Date: 2022-06-30

Final report

Crop protection against slugs and cabbage stem flea beetles when establishing winter oilseed rape

Project number: O-18-20-165

Budget: 2 865 890 SEK

Project period: 2019-01-01 – 2021-12-31

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A Spanish slug (*Arion vulgaris*) feeding on a winter oilseed rape leaf. Photo: Sara Emery.

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Part 1.1: Summary/Abstract

Slugs (primarily the grey field slug *Deroceras reticulatum)* and cabbage stem flea beetle (*Psylliodes chrysocephala*, CSFB) are important pests damaging winter oilseed rape (WOSR) in the establishment phase. Integrated pest management strategies are needed that can deliver efficient and environmentally friendly crop protection strategies to growers. The aims of this project were to (1) evaluate if companion cropping can protect establishing WOSR crops from slugs and CSFB, (2) explore how soil tillage can be performed to suppress slugs while promoting their natural enemies and (3) analyze CSFB long-term population dynamics. Companion cropping with legumes was evaluated in a greenhouse trial and a field experiment in southern Sweden. Slugs and their main natural enemies, carabid beetles, were studied in 30 fields with different tillage regimes over two seasons in western Sweden, and CSFB long term population dynamics was explored by analyzing samples collected from more than 3000 fields over fifty years in southernmost Sweden. Intercropping WOSR with legumes reduced damage and abundance of CSFB. For slugs, damage to WOSR was reduced in the greenhouse by intercropping with spring faba bean, whereas in the field neither tested legume affected damage. Carabid abundance, species richness and diversity decreased in fields with ploughing after the soil tillage operation, but then recovered by the next season to levels similar to those in the other tillage treatments. Carabids predated on slugs but to a limited extent, perhaps due to low slug abundance. Slug abundance and damage was too low in the WOSR seedling stage to powerfully assess differences between tillage treatments. Slug abundance did not differ between tillage treatments in the following season, when their abundances again had increased to levels where differences could be compared across treatments. Cabbage stem flea beetle had strong, regular and synchronous population cycles with a period of 8 years. Our project shows that intercropping with legumes can be used a strategy to control CSFB and that soil tillage can be used as strategy to control slugs without long term negative effects on carabid beetles. Further, CSFB abundance can be predicted, which means that pest management tactics can be tailored to projected pest pressure, for example by using insecticide treated seeds or reducing WOSR crop area in years with high expected pest pressure.

Sniglar (främst åkersnigel, *Deroceras reticulatum*) och rapsjordloppa (*Psylliodes chrysocephala*) är viktiga skadedjur som skadar höstraps i etableringsfasen. Det behövs integrerade växtskyddsstrategier som kan leverera effektiva och miljövänliga växtskyddsstrategier för dessa skadegörare. Syftet med detta projekt var att (1) utvärdera om samodling med baljväxter kan skydda höstraps mot skador av sniglar och rapsjordloppor (2) utforska hur jordbearbetning kan utföras för att motverka snigelskador samtidigt naturliga fiender i form av jordlöpare främjas och (3) analysera rapsjordloppans långsiktiga populationsdynamik. Samodling av höstraps med baljväxter utvärderades i ett växthusförsök och ett fältförsök i södra Sverige. Sniglar och deras huvudsakliga naturliga fiender, jordlöpare, studerades i 30 fält med olika jordbearbetning under två säsonger i Västergötland, och rapsjordloppans långsiktiga populationsdynamik undersöktes genom att analysera prover insamlade från mer än 3000 fält under femtio år i Skåne. Samodling av höstraps med baljväxter minskade skador och förekomst av rapsjordloppa. För sniglar minskade skadorna på höstraps i växthusförsöket vid samodling med våråkerböna, medan ingen av de testade baljväxterna påverkade skadan i fält. Jordlöparnas förekomst, artrikedom och diversitet minskade i fält med plöjning efter jordbearbetningstillfället, men återhämtade sig sedan till nästa säsong till nivåer som liknade de andra jordbearbetningsmetoderna. Jordlöparna åt sniglar men i begränsad utsträckning, kanske på grund av låg snigelförekomst. Förekomsten av sniglar och snigelskador på groddplantorna var för låg i höstrapsfälten för att på ett kraftfullt sätt kunna bedöma skillnader mellan jordbearbetningsmetoderna. Förekomsten av

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sniglar skilde sig inte mellan jordbearbetningsmetoderna under den följande säsongen, då sniglarnas förekomst åter hade ökat till nivåer där skillnaderna kunde utvärderas mellan behandlingarna. Rapsjordloppan hade en stark, regelbunden och synkron cyklisk populationsutveckling med en period på 8 år. Vårt projekt visar att samodling med baljväxter kan användas som en strategi för att kontrollera rapsjordloppa och att jordbearbetning kan användas som en strategi för att bekämpa sniglar utan långsiktiga negativa effekter på jordlöpare. Rapsjordloppans förekomst kan också prognosticeras, vilket innebär att skadedjurshanteringen kan skräddarsys till det förväntade skadedjurstrycket, till exempel genom att använda insekticidbehandlade frön eller minska arealen höstraps under år med höga förväntade förekomster av rapsjordloppa.

Part 1.2: Main report

Introduction

Crop protection is becoming more important as the availability of chemical pesticides continuously decrease. Integrated pest management strategies are needed that can deliver efficient and environmentally friendly crop protection strategies to growers. In winter oilseed rape (WOSR, *Brassica napus*), slugs (primarily the grey field slug *Deroceras reticulatum)* and cabbage stem flea beetles (*Psylliodes chrysocephala*, CSFB) are important pests attacking the crop in the establishment phase (SJV 2017, Ortega-Ramos et al. 2022).

Winter oilseed rape can be severely damaged by slugs, primarily the grey field slug (*Deroceras reticulatum*), feeding on the seedlings. Moist weather, high soil moisture and soil organic matter content as well as coarse textured clay soils are favorable factors for slugs (SJV 2017).

Cabbage stem flea beetle adults colonize newly sown WOSR fields in the autumn, causing feeding damage to the seedlings. Larvae develop inside WOSR stems, which also can damage the plant, and adult CSFB hatch from the crop plants in the following spring (Ortega-Ramos et al. 2022).

An increasingly popular cropping strategy is to establish WOSR with frost-sensitive legumes that are winter-killed. The legumes add biological fixed nitrogen and can improve weed and pest control (Cadoux et al. 2015). The first aim of this project was to evaluate if companion cropping with legumes can protect establishing WOSR crops from slugs and CSFB.

Carabid beetles (Coleoptera: Carabidae) are potentially important natural enemies of slugs (Symondson et al. 1996, 2002, Bohan et al. 2000), but their role for slug control in Sweden has not been examined. By studying slugs, carabids and their predation of slugs in commercial WOSR fields over two seasosn, the second aim of our project was explore how soil tillage can be designed to suppress slugs while promoting carabid beetles.

Long term monitoring data for pests currently collected and stored by the Swedish Board of Agriculture is a promising resource to advance our understanding of best integrated pest management practices. Cabbage stem flea beetle has been systematically monitored for more than half a century in Scania in southernmost Sweden. The third and last aim of this project was to analyze CSFB long term population dynamics and how abundance is affected by crop management and climate.

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Materials and methods

Companion cropping

We tested the effect of intercropping on slug preference in the greenhouse. Five WOSR plants per cage were sown in 45 cages. In 15 cages each, we additionally sowed five spring faba (OSR + Sfaba) or berseem clover (OSR + Clover) plants. Adult slug specimens of *D. reticulatum* were collected around Uppsala in the autumn of 2019 and starved prior the experiment. One slug was placed in each of the 45 cages when the WOSR had two to three true leaves. On each OSR plant we assessed slug damage after seven days from 0–10 corresponding to 0–100% damage.

We tested the effect of intercropping WOSR with legumes on both CSFB and slug damage in a field experiment at the SITES (Swedish Infrastructure for Terrestrial Ecosystem Science) Lönnstorp field research station in southern Sweden in 2019. A randomized complete block design was used, with four replicates of each treatment, and a 12 m by 6 m plot size with 0.5 m between plots and 12 m between blocks. The experiment had five treatments, winter OSR sole crop (OSR) and winter OSR intercropped with berseem clover (*Trifolium alexandrinum* L., OSR + Clover), spring faba bean (*Vicia faba* L. var. *minor*, OSR + Sfaba), winter faba bean (*Vicia faba* L. var. *minor*, OSR + Wfaba) or winter peas (*Pisum sativum* L., OSR + Wpeas).

Winter OSR was sown with a 50 cm row distance on August 29, 2019 (cv. Mentor, 2.8 kg/ha, corresponding to approximately 50 seeds/m^2). Berseem clover (cv. Tigri, 20 kg/ha, 600 seeds/m^2) was sown with a broadcast seeder just before sowing the winter OSR, on the same day August 29. Spring faba bean (cv. Boxer, 110 kg/ha, 20 seeds/m^2) was sown with a 50 cm row distance in between OSR rows on September 9. Winter faba bean (cv. Hiverna, 130 kg/ha, 25 seeds/m^2) and winter peas (cv. EFB33, 120 kg/ha, 100 seeds/m^2) were sown with a 50 cm row distance, in between OSR rows on October 7.

We assessed 15 OSR plants per plot for slug and CSFB damage from 0–10, corresponding to 0–100% damage. Slug and CSFB damage were quantified in OSR, OSR + Clover and OSR + Sfaba on September 26, 2019 at crop stage BBCH 12–13, as these treatments are most likely to impact early season pests. Slug and CSFB damage assessments were conducted a second time on November 4, 2019, at crop stage BBCH 15 in all five OSR treatments. We additionally counted CSFB larva in OSR, OSR + Clover and OSR + Sfaba on March 2, 2020 (BBCH 30–32). Ten plants per plot were collected, dissected, and the number of CSFB larvae per plant were counted in the laboratory.

We analyzed percent differences in percent damage from slugs and CSFB, and abundance of CSFB larva in spring using linear mixed models, with treatment as a fixed effect and plot within block as a random effect. For slug damage to OSR seedlings in the greenhouse, treatment was a fixed effect and cage was a random effect. Tukeys test was used to identify significant differences between treatments in all models. We performed statistical analyses in R version 4.0.3 (R Development Core Team 2020), with the lme4 and multcomp packages.

Tillage, slugs and carabids

We identified and selected 30 winter wheat fields (one winter barley field was included) in Västergötland early summer 2020 that we going to be sown with WOSR following harvest.

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The fields were evenly distributed between farmers ploughing, using reduced tillage or no soil tillage (direct seeding) prior to sowing WOSR. In each field we monitored slugs and carabids in both the winter wheat in 2020 and the WOSR in 2020-2021. Carabids were sampled using pitfall traps in June, July and September 2020 and May, June and July 2021. Slugs were sampled using slug boards in July and September 2020 and May and July 2021. We assessed slug damage to WOSR in September 2020 by scoring visual damage to seedlings. In July and September 2020 we additionally sampled live carabid beetles using pitfall traps. In the laboratory we collected regurgitates from 2063 beetles representing the most common species, that were analyzed for their gut content using multiplex-PCR.

We used linear mixed models in R to analyze the effect of sampling occasion and tillage method on slug abundance, slug damage (sampling occasion not included in models, as damage only was assessed once) and carabid abundance, species richness and Shannon diversity.

Cabbage stem flea beetle population dynamics

Cabbage stem flea beetle has been monitored in southern Sweden since 1969. We analysed data collected over 50 years: 1969-2018. Our database included observations from 3045 field in Scania. In each field, an average of 29 crop plants (range: 5-105) were cut at the base between December and April. Plants were brought to the laboratory, where petioles and stalks were dissected and the number of live *P. chrysocephala* larva per plant were counted. We divided data into five subregions: (1) southeast, (2) southwest, (3) west, (4) northeast, and (5) northwest (Fig. 2). Our database additionally contained information on crop planting date.

We calculated accumulated larval degree days and number of cold days for each sample based on their documented importance for *P. chrysocephala* (Johnen and Meier 2000, Mathiasen et al. 2015a). We used weather data from a nearby weather station to calculate larval accumulated degree days (lower and upper thresholds of 3 and 25°C, respectively) and the number of cold days with a minimum temperature below -10 °C. We started calculating larval degree days after first adding one week to the sowing date for egg laying to start and then 185 degree days (lower threshold 5 °C) for egg development (Mathiasen et al. 2015b).

Statistical analyses were conducted in the R software environment for statistical computing and graphics, version 4.0.3 (R Development Core Team 2020). We tested for and estimated population cycle length with the autocorrelation function (ACF) plots using 95% confidence intervals for each subregion, as well as for the global dataset. Wavelet transform analysis was used to calculate the wavelet spectrum of each subregional as well as the global time series using the wt function from the biwavelet R package. The synchronicity across the five populations of *P. chrysocephala* from each subregion was analyzed using wavelet phasor mean fields withthe wsyn package.

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Results

Companion cropping

In the laboratory experiment, slug damage to oilseed rape seedlings where highest in the WOSR sole crop and lowest when companion cropped with spring faba bean (Table 1). Damage when intercropped with berseem clover was intermediate and not statistically different from any of the other treatment (Table 1). In the field experiment, slug damage was similar in all treatments, both in September and November (Table 1).

Table 1. Slug and cabbage stem flea beetle damage to oilseed rape (OSR) in winter OSR sole crop or winter OSR intercropped with berseem clover (+Clover), spring faba bean (+Sfaba), winter faba bean (+Wfaba) or winter peas (+Wpeas). Numbers are mean percent leaf area injury with standard errors within parenthesis. Treatments not sharing the same letter are significantly ($p < 0.05$) different from each other. Table has been adapted from Emery et al. 2021.

	OSR	OSR + Clover	OSR + Sfaba	OSR + Wfaba	OSR + Wpeas
Slugs (Sept)	17.6 (1.2)^{a}	15.1 (1.1)^{a}	15.6 (1.1)^{a}	NA	NA
Slugs (Nov)	10.5 (0.5)^{a}	10.8 (0.5)^{a}	10.3 (0.3)^{a}	10.0 (0.4)^{a}	11.9 (0.7)^{a}
Slugs (Lab)	25.5 (2.8)^{b}	18.9 (2.5)^{ab}	15.3 (2.2)^{a}	NA	NA
CSFB (Sept)	25.8 (1.4)^{b}	18.7 (1.2)^{a}	21.3 (1.0)^{ab}	NA	NA
CSFB (Nov)	9.5 (0.3)^{c}	4.5 (0.7)^{a}	9.0 (0.4)^{bc}	8.3 (0.5)^{bc}	7.5 (0.6)^{b}
CSFB (March)	5.5 (0.6)^{b}	3.6 (0.4)^{a}	2.6 (0.3)^{a}	NA	NA

Tillage, slugs and carabids

Slug abundance was significantly lower in September in the WOSR seedling stage compared to the other sampling occasions (Figure 1). Slug abundance did not differ between tillage treatments (Figure 1). Slug damage to WOSR seedlings was below 1% in all tillage treatments and was not statistically different from each other.

Carabid abundance, species richness and Shannon diversity showed a seasonal pattern in all tillage treatments, with lowest numbers in September 2020 (Figure 2). September 2020 was the only sampling occasion where there were effects of the tillage treatment, with generally higher abundance, richness and Shannon diversity of carabids in treatments with reduced or no tillage (Figure 2).

Of the prey items tested for, carabid were mainly consuming earthworms and springtails. Three percent of the carabids had consumed slugs, with *Harpalus rufipes* and *Pterostichus melanarius* being the species most often testing positively.

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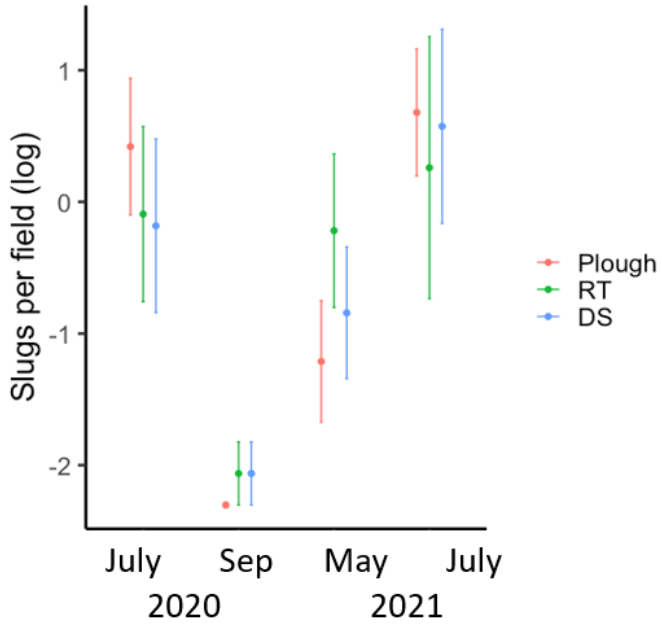

Figure 1. Mean slug abundance in the three tillage treatment at four sampling occasions in 2020-2021. Slug abundance did not vary significantly across tillage treatments. Plough = ploughing, RT = reduced tillage, DS = direct seed. Error bars display standard errors.

Cabbage stem flea beetle population dynamics

Cabbage stem flea beetle exhibited strong 8-year population cycles that were stable over the period and highly synchronous across the region (Figure 3), although periodicity was somewhat stronger in the southwest compared with the northeast. Later sowing, fewer larval accumulated degree days and number of days with strong frosts with temperatures below -10°C during winter were all significantly correlated with lower CSFB density (Figure 4).

Discussion

Intercropping WOSR with legumes reduced damage and abundance of CSFB, which is line with other research performed concurrently (Breitenmoser et al. 2022). The explanation for this result could be that the intercropped legumes complicate visual or olfactory host plant location for the pest. For slugs, damage to WOSR was reduced in the greenhouse by intercropping with spring faba bean, whereas in the field neither legume affected damage. Slug abundance and damage in the field experiment was low, which could explain these discrepancies.

Carabid abundance, species richness and diversity decreased in fields with ploughing after soil tillage, but then recovered by the next season to levels similar to those in the other tillage treatments. Carabids predated on slugs but to a limited extent, perhaps due to the low slug abundance. Slug abundance and damage was too low in the WOSR seedling stage for us to powerfully assess differences between tillage treatments, but from other research it is known that soil tillage reduce slug crop crop damage by directly killing slugs and redistributing them deeper into the soil (Symondson et al. 1996, Glen et al. 2004). Slug abundance did not differ between tillage in the following season, when their abundances again had increased to levels where differences could be meaningfully compared across treatments.

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We showed that cabbage stem flea beetle had strong, regular and synchronous population cycles in Scania, southernmost Sweden with a period of 8 years. Intraspecific competition between CSFB larvae inside the plant, or natural enemies are potential drivers of this cyclicity and synchronicity, whereas bottom-up effects through changes in host plant quantity or quality seems more unlikely. There is some evidence that these cycles also occur in Denmark and northern Germany, but not in the United Kingdom (Crop Monitor 2021, Erichsen 1993, Kristensen and Skovgard 2018, Nilsson 2002). The cyclic population dynamics for CSFB means that pest damage can be forecasted before the crop is sown. Pest management tactics can therefore be planned, implemented and tailored to projected pest pressure, for example by using insecticide treated seeds or reducing WOSR crop area in years with high expected pest pressure.

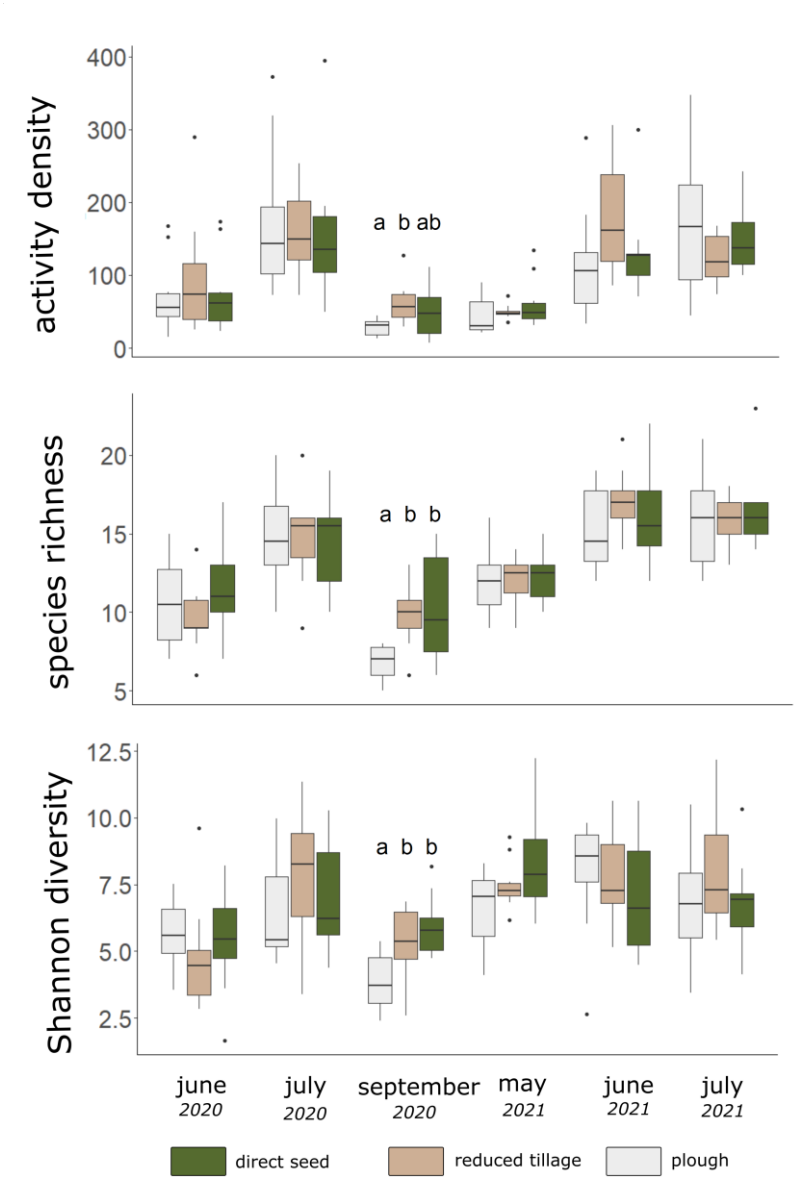

Figure 2. Carabid abundance, species richness and Shannon diversity across three tillage treatments and six sampling occasions 2020-2021. Shown are boxplots with medians and quartiles. Points indicate extreme values. Different letters indicate statistically significant ($p < 0.05$) differences between tillage treatments.

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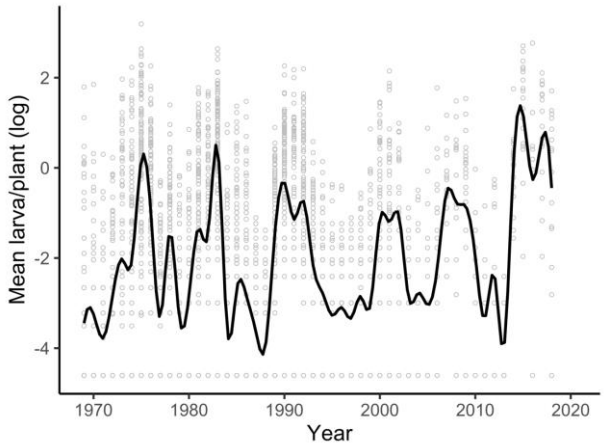

Figure 3. Mean number of *P. chrysocephala* larva per plant in a winter oilseed rape field within in Scania, Sweden 1969-2018. The black line indicates the mean each year and data points individual samples and variation around the mean.

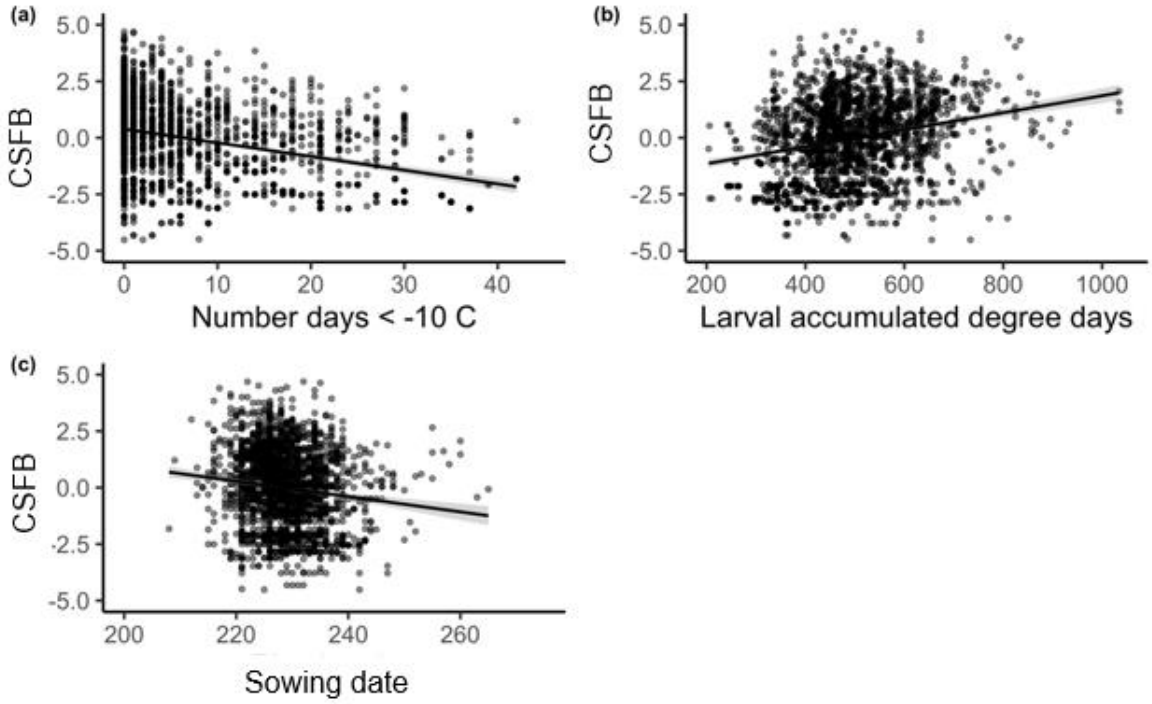

Figure 4. Relationships between the number of cold days during winter (y = -0.06x + 0.39, R2 = 0.1, p < 0.001), larval degree days (y = 0.004x – 1.91, R2 = 0.06, p < 0.001) and sowing date (day number, y = -0.03x + 7.63, R2 = 0.02, p < 0.001) and cabbage stem flea beetle larval abundance (model residuals). Data points display variation around the trend line.

Conclusions

Intercropping with legumes can reduce abundance and damage by CSFB in WOSR. Intercropping also shows promise to reduce slug damage, but further field experiment with higher slug pressure are needed to confirm this.

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Carabids predate on slug and that their abundance, richness and diversity decline immediately following ploughing, but these properties seem to recover by the next season. In a similar way, our data indicate that slug abundance does not increase in the long term following reduced or avoided soil tillage. No conclusions regarding short-term effect of tillage on slug abundance or crop damage could be drawn from this project due to low slug pressure in the WOSR seedling stage.

Cabbage stem flea beetle has strong, regular and synchronous population dynamics in Scania, southernmost Sweden, with 8 years between the population peaks. The number of CSFB larvae per plant decline when the crop is sown later and if there are many days with strong frost during the winter, and they increase if the autumn is mild.

Relevance and recommendations

Intercropping with spring faba bean reduced slug damage to winter oilseed rape in the laboratory, but not the in the field. Therefore, further experiment are needed before intercropping can be recommended as a control strategy for slugs. We find, however, support from our field experiment that intercropping with legumes reduce larval damage by CSFB to winter oilseed rape. Further experiments where oilseed-legume intercropping is tested and developed by farmers themselves, are needed before application in commercial farming. Such experiments are underway in an ongoing SLF project (O-21-20-636).

Our results show that carabid eat slugs and that carabid abundance and richness will temporarily decline following ploughing, whereas we could not evaluate short-term effects of soil tillage on slugs and their damage. There is, however, strong support from other research that slug abundance and damage will decrease in the short term following soil tillage. We did not find any evidence that neither slugs nor carabids were affected by tillage in the following season. In conclusion, we can therefore recommend soil tillage as a method to control slugs without any persistent negative effects on natural enemies.

Cabbage stem flea beetle has strong population cycles, which are synchronized in Scania. Sowing the crop later will reduce the number of larvae per plant, but should be weighted against other benefits of early establishment. Mild autumns and winters without strong frosts favor build-up of CSFB populations. This knowledge can be applied for forecasting of pest pressure. Farmers can for example, use insecticide treated seeds or reduce WOSR crop area in years with high expected cabbage stem flea beetle pest pressure.

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Result dissemination:

Scientific publications, *published*	Emery, S. E., P. Anderson, G. Carlsson, H. Friberg, M. C. Larsson, A.-C. Wallenhammar & O. Lundin. 2021. The potential of intercropping for multifunctional crop protection in oilseed rape (Brassica napus L.). Frontiers in Agronomy 3: 782686. https://doi.org/10.3389/fagro.2021.782686

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Scientific publications, *submitted*	Emery, S., M. Klapwijk, R. Sigvald, R. Bommarco, O. Lundin. Consistent and spatially synchronous 8-year population cycles of cabbage stem flea beetle in Swedish agroecosystem.
Scientific publications, *manuscript*	Emery, S., R. Bommarco, O. Lundin. Carabid predation of slugs under different tillage regimes.
Conference publications/ presentations	Emery, S., M. Klapwijk, R. Sigvald, R. Bommarco, O. Lundin. 2022. Consistent and spatially synchronous 8-year population cycles of cabbage stem flea beetle in Swedish agroecosystem. International Congress of Entomology. Talk. Helsinki. July 21.
	Emery, S., M. Klapwijk, R. Sigvald, R. Bommarco, O. Lundin. 2022. Regular and synchronous population cycles of cabbage stem flea beetle facilitate integrated pest management. International Organisation for Biological and Integrated Control – Working Group Integrated Control in Oilseed Crops. Talk. Online. May 17.
Other publications, *media etc.*	Lundin, O. 2021. Påverkan av markbearbetning på sniglar och deras naturliga fiender. Svensk Frötidning 6: 10-12. https://sfo.se/kunskap/paverkan-av-markbearbetning-pa-sniglar-och-deras-naturliga-fiender/
	Lundin, O. 2021. Höstraps i samodlingssällskap gav både plus och minus. Svensk Frötidning 5: 20-23. https://sfo.se/wp-content/uploads/2021/07/02266.pdf
Oral communication, *to sector, students etc.*	Effects of tillage on slugs and their natural enemies. Talk on a regional crop production conference. Organized by the Swedish Rural Economy and Agricultural Societies. Uddevalla.
	How can research address increasing problems with insect pests? Talk on the subject committee for crop protection. Organized by the Swedish University of Agricultural Sciences. Alnarp.
	Crop protection against insect and slugs in oilseed rape and grain legumes. Crop protection day for crop advisors. Organized by the Swedish University of Agricultural Sciences. Uppsala

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