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SPATIO-TEMPORAL DISTRIBUTIONS AND ASSOCIATIONS OF CABBAGE STEM WEEVIL (*CEUTORHYNCHUS PALLIDACTYLUS* MARSHAM, 1802) AND POLLEN BEETLE (*BRASSICOGETHES AENEUS* FABRICIUS, 1775) IN WINTER OILSEED RAPE

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Abstract

HLAVJENKA VOJTĚCH, SEIDENGLANZ MAREK, ŠAFÁŘ JAROSLAV. 2017. Spatio-temporal Distributions and Associations of Cabbage Stem Weevil (*Ceutorhynchus Pallidactylus* Marsham, 1802) and Pollen Beetle (*Brassicogethes Aeneus* Fabricius, 1775) in winter oilseed rape. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 65(3): 839–847.

From 2013 to 2015 the within-field spatio-temporal distributions and interactions between *Ceutorhynchus pallidactylus* and *Brassicogethes aeneus* in winter oilseed rape crops were assessed using SADIE analysis (Spatial Analysis by Distance IndicEs) and Quick association analysis. A significant tendency for aggregation was recorded in *C. pallidactylus* males in the crop in both 2013 and 2015. Females showed a high tendency towards aggregation only in 2013. Both sexes were significantly associated in crops in 2013 and 2015. *B. aeneus* adults showed a high tendency towards aggregation in all three years. From the three seasons the cumulative distributions (based on the sums of insect catches in traps for the individual dates) of males and females of *C. pallidactylus* were significantly associated with the cumulative distribution of *B. aeneus* adults only in 2013. Contrary to this, an almost significant dissociation between cumulative distributions of *C. pallidactylus* males and *B. aeneus* adults was recorded in 2014.

Keywords: SADIE analysis, quick association analysis, pests, brassica napus

INTRODUCTION

Oilseed rape (*Brassica napus* L.; Brassicaceae) crops are host to a diverse community of invertebrates, including many pest and beneficial species (e.g. Kazda, 1958; Láska and Kocourek, 1991; Bezděk, 1997; Alford *et al.*, 2003; Tóth, 2013). For future advances in integrated pest management (IPM) strategies, including improved risk assessment and the possibility of improved targeting of biological control, more understanding is needed of the distribution and within-field spatio-temporal interactions of the main pests and their naturally occurring biological control agents (natural enemies) (Warner *et al.*, 2000; Ferguson *et al.*, 2006).

The spatial relationships between insects and crop plants remain an aspect of pest/host-plant ecology which has so far received insufficient attention. The spatial distribution of insect pests infesting oilseed rape was the subject of studies by Free and Williams (e.g. Free and Williams, 1978; 1979 a, b). Insects were sampled along a line transect into crops, and from discrete points at the edges and centres of crops, to determine pest distribution during their immigration and infestation of the crop (Warner *et al.*, 2000). However, there is still a lack of detailed information on the spatial heterogeneity of pest populations in oilseed rape at crop level, and the effect of this heterogeneity on crop yield and its implications for sampling and decision-making in integrated pest management (IPM). Spatial information is also needed for studies of insect movements into and within crops (Murchie *et al.*, 1999) to underpin the development of pest control strategies in which the application of pesticides may be spatially targeted to minimize their negative impact on beneficial insects (Evans *et al.*, 1998). The spatial distribution pattern for an insect species was often measured using the relationship between the variance and mean in the past (Taylor, 1984; Clark and Perry, 1994) and spatial association between different populations in crops has been measured using a correlation coefficient. These approaches do not utilize information about the location of insect counts. In the past decade, the development of novel spatial statistics, notably Spatial Analysis by Distance IndicES (SADIE, Perry, 1998 a, b) has enabled spatial information in a two dimensional array of sample counts to be used as part of the analysis, giving a much more detailed and informative picture of the pattern of crop colonization by insect species. This method enables any association between different species to be analysed (Williams and Ferguson, 2010; Ferguson *et al.*, 2000).

Psylliodes chrysocephala (L., 1758), *Ceutorhynchus pallidactylus* (Marsham, 1802), *C. napi* Gyllenhal, 1837, *C. assimilis* (Paykull, 1792), *Brassicogethes aeneus* (F., 1775) and *Dasineura brassicae* (Winnertz, 1853) are considered to be the most important insect pests in winter oilseed rape crops in Europe (Williams and Ferguson, 2010). In this paper we focus on two of the six key insect pests: cabbage stem weevil (*C. pallidactylus*; Coleoptera: Curculionidae) and the pollen beetle (*B. aeneus*; Coleoptera: Nitidulidae). *C. pallidactylus* adults emerge from hibernation in the spring (March/April) and migrate to rape crops. After a period of feeding and mating the females lay their eggs into the petioles or mid-ribs of the leaves (March to June). The harmful stage is the larva. There are three larval instars; first and second instars tunnel inside the leaf petioles and midribs, but later move into the stems. *B. aeneus* adults also emerge from overwintering sites in spring (March–June). When temperatures exceed 12–15°C, they migrate to oilseed rape plants. Just the immigrating adults of this species form the harmful stage. They seriously damage flower buds (Alford *et al.*, 2003; Williams and Ferguson, 2010).

The first objective of this paper is to describe the within-field spatial distributions of *C. pallidactylus* and *B. aeneus* adults in winter oilseed rape crops using SADIE analysis.

The second objective of this paper is to investigate if there are some spatial associations between *B. aeneus* adults and both sexes of *C. pallidactylus* (interspecific association) and also between both sexes of *C. pallidactylus* (intraspecific association) in crops using Quick association analysis.

MATERIALS AND METHODS

Crop site and insect sampling

The trials were located in Rapotín (near the town of Šumperk; Olomouc region, Czech Republic), carried out under field conditions in the course of 2013–2015. Uniformly managed (same conventional tillage operations, same fertilizing and same pre-emergence herbicides used) winter oilseed rape fields were used as experimental crops in all three years. The acreages of the rape fields differed in the individual years: 1.4-ha (2013), 0.8-ha (2014, 2015). Fields had regular rectangular shapes in all three years.

Adults of *C. pallidactylus* and *B. aeneus* were sampled from April 8th (BBCH 50) to May 3rd (BBCH 62) in 2013, from March 3rd (BBCH 32) to April 11th (BBCH 55) in 2014 and from March 23rd (BBCH 13) to June 3rd (BBCH 72) in 2015 using yellow water traps (diameter: 20 cm; water + several drops of detergent; YWT) installed across the crop in a rectangular grid formation ($6 \times 6 = 36$ sampling places in 2013, $5 \times 5 = 25$ places in 2014 and 2015). Each YWT (individual sampling point) was designated by its x and y coordinates. The distances between individual sampling places were different in 2013 compared to 2014 and 2015 due to differences in acreages of the fields and numbers of YWT placed in grids (as shown in Figs. 1–6). The YWT were emptied twice a week. At each date of insect sampling the mean growth stage (BBCH scale was used according to Bečka *et al.*, 2007) of the winter oilseed rape crop was recorded.

The adult catches were preserved in bottles, filled with 70% ethyl alcohol until they could be sorted. In the laboratory species and sexes were identified. For distinguishing females from males of *C. pallidactylus* a previously published method (Seidenglanz *et al.*, 2013) was used.

Analysis of pest distribution and association

Spatial distributions of adults of *C. pallidactylus* and *B. aeneus* were analysed using the statistical methodology termed SADIE (Perry, 1995; 1998 a, b). This technique enables the spatial characteristic of observed distributions to be assessed and compared by randomisation procedures, using indices and a test of randomness. To describe the spatial pattern in single sets of 36 counts (25 counts in 2014 and 2015) of adult insects caught in the YWTs, two indices were used – the main SADIE index I_a and one subsidiary index J_a. In the case of non-random distribution of individuals of the tested population in crops, indicating a significant tendency to aggregate in clusters (patch or gap clusters) the value of I_a is higher than unity ($I_a > 1$ for $p < 0.05$). The index J_a discriminates between patterns, where there is one major cluster, for which its value is greater than unity, and two or more clusters, for which its value is equal to or less than unity ($J_a \approx 1$ for

$p < 0.05$). SadieShell software version 2.0 was used for computing the indices.

To assess association in distributions of two populations (♂ × ♀ of *C. pallidactylus* and ♂, ♀ of *C. pallidactylus* × *B. aeneus*) Quick Association analysis was used (version 2.0). The software calculates an overall spatial association index (X), based on the similarity of the local clustering indices previously stated for the two compared populations (Ferguson *et al.*, 2006). Values of X are >0 for distributions that are associated (for $p < 0.025$), around zero for distributions positioned at random with respect to another, and <0 for distributions that are dissociated ($p > 0.975$). The mean number of pests caught in YWT and the standard error of the mean (SE) were calculated using standard routine statistical methods. For graphic visualisation of SADIE results Statistica software v. 12 (STATSOFT, Inc. 1984–2015) was used.

RESULTS

Spatio-temporal distribution and association of *C. pallidactylus* in 2013

A total of 8270 adults of *C. pallidactylus* (of which 25.16 % were females) were caught in the 36 YWTs from April 8th to May 3rd. Only a few individuals were caught thereafter (the catches made after May 3rd were not included in the SADIE analysis). The major part of flight activity lasted for two weeks (from April 15th to 30th; BBCH 53–60) and relatively high catches were recorded on April 15th and 18th (for females also on April 25th). The peak for female flight activity (mean: 25.13 individuals per trap) was recorded on the same day as for the males (mean: 92.33 individuals per trap) on April 18th. Then the male catches declined continuously up to May 3rd (mean: 4.91 ♂) whereas the females activity on April 25th and May 3rd showed a slight increase but thereafter also gradually decreased.

SADIE analysis of the distribution of males indicated strong aggregation ($I_a > 1$ for $p < 0.05$) on April 15th, 18th and 22nd. Females were significantly aggregated for four dates: April 15th, 18th, 25th and 30th (Tab. I). On April 18th males and females were aggregated into two patch clusters localized on the west and east sides of the field. A gap cluster was localized on the south-west of the field ($I_a > 1$ and $J_a \approx 1$ for $p < 0.05$; Figs. 1–2, Tab. I). Females also showed aggregation into more than one cluster on April 25th (Tab. I). Significant overall spatial associations (positive value of X for $p < 0.025$) between the *C. pallidactylus* sexes were recorded from April 15th to May 3rd.

From the sums of catches gathered in 36 YWTs from April 8th to May 3rd it is clear that there was a significant tendency for males and females to aggregate in crops ($I_a > 1$ and $J_a \approx 1$ for $p < 0.05$; Tab. I). The distributions of both sexes were significantly associated in the crop from April 15th to May 3rd as well as sums of these catches (X for $p < 0.025$; Tab. II).

Spatio-temporal distribution and association of *C. pallidactylus* in 2014 and 2015

A total of 339 adults of *C. pallidactylus* (of which 35.10 % were females) were caught in 25 YWTs from March 3rd to April 11th 2014. In 2015, a total of 1313 adults (of which 59.86 % were females) were caught from March 23rd to June 6th.

In 2014, males were randomly distributed within the field (I_a for $p > 0.05$) during the whole period of monitoring (March, April). The females showed significant aggregation only on April 7th ($I_a > 1$ for $p < 0.05$). The highest catches for both sexes were recorded on March 13th (BBCH 39) and April 1st (BBCH 53), but relatively low numbers of individuals occurred in YWTs (2.24 ♂, 1.20 ♀ and 2.96 ♂, 1.24 ♀, respectively). The overall spatial indices (X) were non-significant ($p > 0.025$; $p < 0.975$) for both sexes from March 3rd to April 7th (Tab. II).

In 2015, the major flight activity period for both sexes lasted roughly two weeks (from April 13th to 29th BBCH 39–55). The males were non-randomly distributed on three dates, April 13th, 15th and May 1st ($I_a > 1$ for $p < 0.05$). The females showed aggregation in one major patch cluster and one or more adjacent patch clusters ($I_a > 1$ for $p < 0.05$ and $J_a \approx 1$ for $p < 0.05$) situated along the south (south-east) margin of the field on April 15th (Fig. 4) and 21st (Fig. 6). On April 15th the males showed aggregation in one major patch cluster and in another adjacent patch cluster, (Fig. 3) localized in similar areas of the field as those for females. Both sexes were significantly spatially associated on April 15th and 21st (Tab. II).

The aggregation indices (I_a and J_a for $p < 0.05$) calculated from the sums of male and female catches gathered in 25 YWTs from March 3rd to April 11th, 2014 and March 23rd to June 3rd, 2015 were significant only for males in 2015 ($I_a > 1$ and $J_a \approx 1$ for $p < 0.05$; Tab. I).

The overall spatial association index X (based on sums of catches from March 13th to April 7th in 2014; from April 13th to May 11th in 2015) for both sexes was significant only in 2015 (X for $p < 0.025$; Tab. II).

Spatio-temporal distribution of *Brassicogethes aeneus* and its spatial association with *C. pallidactylus* in OSR crops 2013–2015

The first adults of *Brassicogethes aeneus* were caught in YWT on April 11th, 2013 (mean 0.02 adults per trap; BBCH 52). Subsequently, numbers of these beetles increased and very high catches were recorded from April 22nd to April 30th (more than 400 adults per trap; BBCH 55–60). Thereafter the numbers of adults in traps showed a slight decrease (Tab. I).

The spatial distribution proved to be non-random ($I_a > 1$ for $p < 0.05$) on April 22nd and May 3rd, 2013, when beetles always showed aggregation in

I: *Results of fly activity and SADIE for adults of C. pallidactylus and B. aeneus. Aggregation indices (I_a, J_a) characterize their spatio-temporal distributions within the winter oilseed crops in 2013, 2014 and 2015 (used grids: 6 × 6 = 36 traps/2013, 5 × 5 = 25 traps/2014 and 2015).*

Date of sampling	**No. of** *Ceutorhynchus pallidactylus*		**No. of** *Brassicogethes aeneus*	**SADIE index** *Cetorhynchus pallidactylus*				**SADIE index** *Brassicogethes aeneus*	
	Mean per trap (SE)		**Mean per trap (SE)**	I_a^1 ♂	J_a^2 ♂	I_a^1 ♀	J_a^2 ♀	I_a^1	J_a^2
	♂	♀							
8.4.2013	0.02 (0.02)	0.02 (0.02)	×	×	×	×	×	×	×
11.4.2013	0.13 (0.05)	×	0.02 (0.02)	×	×	×	×	×	×
15.4.2013	35.27 (3.68)	7.38 (0.90)	8.19 (0.58)	**2.20***	1.01	**2.01***	1.01	0.89	1.01
18.4.2013	92.33 (6.69)	25.13 (2.00)	109.97 (6.93)	**1.53***	**1.05***	**1.54***	1.07*	0.92	0.98
22.4.2013	10.19 (1.24)	4.58 (0.68)	411.33 (28.27)	**1.41***	0.97	1.19	1.01	**1.77***	1.05
25.4.2013	14.33 (1.69)	10.91 (1.07)	433.63 (22.87)	1.05	1.09	**1.33***	**1.11***	1.30	1.00
30.4.2013	5.36 (0.63)	3.83 (0.38)	440.00 (21.81)	0.95	0.93	**1.45***	0.99	1.30	0.99
3.5.2013	4.91 (0.64)	6.02 (0.81)	359.97 (30.73)	1.20	1.08	0.97	1.05	**1.50***	1.03
Σ	162.54 (10.55)	57.87 (3.51)	1763.11 (63.19)	**1.88***	**1.09***	**1.92***	**1.06***	**1.39***	1.00
3.3.2014	0.96 (0.17)	0.20 (0.10)	×	1.11	0.97	×	×	×	×
7.3.2014	0.16 (0.07)	0.12 (0.06)	0.08 (0.05)	×	×	×	×	×	×
10.3.2014	0.64 (0.17)	0.04 (0.04)	0.08 (0.05)	×	×	×	×	×	×
13.3.2014	2.24 (0.21)	1.20 (0.23)	0.12 (0.06)	1.09	1.01	1.18	1.00	×	×
17.3.2014	0.24 (0.16)	×	0.52 (0.15)	×	×	×	×	**1.36***	1.17
20.3.2014	0.48 (0.13)	0.16 (0.07)	1.08 (0.27)	1.01	1.02	×	×	1.29	0.90
24.3.2014	0.56 (0.15)	0.44 (0.17)	14.48 (1.52)	0.90	0.80	0.79	0.79	**1.77***	0.90
27.3.2014	0.12 (0.06)	0.32 (0.14)	8.76 (1.36)	×	×	×	×	**1.91***	1.01
1.4.2014	2.96 (0.45)	1.24 (0.21)	59.64 (5.63)	1.04	0.92	0.95	0.92	**1.73***	0.94
3.4.2014	0.28 (0.09)	0.12 (0.06)	31.52 (3.53)	×	×	×	×	**1.87***	0.97
7.4.2014	1.00 (0.23)	0.92 (0.19)	149.88 (10.64)	1.31	0.99	**1.51***	1.03	1.14	1.04
11.4.2014	0.20 (0.10)	0.12 (0.06)	16.48 (1.46)	×	×	×	×	0.82	1.08
Σ	9.84 (0.91)	4.88 (0.63)	282.64 (19.90)	1.13	0.93	0.90	0.92	**1.59***	0.99
23.3.2015	0.28 (0.10)	×	×	×	×	×	×	×	×
26.3.2015	0.84 (0.20)	0.04 (0.04)	×	×	×	×	×	×	×
30.3.2015	0.04 (0.04)	×	×	×	×	×	×	×	×
13.4.2015	6.08 (0.86)	7.28 (0.88)	17.76 (1.99)	**1.64***	1.00	1.01	1.07	1.23	1.10
15.4.2015	3.08 (0.47)	5.04 (0.67)	1.20 (0.18)	**1.44***	**1.16***	**1.48***	**1.06***	0.89	1.08
21.4.2015	1.12 (0.24)	3.12 (0.42)	19.92 (2.75)	1.17	0.90	**1.56***	**1.04***	**1.75***	**1.03***
29.4.2015	7.16 (1.40)	13.40 (2.21)	115.20 (13.61)	1.14	1.19	0.94	1.06	**1.42***	**1.10***
1.5.2015	1.16 (0.34)	0.08 (0.05)	26.88 (3.67)	**1.35***	1.40	×	×	1.16	1.15
4.5.2015	0.20 (0.08)	0.12 (0.06)	90.04 (8.83)	×	×	×	×	**1.34***	**1.07***
7.5.2015	0.48 (0.14)	0.88 (0.20)	156.32 (13.43)	1.05	1.08	1.23	1.03	1.17	1.12
11.5.2015	0.48 (0.15)	0.92 (0.20)	165.72 (16.62)	1.20	0.99	1.08	1.12	1.24	1.10
14.5.2015	0.12 (0.06)	0.32 (0.14)	174.68 (13.41)	×	×	×	×	**1.37***	1.04
18.5.2015	×	0.12 (0.08)	153.44 (15.75)	×	×	×	×	1.19	1.08
21.5.2015	×	×	90.08 (8.75)	×	×	×	×	**1.61***	1.01
25.5.2015	×	0.04 (0.04)	83.16 (6.58)	×	×	×	×	1.04	1.04
29.5.2015	0.04 (0.04)	0.04 (0.04)	88.00 (12.85)	×	×	×	×	**1.85***	1.08
3.6.2015	×	0.04 (0.04)	46.16 (5.30)	×	×	×	×	1.17	1.01
Σ	21.08 (2.48)	31.44 (2.85)	1228.56 (80.42)	**1.46***	1.07	1.17	1.03	1.00	1.06

Values of Ia > 1 indicate aggregation within the sample area (*red bold values $p < 0.05$)
Values of Ja ≈ 1 indicate the presence of multiple clusters when Ia > 1 (*red bold values $p < 0.05$)
The number of insect individuals caught in YWT was not sufficient (values marked ×) for evaluation by SADIE analysis and Quick association analysis (for Tab. II too)

one major patch cluster (J_a for $p > 0.05$). Despite the random distribution of females of *C. pallidactylus* in the crop on April 22nd, 2013 there was significant spatial association between *B. aeneus* adults and the females of *C. pallidactylus* on that date ($X = 0.42$ for $p < 0.025$). Non-significant dissociation indices (negative values of X for $p < 0.975$) between adults of *B. aeneus* and both sexes of *C. pallidactylus* were recorded from April 25th to May 3rd (Tab. II).

In 2014, the first adults of *B. aeneus* were caught on March 7th (mean 0.08 adults per trap; BBCH 33); flight activity increased somewhat from March 24th to April 11th, but in comparison with the previous and following seasons the catches remained relatively low during the duration of monitoring (Tab. I). The distribution of *B. aeneus* adults was non-random ($I_a > 1$ for $p < 0.05$) on March 17th and from March 24th to April 3rd when beetles always showed significant aggregation in one major patch cluster (J_a for $p > 0.05$). On the day when the highest flight activity was recorded (April 7th), the distribution of beetles in the crop was random (I_a for $p > 0.05$). Some negative dissociation indices (X for $p < 0.975$) between adults *B. aeneus* and both sexes of *C. pallidactylus* were recorded in the first week of April (Tab. II).

In 2015, the first *B. aeneus* adults were caught on April 13th (mean 17.76 per trap; BBCH 39). The first peak of flight activity (on average more than 100 beetles per trap) was recorded on April 29th (BBCH 55). Relatively high flight activity (on average more than 150 individuals per trap) was recorded from May 7th to 18th (BBCH 60–67).

The distributions of *B. aeneus* adults were non-random ($I_a > 1$ for $p < 0.05$) on April 21st, 29th and May 4th, 14th, 21st and 29th (Tab. I). The presence of two or more patch clusters ($J_a \approx 1$ for $p < 0.05$) was recorded on April 21st, 29th and on May 4th (the situation from April 21st is shown in Fig. 5).

Significant overall spatial association indices (positive value of X for $p < 0.025$) between distributions of *B. aeneus* adults and males of *C. pallidactylus* were recorded on April 13th, 21st and May 1st (Tab. II). Distributions of *B. aeneus* adults and females of *C. pallidactylus* were significantly associated on April 21st (value of X was 0.67 for $p < 0.025$) and non-significantly dissociated (negative values of X for $p < 0.975$) on May 7th (Tab. II).

The aggregation indices (I_a and J_a for $p < 0.05$; calculated from the sum of *B. aeneus* adult catches gathered in 36 YWTs from April 8th to May 3rd, 2013; in 25 YWTs from March 3rd to April 11th, 2014; March 23rd to June 3rd, 2015) were significant in 2013 and 2014 (X for $p < 0.025$; Tab. I).

It is clear from the analyses of *B. aeneus* and both sexes of *C. pallidactylus* distributions derived from the sums of their catches in YWT in 2013 (April 15th to May 3rd), 2014 (March 3rd to April 7th) and 2015 (April 13th to May 11th) that distributions of both insect pests in crops were significantly spatially associated only in 2013 (X for $p < 0.025$ Tab. II).

DISCUSION

As shown in many previous studies (Alford *et al.*, 2003; Seidenglanz *et al.*, 2009; Junk *et al.*, 2012; Vaitelyté *et al.*, 2013; and many others) the dates when migration of *C. pallidactylus* and *B. aeneus* adults in winter oilseed rape crops commences, peaks and ends can fluctuate very considerably from year to year depending on a variety of environmental (especially meteorological) factors. In Europe, different ways of timing insecticidal sprays against these pests are based on continual monitoring of some simple-to-measure meteorological factors (individually or in combination) (in detail in Junk *et al.*, 2012). Great seasonal differences during the course of fly activity of these two insect pests also appeared in the three years of this study.

Klukowski (2006) investigated the colonisation of winter rape crops by *C. pallidactylus*. He found that the males arrived before the females. This tendency was also documented by Seidenglanz *et al.* (2009). However, in this study the first *C. pallidactylus* females appeared in the crop (more precisely in YWTs located in crops) at the same time as the males in 2013 and 2014. In 2015 the first females appeared with a negligible delay after the males (3 days). Peaks of flight activity were also achieved by both males and females at the same dates in all three seasons. So, on the basis of this study it is not possible to confirm the previous results that females of *C. pallidactylus* enter the winter oilseed rape crops markedly later than males. On the other hand in this study the proportions of females were increasing in YWTs during the period of fly activity of the adults in the crop, in agreement with the findings of Seidenglanz *et al.* (2009) and Johnen *et al.* (2010).

Klukowski (2006) also studied changes in within-field spatial distribution patterns of male and female *C. pallidactylus* in the course of crop colonisation. He concluded that both sexes were aggregated and more concentrated at the crop edge and that the females tended to stay at the crop edge for longer than the males before spreading further into the crop during the egg-laying period. Both sexes however, remained essentially edge-distributed and the crop centres never became as heavily infested as the edges. In this study a higher tendency for aggregation in females was observed only in 2013 and 2014 but in 2015, males showed a higher and longer tendency towards aggregation in the crop than females. In agreement with Klukowski (2006) the crop centres remained markedly less infested in all three years but on the other hand the patch and gap clusters showed various shapes, sizes and directions in the individual seasons and often penetrated from the edges into deeper zones of the crop. So it is also possible to say (contrary to Klukowski, 2006) that margins need not be more infested than the central zones in all cases. However, Klukowski (2006) studied the spatial distribution of *C. pallidactylus* adults in

II: *Overall measures of spatial association (X) and their probability levels (p) stated for the three selected pairs of distributions (males × females of C. pallidactylus, males of C. pallidactylus × B. aeneus adults and females of C. pallidactylus × B. aeneus adults) in winter oilseed rape crops in 2013, 2014 and 2015.*

Date of sampling	**BBCH scale**	*C. pallidactylus* ♂ × ♀.		*C. pallidactylus* ♂ × **adults** *B. aeneus*		*C. pallidactylus* ♀ × **adults** *B. aeneus*	
		X	**p**	**X**	**p**	**X**	**p**
15.4.2013	53	**0.66***	**<0.001**	0.33	0.055	0.30	0.043
18.4.2013	53–54	**0.86***	**<0.001**	0.06	0.374	0.08	0.328
22.4.2013	55	**0.53***	**0.001**	0.11	0.289	**0.42***	**0.009**
25.4.2013	55–57	**0.49***	**0.002**	–0.03	0.564	–0.01	0.509
30.4.2013	60	**0.44***	**0.003**	–0.19	0.859	–0.18	0.834
3.5.2013	62	**0.45***	**0.012**	–0.14	0.791	–0.08	0.340
Σ		**0.81***	**0.000**	**0.53***	**0.002**	**0.57***	**0.000**
13.3.2014	39	–0.07	0.646	×	×	×	×
20.3.2014	50	×	×	0.26	0.124	×	×
24.3.2014	51	0.41	0.040	0.16	0.295	0.28	0.160
1.4.2014	53	0.30	0.073	–0.18	0.769	0.33	0.080
7.4.2014	55	0.21	0.177	–0.02	0.542	–0.20	0.819
Σ		0.31	0.058	–0.39	0.958	0.13	0.257
13.4.2015	39	0.32	0.088	**0.47***	**0.024**	0.40	0.065
15.4.2015	39	**0.47***	**0.016**	0.26	0.115	0.42	0.036
21.4.2015	50	**0.51***	**0.013**	**0.55***	**0.005**	**0.67***	**0.006**
29.4.2015	55	0.34	0.060	0.30	0.084	0.00	0.484
1.5.2015	57	×	×	**0.75**	**<0.001**	×	×
7.5.2015	60	0.26	0.153	0.12	0.164	–0.16	0.776
11.5.2015	63	0.11	0.308	0.16	0.228	0.20	0.211
Σ		**0.74***	**0.001**	0.03	0.437	0.40	0.043

Values of positive X indicated significant association for (*red bold values p<0.025)
Values of negative X indicated significant dissociation for (p>0.975)

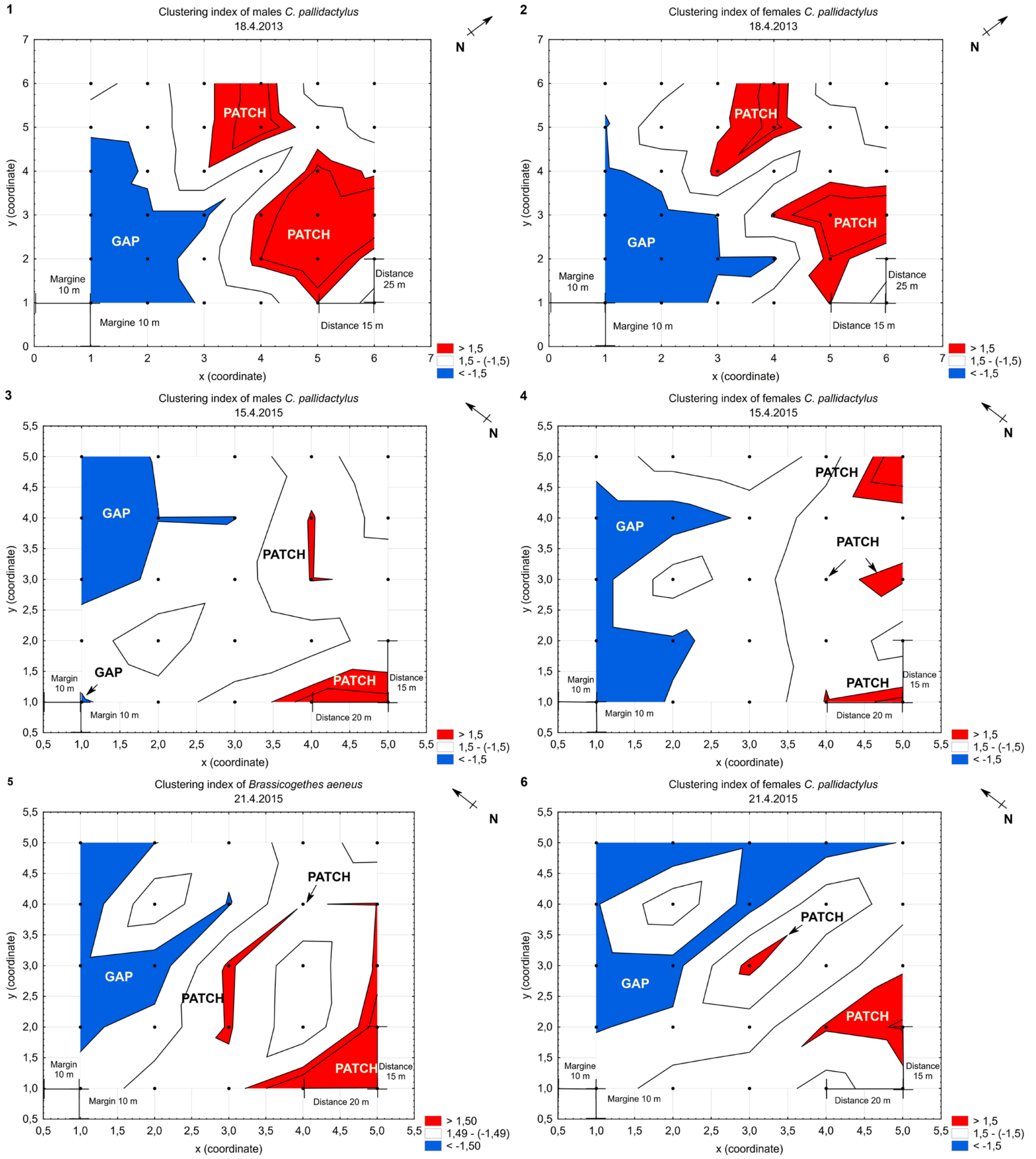
crops with markedly greater acreages in comparison with the ones used in this study.

According to Free and Williams (1979a) and Williams and Ferguson (2010) *B. aeneus* adults infest plants at the edges of a rape crop and later those nearer the centre. As the beetle populations spread towards the crop centres the proportion at the edges diminishes. These assertions are in agreement with the results recorded in this study when the edge effect was not so apparent. Patch clusters were often localized along the field margins but they also penetrated (the directions and sizes of the clusters were different in the individual seasons) into deeper parts of fields practically from the beginning of crop colonisation (Fig. 5). However, this still confirms Williams and Ferguson (2010) that the edge effect should be more pronounced on large crops than on small ones (as in the case of this study).

The cumulative distributions (based on the sum of catches from the individual dates; Tab. II) of males and females of *C. pallidactylus* were significantly associated in 2013 and 2015, but not in 2014 in this study. The 2014 season differed from the other two in having very low flight activity of *C. pallidactylus* adults (low catches in YWT). Ferguson *et al.* (2000) studied spatial associations between the distribution of *C. assimilis* (closely related species to *C. pallidactylus*) females and males in winter oilseed rape crop. *C. assimilis* occurs in winter oilseed rape somewhat later than *C. pallidactylus*. According to them (Ferguson *et al.* 2000) the cumulative distributions of both sexes were almost identical in the crop. They also show evidence of spatial associations between males and females of *Psylliodes chrysocephala* (another important insect pest of oilseed rape) in crops (Ferguson *et al.* 2006). So we may conclude that females and males of *C. pallidactylus* should be spatially associated in crops when their abundances are not too low (2014).

From the three seasons the cumulative distributions (based on the sum of catches from the individual dates; Tab. II) of males and females of *C. pallidactylus* were significantly associated with the cumulative distribution of *B. aeneus* adults only in 2013 in this study. Contrary to that, in 2014 an almost significant dissociation (X = –0.39 for p = 0.958) between cumulative distributions of *C. pallidactylus* males and *B. aeneus* adults was recorded. In all other cases there were no spatial associations between cumulative distributions of both species in crops (females of *C. pallidactylus* × *B. aeneus* adults in 2014; both sexes of *C. pallidactylus* × *B. aeneus* adults in 2015). In 2013 and 2015 some cases of significant association between *C. pallidactylus* (three times for males and twice for females) and *B. aeneus* adults were recorded on several dates.

In cases where the adults of *C. pallidactylus* and *B. aeneus* were spatially associated (2013, 2015) they both moved preferentially to areas where their host plants (resource) had a higher quality. It need not be the case though that they prefer the same zones in a crop. In some cases both species may prefer completely different zones in the crop. Ferguson *et al.* (2006) studied the spatial association between *C. pallidactylus* and another stem miner *Psylliodes. chrysocephala* in winter oilseed rape crops. They found significant spatial dissociation between them in crops. However, in contrast to *C. pallidactylus* and *B. aeneus*, both the stem miners (*C. pallidactylus* and *P. chrysocephala*) are niche competitors.



1: 1–6: *Graphical illustration of SADIE results for males and females of C. pallidactylus on April 18th, 2013 (Figs. 1, 2.) and April 15th, 2015 (Figs. 3, 4.), for adults of B. aeneus and females of C. pallidactylus on April 21st, 2015 (Figs. 5, 6.). Red (patch) and blue (gap) areas were identified by SADIE as patch and gap clusters in pest distributions. The black points mark the sampling places arranged in a rectangular grid (6 × 6 points: 36 sampling places in 2013 and 5 × 5 points: 25 sampling places in 2014 and 2015).*

CONCLUSION

The sampling of *C. pallidactylus* and *B. aeneus* at regular time intervals from points on a grid across a whole crop and the analysis of these data by SADIE have produced a much more detailed picture of the pattern of crop colonization processes by these pests. The Quick association analysis to compare whether the two distributions (in this study: males × females of *C. pallidactylus* and × adults of *B. aeneus*) of pests occur close together or are segregated from one another.

Males and females of *C. pallidactylus* and *B. aeneus* adults are non-randomly distributed in crops during the winter oilseed rape colonization. On the basis of sums of catches gathered in YWTs, both pests showed a significant tendency to form one or more patches and (or) gap clusters (different shapes, sizes and directions), particularly in 2013. Patch and gap clusters were possible to locate on the field margins but they often encroached into central zones of the crop too.

Distributions of males and females of *C. pallidactylus* during crop colonisation of winter oilseed rape show significant association. Contrary to this, adults of *B. aeneus* and *C. pallidactylus* (males and females) are not directly associated in crops – *B. aeneus* adults probably do not search for the places where *C. pallidactylus* adults aggregate.

A knowledge of within-field spatio-temporal changes in the distribution patterns of *B. aeneus* and *C. pallidactylus* adults in oilseed rape crops and their spatial associations (inter/intraspecific interactions respectively) can improve both the timing and targeting of insecticide applications and diminish the negative impacts of such applications on the environment.

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