Dispersal and spatiotemporal distribution of Protapion fulvipes in white clover fields: implications for pest management

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Abstract
Yield loss caused by insect pests remains a substantial problem in agriculture. Chemical control, with potential negative effects on non-target organisms, is still the main tool for pest management. For pest species with limited dispersal capacity, rotation of the crop in time and space has potential as an alternative management measure. This is particularly important in organic farming, where most agrochemicals are prohibited, but also relevant as a complementary pest management strategy in conventional agriculture. Clover is an important crop used for animal feed and as green manure; however, seed-eating weevils can severely limit the seed yield. We hypothesized that the previous year’s clover seed fields constitute the major sources of weevil pests. Consequently, a greater distance to, and a smaller pest load from, this source should reduce the number of weevils colonizing the new seed fields. To map population dynamics and dispersal range of Protapion fulvipes, an economically important seed weevil specialized on white clover, we conducted field studies over four years in 45 white clover seed fields. We found that P. fulvipes overwinters close to its source field and disperses to new fields in early spring the following year. Pest abundance increased with pest load in the previous year’s seed field, but decreased by 68% per km distance to the previous year’s field. Thus, separation of seed production fields between years by 2–3 km would create a spatiotemporal pest management tool to reduce the pest infestation below the estimated economic injury level.

Keywords Trifolium repens · White clover seed weevil · Agricultural pest · Dispersal distance · Clover seed production

Key Message
- The seed pest Protapion fulvipes severely limits white clover seed yields
- We mapped dispersal and spatiotemporal distribution of the pest over 4 years in 45 seed fields
- Weevil population and field size one year influence the weevil population the following year
- Pest abundance decreases, and seed set increases, with distance to the previous year’s field
- Moving the seed field 2–3 km between years would allow spatial escape from the weevil pest

Introduction
Despite extensive use of chemical control, crop yield losses caused by pests have remained proportionally constant or even increased in some areas in the latter half of the last century (Oerke et al. 1994; Pimentel et al. 1991; Oerke 2006). Although integrated pest management today could offer a wide diversity of measures (Kogan 1998; Zalucki et al. 2009), the reality is that pest control still depends mainly on insecticides (Dent 2000; Rebek et al. 2012). To design appropriate integrated pest management (IPM) measures that can reduce the pest load below the estimated economic
injury level, where insecticides are redundant, knowledge about pest biology is crucial.

Growing the same crop in the same field for several years in a row is known to cause accumulation of crop diseases, pests and depletion of nutrients (Bullock 1992; Mohler and Johnson 2009). For pest species with limited dispersal capacity, moving the resource in time and space by crop rotation can be an important measure to control population buildup (Bullock 1992; Dent 2000; Mohler and Johnson 2009). This measure, however, requires knowledge on pest dispersal ability. We know that insects disperse and migrate to escape unfavorable environmental conditions or in search of resources such as suitable habitat, food, mates and oviposition sites (Wolfenbarger 1946; Dingle and Drake 2007; Dingle 2014; Chapman et al. 2015). Their dispersal distances differ, ranging from very short distances (Wolfenbarger 1946) to several hundreds of kilometers (Kim and Sappington 2013). Nevertheless, for many insect species, dispersal distances are unknown.

The white clover seed weevil Protapion fulvipes, Geoffroy, 1785 (Coleoptera: Apionidae) is an economically important pest, which substantially reduces seed yields of white clover, Trifolium repens L. (Göngö 1997; Langer and Rohde 2005; Hansen and Boelt 2008); an important forage legume for grazing livestock in many temperate areas (Baker and Williams 1987). As clover efficiently fix atmospheric nitrogen, it is an essential crop in the organic farming sector, where inorganic fertilizers are prohibited (Stockdale et al. 2001). However, predation by seed-eating weevils is limiting yields particularly in organic clover seed production (Langer and Rohde 2005; Lundin et al. 2017).

Overwintering adult P. fulvipes emerge in early spring, around mid-April, after which they locate host plants, T. repens and T. hybridum L. (Markkula and Myllymaki 1957, 1964; Göngö 1997), to feed on until sexually mature (Freeman 1967). After mating, females oviposit inside clover inflorescences (Freeman 1967; Göngö 1997). Hatched larvae feed on ovules and developing seeds (Jones 1950; Freeman 1967), pupate, and in late summer, often coinciding with clover seed harvest, adults emerge from inflorescences and feed from clover leaves before dispersing to overwintering areas (litter or tall grass, Ohlsson 1968; Freeman 1967). At northern latitudes, P. fulvipes has only one generation per year (Jones 1950; Freeman 1967). The substantial variation in weevil abundance among fields and years (Freeman 1967; Nyabuga et al. 2015) may be caused by varying survival rates following winter conditions (Freeman 1965) as well as by proximity to previous year’s clover fields (Langer and Rohde 2005). Protapion spp. weevils have been reported to easily move up to 500 m (Ohlsson 1968; Krüss and Tscharntke 1994), and the suggested pest control method of growing white clover on fields away from any insect sources, e.g., previous year’s clover field (Bovien and Jørgensen 1934, 1936), would work only if fields are separated by long enough distances. Langer and Rohde (2005) reported that presence of an immediate pest source within 1 km was linked to a higher number of weevils, indicating the potential for spatial escape in IPM, but did not provide any detail about the general relationship between inter-field distance and pest pressure. In Sweden, growers are advised to wait five years before growing white clover in the same field again, and recently an advice of a distance of at least 500 m between fields in subsequent years has been added (Swedish Board of Agriculture, 2019). Comparatively, Lundin et al. (2016) showed that abundance of Protapion trifolii (L.), attacking red clover Trifolium pratense L., tended to increase when a red clover field was within 800 m from a previous year’s field. However, P. trifolii, in contrast to P. fulvipes, flies minimally and primarily walks during dispersal (Bovien and Jørgensen 1936), indicating that P. fulvipes might have a greater dispersal range than P. trifolii. Hence, research on the potential to use spatial escape as a pest control measure in white clover, and an accurate distance recommendation, is still needed.

Our aim was to investigate the potential to reduce pest pressure and yield loss in white clover seed production using spatial planning. We did this by studying spatiotemporal dynamics of P. fulvipes and its influence on seed set over four years in 45 white clover seed fields in southern Sweden. In Sweden, seeds are sown into a cereal crop the year before the seed harvest year (Svensk raps 2018) (Fig. 1). It is, however, unclear whether the weevils locate clover seedlings present in the new seed field already in the autumn or in the following spring. We hypothesized that the previous year’s clover seed field constituted the major pest source for new seed fields in the agricultural landscape and that a longer distance to this source would significantly reduce the number of weevils colonizing the new seed field. We also predicted a positive relationship between source field size, its abundance of seed weevils and the potential number of seed weevils dispersing from the source field (i.e., pest load). Our study addressed the following specific questions: (1) Do clover seed weevils overwinter by the recently harvested seed field or by the newly sown seed field? (2) Does the pest load from previous year’s seed field influence subsequent attacks and seed loss in the new, adjacent seed field? (3) Can an increased distance between subsequent fields mitigate the pest pressure and benefit seed production?

Material and methods

Study system

In total, 45 fields were included in the study that took place in the southernmost part of Sweden, Scania, in 2014–2017.
Fields were sown with white clover for commercial seed production and varied in management practices (i.e., organic or conventional), cultivars, field sizes and in distance to the previous year’s closest white clover seed field (Table S1.1). Twenty-one fields were organically managed without use of any pesticides, whereas 24 fields were conventionally managed with application of different pesticides to control weevils (Table S1.1). In each year, 12 fields (three organic, nine conventional), 14 fields (nine org., five conv.), 13 fields (seven org., six conv.) and six fields (two org., four conv.) were part of the study, respectively (Fig. S1.1). With exceptions for sampling of overwintering weevils (Sect. Overwintering sites), all fields were included in the sampling. Field locations followed the farmers’ crop rotation and schedules and were thus beyond our control. A timeline for the management of clover seed fields and timing of our monitoring with emergence traps, pan traps and inflorescence harvest is shown in Fig. 1.

For in-field sampling (immigrating weevils and flower heads), we established plots of each 24 m × 50 m, containing a 50-m transect in parallel to and 8–12 m from the field border. In organic fields we had one plot, whereas in conventional fields we had two plots, of which one was kept unsprayed in order to single out the effect of insecticide spraying from other management differences.

**Overwintering sites**

To investigate whether weevils overwinter close to their native field (source field) or the field to be harvested in the subsequent year (study field) (i.e., to disentangle when weevils locate the new seed fields), we caught insects when emerging from overwintering. A preliminary survey was done in 2013 to test the suitability of soil emergence traps in sampling overwintering weevils and to establish the locations of overwintering sites, i.e., inside or right outside clover fields (see Supplementary Information 2 for details). We then selected five (four conventional and one organic), seven (four conv. and three org.) and seven (four conv. and three org.) of our study fields for sampling of overwintering weevils over the years 2015, 2016 and 2017, respectively (Table S1.1–S1.2). Data from 2014 were here only used as source field data to the study fields in 2015. Based on the preliminary survey, we subsequently placed soil emergence traps (BT2006 and BT2003, Bugdorm, MegaView Science Company, Taiwan, 60 × 60 × 60 cm) outside white clover fields in early spring, April 15–21 of 2015 and 2016, and in March 25–27 of 2017 due to an unusually warm spring this year. By each study field, five sets with three emergence traps were placed at different distances from the source field (Fig. 2, exceptions in Table S1.2). i) Two sets were placed in the grassy margin right outside the study field; one on the side farthest away from the source field, and one on the side closest to the source field. ii) Two sets were placed by the source field, in the same positions as in the previous year (i.e., when the field was a study field). iii) The fifth set of traps was placed at the midpoint halfway between the study field and its source field (Fig. 2), usually in a grassy field margin, otherwise in a similar grassy area. Trap flaps were dug down and secured, so that all insects overwintering in...
the covered area would crawl toward the light and end up in a 500-ml polyethylene bottle partly filled with 1/3 each of water, glycol and ethanol. Bottles were emptied and refilled every third week (in total three times). Traps were removed when no more insects were emerging (June 15–17, 2015; June 20–24, 2016; June 20–22, 2017). Sampled weevils were identified to species, counted and summed per trap.

Immigrating weevils

In order to investigate the immigration of weevils into a seed field, pan traps were placed in the study fields, following methods in Lundin et al. (2017), before onset of flowering in May 2014–2017 (Table S1.3). Three pan traps were placed along the transect inside each plot in the study field, with a distance of 12.5 m between traps. Pan traps were fastened with tent pegs and marked with flags for ease of location. The traps were filled to approximately ¾ with water containing a drop of liquid detergent and emptied once per week for 7–8 weeks (May 23–July 16, Table S1.3). In the analyses (and from here on), we only included pan trap catches from the first five emptying rounds (May 23–June 25) because we aimed to establish immigration of weevils rather than the later reproduction and emergence of weevils from flower heads (see motivation and details in Fig. S1.2–S1.3; Box S1.1).

Incubation of flower heads for weevil emergence, assessment of flower density, seed set and pest load

Late in crop bloom each year (July 10–21, Table S1.3), we determined the inflorescence density by counting flowering and withered flower heads in three 0.25-m² squares along each transect. At the same occasion, we collected inflorescences for incubation and emergence of clover weevils and assessment of seed set following methods described in Lundin et al. (2012, 2017). We picked 120 withered flower heads along each transect and incubated them in poster tubes with a transparent tube connected to the lid. After leaving the samples at room temperature for at least six weeks, we counted and identified the emerged weevils. To assess seed set, we counted the number of florets per flower head on 20 flower heads per transect, and the number of seeds per floret from 100 florets per transect (five florets from each of the 20 flower heads).

For analysis, the inflorescence density (from previous year’s field) was used together with field size and the number of weevils emerging from flower heads to calculate the pest pressure (pest load) from the source field (pest load...
from source field = mean number of flowering and withered inflorescences per m² × area of field in m² × mean number of emerged weevils per inflorescence). In conventional fields, weevils from the insecticide-treated plot (the plot treated as the rest of the field) were the basis for calculating the pest load.

**Statistical analyses**

Statistical analysis was performed using R version 4.0.4 (R Core team 2021), and results were obtained from type II Analysis of Deviance Table using Wald Chi-square tests (package car and lmerTest, function Anova, Fox and Weisberg 2011; Kuznetsova et al. 2017). Post hoc tests featuring multiple comparisons between groups were computed using estimated marginal means (function emmeans in package emmeans, Lenth 2019). The estimated marginal means show the mean response for each factor adjusted for the other variables in the model. Tests performed to answer our research questions are detailed in Table 1. Dependent variables included number of weevils in emergence traps, in pan traps and hatched from flower heads as well as number of seeds per pod. Fixed variables included combinations of management, field type and distances between fields, pest load, number of weevils, year and relevant interactions (see Table 1 for details). As random factors, we used either Farm and Field identity or Geographical area and Field identity (Table 1). For all models, we confirmed that covariates were not collinear and that model assumptions were met by checking for distribution of residuals, equal variance between groups, independence, and no overdispersion (Zuur et al. 2009, 2010). To ensure spatial independence, we included a random variable to group data collected close in space. This was either a density-dependent spatial clustering at 4 km (computed in ArcGIS PRO, ESRI 2020), or Farm (an area with fields belonging to one farmer), depending on what provided a better model fit (AIC and residual patterns). Results were very similar independent of this spatial grouping factor, and for analyses with fewer fields (e.g., emergence trap data), these groupings were identical. In addition, we checked for spatial autocorrelation using package DHARMa (Hartig 2021). In two cases of missing emergence trap samples among the three emptying rounds, all data from the emergence trap in question were excluded from the analysis and an offset included in the analysis. In cases of missing pan trap samples (when traps had been trampled or run over, etc., 40 out of 1035 cases), we used imputation to calculate values for the missing traps using the mean from remaining traps emptied during that round in the same transect. To get one value per transect, we then summed the number of weevils caught per trap and took the mean per transect.

Distances to source fields were determined based on land-use data from the Integrated Administration and Control System (IACS) from the Swedish Board of Agriculture and confirmed by information from the farmers. According to this, the source field was the closest clover field in the surrounding of the study field. Distances used in the analyses were measured as i) from the source field to each set of emergence traps and ii) between the closest points of the source field and the study field (Fig. 2), using ArcGIS software 10.6 (ESRI 2017). The distance (ii) between source and study field (Table S1.1) did not differ depending on management practice (Wilcoxon: W=729, p=0.26). Neither was it correlated with the proportion area covered by clover seed fields within a radius of 1 km (spearman corr: p=0.28), 3 km (p=0.51) and 6 km (p=0.54), nor to the amount of clover seed fields plus other potential clover weevil sources (leys and mixtures with clover) within 1 (p=0.35), 3 (p=0.69) and 6 (p=0.60) km. We also controlled for and found no correlation between this distance measure and the proportion semi-natural land within a radius of 1 km (p=0.26), 3 km (p=0.33) and 6 km (p=0.28) from the study fields. Semi-natural land included uncultivated permanent grasslands, fallows and field border areas. The distance (i) between emergence traps and source field was, however, generally larger at conventionally managed farms (Wilcoxon: W=8930, p=0.033). Pest load was higher at organic fields, LM: χ² = 16.88, p < 0.001). Hence, management was not included in the same analyses as distance and pest load. Every time pest load was included in a model some data were excluded, as the calculation of pest load required data from a previous year (i.e., data from the first year of a farmers set of fields could not be included in the analysis).

**Results**

In addition to the statistical test results presented below, summary output and analysis of deviance tables for all models are presented in Table S3.1.

**Weevil species composition**

Clover-associated weevils emerging from traps at overwintering sites during all years were to a large extent Protapion spp., 82% (of which 99% P. fulvipes), but also Sitona spp., 14%, and Hypera spp., 4%, were caught. Seventy-six percent of P. fulvipes, 83% of Hypera spp., and 67% of Sitona spp. individuals were found at the source field, whereas 13% of the P. fulvipes were found at the study field and 11% at midpoints (Table 2). Pan trap catches showed a similar pattern: 92% of trapped weevils belonged to the genus Protapion (of which 99% were P. fulvipes, Table 2), 6% to Sitona, and 2% to Hypera. Of the weevils emerging from harvested flower heads, 98.5% were Protapion spp. (of which 99.97% P.
| Testing | Test | Dependent variable | Independent fixed variables | Random variables | Family (link) | Comment | Number of observations |
|---------|------|-------------------|----------------------------|------------------|--------------|---------|-----------------------|
| (1) Difference in the number of weevils overwintering right outside the source field, compared to the study field or at midpoints | GLMM | Number of *P. fulvipes* per emergence trap placement (a set of 3 traps) | Field type + Management + Field type:Management + Year | Farm / Field identity | Negative binomial (log) | Offset (log(number of emergence traps per placement)) | Obs: 89, groups: FieldID: Farm, 19; Farm, 7 |
| (2) If the number of overwintering weevils at midpoints and study fields was dependent on the distance and pest load from the source field | GLMM | Number of *P. fulvipes* per emergence trap placement (a set of 3 traps) | Pest load + Distance + Pest load:Distance + Year | Farm / Field identity | Negative binomial (log) | Offset (log(number of emergence traps per placement)) | Pest load and distance standardized to zero mean and unit variance Management practice was not included in the main model but the data was also analysed separately for the different management practices | All: Obs: 51, groups: FieldID: Farm, 18; Farm, 7 Organic: Obs: 18, groups: FieldID: Farm 6; Farm, 3 Conventional: Obs: 33, groups: FieldID: Farm, 12; Farm, 4 |
| (3) If there was a relationship between pan trap catches at a study field, and distance from the source field and its pest load | GLMM | Number of *P. fulvipes* per pan trap | Pest load + Distance + Pest load:Distance + Year | Farm / Field identity | Negative binomial (log) | Pest load and distance standardized to zero mean and unit variance Management practice was not included in the main model but the data was also analysed separately for the different management practices | For conventional fields, data from pan traps in the unsprayed plot were used | Obs:75, groups: FieldID: Farm, 25; Farm, 12 |
| (4) If the number of weevils in soil emergence traps at the study field reflected the number of weevils found later on in pan traps | LM | Log (1 + mean number of *P. fulvipes* in pan traps (first summed per trap)) | Log (1 + mean number of *P. fulvipes* in soil emergence traps at the study field) + Management | Gaussian | | For conventional fields: data from pan traps in the unsprayed plot were used. Due to few observations, the number of variables was reduced to attain the best model fit (AIC, residual patterns); hence, year and grouping variable were omitted. Results were very similar either way | Obs: 19 |
| Testing                                                                 | Test  | Dependent variable                                      | Independent fixed variables                                                                 | Random variables               | Family (link)       | Comment                                                                                      | Number of observations |
|------------------------------------------------------------------------|-------|----------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------|-------------------|---------------------------------------------------------------------------------------------|------------------------|
| (5) Whether the number of P. fulvipes in pan traps could predict the   | LMM   | Number of emerged weevils from flower heads per transect | Mean number of weevils in pan traps per transect (first summed per trap) + Management-      | Geo.Area/Field identity       | Gaussian          | Offset (log(number of incubated inflorescences))                                           | Obs: 69, groups:       |
| number of weevils that would later emerge from flower heads in the    |       |                                                          | Treatment + Year                                                                           |                                |                   | Geo.Area, 45; Geo.Area, 19                                                                    | Geo.Area, 19           |
| field                                                                   |       |                                                          |                                                                                           |                                |                   |                                                                                             |                        |
| (6) If seed set (measured as mean number of seeds per pod) could be    | LMM   | Number of seeds per pod (mean per plot)                  | Mean number of weevils in pan traps (as above) + Management-Treatment + Number of weevils   | Geo.Area/Field identity       | Gaussian          | Obs: 69, groups: Geo.Area, 45; Geo.Area, 19                                               | Obs: 69, groups:       |
| predicted by the number of weevils in pan trap catches                 |       |                                                          | in pan traps: Management-Treatment + Year                                                  |                                |                   | Geo.Area, 45; Geo.Area, 19                                                                    | Geo.Area, 19           |
| (7) If seed set (measured as mean number of seeds per pod) could be    | LMM   | Number of seeds per pod (mean per plot)                  | Number of emerged weevils + Management-Treatment + number of emerged weevils: Management-   | Geo.Area/Field identity       | Gaussian          | Obs: 69, groups: Geo.Area, 45; Geo.Area, 19                                               | Obs: 69, groups:       |
| predicted by the number of weevils emerging from flower heads          |       |                                                          | Treatment + Year                                                                           |                                |                   | Geo.Area, 45; Geo.Area, 19                                                                    | Geo.Area, 19           |
| (8) If seed set was related to distance and pest load from the         | LMM   | Number of seeds per pod (mean per plot)                  | Distance + Pest load + Distance: Pest load + Year                                          | Geo.Area/Field identity       | Gaussian          | Pest load and Distance standardized to zero mean and unit variance                           | Obs: 40, groups:       |
| previous year’s closest clover field                                   |       |                                                          |                                                                                           |                                |                   | Obs: 25; Geo.Area, 11                                                                         | Geo.Area, 11           |
| (9) If the number of weevils emerging from flower heads at the end of  | LMM   | Number of emerged weevils from flower heads per transect | Distance + Pest load + Distance: Pest load + Year                                          | Geo.Area/Field identity       | Gaussian          | Pest load and Distance standardized to zero mean and unit variance                           | Obs: 40, groups:       |
| the season was related to distance and pest load from the previous     |       |                                                          |                                                                                           |                                |                   | Obs: 25; Geo.Area, 11                                                                         | Geo.Area, 11           |
| year’s closest clover field                                            |       |                                                          |                                                                                           |                                |                   | Offset (number of incubated inflorescences) included or not does not change result or       |                        |
|                                                                         |       |                                                          |                                                                                           |                                |                   | model fit                                                                                   |                        |

Field type = Source field, midpoint or study field, Management = organic or conventional, ManagementTreatment = management practice combined with insecticide treatment (organic untreated, conventional untreated or conventional treated), Geo.Area = Geographical area, LM = Linear model, LMM = Linear mixed model, GLMM = Generalized mixed model (package lme4; Bates et al. 2015), Obs = Number of observations
fulvipes) and 1.5% Hypera spp. In addition, a few individuals of P. trifolii and Sitona spp. were found in this material (Table 2).

Weevil abundance

Overwintering weevils

There were more weevils overwintering outside source fields than outside study fields and at midpoints (χ²=27.48, p<0.001), but no difference in between midpoints and at study fields (Fig. 3). The number of weevils was also higher in emergence traps at organic fields than at conventional fields (χ²=12.04, p<0.001; Fig. 3), although there was some variation between farms (Fig. S3.1). There was no interaction effect between trap placement and management practice (χ²=1.67, p=0.43).

The number of P. fulvipes in emergence traps decreased with distance (χ²=6.65, p=0.001) and increased with pest load from previous year’s clover seed field (χ²=11.39, p<0.001; Fig. 4a), but there was no interaction effect of distance and pest load (χ²=3.23, p=0.073). When analysing the data from organic vs. conventional fields separately, the number of weevils in emergence traps at organic fields showed a similar pattern; the number of weevils decreased with distance (χ²=6.77, p=0.009) and exhibited a near-significant increase with pest load (χ²=3.55, p=0.060) from the source field and with no interaction effect between these two variables (χ²=0.12, p=0.73). For data on weevils at conventional fields, we found an interaction effect between pest load and distance (χ²=17.51, p<0.001), showing that when pest load was high, the number of weevils in emergence traps decreased with distance, whereas with low pest load there was no effect of distance, as the numbers were uniformly low from the source field (Fig. S3.2).

We observed that one organic field had pest loads that were more extreme than the rest, and when performing the same analyses without observations from this field, we found an interaction between distance and pest load when including both conventional and organic fields (χ²=9.43, p=0.002).

Again, the interaction showed that when pest load was high, weevils in emergence traps decreased with distance (Fig. S3.2). When only including organic fields there was no interaction and no effect of pest load, but again the number of weevils decreased with distance (Table S3.1).

Immigrating weevils and the new weevil generation

The number of P. fulvipes caught in pan traps decreased with 68% (calculation in Box S3.1) per km distance from

![Fig. 3 Model estimated means and confidence limits (95%) for the number of P. fulvipes per emergence trap in field margins of source fields, at midpoints, and at study fields in relation to management practice (black=organic, gray=conventional). Pairwise comparisons are indicated by letters, and comparison between management practices by stars. Means sharing a letter are not significantly different. ***p<0.001; **p<0.01; *p<0.05; n.s., p>0.05](image-url)
the source field (GLMM: $\chi^2_1=24.29$, $p<0.001$; Fig. 4b) and increased with pest load ($\chi^2_1=10.86$, $p<0.001$), but there was no interaction effect of pest load and distance ($\chi^2_1=0.93$, $p=0.34$). The number of weevils found in pan traps (immigrating weevils) was positively related to the number of $P. fulvipes$ in soil emergence traps (overwintering weevils) earlier in the season at the same study field (LM: $F_1=48.64$, $p<0.001$; Fig. 5a), independent of management ($F_1=2.07$, $p=0.17$). We also found that the number of weevils emerging from flowers at the end of crop bloom (the new generation) was positively related to the number of $P. fulvipes$ in pan traps (LMM: $\chi^2_1=6.49$, $p=0.011$; Fig. 5b). Management type and insecticide treatment also affected the number of emerging weevils ($\chi^2_2=28.95$, $p<0.001$), and post hoc test showed that insecticide-treated conventionally managed plots had the least number of weevils, while numbers in untreated conventional and organically managed plots were not statistically different. There was no interaction effect of management and trapped weevils on the number of weevils emerging from flower heads ($\chi^2_2=2.35$, $p=0.31$).

Seed production and weevils

Seeds per pod were negatively related to pan trap weevil catches (Table 3; Fig. 5c) and to the number of weevils emerging from flower heads (Table 3; Fig. 5d). Management practice and insecticide treatment also affected seeds per pod (Table 3). Post hoc tests showed that there were more seeds per pod in conventional treated plots compared to organic plots, whereas the estimated number of seeds in untreated conventional plots was in between these values (Fig. S3.3).

Because abundance of weevils in emergence traps and in pan traps was related to distance to source field and to the number of later emerging weevils from flowers, as well as to seed set, we tested whether 1) the number of weevils emerging from flower heads at the end of the season and 2) the number of seeds per pod were related to distance from the source field. Results showed that the number of weevils emerging from flower heads was negatively related to distance (Fig. 5e) and positively related to pest load from the source field (Table 3). The opposite was true for seed set, where the number of seeds per pod was positively related to distance (Fig. 5f) and negatively related to pest load from the source field (Table 3). When running the same models but excluding one observation from a field situated 6 km from its source field, there was an interaction between distance and pest load on the number of weevils emerging from flower heads (Table 3), with distance having a negative effect when pest load was high (Fig. S3.4), whereas the number of seeds per pod was no longer significantly positively related to distance (Table 3); however, the trend was still positive (Fig. S3.4).

Discussion

We mapped spatial and temporal population dynamics of white clover seed pests, using soil emergence traps, pan traps and incubation of inflorescences, in order to explore the potential for reducing crop yield loss through spatial planning. We found that the major seed pest, $P. fulvipes$ and other clover-associated weevil pests primarily overwintered adjacent to the source field, i.e., the field grown with clover the
year before the weevils emerge after winter. Pest abundance in clover seed fields to be harvested in a given year increased with pest load and decreased with distance from the previous year’s clover seed field. The current estimated economic injury level for Protapion in red clover seed production is one weevil per pan trap and week (Lundin et al. 2012). To reduce the pest infestation to this level would require a distance of 2–3 km between white clover fields among years.

**Protapion fulvipes as the major seed pest in southern Sweden**

In line with Nyabuga et al. (2015), we found that *P. fulvipes* was the most abundant clover herbivore in and around white clover fields and that there were few individuals of other *Protapion* species present. We also found the more destructive *Hypera* spp. at our study sites, but congruent with Lundin et al. (2017) we found fewer *Hypera* spp. compared to Langer and Rohde (2005) (1.5% vs. 7% of weevils emerging from inflorescences). The combined results strongly suggest that *P. fulvipes* is the main pest of importance for white clover seed production in southern Sweden, with *Hypera* weevils constituting a considerably lesser problem in clover seed production in southern Sweden compared to in Denmark.

**Overwintering and mobility of *P. fulvipes* in relation to age of clover fields**

A pest’s host specificity and mobility, as well as its persistence through dormant life stages, are key factors that...
determine whether crop rotation, i.e., movement of a crop in time and space, is an efficient pest management strategy (Mohler and Johnson 2009). Although *P. fulvipes* is specific in its host range (Markkula and Myllymaki 1957, 1964; Gønget 1997), there is little information as to where this pest overwinters, when it locates the new clover seed field, and how mobile it is. According to Gønget (1997), *P. fulvipes* is an active flyer, more so than relatives such as *P. apricans*, *P. assimile* and *P. trifolii* (Bovien and Jørgensen 1936).

The premise for this four-year study involving 45 white clover fields was based on the assumption that last year’s clover field constituted the main source of clover weevils in the agricultural landscape under study. Similar to Ohlsson (1968), we observed that *P. fulvipes* remained close to the field from where they emerged when overwintering. The weevils overwintered to a higher extent adjacent to the source fields compared to margins of study fields or in the landscape between fields. Furthermore, there was no difference in weevil abundance in emergence traps placed halfway between fields compared to emergence traps close to(112,938),(987,947)

### Influence of pest load and distance on *P. fulvipes* abundance and white clover seed loss

We show that both distance to, and magnitude of the pest load of the source field affect the number of *P. fulvipes* close to and inside the study field. The number of weevils fell below the estimated economic injury level of one weevil per pan trap and week (Lundin et al. 2012) at 2–3 km distance from the previous year’s clover seed field. Our results, demonstrating that weevil abundance in the landscape is closely related to the presence of individual white clover seed fields, provide strong evidence that the seed fields constitute the main sources of white clover weevils in the agricultural landscape context. Similarly, abundance of the red clover pest *P. trifolii* tended to be higher in the presence of a red clover field within 800 m from the previous year’s field (Lundin et al. 2016), and higher numbers of *P. fulvipes* in white clover fields were previously found in the presence of a source field within 1 km (Langer and Rohde 2005).
Because *P. fulvipes* and its host plants are widespread both in the wild and in leys in the agricultural landscape, the presence of various sources of white clover could have influenced our results. Langer and Rohde (2005) suggested that substantial amounts of feral clover in their Danish study area would support background levels of clover seed weevils high enough to preclude relocation of seed fields as a viable strategy to prevent economically relevant crop damage. However, the area where our study was conducted was consistent with the presumptions: the proportion of wild and feral clover was low, and through the IACS database we had information regarding the positions of all clover fields or leys in the area. Congruently, we found no correlation between the amount of clover fields or semi-natural areas in the landscape and our distance measures. Moreover, leys are generally not allowed to mature long enough before harvest to constitute a significant source of seed weevils; thus, any potential leys not reported in the IACS system were presumably not important.

Furthermore, we found that the number of weevils immigrating into the field early in the season positively correlated with the number of weevils that later emerged from inflorescences, and that these in turn were negatively related to the number of seeds per pod at harvest. This is in line with results by Lundin et al. (2017). We found most seeds per pod in conventionally treated plots, whereas the greatest loss to seed-eating weevils occurred in organic fields. We also showed that seeds per pod could be predicted directly by pan trap catches and that seed set increased with distance to, and decreased with pest load of, the previous year’s field. In contrast, the number of weevils emerging from flower heads decreased with distance and increased with pest load. It should be noted that most of our observations were within 3 km of the source field. Excluding the only observation with a longer distance to the source field (6 km), i.e., testing the relationship for shorter distances only, showed that number of weevils emerging from the flowers was decreasing more with distance when pest load was high than when it was low. For shorter distances, the influence on seed set was not significant (but the trend was still the same). In order to directly predict the effect of distance on seed set, we would need more observations at different distances from the source field. In any case, our results confirm that *P. fulvipes* causes significant damage to clover seeds (Langer and Rohde 2005; Lundin et al. 2017) and affirm that preventing weevils from finding the new seed field in the first place is of great importance.

**Spatial planning as a control measure of *P. fulvipes***

Using mark-recapture experiments, Follet et al. (1996) suggested that an effective crop rotation to reduce Colorado potato beetle population densities from year to year would require a distance of ≥ 500 m from previous year’s potato field. Our study demonstrates that the distances between white clover seed fields in consecutive years constitute an important factor in limiting the pressure of seed-eating *Protapion* weevils, and spatial planning could therefore provide a means to increase yields in general, and especially for organic growers. Increasing distances between white clover seed fields in consecutive years appears to provide continuously increasing benefits at least up to a distance of a few kilometers. We therefore recommend a general implementation of spatial planning to keep seed production fields with a large enough distance between fields in different years, in order to spatially escape pest weevils and prevent population build-up. If the areas of individual farms are too small to allow for sufficient separation between years, overcoming this would require grower cooperation. To cope with problems with pollen beetles in oil seed rape, researchers have suggested crop rotation management at the landscape scale, an intervention occurring at scales greater than farm units (Skellern and Cook 2018a, b).

In our recommendations, we have not considered the potential effects of spatial separation of clover fields on natural enemies and other factors that may alleviate pest pressure. Natural enemies are especially important in organic farming, as insecticide use in conventional farming keeps pests under control. Bianchi et al. (2006) concluded that diversified landscapes hold most potential for conservation of biodiversity and thereby sustaining pest control function (i.e., control by natural enemies). Natural enemies of several *Protapion* species, including *P. fulvipes*, have been shown (in red clover) to be more sensitive to lack of connectivity between habitats than their hosts (Kruess and Tscharntke 1994). Current knowledge regarding maximizing ecosystem services in pollination and pest control would suggest keeping smaller seed production fields, to reduce the pest population buildup, contributing to a more diversified landscape, and reducing the distance to areas harboring pest natural enemies. However, if the same area for seed production is required, dividing the total area into greater numbers of smaller fields could mean smaller distance between fields, which would facilitate pest dispersal between seasons. The practice with smaller fields and maintained sown area would therefore only work positively for seed yield if the natural enemies of *P. fulvipes* are efficient at controlling the pest. The effect of natural enemies on *P. fulvipes* in white clover seed production, with different spatial allocation patterns of seed fields, needs to be further investigated.

**Summary and conclusion**

In this four-year study on 45 white clover fields, pest weevils overwintered to a higher extent adjacent to the source field (the previous year’s seed field), compared to adjacent to the
study field (the current year’s seed field). As the season progressed, there was increased immigration of *P. fulvipes* into the study fields. A higher pest load, i.e., the total number of weevils hatched, of the source field in one year resulted in an increase of weevils in nearby study fields in the following year. Higher pan trap catches, i.e., higher numbers of immigrating weevils, in turn resulted in higher pest loads in the mature flower heads and severely reduced numbers of seeds per pod during harvest. This effect was greatly mitigated by insecticide treatments in conventional fields, but also by increased distances between fields in subsequent years. An increased distance from the source field resulted in a decreased number of overwintering weevils near the study field, as well as a reduced number of weevils caught in pan traps or emerging from inflorescences inside the study field later in the season. This points to spatial management as a promising IPM tool to mitigate local population buildup of pests; particularly within organic farming, where insecticide use is not an option, but also as an alternative to insecticide use in conventional farming. We suggest that reducing the size of and separating seed production fields with 2–3 km, and/or only growing white clover for seed every other year, would result in both spatial and temporal escapes from the seed-predating weevil pest. However, it should be kept in mind that alternate growing over years or greatly increasing distances between clover fields beyond 2–3 km between years could be a disadvantage for pollinators and pollination of the crop (Rundlöf et al. 2014; Lundin et al. 2017).

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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