

## Early flowering field pea variety (*Pisum sativum* L.) as a trap crop for pea weevils (*Bruchus pisorum* L.)

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**Abstract:** *Bruchus pisorum* L., is an economically important pest of field pea (*Pisum sativum* L.) crops worldwide, however, no fully effective methods (conventional, alternative) of its control exist now. To fill this knowledge gap, possibilities of trap cropping were tested in the years 2015–2017. Two field pea varieties differing in the start of flowering, and one spring barley variety, were used to design four different types of crops, that were compared in large plot trials (2.82 ha). It was hypothesized the females would prefer zones planted with early flowering variety for oviposition. In all three seasons, females placed more eggs on pods in the parts of plots planted with the early flowering variety, and positive aggregation of the eggs and infested seeds were recorded there too. In cases where the egg-laying period is shorter than 14–21 days, the early flowering variety located at the margins should serve efficiently as a trap crop.

**Keywords:** integrated pest management; insect pest control; spatio-temporal distribution; SADIE; movement in landscapes; decrease in insecticide consumption

Insect pests are one of the major constraints of field pea production (Clement et al. 2000), among which the pea weevil, *Bruchus pisorum* L., is an economically important pest of field pea worldwide (Mendesil et al. 2016). Although the damage of seeds induced by *B. pisorum* can result in high losses of yield in warm regions (up to 40% in Australia, according to Smith 1990, or up to 59% according to Teka 2002 and Seyoum et al. 2012 in Ethiopia), in central, western, and northern parts of Europe, the yield loss usually does not exceed 5% (Seidenglanz et al. 2011a). The main concerns of pea weevil for farmers in Europe are mainly

based on the fact that even relatively slight infestation causes a significant decrease in the valuation of the harvest grain (Aznar-Fernández et al. 2018). Infested seeds show reduced germination (Nikolova & Georgieva 2015) and contain the alkaloid cantharidine, which makes the damaged seeds hazardous to human health (Grigorov 1976). Therefore, the infestation renders pea seeds both unsuitable for use as seed stock (very low levels of infestation are accepted by state authorities in Europe) and for human consumption (processors strictly accept only null levels of infestation in Europe, Seidenglanz et al. 2011a).

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Adult females attach eggs mainly on the surface of green pods. The eggs are orange-yellow and are 1.5 mm long and 0.6 mm wide (Brindley 1933; Smith & Hepworth 1992). The mean number of eggs per affected pod can vary from one to 33 under natural conditions (Smith & Hepworth 1992). The hatched larvae burrow through the pod wall and enter the immature seed, where they feed extensively on the seed contents while developing into fully mature, pre-emergent adults. Management of pea weevil is traditionally aimed at controlling adult beetles in the crop before they lay their eggs on pods (Horne & Bailey 1991; Smith & Hepworth 1992; Smith 1992). Monitoring of adults is often based on the usage of sweep nets, and threshold values move from one to two adults per 25 sweeps in Europe, the USA, and Australia (Blodgett 2006; Seidenglanz et al. 2011a; Baker 2016). However, there is still the great problem of making a good decision about the requirement and timing of insecticide application. It is not easy or reliable to monitor adults in pea fields using sweep nets just before flowering or while flowering (Seidenglanz et al. 2011a). It is technically complicated to move inside a pea crop for the evaluator and in addition to this, pea weevils show a clumped distribution, which decreases the value of the monitoring. This leads to poor timing of insecticide applications, which often miss the beetles themselves, as well as the period of egg-laying. The monitoring of eggs seems to be more useful. Spraying is then carried out at the time when the first eggs are seen on the pods (Horne & Bailey 1991; Seidenglanz et al. 2011a).

Usually, pyrethroids or neonicotinoids, or combinations of the active ingredients are used against the pest. From the neonicotinoids insecticides, only acetamiprid is available in the EU now, thiacloprid was banned from applying under field conditions in 2020. However, the effects of the applications are frequently inadequate. In some regions and seasons (warm regions: Australia, southern Europe), repeated sprayings are needed (Michael et al. 1990a; Baker & Phillips 1992). More often, spraying is carried out at a time when crops are in the flowering stage, so the negative effects of such applications on honeybees and other non-target and beneficial organisms are inevitable (Sharma et al. 2005; Teshome et al. 2015).

Field pea genotypes (commercial varieties, gene bank accessions, breeding materials) can differ substantially in the levels of attractiveness for ovipositing females (Matlosz & Kaniuczak 2001; Mendesil

et al. 2016; Aznar-Fernández et al. 2018; Aznar-Fernández & Rubiales 2019) and in the levels of larvae mortality during their development in plant tissues (Clement et al. 2009; Nikolova 2015, 2016). Even though *Pisum* accessions identified as being resistant have been reportedly incorporated into breeding programmes in Russia, EU, USA, Australia, and in Africa (Hardie & Clement 2001; De Sousa-Majer et al. 2007; Clement et al. 2009; Teshome et al. 2015; Aznar-Fernández et al. 2018; Kaplin et al. 2019), no field pea varieties resistant to this pest are available to farmers worldwide.

In such a situation, some effective alternative approach to pea weevil control is vitally necessary. One of the alternatives, which should have potential and on which this study is focused, is the system of trap cropping. According to the Shelton and Badenez-Perez review (2006), there are only ten cases of successful applications of trap cropping in agricultural and forest systems. Holden et al. (2012) noted another five successful applications. Although in the case of the pea weevil, there is a certain potential for use of trap cropping (Michael et al. 1990b; Sharma et al. 2005), successful cases relating to the insect pest remain poorly described. The *B. pisorum* egg-laying females may be a good subject for this type of management, due to their migration across the landscape, their host-finding behaviour (females need to ingest pea pollen to complete their development and to begin egg-laying; Clement 1992), and their good ability to distinguish among more and less suitable field pea genotypes for laying eggs (Mendesil et al. 2016; Aznar-Fernández et al. 2018; Aznar-Fernández & Rubiales 2019).

Unlike in warm regions, such as southern parts of Europe, Ethiopia, and Australia, where the egg-laying period of the pea weevil can continue for four or more weeks in some seasons (Michael et al. 1990a, b; Baker & Phillips 1992; Smith & Hepworth 1992; Smith 1992), in the Czech Republic (and in general in central, western and northern parts of Europe), the population of females present in one locality (i.e. within one field) usually lay eggs approximately for 14–21 days (Seidenglanz et al. 2011a).

We hypothesise that under field conditions *B. pisorum* females should prefer varieties with earlier onset of flowering and the earlier formation of pods for egg-laying and that they should start egg-laying on these plants when they have a choice at a locality.

The main objective of this study was to determine whether the design (composition and deployment)

of a field pea crop can affect within-field distribution patterns of *B. pisorum* eggs and seeds infested by the insect pest. The second objective is to determine if combining two field pea varieties in one field, which differ substantially regarding the time of onset of flowering, can result in the decrease of seed infestations induced by *B. pisorum* in the variety with the delayed start of flowering. Finally, determine whether the earlier field pea variety could effectively serve as a trap crop for *B. pisorum* egg-laying females and could prevent females from migrating to (and laying eggs on) the delayed variety crop (main crop) located in the vicinity.

## MATERIAL AND METHODS

**Trial design.** Distributions of pea weevil (*B. pisorum* L.) eggs, and infested seeds, were assessed under field conditions in years 2015, 2016, 2017 at four differently designed field pea crops (plots), which were surrounded by spring wheat (location: the north-east Czech Republic, Olomouc region, near the town of Šumperk; 2015: 49°59'02.1"N 16°59'45.6"E; 2016: 49°59'23.5"N 16°59'52.4"E; 2017: 50°00'18.7"N 17°01'04.7"E). The four plots were located side by side in one field. The experimental design was the same during all three seasons. Two field pea varieties were used, Cysterski and Protecta. The Cysterski variety usually starts flowering 7–14 days earlier than Protecta. Plot margins and inner strips planted with the Cysterski variety served as a trap crop and parts with the Protecta variety as the main crop (designed either as a mono-crop in plots FPIIA = field pea II type A, FPI = field pea I, and FPIIB = field pea II type B, or mixed

with spring barley in FP/B plot; FP/B = field pea intercropped with spring barley) (Table 1, Figure 1). Before the first assessment, 20 (in plots FPI, FPIIA, FP/B) or 35 (in plot FPIIB) assessment places (APs) were marked out in every plot. So, across the whole trial field (all four plots together), 95 APs (5 × 19) were distributed. The APs were located in a rectangular grid formation (Figure 2), with *x* and *y* coordinates. All assessments were then made on randomly selected plants growing near the individual APs. No insecticides or fungicides were applied during the vegetation period. One pre-emergent application of herbicide was made every year to all plots (1 183 g of pendimethalin/ha). In each of the

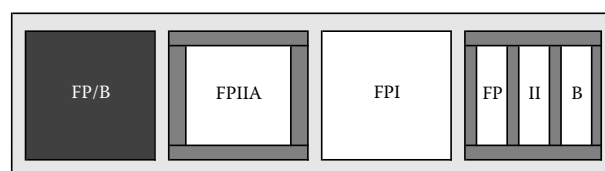


Figure 1. Trial design

Black area (FP/B) – a mixture of field pea v. Protecta (60% of normal seeding rate) and Azit spring barley variety (40% of normal seeding rate); dark grey area – Cysterski field pea variety, trap crop in this study; light gray area – spring wheat crop (Alicia variety, normal seeding rate); white area – Protecta field pea variety, principal crop for this study. The plots had the same composition, arrangement, and area (64 m × 104 m = 6 656 m<sup>2</sup> per plot) in the three years (2015–2017). The area of the inner Protecta zone in FPIIA: 52 m × 92 m = 4 784 m<sup>2</sup>. The area of one inner Protecta zone in FPIIB: 17 m × 98 m = 1 666 m<sup>2</sup>. The time of sowing was the same for all plant species used in the trial in each of the three years: April 24, 2015; April 26, 2016; May 2, 2017. The distance between the individual plots was 2 m

Table 1. Description of the four field pea plots and varieties used in the trials during the years 2015, 2016 and 2017

Plot (treatment)	Crop composition on plot	Description of the crop composition/design
FPI	one field pea variety	Protecta variety (recommended for organic agriculture, normal leaf variety, white flowers, yellow seeds, TSW: 210–230 g)
FPIIA	two field pea varieties combined on plot; design A	v. Protecta and v. Cysterski combined: Cysterski, (the earliest variety registered in CZ, semi-leafless variety, white flowers, yellow seeds, TSW: 220–230 g) usually starts its flowering period 7–14 days earlier than v. Protecta; v. Cysterski was arranged in 6 m wide strips surrounding the inner Protecta crop; see also Figure 1
FPIIB	two field pea varieties combined; design B	v. Protecta and v. Cysterski combined again: v. Cysterski was arranged in 3 m wide strips located on plot margins and also inside the plot; v. Protecta crop was divided into three smaller sections on the plot; see also Figure 1
FP/B	field pea and spring barley combined	Field pea v. Protecta (60% seeding rate) and spring barley v. Azit (40% seeding rate) mixture

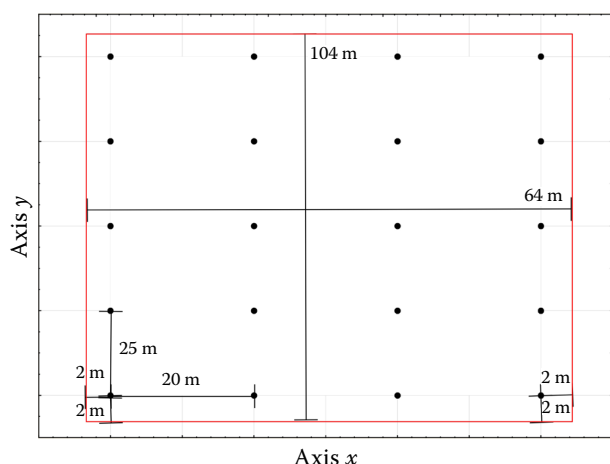


Figure 2. Trial design – rectangular grid formation

The black points indicate assessment places (AP) in plots FP/B, FPIIA and FPI. A rectangular grid consisting of 20 APs ( $= 5 \times 4$ ) was used in these plots. A different rectangular grid with 35 APs ( $= 5 \times 7$ ) was used in the plot FPIIB. More APs were necessary for that plot, due to the alternating strips of the two field pea varieties there, and the APs had to be placed in all Cysterski strips and all inner zones with Protecta

three years winter wheat (commercial crop) served as a previous crop.

**Data sampling.** *B. pisorum* eggs abundance at the plots, started to be assessed from the date when the first open flowers on plants in the Cysterski zones appeared (June 13, 2015; June 17, 2016; June 14, 2017). The assessments were repeated once per week. The plants occurring nearby the APs were examined. At the start, the eggs were assessed on the flower calyces (pods were not present yet), later, on the pods of the two bottom nodes. In this paper, the abundances of eggs recorded at the peak of the egg-laying period were analysed. The peak is defined as the date when the highest number of eggs on pods was recorded in the season: July 2, 2015; June 1, 2016 and June 4, 2017. At those dates, the eggs were counted on the pods located on the two bottom nodes of the racemes (most eggs are usually placed there; Seidenglanz et al. 2018). Five (2016) or ten (2015, 2017) plants per AP (this usually means 20 or 40 pods per AP) were examined. The plants or eggs were not damaged during these assessments. The total number of eggs attached to the two bottom nodes of the five or 10 plants per AP was determined. At the time of full crop maturity, 20 pods (again from the two bottom nodes) per AP were sampled (minimally from five randomly

selected plants per AP). In the laboratory, the total number and percentage of seeds infested by *B. pisorum* were determined for individual pods gathered from each AP. This assessment was carried out approximately two months after pod sampling, to enable to complete the development of *B. pisorum* larvae and pupas in the seeds. During the time the pod samples were stored in marked paper bags at a dry place under common room temperatures (17–27 °C). For the purposes of the study, an infested seed was defined as a seed, in which *B. pisorum* achieved at least the last larval stadium (seeds with prepared exit holes for an adult).

**Data analysis.** The abundances of *B. pisorum* eggs and the levels of seed infestation were statistically analysed using one-way ANOVA followed by the post hoc Tukey test (Statistica software version 12; StatSoft CR s.r.o.) to determine whether there were any significant differences in the mean levels of infestation among the compared plots and/or their parts (Figure 1).

Spatial patterns in the grid data (counts of eggs and infested seeds recorded at the individual APs) were analysed, using spatial analysis of distance indices (SADIE; Perry 1995; Perry 1998; Pearce & Zalucki 2006). The indices (results of SADIE) quantify the degree of non-randomness within a set of data. The null hypothesis tested is that the counts within the grid are arranged randomly with respect to each other. An index of aggregation ( $I_a$ ) equals 1, when the counts are arranged randomly in the grid. If  $I_a$  is greater than 1, the counts are aggregated into clusters.  $I_a$  and its associated probability ( $P_a$ ) indicate the overall degree of clustering ( $P_a$  is the probability that the observed counts are arranged randomly among the given APs and is considered significant when  $< 0.05$ ). Clusters can be given in the form of patches or gaps. Patches are defined as neighbourhoods of APs with counts (of eggs and/or infested seeds) that are larger than the overall grid mean. The index  $V_i$  and its associated probability  $P_i$  indicate the degree of patchiness. If the index  $V_i$  is greater than 1, some APs belong to a patch. Gaps are defined as neighbourhoods of APs with counts smaller than the overall grid mean. The index  $V_j$  and its associated probability  $P_j$  indicate the presence of gaps. If  $V_j$  is equal to minus 1 (–1), then there is a random arrangement of counts; but if  $V_j$  is less than minus 1 ( $< -1$ ), then gaps are present. For each distribution, SADIE determined the spatial characteristics of the

observed arrangement of counts by comparing it to randomised permutations of the same counts amongst the sampled locations (APs). The complete sets of the SADIE indices were derived both for the whole trial field (plots FP/B, FPIIA, FPI and FPIIB, assessed together as one plot; grid  $5 \times 19 = 95$  APs), and also for two plots assessed individually (FPIIA and FPIIB) in each of the three years, indicating whether the APs are located in an area where counts are randomly distributed or whether it falls within a patch or a gap in the distribution of counts. In some cases (distribution of infested seeds in 2015 and 2017), the location of patch and gap clusters in grids (plots) is illustrated by contour maps [gaps = places with clustering indices  $\leq -1.5$ , blue zones on maps; patches = places with clustering indices  $\geq +1.5$ , red zones on maps; Surfer (version 15, Golden Software LLC) was used; construction of maps according to Perry 1995; Perry 1998].

## RESULTS

**Differences in abundances of eggs.** The differences in the mean numbers of eggs recorded on bottom pods are listed in Table 2. In all three years, *B. pisorum* females preferred zones with Cysterski for laying eggs. The 6 m wide strips with Cyster-

ski located on the margins of plot FPIIA showed significantly higher attractiveness to pea weevil females than all the Protecta zones in the trial (plot FP/B, inner part of FPIIA, plot FPI, three inner parts of FPIIB) in all three years. The 3 m wide strips located on the margins and in inner parts of plot FPIIB showed high attractiveness to *B. pisorum* females in 2015 and 2017, but in 2016 the numbers of eggs recorded on plants in this zone were not significantly higher than in all the Protecta zones. If we look at all four plots together as one field (FP/B + FPIIA + FPI + FPIIB; total area:  $271 \text{ m} \times 104 \text{ m} = 2.82 \text{ ha}$ ; Figure 1), it is possible to state that, in 2015 and 2017, females clearly distinguished between the zones with Cysterski and Protecta, regardless of the type of plot and type of crop (monocrop or mixture). In 2016, they showed a distinct preference; only the 6 m wide Cysterski strips were markedly more attractive to them. In all three years, only the zones with Protecta surrounded with 6 m wide Cysterski strips (inner part of plot FPIIA) proved to be safely hidden from egg-laying females.

**Distribution of eggs.** Spatial distributions of eggs in the plots, even through some parts of the plots (zones with Protecta or Cysterski in combined plots), were not uniform. Even in more attractive Cysterski strips, there were some zones (= some parts of the strips) that females greatly

Table 2. Differences in mean numbers of eggs placed by pea weevil (*Bruchus pisorum*) females on bottom pods in different zones (Protecta vs. Cysterski) of plots in 2015, 2016 and 2017

Plot or plot zone	Assessment: July 2, 2015			Assessment: July 1, 2016			Assessment: July 4, 2017		
	mean <i>n</i> of eggs/2 bottom nodes <sup>1</sup>	SD	assessed plants ( <i>n</i> )	mean <i>n</i> of eggs/2 bottom nodes <sup>1</sup>	SD	assessed plants ( <i>n</i> )	mean <i>n</i> of eggs/2 bottom nodes <sup>1</sup>	SD	assessed plants ( <i>n</i> )
FP/B: Protecta + barley (the whole plot, mixture)	0.02 <sup>a</sup>	0.16	200	0.31 <sup>a</sup>	1.04	100	0.01 <sup>a</sup>	0.07	200
FPIIA: Protecta (the inner part of the plot)	0.00 <sup>a</sup>	0.00	60	0.07 <sup>a</sup>	0.25	30	0.00 <sup>a</sup>	0.00	60
FPIIA: Cysterski (6 m wide margin of the plot)	1.33 <sup>b</sup>	2.10	140	1.14 <sup>b</sup>	1.87	70	0.74 <sup>b</sup>	1.98	140
FPI: Protecta (the whole plot)	0.02 <sup>a</sup>	0.28	200	0.30 <sup>a</sup>	0.85	100	0.02 <sup>a</sup>	0.21	200
FPIIB: Protecta (the separated inner parts of the plot)	0.02 <sup>a</sup>	0.13	60	0.30 <sup>a</sup>	0.92	30	0.00 <sup>a</sup>	0.00	60
FPIIB: Cysterski (3 m wide strips)	1.69 <sup>b</sup>	2.72	220	0.44 <sup>ab</sup>	1.63	110	2.02 <sup>c</sup>	4.12	220
	$F_{(5,874)} = 39.764; P < 0.05$			$F_{(5,434)} = 5.059; P < 0.05$			$F_{(5,874)} = 25.777; P < 0.05$		

<sup>a-c</sup>Mean values listed in one column are statistically different when they are marked with different letters

<sup>1</sup>The value expresses the total number of eggs placed by females on pods occurring on two bottom nodes of an assessed fruitful shoot; there are usually four pods on the two bottom nodes

preferred, and others that were almost ignored by them (Figure 3). According to SADIE, distributions of *B. pisorum* eggs were arranged into several clusters (positive = patches even negative = gaps) scattered across the whole trial fields (plots FP/B + FPIIA + FPI + FPIIB analysed together) in 2015 and 2017. The eggs showed strong spatial aggregation even in the plots FPIIA and FPIIB analysed solely in 2015 and 2017. In 2016, the situation regarding the distribution of eggs proved to be different. The counts of eggs did not show a significant spatial pattern – they were arranged randomly throughout the whole trial field, even in the two plots (FPIIA, FPIIB) assessed alone. The SADIE results confirmed that egg-laying females of *B. pisorum* behaved differently in 2016 from in 2015 and 2017 (Table 3).

**Differences in the levels of seed infestation.** In all three years, the levels of seed infestation were higher in strips planted with Cysterski, in comparison with the plots (or parts of plots) with late flowering variety Protecta. In 2015 and 2016, the levels of seed infestation in both Cysterski zones (FPIIA and FPIIB) were similar (they did not differ significantly). In 2017, the seeds harvested from 3 m wide strips were significantly more infested than the seeds from the 6 m wide margin of the FPIIA plot. The inner part of plot FPIIA with the Protecta variety showed levels of seed infestation below the value of 1% in all three years. Therefore, this was the best-protected area in the trial field. The other zones with Protecta (plots FP/B and FPI, inner parts in FPIIB) were not so safely protected –

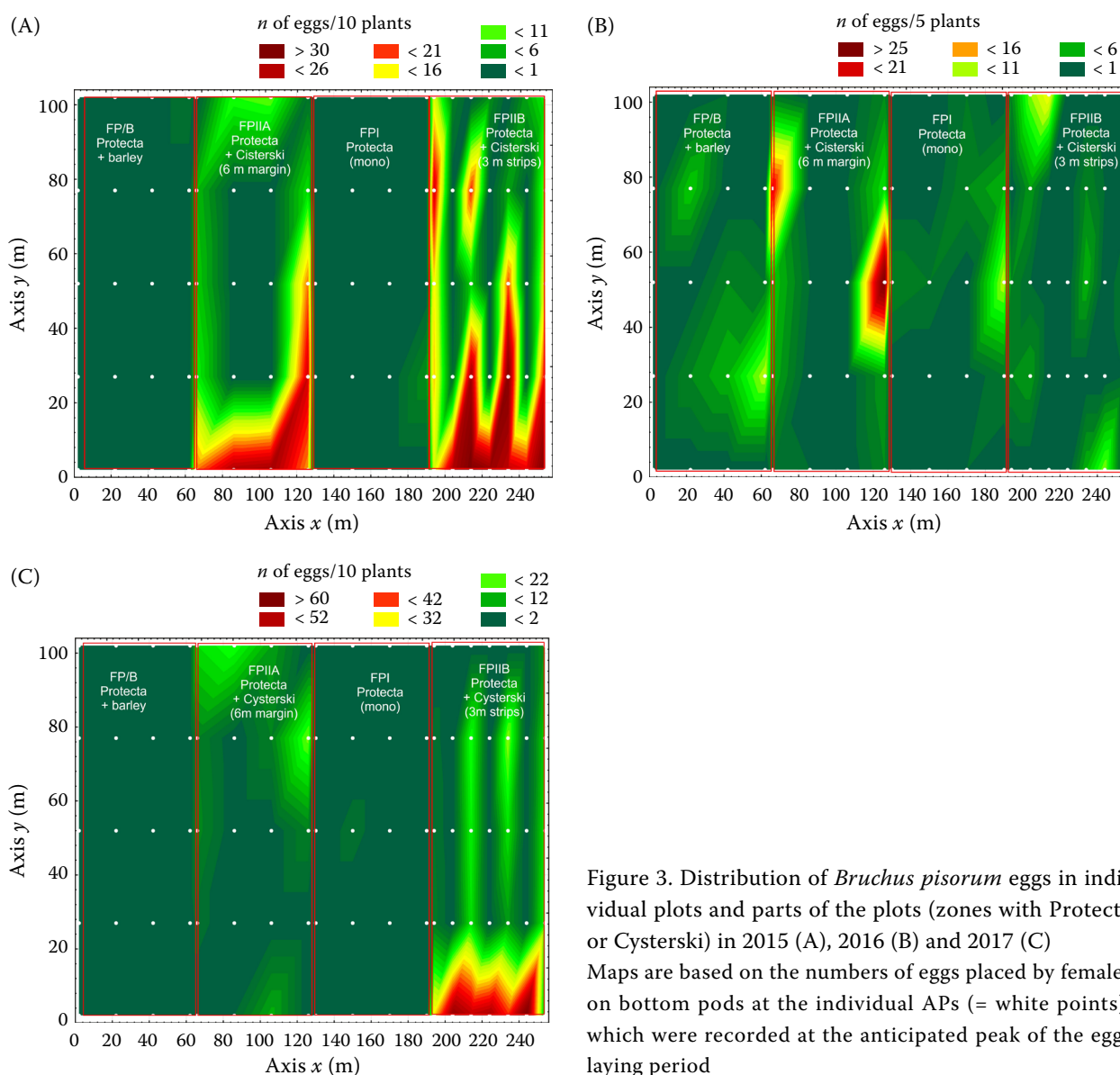


Figure 3. Distribution of *Bruchus pisorum* eggs in individual plots and parts of the plots (zones with Protecta or Cysterski) in 2015 (A), 2016 (B) and 2017 (C) Maps are based on the numbers of eggs placed by females on bottom pods at the individual APs (= white points), which were recorded at the anticipated peak of the egg-laying period

Table 3. Results of SADIE describing distribution patterns of *Bruchus pisorum* eggs in crops

Date of assessment	Crop	$I_a (P_a)$	$V_j (P_j)$	$V_i (P_i)$
July 2, 2015	FP/B + FPIIA + FPI + FPIIB	<b>2.46</b> (0.000 2)	–2.22 (0.000 5)	2.49 (0.000 3)
	FPIIA	<b>1.58</b> (0.012 8)	–1.49 (0.025 6)	1.40 (0.012 8)
	FPIIB	<b>2.11</b> (0.000 2)	–2.24 (0.000 2)	2.00 (0.001 5)
July 1, 2016	FP/B + FPIIA + FPI + FPIIB	0.79 (0.865 1)	–0.78 (0.893 7)	0.82 (0.828 9)
	FPIIA	0.90 (0.656 1)	–0.91 (0.634 2)	1.03 (0.336 9)
	FPIIB	1.11 (0.244 7)	–1.10 (0.270 5)	1.30 (0.114 6)
July 4, 2017	FP/B + FPIIA + FPI + FPIIB	<b>2.44</b> (0.000 2)	–2.27 (0.000 3)	2.18 (0.000 7)
	FPIIA	<b>1.37</b> (0.041 7)	–1.38 (0.041 1)	1.54 (0.011 7)
	FPIIB	<b>2.37</b> (0.000 2)	–2.23 (0.000 0)	2.54 (0.000 0)

$I_a$  – if the values are greater than 1 for  $P < 0.05$  (bold values), the counts (numbers of eggs on bottom pods) are aggregated into clusters;  $V_i$  – there are significant patches in the distribution if the values are greater than 1 for  $P < 0.05$ ;  $V_j$  – there are significant gaps in the distribution if the values are lower than –1 for  $P < 0.05$

The distributions were analysed for all four plots together as one field (FP/B + FPIIA + FPI + FPIIB; total area: 271 m × 104 m = 2.82 ha; grid 5 × 19 = 95 APs), and also for the plots FPIIA (grid 5 × 4 = 20 APs) and FPIIB (grid 5 × 7 = 35 APs) alone (in both cases area: 64 m × 104 m = 0.67 ha) in all three seasons (2015–2017)

at least in one of the three years, in the case of Protecta in plot FPI (Table 4).

**Distribution of infested seeds.** When all four plots were assessed together, the distribution of infested seeds showed strong spatial aggregation in 2015 and 2017 (Table 5). The patches were located especially in strips planted with early flowering variety Cysterski – parts of plots FPIIA and

FPIIB (Figure 4). In 2016, there was a random distribution of infested seeds across the whole trial field. A significant spatial pattern in FPIIA (when the plot was analysed individually) was recorded only in 2015. Contrary to that, in FPIIB the infested seeds showed non-random distribution in all three years, and there were significant patches and gaps in the distribution (Table 5).

Table 4. Differences in mean proportions of infested seeds induced by *Bruchus pisorum* in bottom pods sampled from different zones (Protecta vs. Cysterski) of plots in 2015, 2016 and 2017

Plot or plot zone	Assessment: September 2015			Assessment: October 2016			Assessment: October 2017		
	proportion of infested seeds/1 bottom pod (%) <sup>1</sup>	SD	assessed pods ( $n$ ) <sup>1</sup>	proportion of infested seeds/1 bottom pod (%) <sup>1</sup>	SD	assessed pods ( $n$ ) <sup>1</sup>	proportion of infested seeds/1 bottom pod (%) <sup>1</sup>	SD	assessed pods ( $n$ ) <sup>1</sup>
FP/B: Protecta + barley (the whole plot, mixture)	0.10 <sup>a</sup>	1.41	400	1.93 <sup>ab</sup>	10.49	400	2.97 <sup>b</sup>	10.25	400
FPIIA: Protecta (the inner part of the plot)	0.36 <sup>a</sup>	2.91	120	0.79 <sup>a</sup>	5.38	120	0.56 <sup>a</sup>	3.75	120
FPIIA: Cysterski (6 m wide margin of the plot)	5.81 <sup>b</sup>	11.48	280	3.77 <sup>b</sup>	14.81	280	4.23 <sup>b</sup>	14.06	280
FPI: Protecta (the whole plot)	0.25 <sup>a</sup>	2.01	400	1.63 <sup>ab</sup>	8.62	400	0.62 <sup>a</sup>	4.71	400
FPIIB: Protecta (the separated inner parts of the plot)	0.12 <sup>a</sup>	1.30	120	1.89 <sup>ab</sup>	8.25	120	3.22 <sup>b</sup>	10.00	120
FPIIB: Cysterski (3 m wide strips)	5.70 <sup>b</sup>	10.06	440	2.21 <sup>ab</sup>	10.25	440	10.55 <sup>c</sup>	22.27	440
	$F_{(5,1754)} = 54.324, P < 0.05$			$F_{(5,1754)} = 2.1324, P < 0.05$			$F_{(5,1754)} = 26.104, P < 0.05$		

<sup>a–c</sup>Mean values listed in one column are statistically different when they are marked with different letters

<sup>1</sup>Approx. two months after sampling ripe pods, the portion of infested seeds for each of the pods was recorded – in this way we increased the value of  $n$  for statistical analysis



Table 5. Results of SADIE describing distribution patterns for the levels of seed infestation induced by *Bruchus pisorum* in crops

Season	Crop	$I_a (P_a)$	$V_j (P_j)$	$V_i (P_i)$
2015	FP/B + FPIIA + FPI + FPIIB	<b>2.11</b> (0.000 7)	–1.92 (0.003 4)	2.19 (0.000 2)
	FPIIA	<b>1.45</b> (0.028 3)	–1.39 (0.039 2)	1.43 (0.026 6)
	FPIIB	<b>2.05</b> (0.000 7)	–2.37 (0.000)	1.95 (0.001 7)
2016	FP/B + FPIIA + FPI + FPIIB	0.99 (0.423 5)	–0.99 (0.403 1)	1.08 (0.263 3)
	FPIIA	0.77 (0.489 4)	–0.98 (0.456 2)	0.89 (0.747 3)
	FPIIB	<b>1.60</b> (0.017 1)	–1.66 (0.013 6)	1.52 (0.034 7)
2017	FP/B + FPIIA + FPI + FPIIB	<b>1.96</b> (0.001 5)	–1.76 (0.007 5)	1.68 (0.015 8)
	FPIIA	1.03 (0.352 4)	–1.01 (0.391 0)	1.02 (0.364 0)
	FPIIB	<b>1.87</b> (0.001 5)	–1.75 (0.004 9)	2.13 (0.000 0)

$I_a$  – if the values are greater than 1 for  $P < 0.05$  (bold values), the counts (portions of infested seeds in pods) are aggregated into clusters;  $P$  – associated probability;  $V_i$  – there are significant patches in the distribution if the values are greater than 1 for  $P < 0.05$ ;  $V_j$  – there are significant gaps in the distribution if the values are lower than –1 for  $P < 0.05$

The distributions were analysed for all four plots together as being one field (FP/B + FPIIA + FPI + FPIIB; total area: 271 m × 104 m = 2.82 ha; grid 5 × 19 = 95 APs), and also for the plots FPIIA (grid 5 × 4 = 20 APs) and FPIIB (grid 5 × 7 = 35 APs) alone (in both cases area: 64 m × 104 m = 0.67 ha) in all three seasons (2015–2017)

**Interannual differences in *B. pisorum* egg-laying behaviour and preferences.** In 2016, the season when the effect of early flowering variety Cysterski as a trap crop was lower, the pea plants were heavily damaged by fungal pathogens infecting the roots (*Pythium* spp., *Aphanomyces euteiches* and *Fusarium oxysporum* f. sp. *pisi*), and

were later also invaded by pea aphids (*Acyrtosiphon pisum* Harris, 1776) – this occurred before *B. pisorum* appeared in the locality. In the zones occupied by the early flowering variety (Cysterski), the symptoms of damage were more apparent, than in zones with the late flowering variety Protecta (Protecta showed a somewhat higher lev-

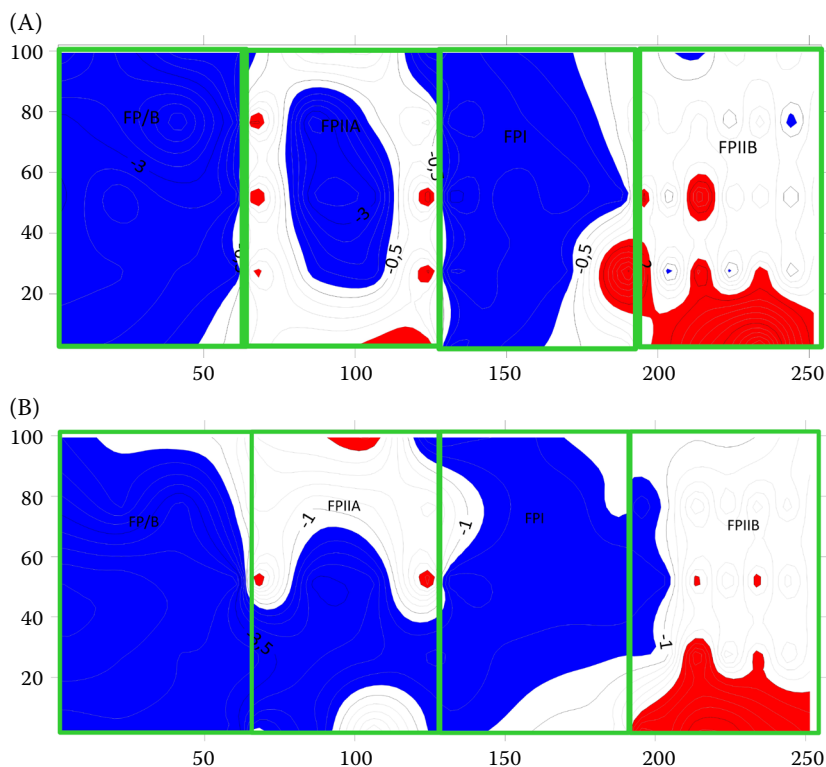


Figure 4. Results of SADIE for all four plots (FP/B + FPIIA + FPI + FPIIB) analysed as one grid, for seasons 2015 (A) and 2017 (B)

Blue zones – gaps; red zones – patches



el of tolerance to the harmful organisms). In the season, Cysterski inflorescences had fewer fruitful nodes and fewer flowers, and the onset of flowering was delayed. *B. pisorum* females probably did not perceive the early flowering variety as significantly more attractive than zones planted with the late flowering variety. According to SADIE results, there were random distributions of eggs and infested seeds across the trial field. The females probably tended to leave the Cysterski strips earlier, because the plants of the variety were poor in general, and the time period between the onset of flowering of the two varieties was substantially shorter in 2016 than in 2015 or 2017. The early flowering variety showed both a lower level of attractiveness (smaller differences in mean numbers of eggs on pods among the compared parts of plots), as well as a decreased level of retention (smaller differences in mean portions of infested seeds between the adjacent Cysterski and Protecta zones) in 2016 than in 2015 and 2017. Despite this fact, the 6 m wide strips of Cysterski were apparently somewhat more distinguishable by females, than the 3 m wide strips and were able to hold (= to arrest) them for a longer time. Probably because of this, even in that year (2016), the level of seed infestation in the inner Protecta zone of the plot FPIIA, stayed relatively low (below the 1% level).

In 2017, the 3 m wide strips of Cysterski again failed as an effective trap crop (at least partly), but the situation was markedly different from 2016. The crops of both varieties grew and developed well, the onset of flowering in Cysterski zones being about 10–14 days earlier than in the Protecta zones. However, the weather conditions during the spring and, at the beginning of summer were unusually warm for the region, the population of *B. pisorum* adults was more abundant than in the two previous seasons and the migration from the surroundings into the trial field was also lengthier. Because of this, the egg-laying period was also prolonged. Although the Cysterski zones attracted females significantly more than the Protecta zones, which was confirmed by the differences in the numbers of eggs on Cysterski and Protecta pods recorded at the peak of the egg-laying period, and the 3 m wide strips were even significantly more attractive than the 6 m wide strips, the crucial factor was the level of arrestment in this season. The 6 m wide strips probably

have a better ability for arresting *B. pisorum* females for a longer period than the 3 m wide strips. This was the most important factor for trap crop effectiveness in 2017. The SADIE results (based on the distribution of infested seeds) indicate a higher level of retention of females in the 6 m wide Cysterski strips, in comparison with the 3 m strips, not only in 2017 but also in 2015. This is apparent from the positions and sizes of patches and gaps distributed across the trial field. There are large patches (indicating a high level of attractiveness), but only small and infrequent gaps in the plot with 3 m wide strips of Cysterski (2015, 2017). This indicates a lower ability to prevent emigrations of *B. pisorum* females from the Cysterski zones to the Protecta zones in their vicinity. Contrary to this, in the vicinity of the 6 m wide strips of Cysterski, markedly larger gaps (located in Protecta parts) were visible in 2015 and 2017. That is apparent from Figure 4.

## DISCUSSION

The early flowering variety Cysterski zones covered approx. 11% of the whole trial field, but in specific plots, the levels of trap crop coverage in relation to the area of the adjacent late-flowering Protecta zones differed substantially. The best-protected area against *B. pisorum* infestation in the trial field was the Protecta zone, surrounded by a 6m wide margin of Cysterski. Another relatively safe zone, well hidden from *B. pisorum* in 2015 and 2017, was the plot that should simulate a common field pea monoculture. In fact, Protecta in this plot probably benefitted from its position in the trial field – the plot was surrounded by Cysterski strips on both sides (a 6 m wide strip on the left and a 3 m wide strip on the right). So, the late-flowering variety Protecta in this plot was in a similar situation to the Protecta completely surrounded by 6 m wide Cysterski strip. The Protecta crop, divided into relatively small zones surrounded by 3 m wide strips of trap crop (early flowering variety Cysterski), was not so safely protected from *B. pisorum* infestation. And the presence of another plant species in the crop (barley as a companion plant in a mixture with Protecta) seems to have no (or marginal) effect on the final levels of seed infestation induced by *B. pisorum*.

In herbivory insects that use olfactory or visual cues to find plants, the actual aggregation in the trap crop should usually be a combination of attraction and arrestment (Banks & Ekbom 1999; Smith & McSorley 2000; Shelton & Badenes-Perez 2006). So, to be effective as a trap crop, an early flowering field pea variety initially must achieve a sufficient level of attractiveness for migrating *B. pisorum* females, and then maintain a high level of retention of them for a sufficiently long time. Optimally, a sufficiently long time means the total duration of the egg-laying period of the pea weevil population present within the field. To fulfill the first condition, the variety intended as a trap crop should start flowering substantially earlier than the later principal variety located in the vicinity. Such variety also must serve as a good source of pollen to initiate the start of egg-laying (Clement 1992) precisely in the zone. However, the strength of arrestment seems to be the more important parameter influencing the effectiveness of a trap crop for insects with post-alighting host-recognition behaviour (Bukovinsky et al. 2005; Potting et al. 2005; Holden et al. 2012). It is obvious from the results presented in this paper, that the *B. pisorum* egg-laying activity was not limited only to the zones intended as a trap crop but that finally extended also into the zones designed as a principal crop (late-flowering variety). The findings confirm the results of Holden et al. (2012), which demonstrate the importance of retention (= arrestment) as a factor for successful usage of trap cropping systems. Based on their simulation model, in the case when the target insect pest is relatively mobile (and this is the case of *B. pisorum* adults) and the trap crop coverage is more than 2% of the landscape, trap cropping is only effective if trap crop retention is very high. According to Holden et al. (2012), it is more important for the successful usage of a trap crop to have a higher level of arrestment than to invest in a further increase of its attractivity (if the level is not too low, of course).

Based on our results, it seems that the simple increase of plant diversity, i.e. the addition of one non-host plant species (spring barley, in the case of this study) to a field pea crop probably has no (or only a small) effect on pea weevil behaviour. Even though the effect of plant diversity on the final levels of seed infestation induced by *B. pisorum* seems to be negligible, some authors (Hele-

nus 1991; Bedoussac et al. 2010; Seidenglanz et al. 2011b) demonstrated in their studies that pea aphid (*Acyrtosiphon pisum* Harris, 1776) colonies usually decline in mixtures of field peas (or other legumes) and cereals significantly earlier than in field pea monocultures. Therefore, increased plant diversity in field pea crops (mixture of field peas with cereals) has different effects on the two main insect pests of field peas, pea weevils and pea aphids. According to Root's (1973) hypothesis, crop diversity would tend to reduce densities of monophagous herbivores (*B. pisorum* is strictly monophagous) rather than polyphagous ones (the range of the pea aphid's host plants is wider, but it is not a polyphagous species). According to Andow (1991), only 8% of monophagous herbivores had higher densities in polyculture, whereas 59% had lower densities. The behaviour of pea weevils is shown to be slightly in contrast with these general expectations.

In general, the results of this study confirm the practical potential of combining two field pea varieties for the development of a successful trap cropping system for the pea weevil. In fact, some authors have already considered the usage of this access. But in practice, this method has not yet been used in field peas (Michael et al. 1990a; Smith & McSorley 2000; Sharma et al. 2005; Reddy et al. 2017). Some successful trap crop systems, in which the trap crop is the same species as the main crop, have been developed in legumes. Early maturing varieties of soybean have been used as a trap crop to reduce densities of the green stink bug *Nezara viridula* (L.) on soybean (McPherson & Newsom 1984; Todd & Schumann 1988). In general, manipulation of crop phenology within a given crop species is an important approach to trap cropping that uses mixed cultivars of the same crop species, or even early plantings of the same cultivar as the intercropped plants (Smith & McSorley 2000; Gripenberg et al. 2010).

It seems it would be practical in commercial breeding programmes to aim at the production of field pea varieties with markedly earlier onset of the flowering period too and make them available for farmers. Such varieties should be possible to use as trap crops for *B. pisorum*. The reason is that commonly available commercial varieties in European catalogues are relatively uniform in such traits as the time of the beginning of flowering and the length of the flowering period.

The following may be concluded, based on our findings:

The design (composition and deployment) of a field pea crop affects within-field distribution patterns of *B. pisorum* eggs and seeds infested by the insect pest.

Combining two field pea varieties which differ from one another substantially regarding time of onset of flowering, in one field, results in the decrease of seed infestations induced by *B. pisorum* in the variety with the delayed start of flowering.

The early flowering field pea variety could serve effectively as a trap crop for *B. pisorum* egg-laying females and could prevent the females from migrating to (and egg-laying on) the late flowering variety crop (main crop) located in the vicinity in regions (or in seasons) where (when) the periods of egg-laying of *B. pisorum* are not too long (14–21 days). This should be common to the central, western, and northern parts of Europe. The use of an early flowering field pea variety as a trap crop has practical potential when the onset of flowering (and also the time of formation of the first pods) in the principal variety is substantially delayed (10–14 days).

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