earliest onset of bud formation on the marginal strips of a field. Acceleration of the phenophase of oilseed rape at field edges can be achieved by earlier sowing, reducing the doses of morphoregulators and lower dosing of nitrogen-based fertilisers. We propose that the cultivar preferred by the pest should be sown along the edges of a field with a width of a one-track line (approx. 24 m), which corresponds to the area of the sprayers used in this study. The cultivar less preferred by pests (i.e., the white-flowering cultivar) should be sown inside the plot. In plots larger than 20 ha, it is recommended to establish one or more central strips of the preferred cultivar, preferably in the longitudinal direction of the field.

In our four-year trial, we found that oilseed rape cultivars of different colours and phenologies have different attractiveness for PBs and CSWs. These findings can be used as a part of a push-pull control-based strategy. This can lead to a reduction in a limited range of pesticides and, as a result, protect natural enemies, which are important in pest population reduction. This control strategy, which considers cultivaral differences, works together with other approaches in an IPM system.

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