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The effects of reduced tillage and earlier seeding on flea beetle (*Phyllotreta* spp.) crop damage in spring oilseed rape (*Brassica napus* L.)

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Abstract

The restriction on seed treatments containing neonicotinoid insecticides in the European Union has brought crop protection into focus for oilseed rape (*Brassica napus* L.). In spring sown oilseed rape, neonicotinoid seed treatments have mainly been used for protection against flea beetles (*Phyllotreta* spp.), and there is now a need to evaluate alternative control methods. We investigated the effect of reduced tillage and altered seeding date on flea beetle crop damage in spring oilseed rape in eight field experiments over three years in south central Sweden. The average proportion of cotyledon area damaged by flea beetles was not affected by the tillage treatment. Proportion of crop damage was, however, lower in early seeded compared to late seeded plots (0.21 compared to 0.28). We conclude that earlier seeding holds promise to be incorporated into an integrated pest management program for flea beetles in spring oilseed rape, whereas further research on reduced or zero tillage strategies for flea beetle control is warranted.

Key words: integrated pest management, non-inversion tillage, neonicotinoids, canola

1. Introduction

The restriction on seed treatments containing neonicotinoid insecticides in the European Union due to the questioned bee safety of these compounds (EU 2013, Lundin et al. 2015, Rundlöf et al. 2015), has brought crop protection into focus for oilseed rape (*Brassica napus* L.) (Dewar 2017, Zhang et al. 2017). In spring sown oilseed rape (hereafter SOSR; we also use this term for spring sown canola varieties of *B. napus*), neonicotinoid seed treatments have mainly been used in the European Union for protection against flea beetles (*Phyllotreta* spp.) (Ekbom 2010, Ekbom and Müller 2011). Several species of Phyllotreta attack the crop, and damage incurred during crop emergence and the first weeks following seeding can be critical for crop establishment (Ekbom 2010, Sekulic and Rempel 2016, Knodel 2017). The economic threshold is reached when 25-30% of the cotyledon area is damaged (Ekbom 2010). There is a need to develop alternative control methods for protection against flea beetle crop damage (Ekbom and Müller 2011).

Altering the tillage regime is one promising option to reduce crop damage caused by flea beetles in SOSR. Two North American studies have found that zero tillage reduces flea beetle abundance or crop damage caused by flea beetles in SOSR in comparison with conventional tillage regimes (Milbrath et al. 1995, Dosdall et al. 1999). While Milbrath et al. (1995) suggest that the increased amounts of crop residues under zero tillage increase structural complexity and interfere with flea beetle host plant location, Dosdall et al. (1999) instead suggest that the crop residues decrease flea beetle activity due to a cooler and more humid microclimate. Agronomic and climatic constraints, however, limit the feasibility and uptake in practice of zero tillage in northern Europe (reviewed by Soane et al. 2012, see also Arvidsson et al. 2014). Therefore, it would be valuable to evaluate how flea beetle crop damage is affected by tillage regimes which lie in-between the extremes of conventional tillage and zero tillage, i.e. different types of reduced or non-inversion tillage.

Altering the seeding date is another potential control option for flea beetles in SOSR. The effect of seeding date on flea beetle crop damage seems, however, to be complex (Cárcamo et al. 2008). Lower flea beetle abundance and/or crop damage has been found in both early and late seeded SOSR (Lamb 1984, Milbrath et al. 1995, Cárcamo et al. 2008, Knodel et al. 2008, Pavlista et al. 2011), and no difference in flea beetle crop damage between early and late seeded SOSR has also been observed (Dosdall and Stevenson 2005). The variable results could, at least partly, be explained by that Phyllotreta species differ in their emergence phenology, and that Phyllotreta species composition varies among growing regions (Cárcamo et al. 2008). All the aforementioned studies were conducted in North America, where the Phyllotreta species complex attacking SOSR only partly overlaps with that in Europe (Ekbom 2010), pointing to a need to evaluate the effect of seeding date on flea beetle damage in European SOSR crops.

We aimed to explore alternative controls options that can contribute to integrated pest management of flea beetles in SOSR. More specifically, we ask how reduced tillage and alternate seeding dates affect flea beetle crop damage.

2. Material and methods

We investigated the effect of reduced tillage and altered seeding date on crop damage caused by flea beetles in SOSR in eight field experiments over three years near the city of Uppsala in south central Sweden. This area is historically known to experience SOSR crop damage caused by flea beetles (Ekbom and Müller 2011). Two experiments were conducted in 2014, and another three experiments each were conducted in 2015 and 2016 (Table 1). The distances between experiments within each year were 0.2-2 km. Soil types were light to heavy clays. Pre-crops were wheat, barley or oat.

Table 1. Seeding (S) dates and plant assessment (PA; plant density and cotyledon damage) dates for the experiments. # = experiment number (1-8). S, early = date for early seeding. S, late = date for late seeding. PA, early = date for plant assessment in early seeded plots. PA, late = date for plant assessment in late seeded plots. N/A = no data available.

#	Year	Pre-crop	S, early	S, late	PA, early	PA, late
1	2014	Winter wheat	22 April	6 May	2 June	9 June
2	2014	Spring wheat	26 April	6 May	2 June	9 June
3	2015	Winter wheat	23 April	4 May	2 June[a]	N/A
4	2015	Winter wheat	23 April	4 May	2 June[a]	N/A
5	2015	Oat	23 April	4 May	2 June[a]	N/A
6	2016	Spring barley	6 May	18 May	3 June	15 June
7	2016	Spring barley	6 May	18 May	3 June	15 June
8	2016	Spring barley	6 May	18 May	3 June	15 June

[a] crop damage was assessed on 2 June, whereas plant density was measured on 10 June

In each experiment, we compared four tillage methods, repeated in four plots in complete randomized blocks: Conventional – mouldboard ploughing in autumn, Reduced 1 – shallow disc cultivation twice in autumn, Reduced 2 – shallow disc cultivation once in autumn and once in spring, and Reduced 3 – shallow disc cultivation twice in spring. Spring harrowing was performed once or twice in the treatment Conventional, and 2015-2016 also in the treatment Reduced 1. Mouldboard ploughing was performed to a depth of 20-22 cm, disc cultivation to a depth of 4-7 cm, and spring harrowing to a depth of 3-4 cm. Seeding date was a split-plot factor with two levels within each tillage plot: early seeding or late seeding. Early seeding was carried out as soon as good seeding conditions were reached in conventionally tilled plots. Late seeding was carried out 10-14 days later (see Table 1) when the soil was somewhat drier, in order to achieve good conditions for seeding in shallowly spring cultivated plots. Photos of the different tillage treatments are presented in the Supplementary Material.

Tillage main plot sizes were 12 m by 20 m, and the seeding date split-plots were 6 m by 20 m. In 2014 only, seeding date split plots were further subdivided in split-split-plots either with or without seed treatments (see details below), but here we report data for seed treated plots only in order to keep treatments consistent across all three study years.

Plots were seeded with SOSR of the cultivars Majong (SW, 2014-2015) or Mirakel (NPZ, 2016) at a rate of 7.2-8.0 kg per ha depending on year. Sowing was carried out with a seed drill with disc coulters (Väderstad Rapid - system disc, working depth 2.5-4.5 cm). The original and main focus of the experiments was to compare SOSR establishment with different tillage regimes under optimal control of flea beetles. Seeds were, therefore, coated with seed treatments that included neonicotinoid insecticides, either Elado (Bayer; 25 ml per kg seed: 400 g l^{-1} clothianidin, 80g l^{-1} β-cyfluthrin) in 2014 and 2015 or Cruiser OSR (Syngenta; 15 ml per kg seed: 280 g l^{-1} thiamethoxam, 8 g l^{-1} fludioxonil, 32.3 g l^{-1} metalaxyl-M) in 2016. Experiments were in some cases (June 1 and June 8 in both experiments in 2014, and on June 7 and June 14 in all three experiments in 2016) also sprayed with the pyrethroid insecticide Sumi-Alpha (Sumitomo Chemicals; 0.30-0.35 l ha^{-1}, 50 g l^{-1} esfenvalerate) to control flea beetles or pollen beetles before we assessed flea beetle damage. Sumi-Alpha is one of several pyrethroid compounds that were used in Sweden for flea beetle control in 2014-2016. Despite these chemical control efforts, flea beetle damage was readily observed and quantifiable (see Results), meaning that we still could fulfill the goals of this study.

We assessed plant density and cotyledon damage once per plot in early to mid-June when the crop had approximately two fully developed true leaves (Table 1). This captures the most critical period for flea beetle crop damage, and damage later in the season rarely has any economic impacts (Dosdall and Mason 2010). Early and late seeded plots in the same experiment were assessed on different dates to standardize plant growth stage (Table 1). Late seeded plots were not assessed in 2015; the seeding date analysis, therefore, relies on data from a subset of 5 experiments and 2 years (Table 1). Crop plant density was measured in four 0.25 m^2 quadrats per plot. Cotyledon damage was visually observed and assessed on 20 plants (40 plants in 2015) per plot. We classified flea beetle damage into five categories: 0 = 0 % of cotyledon area damaged, 1 = 1-10 %, 2 = 11-30 %, 3 = 31-60 %, and 4 = 61 % or more of cotyledon area damaged (Ekbom and Kuusk 2005). Flea beetles cause characteristic damage to leaves (Brandt and Lamb 1993), and, with rare exceptions, damage observed on cotyledons was attributable to flea beetles. We excluded data from two plots in 2014 that contained large weed populations of charlock mustard (*Sinapis arvensis* L.) which is an alternative host plant for Phyllotreta flea beetles. Cotyledon damage classifications were converted to proportions using the center point in each damage class (0 = 0, 1 = 0.055, 2 = 0.205, 3 = 0.455 and 4 = 0.805). All data were averaged per plot prior to statistical analyses.

Plant density and cotyledon damage data was analyzed using a general linear mixed model (PROC MIXED) in SAS 9.4 for Windows (SAS Institute Inc., Cary, NC). Plant density was analyzed untransformed, whereas proportion of cotyledon damage was arcsine square root transformed to achieve approximately normal distribution of model residuals. Tillage, seeding date and year were fixed factors in the analysis, and we also included the interaction between tillage and seeding date. Experiment, block within experiment, and tillage within block and experiment were random factors. Degrees of freedom were estimated with the Kenward-Roger method, and the nobound option allowed negative within subject variances to be estimated (Littell et al. 2006). We sequentially simplified models by removing non-significant ($p > 0.05$) terms. In cases when the overall effect of a factor was statistically significant ($p < 0.05$), we compared the levels within each factor pairwise with Tukey adjustment.

3. Results

Plant density varied significantly across tillage treatments, seeding dates and years. Plant density was 102 plants m^{-2} in Conventional, 100 plants m^{-2} in Reduced 1, 95 plants m^{-2} in

Reduced 3 and 89 plants m^{-2} in Reduced 2. Early seeded plots had higher plant densities (111 plants m^{-2}) than late seeded plots (82 plants m^{-2}). Plant density was 95 plants m^{-2} in 2014, 77 plants m^{-2} in 2015 and 117 plants m^{-2} in 2016. Plant density test statistics with pairwise comparisons and estimates are presented in Table S1.

The average proportion of cotyledon area damaged by flea beetles was not affected by the tillage treatment or the interaction between tillage and seeding date. Proportion of cotyledon damage was, however, lower in early seeded compared to late seeded plots (0.21 compared to 0.28). Proportion of cotyledon damage also varied between years. Average damage was 0.46 in 2014, 0.25 in 2015, and 0.082 in 2016. Cotyledon damage test statistics with pairwise comparisons and estimates are presented in Table 2. Crop damage data are presented separately for each experiment in Table S2.

Table 2. Test statistics and estimates for proportion of SOSR cotyledon area damaged by flea beetles in eight experiments 2014-2016. Estimated numbers are back transformed least square means, with 95 percent confidence intervals within parentheses. See Materials and methods for further explanation of variables tested. Factor levels denoted with different letters are significantly ($p < 0.05$) different.

Variable	F_{df}	p	Factor level	Estimate	
Year	$18.4_{2,4.90}$	0.0053	2014	0.46 (0.31-0.61)	a
			2015	0.25 (0.15-0.37)	a
			2016	0.083 (0.027-0.16)	b
Tillage (T)	$1.62_{3,52.9}$	0.19	Conventional	0.26 (0.19-0.33)	
			Reduced 1	0.25 (0.19-0.33)	
			Reduced 2	0.24 (0.17-0.31)	
			Reduced 3	0.24 (0.18-0.31)	
Seeding (S)	$19.9_{1,86.8}$	<0.0010	Early (E)	0.21 (0.15-0.28)	a
			Late (L)	0.28 (0.22-0.36)	b
T × S	$2.42_{3,106}$	0.070	Conventional: E	0.24 (0.17-0.31)	
			Reduced 1: E	0.23 (0.17-0.30)	
			Reduced 2: E	0.19 (0.13-0.26)	
			Reduced 3: E	0.19 (0.13-0.25)	
			Conventional: L	0.27 (0.20-0.35)	
			Reduced 1: L	0.27 (0.20-0.35)	
			Reduced 2: L	0.29 (0.22-0.36)	
			Reduced 3: L	0.30 (0.23-0.38)	

4. Discussion

The average proportion of cotyledon area damaged by flea beetles was not affected by the tillage treatment. The increased amount of crop residues in zero tillage regimes has been suggested to reduce flea beetle crop damage (Milbrath et al. 1995, Dosdall et al. 1999). The amount of crop residues in our reduced tillage regimes was, however, limited in all treatments (see Figure S1), and this likely contributed to the small differences in crop damage. There was a limited potential for crop residues in reduced tillage treatments to affect host plant location and create a more unfavorable micro-climate for flea beetles. Another factor contributing to the smaller than expected damage differences might have been that plant density generally was somewhat lower in the reduced tillage treatments, and a lower plant density tends to increase flea beetle damage per plant (Dosdall et al. 1999, Dosdall and Stevenson 2005). We conclude that there is scope to further explore tillage methods for flea beetle control in SOSR that result in more crop residues. Such efforts must, however, be balanced against a need for minimized tillage and the increased amount of crop residues to not negatively affect crop germination or emergence (Soane et al. 2012, Arvidsson et al. 2014). Moreover, the effect of tillage was heterogeneous across the individual experiments (Table S2), and further investigations are needed to unravel the reasons for this variation. This heterogeneity across experiments, coupled with the fact that late seeding dates were not sampled in all experiments, might also have led to the trend for a tillage by seeding date interaction, despite that the effect of tillage on crop damage seemed fairly constant between the two seeding dates within each experiment (Table S2).

An earlier seeding resulted in less crop damage caused by flea beetles. Earlier seeding also led to higher plant density. To disentangle whether a later seeding date had direct negative effects on plant density, or whether the lower plant density in later seeded plots was caused by increased flea beetle damage, it would be necessary to include insect pest control as an additional experimental treatment in future studies. We suggest two interrelated explanations for why earlier seeding might decrease flea beetle crop damage. One is that SOSR partly escape attacks early in spring if all flea beetles have not yet emerged or emigrated from their overwintering sites when the crop emerges. Spring emergence has been examined for flea beetles attacking SOSR in Canada (Ulmer and Dosdall 2006), but emergence patterns of the European flea beetle fauna need to be better understood in order to explore if SOSR can be established while spring emergence of *Phyllotreta* spp. is not yet complete. The other explanation for less crop damage with early seeding is that warm and dry weather, which favors flea beetle feeding activity (Burgess 1977), is less likely early in spring. If this explanation is prevailing, earlier seeding will only increase the likelihood of lower crop damage, but not guarantee it, because weather might indeed in some years be warmer and drier early in spring compared to later. Shifting to seeding OSR in autumn instead of in spring could be used as a strategy to further limit crop damage caused by Phyllotreta flea beetles (Dosdall and Stevenson 2005). However, in our study area and elsewhere at high latitudes, where growing seasons are short and winters can be harsh, autumn sown OSR stand establishment can be challenging or in some contexts not possible, limiting the feasibility of this approach (Ekbom 2010). The generality of our finding that earlier seeding in spring decrease flea beetle crop damage should, however, be tested in more locations and years given the inconclusive results found in earlier studies on the effect of seeding date on flea beetle abundance and/or crop damage in SOSR (Lamb 1984, Milbrath et al. 1995, Dosdall and Stevenson 2005, Cárcamo et al. 2008, Knodel et al. 2008, Pavlista et al. 2011).

We found that flea beetle crop damage varied largely from year to year, with damage clearly above the economic threshold in 2014, around the threshold in 2015, and clearly below it in 2016. In fact, the yearly variation in crop damage dominated over any effects of the treatments in the experiments. Despite considerable research efforts, the population dynamics and long term outbreak patterns for flea beetles are still poorly understood. Further research on forecasting flea beetle crop damage is needed as part of an integrated pest management program to adaptively manage flea beetles in SOSR (Sekulic and Rempel 2016). We conclude that earlier seeding holds promise to be incorporated into such a program, whereas further research on reduced or zero tillage strategies for flea beetle control is warranted.

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Supplementary Material

Conventional Reduced 1

Reduced 2 Reduced 3

Figure S1. Photos of different tillage treatments assessed: Conventional (upper left) – mouldboard ploughing in autumn and harrowing in spring, Reduced 1 (upper right) – shallow disc cultivation twice in autumn, Reduced 2 (lower left) – shallow disc cultivation once in autumn and once in spring, and Reduced 3 (lower right) – shallow disc cultivation twice in spring. The quadrat displayed in each photo has a side of 0.5 meter.

Table S1. Test statistics and estimates for SOSR plant densities (plants per square meter) in eight experiments 2014-2016[1]. Estimated numbers are least square means, with 95 percent confidence intervals within parentheses. See Materials and methods of main article for further explanation of variables tested. Factor levels denoted with different letters are significantly ($p < 0.05$) different.

Variable	F_{df}	p	Factor level	Estimate	
Year	$6.93_{2,5.26}$	0.034	2014	95 (71-118)	ab
			2015	77 (58-96)	a
			2016	117 (98-137)	b
Tillage (T)	$5.39_{3,96.4}$	0.0018	Conventional	102 (90-114)	a
			Reduced 1	100 (87-112)	a
			Reduced 2	89 (77-101)	b
			Reduced 3	95 (83-107)	ab
Seeding (S)	$93.6_{1,105}$	<0.0010	Early	111 (99-123)	a
			Late	82 (70-94)	b
T × S	$0.27_{3,127}$	0.85	Conventional: E	118 (105-130)	
			Reduced 1: E	113 (101-126)	
			Reduced 2: E	105 (92-117)	
			Reduced 3: E	108 (96-121)	
			Conventional: L	86 (72-99)	
			Reduced 1: L	86 (73-100)	
			Reduced 2: L	73 (60-86)	
			Reduced 3: L	82 (69-95)	

[1] Only early seeded plots were assessed in 2015.

Table S2. Estimated average proportion of cotyledon damage in each experiment and treatment. Data are back transformed least square means, with 95 percent confidence intervals within parentheses. See Materials and methods of main article for further explanation of variables tested. N/A = no data available.

		Experiment							
Variable	Factor level	1	2	3	4	5	6	7	8
Year		2014	2014	2015	2015	2015	2016	2016	2016
Tillage	Conventional	0.38 (0.32-0.44)	0.47 (0.38-0.57)	0.24 (0.11-0.41)	0.38 (0.25-0.52)	0.18 (0.12-0.24)	0.067 (0.032-0.11)	0.097 (0.048-0.16)	0.13 (0.073-0.20)
	Reduced 1	0.40 (0.34-0.46)	0.51 (0.41-0.61)	0.25 (0.11-0.42)	0.44 (0.30-0.57)	0.17 (0.11-0.23)	0.065 (0.031-0.11)	0.086 (0.040-0.15)	0.11 (0.059-0.17)
	Reduced 2	0.40 (0.34-0.46)	0.54 (0.44-0.64)	0.14 (0.043-0.29)	0.26 (0.15-0.39)	0.15 (0.094-0.21)	0.050 (0.021-0.092)	0.080 (0.036-0.14)	0.091 (0.046-0.15)
	Reduced 3	0.40 (0.34-0.46)	0.56 (0.46-0.66)	0.11 (0.024-0.25)	0.24 (0.14-0.37)	0.12 (0.069-0.17)	0.069 (0.034-0.12)	0.084 (0.038-0.14)	0.079 (0.037-0.14)
Seeding	Early (E)	0.37 (0.31-0.43)	0.49 (0.39-0.60)	0.18 (0.076-0.32)	0.33 (0.21-0.46)	0.15 (0.10-0.21)	0.041 (0.015-0.079)	0.081 (0.037-0.14)	0.052 (0.019-0.10)
	Late (L)	0.42 (0.36-0.48)	0.55 (0.45-0.65)	N/A	N/A	N/A	0.089 (0.048-0.14)	0.092 (0.044-0.15)	0.17 (0.10-0.24)
T × S	Conventional: E	0.36 (0.30-0.42)	0.46 (0.36-0.56)	0.24 (0.11-0.41)	0.38 (0.25-0.52)	0.18 (0.12-0.24)	0.045 (0.013-0.095)	0.11 (0.049-0.19)	0.075 (0.021-0.16)
	Reduced 1: E	0.37 (0.31-0.43)	0.48 (0.38-0.58)	0.25 (0.11-0.42)	0.44 (0.30-0.57)	0.17 (0.11-0.23)	0.043 (0.012-0.093)	0.073 (0.026-0.14)	0.059 (0.013-0.14)
	Reduced 2: E	0.37 (0.31-0.43)	0.53 (0.43-0.63)	0.14 (0.043-0.29)	0.26 (0.15-0.39)	0.15 (0.094-0.21)	0.026 (0.004-0.067)	0.070 (0.024-0.14)	0.037 (0.004-0.10)
	Reduced 3: E	0.37 (0.31-0.44)	0.51 (0.41-0.60)	0.11 (0.024-0.25)	0.24 (0.14-0.37)	0.12 (0.069-0.17)	0.053 (0.017-0.11)	0.076 (0.028-0.14)	0.040 (0.005-0.11)
	Conventional: L	0.41 (0.35-0.47)	0.49 (0.39-0.58)	N/A	N/A	N/A	0.094 (0.044-0.16)	0.086 (0.035-0.16)	0.19 (0.10-0.31)
	Reduced 1: L	0.43 (0.36-0.49)	0.54 (0.44-0.63)	N/A	N/A	N/A	0.091 (0.042-0.16)	0.10 (0.044-0.18)	0.17 (0.086-0.28)
	Reduced 2: L	0.43 (0.37-0.49)	0.55 (0.45-0.64)	N/A	N/A	N/A	0.083 (0.036-0.15)	0.091 (0.038-0.16)	0.17 (0.082-0.28)
	Reduced 3: L	0.42 (0.36-0.48)	0.62 (0.52-0.71)	N/A	N/A	N/A	0.087 (0.039-0.15)	0.091 (0.038-0.16)	0.13 (0.055-0.23)