Research Article

Received: 20 November 2022 Revised: 14 March 2023 Accepted article published: 14 May 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7538

Dynamics of pollen beetle (*Brassicogethes aeneus*) immigration and colonization of oilseed rape (*Brassica napus*) in Europe

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Abstract

BACKGROUND: Understanding the dynamics of pest immigration into an agroecosystem enables effective and timely management strategies. The pollen beetle (*Brassicogethes aeneus*) is a primary pest of the inflorescence stages of oilseed rape (*Brassica napus*). This study investigated the spatial and temporal dynamics of pollen beetle immigration into oilseed rape fields in Denmark and the UK using multiple methods, including optical sensors.

RESULTS: In all fields, pollen beetles were found to be aggregated and beetle density was related to plant growth stage, with more beetles occurring on plants after the budding stage than before inflorescence development. Optical sensors were the most efficient monitoring method, recording pollen beetles 2 and 4 days ahead of water traps and counts from plant scouting, respectively.

CONCLUSION: Optical sensors are a promising tool for early warning of insect pest immigration. The aggregation pattern of pollen beetles post immigration could be used to precisely target control in oilseed rape crops.

Supporting information may be found in the online version of this article.

Keywords: monitoring; optical sensors; *Meligethes aeneus*; rapeseed; pesticide reduction; sustainable agriculture; integrated pest management; precision agriculture

1 INTRODUCTION

The immigration dynamics specifying abundance, temporal, and spatial distributions of an economic pest determine the severity of the pest's crop damage.[1] While the pest's population dynamics are subsequently influenced by abiotic and biotic factors,[2] the magnitude of immigration functions as a multiplicative factor for all subsequent development.[3] Understanding the temporal and spatial dynamics of pest immigration into crops enables early detection and timely mitigation and therefore allows for the precise timing and spacing of necessary control management practices.[4,5] These precision interventions could mitigate the overuse of pesticides.[5,6]

Most insect populations exhibit spatial aggregation at some point in their life cycle.[7] This behavior is often driven by resource availability, microclimates, and reproduction.[8] Insect aggregation is correlated with abundance and temporal dynamics.[7] Precision agriculture capitalises on the variability in insect density by managing pests only when and where they occur,[9] rather than treating the whole field i.e., assuming a homogenous distribution of insects in the field. Therefore, if more is known about the patterns of insect abundance in time and space, the amount of insecticide used could be reduced.[4]

Oilseed rape (*Brassica napus* L.; OSR) is the primary oilseed crop grown in Europe[10] with 23.7 million tons produced in 2020.[11,12] This crop is grown primarily for food-grade cooking oil and the meal is used for animal feed; however, it has expanded in acreage

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over the past couple of decades due to growing demand for use as biofuel.[13,14] OSR currently occupies 8.7 million hectares in Europe.[11] Over 90% of European oilseed rape fields are sprayed with insecticides, many of them repeatedly over the growing season;[15] in the UK for example 82.9% of the total area in 2020 sown to OSR (892 119 ha) was treated using a total insecticide weight of 13 839 kg, with 32% treated just once, 28% twice, 21% three times and 19% four or more times.[16] Even slight decreases in this system's insecticide applications would therefore dramatically reduce pesticide use in Europe[17] and contribute to the European Commission's ‘Green Deal’ goals of reducing pesticides by 50% by 2030.

Brassicogethes (syn. *Meligethes*) *aeneus* F. (Coleoptera: Nitidulidae), commonly known as the pollen beetle, is a widely distributed European pest of OSR.[10] This small (1.9 mm long), univoltine beetle[10] immigrates into OSR crops during flower bud development and causes damage by feeding on young buds which may then abscise, leaving podless stalks, thereby reducing seed yield.[18–21] The crop can compensate for damage, or escape it, respectively, in the case of low beetle densities, or if beetles immigrate after the damage susceptible green bud stage, as beetles will then feed from open flowers.[18] Nonetheless, pollen beetles are the major target of insecticides applied in spring in OSR, sometimes requiring more than one treatment for sufficient control.[15] To prevent unnecessary applications of insecticides, action thresholds exist for pollen beetle for each of the major OSR-growing countries in Europe, ranging between 1–15 beetles per plant.[10] Pyrethroid insecticides are the main active ingredient used for control, but resistance of pollen beetle populations to pyrethroids is well documented throughout Europe.[22–24] It is reported that pollen beetles aggregate in the field post immigration.[25] If this is the case, and if aggregations are stable over time, pollen beetles would be a prime target for precision management control strategies[4,26] potentially reducing the amount of pesticide needed.

The aim of this study was to better understand the dynamics of pollen beetle immigration into oilseed rape crops by tracking their arrival and distribution though time in relation to the development of the crop. The study was conducted on three fields in the UK and one field in Denmark. A multiple-method approach using plant scouting, water trapping, and optical sensors was used to determine the spatial and temporal dynamics of pollen beetle immigration. We hypothesied that sensors then traps would capture pollen beetles first as these methods relate to flight[27] and that these measures may not directly relate to counts on plants, at least temporally, as this method reflects plant acceptance of the beetles following landing.

2 METHODS

2.1 Field sites

Observational studies were conducted on Rothamsted Farm, Harpenden, Hertfordshire, UK in the spring of 2015 on three fields: Great Harpenden (4.98 ha; 51.81069817038167, −0.3703752482244993), Little Knott (2.56 ha; 51.80900784362987, −0.37719596539220523) and Long Hoos (4.95 ha; 51.81315117800243, −0.3724778753212932), which were at least 100 m apart, and on an organic farm in Sorø, DK (7.13 ha; 55.483656, 11.493396) in the spring of 2020. Both farms in the UK and Denmark drilled winter OSR cultivars in August of the year prior to evaluation (2014 and 2019, respectively). No insecticide treatments were applied to any of the fields prior to, or during the period of study.

2.2 Pollen beetle sampling

Sampling of pollen beetles was conducted at both sites on multiple occasions on a spatially explicit field scale; all fields were divided into a grid with each cell sized 16.5 × 16.5 m and 15 m x 15 m, for the UK and Denmark, respectively. The study implemented three different methods to identify the spatio–temporal dynamics of pollen beetle immigration; plant scouting (plant beating) was used in all fields in UK and DK, and in addition green water traps and optical sensors were implemented in Denmark.

2.2.1 Plant scouting (beating)

Plant beating was the primary sampling method in this study, as it is the standard protocol for plant scouting used by farmers and researchers and results in a consistent metric during the green bud to flowering growth stage range.[28–30] This method consists of tapping the primary raceme of an OSR plant three times over a tray and counting the dislodged beetles. After counting, the beetles were released in proximity to the plants from which they were collected. Previous work indicates that evaluating the primary raceme is an accurate approximation of evaluating the entire plant.[21] Authors hypothesised plant beating represents infield population dynamics.[26]

At the UK sites, three OSR plants were evaluated for pollen beetles at random from within each cell with Great Harpenden containing 187 cells, Little Knott containing 73 cells, and Long Hoos containing 117 cells. The growth stage of each plant assessed was also recorded (using the BBCH scale[31]). Plants were sampled three times per week from 9th March/2015 (BBCH = 50, budding stage) to 27th April (BBCH = 66, full flowering stage), (except for the week of April 7th, where data at all sites were collected only twice). At the field site in Denmark, three plants were evaluated within each of the 146 cells (Fig. 1) daily from 30/3/2020 (BBCH = 51, green bud stage) to 11th April 2020 (BBCH = 60, flowering started). The centre point of each cell was marked with a flexicane in the UK and virtually marked with ViewRanger™ in Denmark; this method was subsequently used for georeferencing of samples. At both sites, all sampling occurred between 10:00 and 13:00 (local time).

2.2.2 Green water traps

Yellow water traps are commonly used for monitoring insects, including pollen beetles, in OSR.[29,32,33] However, as we did not want to attract beetles to traps that would perhaps not otherwise be there, we used green water traps (DK only) to simulate the green-colored crop (pre-flowering) i.e., to estimate the abundance of beetles naturally immigrating onto plants in the vicinity of the traps.

A five-by-five grid of 25 green water traps (25 cm diameter, 6 cm deep, half-filled with water and a drop of detergent, and suspended on a stake at crop height i.e., adjusted with crop growth) were placed in the field (Fig. 1). Each trap served as the intercept of four cells of the grid used for plant scouting (Section 2.2.1) i.e., were 30 m apart. Pollen beetles in traps were counted and removed daily (30th march–11th April 2020 as 2.2.1), along with the rest of the catch which was not retained. Trap water was replenished as needed. Each trap was hypothesised to approximate the density of beetles landing in that position per day.

2.2.3 Optical monitoring

At the Sorø site in Denmark, six automated near-infrared optical sensors (hereafter called ‘sensors’) (Volito sensor, FaunaPhotonics APS, Støberigade 14, 2450, Copenhagen, SV, Denmark)[34] were

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Figure 1. Set up of field experiment in Sorø, Denmark, showing positions of monitoring methods used to detect pollen beetles (*Brassicogethes aeneus*) during immigration into the oilseed rape crop. The field was divided into a grid with 15 × 15 m cells. Black icons indicate positions of plant scouting (beating) assessments, green circles indicate positions of green water traps (overlayed in a 5 × 5 grid with 30 m between each trap), and white/red icons represent positions of optical sensors (overlayed in 3 × 2 grid with 45 m between each sensor). Field image attributed to Google Earth, ©2019 Google Earth.

placed in a 3 × 2 grid along two linear transects centered on the southern edge of the field (Fig. 1). The transects were spaced 45 m apart. Transects started 30 m from the crop edge, with 45 m spacing between each subsequent sensor. Sensors continuously record the signal of light backscattered by insects flying through a 16 L illuminated measurement volume, for pollen beetle sized insects. This sensor records the signal data of each insect flight event along with metadata containing date, time, temperature, light, and humidity. Wing beat frequency is extracted from the signal[34] and can be subsequently used to estimate insect species. Pollen beetles have a wing beat frequency of 120 Hz,[35,36] so insects within the range of 100–140 Hz were considered pollen beetles. Sensors were run continuously between 30th March and 11th April 2020.

2.2.4 *Plant density*

Crop plant density was evaluated in all fields in UK and DK once at the start of the season to determine the relationship between density and pollen beetle distribution during immigration. The number of OSR plants in four, 0.5 × 0.5 m quadrats were recorded in each cell of the grid and the numbers totaled to estimate the number of crop plants per metre square.

2.3 Analysis

A quasi-Poisson generalized linear model (R 4.2.1 & 'stats' package version 4.2.2) was used to determine if there were differences in pollen beetle density according to plant growth stage (GS) i.e., addressing the hypothesis that insect counts are associated with inflorescence emergence. For this analysis, plants and associated insect counts were categorised according to plant growth stage: GS≤54 (flower buds are not yet visible), GS 55–59 (individual flower buds visible – first petals visible but buds still closed) and ≥60 (first flowers open – onwards). Surfer v. 13 (UK data) and v 24 (DK data) was used to generate visualizations with kriging of the spatial density of plants.

SADIE (Spatial Analysis by Distance IndicEs) analysis[37–39] was used (Epiphy v0.3.4.9000) to assess the spatial aggregation and uniformity of pollen beetle immigration from plant beating counts for each field, split by dates when the average growth stage ranged first between BBCH 55–59 for all fields and ≥60 for UK fields. SADIE graphics were produced using Bick and Forbs (2023).[40]

3 RESULTS

For the UK sites, a total of 49 470 pollen beetles were collected in total by plant sampling at Little Knot (9932), Great Harpenden (20480), and Long Hoos (19058), fields. The start of immigration into the crop was captured for each field, commencing on March 16th (BBCH = 51, green bud stage, Little Knott – 1 insect); and March 11th (BBCH = 51, green bud stage) for both Long Hoos (one insect) and Great Harpenden (one insect), with immigration quickly peaking on 9-10th April (BBCH = 57, individual buds visible on lateral racemes, buds still closed), then gradually declining over time (Fig. 2(a)–(c)). Over the first few days of immigration, beetles were generally first caught on plants on the north–eastern sides of the fields (Fig S1) i.e., downwind of south–eastern prevailing winds on each site.

In order to directly compare the efficiency of the three different methods tested in DK, we compared the date of the first positive pollen beetle identification, the date of the detection of the first major increase in pollen beetle, and the mean number of pollen beetles per trapping unit over the experimental period (12d) (daily means per plant are presented in Fig. 2(d)–(f)). At the Danish

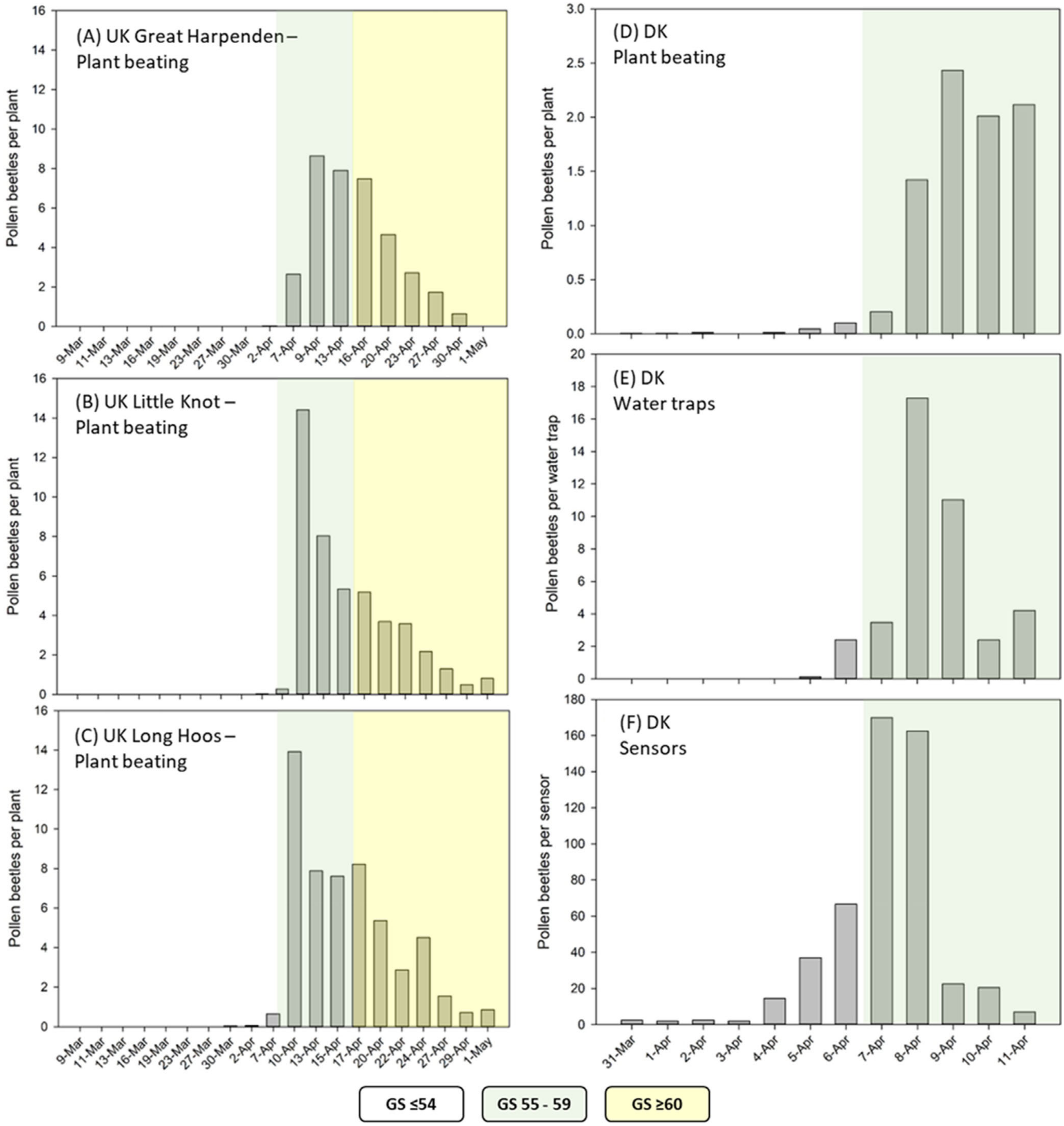

Figure 2. Temporal dynamics of pollen beetle (*Brassicogethes aeneus*) immigration into oilseed rape crops. Insect counts are expressed as pollen beetles per monitoring method (plant scouting (beating method), green water trap, or optical sensor). Plant stage growth stage (GS) is indicated by shaded overlay on each graph (white = GS≤54 (flower buds not yet visible); Green = GS 55–59 (individual flower buds visible – first petals visible but buds still closed); yellow = GS ≥60 (first flowers open – onwards)).

site, 3665 pollen beetles were identified from plant beating, averaging 8.39 pollen beetles per plant over the experimental period (n = 438). A few beetles were identified on the first plant beating assessment on 31st March (BBCH = 51, green bud stage) indicating that assessments did not start early enough to confirm the start of immigration. The first major increase in beetle numbers on plants was recorded on 8th April and numbers increased to the peak on 9th April (BBCH = 57, individual buds visible on lateral racemes, buds still closed), then declined over the following two assessments (Fig. 2(d)). A total of 647 pollen beetles were counted from water traps, averaging 25.88 pollen beetles per water trap (n = 25) over the experimental period. Beetles were detected slightly later (4 days) in the traps than on the plants, with the first beetles detected on 5th April (BBCH = 55, main inflorescence visible but closed stage); but the first major increase in pollen beetle numbers was detected the following day on 6th April

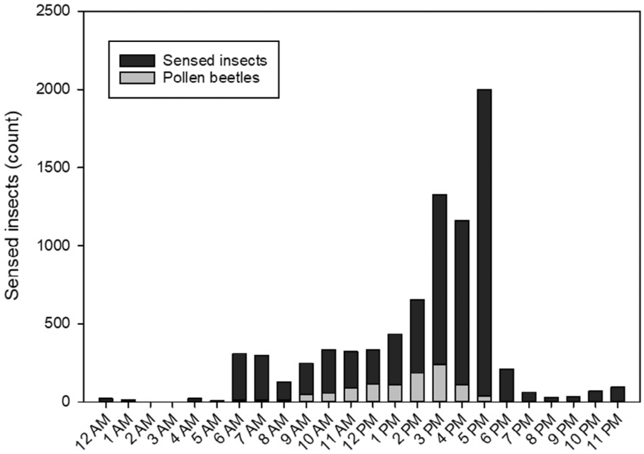

Figure 3. Total number of sensed insects detected over a 12d period (30/3/20–11 April 2020) in an oilseed rape field using optical sensors that were classified as pollen beetles (*Brassicogethes aeneus*) grouped by time of day.

(2 days earlier than on plants) and the peak was detected on 8th April (BBCH = 57, secondary inflorescences yellow but closed stage), (1 day earlier than that detected by plant beating) after which numbers caught declined (Fig. 2(e)). A total of 8441 insects were detected by the sensors, of which 1041 (12.33%) were classified as pollen beetles from their wing beat frequency, averaging 173.5 pollen beetles per sensor (n = 6) over the whole experimental period. Sensors detected pollen beetles on the first day of assessment (31st March) with the first major increase detected on 4th April (4 days earlier than on plants and 2 days earlier than traps). Numbers gradually increased to the peak on 7th April (1 day earlier than traps and 2 days earlier than plan beating) and remained high on 8th April before falling sharply to a relatively stable, low number for the remaining assessments (Fig. 2(f)). The diurnal activity of sensed insects classified as pollen beetles showed distinct flight timing compared with insects not classified as pollen beetles (Fig. 3).

Plant density varied across the field at all sites (Fig. 4(a), (d), (g), (j)); in the UK(Little Knot $\mu = 40.32 \pm 1.12$, Great Harpenden $\mu = 24.86 \pm 0.28$, Long Hoos $\mu = 39.78 \pm 0.56$) and DK ($\mu = 24.62 \pm 0.43$).

Pollen beetle counts on plants were significantly higher after inflorescence emergence i.e., when flower buds were visible (growth stage BBCH ≥55) than prior to this (growth stage BBCH ≤54) across all sites (UK: Little Knott $\mu_{\leq 54} = 2.2$; $\mu_{\geq 55} = 900.91$; *t*-value = 22.13; $P < 0.001$; Long Hoos $\mu_{\leq 54} = 5.67$; $\mu_{\geq 55} = 1727.91$; *t*-value = 27.47; $P < 0.001$; Great Harpenden $\mu_{\leq 54} = 5.33$; $\mu_{\geq 55} = 2554$; *t*-value = 34.31; *P*-value <0.001; Denmark: $\mu_{\leq 54} = 11.71$; $\mu_{\geq 55} = 716.6$; *t*-value = 38.41; $P < 0.001$) (compare white *versus* green regions in Fig. 2).

The SADIE analysis was interpreted for each site. Spatial distribution of pollen beetles was recorded as aggregated if the index of aggregation (Ia) was greater than one, as random if Ia equals one, and as uniform if Ia was less than one.[39] Pollen beetles were found to be significantly aggregated at all sites with indexes of aggregation (Ia) from the SADIE analysis at both the GS 55–59 stage (UK: Little Knot Ia = 1.67; $P < 0.001$; Long Hoos Ia = 1.67; $P < 0.001$; Great Harpenden Ia = 2.16; *P*-value <0.001; Denmark: Ia = 1.66; $P < 0.001$) (Fig. 4(b), (e), (h), (k)) and the GS ≥60 stage (UK: Little Knot Ia = 1.3043; $P = 0.05$; Long Hoos Ia = 1.96; $P < 0.001$; Great Harpenden Ia = 2.64; $P < 0.001$) (Fig. 4(c), (f), (i)). This aggregation occurs primarily on the edge of the field during the GS 55–59 timeframe and within the field at the GS ≥60 stage. The Little Knot site was the only exception, where insects were aggregated in the centre of the field first then aggregated towards the edge. However, the plant density map (Fig. 4(a)) indicates extremely low plant densities in the field centre, which may account for this difference.

4 DISCUSSION

Pollen beetle feeding on developing flower buds in oilseed rape causes injury which may lead to bud abscission, consequently preventing the growth of pods and resulting in the loss of seed yield, which can be economically significant.[41,42] Insecticides are most commonly used to control the beetle and prevent economic loss, but over-use has led to problems with insecticide resistance as well as environmental damage.[22] Our study on the spatio-temporal dynamics of pollen beetle immigration suggests potential for (i) precision agriculture to reduce insecticide use through spatial targeting of pollen beetle aggregations i.e., treating only areas of the crop where pollen beetle density is high, and (ii) optical sensing of pollen beetles for more efficient monitoring in both time and space.

Pollen beetle aggregation was first reported by Free and Williams[19,43] in individual flowers of dandelion (*Taraxacum officinale*) and oilseed rape crop flowers. In the current study we found that the distribution of beetles across the field was not homogenous; significant aggregation of pollen beetles was apparent in different areas of the field, with beetles aggregated towards the edges of the field at the inflorescence development stage (BBCH growth stage 55–59) then aggregated more centrally once flowering started. Using a simple transect sampling strategy, Free and Williams[19] working in the UK, showed that pollen beetles were more numerous at the crop edge than at central points of the transect, especially during April–May during immigration, then reduced in abundance as beetles moved further into the crop during flowering. Nielsen and Axelsen,[25] again using transects, but working in Sweden, showed that pollen beetles were statistically aggregated at high densities, as did Hansen[44] in Denmak, who pointed out that for accurate threshold determination it was important to know the distribution of beetles throughout the field. The advent of geostatistics and analyses methods such as spatial analysis by distance indices (SADIE)[37–39] made this possible. Williams & Ferguson *et al.*,[4,26] studied the spatio-temporal distribution of pollen beetles in a single field in a single year and found, similar to our study on four separate fields, that there were distinct clusters of aggregation and gaps throughout the field, but analyses were not related to growth stage on a temporal scale, as in our study. We found significant aggregations of pollen beetles on plants at the bud stage (these used for feeding by both sexes, mating, and oviposition), compared with plants during the flowering stage (these used for feeding, most often by males).[18,45]

Although insect aggregation offers the opportunity for precision management of crop pests, it also results in poor population estimates resulting from sampling.[22,25] Therefore, improved sampling is needed to properly establish pollen beetle populations to enable accurate application of action thresholds in both time and space. Our results showed that both the damage-susceptible bud stage of the crop and the immigration period of pollen beetles lasted approximately 2 weeks. Monitoring pollen beetle populations during this period to determine threshold breaches could therefore be onerous but models are available to predict timing of pollen beetle immigration flights[46–48] or expected abundance.[49] We also found

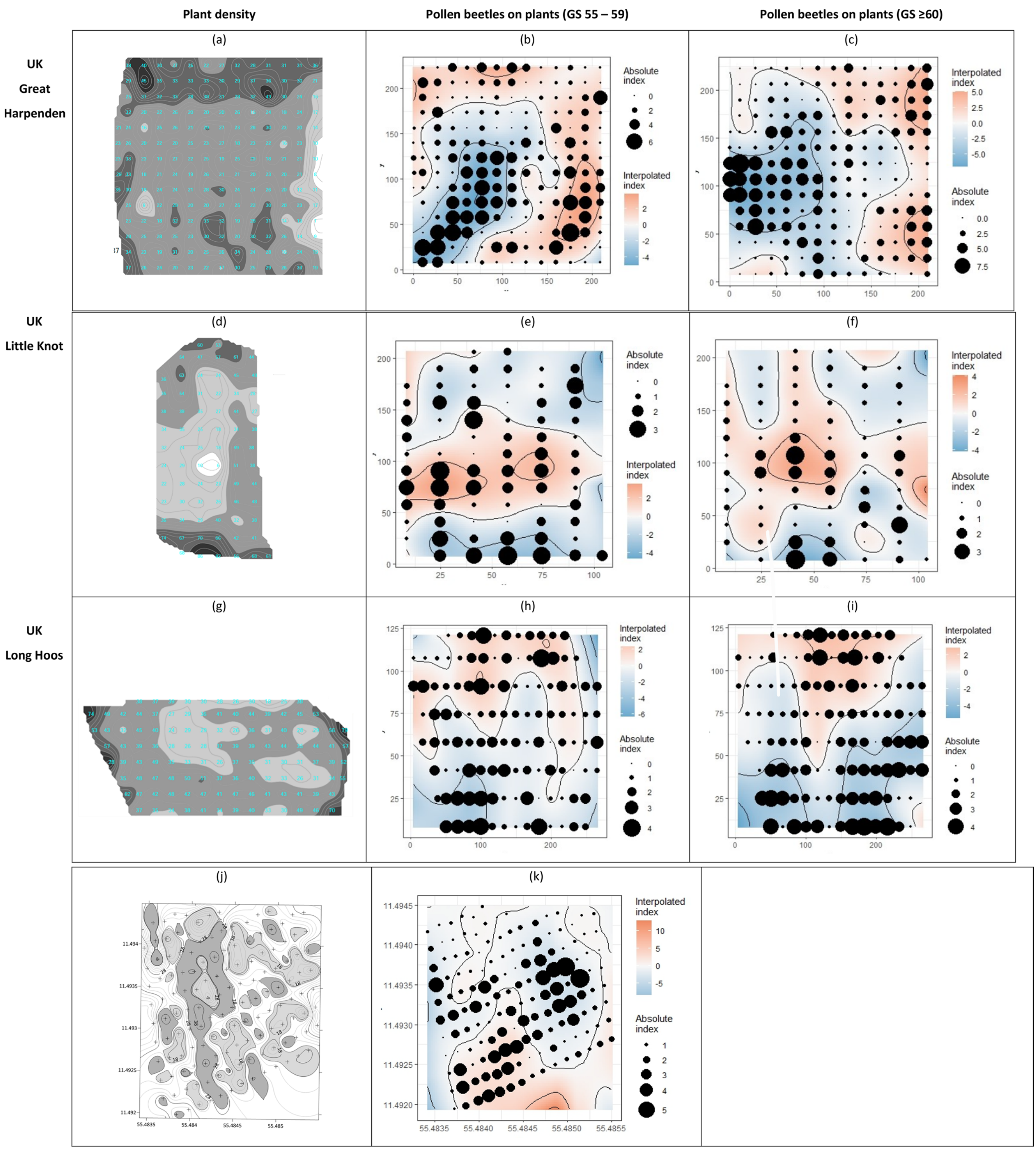

Figure 4. Maps from fields in the UK and Denmark showing: the spatial distribution of oilseed rape plant density (per m^2) (Column 1) and pollen beetle (*Brassicogethes aeneus*) abundance using SADIE (Spatial Analysis by Distance IndicEs) analyses (Columns 2–3), with the top of each plot facing North. Plant density per sample point is shown as a blue numeral; kriged shading represents low (white) – high (black) plant density with data shown on true field shape (Column 1; a, d, g, j). The SADIE maps show pollen beetle distribution with data compressed to a regular grid, grouped by plant growth stage (GS, on BBCH scale) GS 55–59 (individual flower buds visible – first petals visible but buds still closed)) (Column 2; b, e, h, k) and for the UK sites GS ≥60 (first flowers open – onwards) (Column 3; c, f, i). The Red–Blue plots use a 'clustering index' (v) calculated for each point; aggregation (or clusters) occur if the clustering idex is greater than one (v_i), randomness occurs if the clustering index falls between one and negative one, and gaps or uniformity occur when clustering idex is less than negative one (v_j). This is expressed through kriged shading from red (v_i, clusters) to blue (v_j, gaps). Additionally, the absolute value of the clustering index at each grid point is expressed by dot size. Lat:long coordinates shown on axes for DK fields ((j, k) and for UK fields as x:y coordinates (m from crop edge) (b, c, e, f, h, i).

that early temporary aggregation of pollen beetles occurs downwind of the prevailing wind, as shown previously in other studies,[4,26] indicating that immigration occurs on the opposite side of the field to the prevailing wind[50] and provides further data in support of upwind anemotaxis in pollen beetles during host plant location[51–53] as this pattern occurred at all four sites. Therefore, the authors recommend positioning of monitoring devices and focusing monitoring effort downwind of any prevailing wind direction on the field for earlier detection of pollen beetle immigration and localised hotspot development over the bud stage.

The optical sensors recorded an increase in pollen beetles 2 days ahead of water traps and 4 days ahead of plant counts and in terms of early detection and numbers of beetles detected, was the most efficient pollen beetle monitoring method. The sensor recorded 18.6 times and 6.7 times the number of pollen beetles compared to plant counts and water traps, respectively. This finding supports previous work reporting that optical sensors collect one to two orders of magnitude more insects than conventional monitoring methods.[35] While use of optical sensors for tracking insect flight is not a new method,[35,36,54–56] a major study limitation is the inability to validate insect signals as pollen beetles. However, as pollen beetles are early colonisers of oilseed rape fields, and do so in high densities, there is a high likelihood that insects were accurately classified as pollen beetles. Moreover, the flight times of insects classified as pollen beetles were distinct from other sensed insects and match the previously reported flight activity times for this species.[57,58] However, we did not, unfortunately, retain the water trap catch so we were unable to check the proportion of trapped insects that were pollen beetles and relate to the proportion returned by the sensor. Further replicated studies including a mark-release-recapture component *e.g.* as per Sivakoff *et al*.,[59] are proposed by the authors, and adoption of more complex machine learning methods for insect classification of pollen beetle[35] will help to validate the study observations.

5 CONCLUSION

Understanding the dynamics of pollen beetle immigration and crop coloniation informs both monitoring and management strategies. The aggregation of pollen beetles which occurs first on the downwind edge and then in the centre of the crop enables precision management practices to be applied as pollen beetles can be targeted in specific aggregated locations which are related to plant growth stage and density. As this work further supported the idea that pollen beetles immigrate into crops using upwind anemotaxis, early detection *via* sampling and monitoring should correspond with this migration pattern.

As evidenced in this study, sensors provide great promise for agriculture. Optical sensors such as described here provide earlier and more sensitive detection of insects than current monitoring methods. A network of these sensors at a landscape scale could serve as the basis of an interpolated isoline pest map, similar to weather stations. It seems likely that sensors could be used to set economic thresholds for management, providing precision timing data on pests, although sensor data would need to be calibrated for this purpose as current thresholds are expressed in the numbers of beetles per plant.[10] State-of-the-art pollen beetle monitoring calls for counting pollen beetles and/or pollen beetle damage on plants in relation to temperature (as the level of damage is related to temperature).[60] A system that links the number of sensed insects immigrating into a field, the temperature and the crop growth stage would be able to better predict the risk of economic damage and provide an avenue for fully automated pest monitoring. Sensors might even be useful for tracking natural enemies to determine if a pesticide application is necessary considering biocontrol potential, or for identification of pollinators in the field to avoid non-target insecticide effects on these beneficials, thereby contributing to both pesticide reduction and biodiversity protection goals for sustainable agriculture.

ACKNOWLEDGEMENTS

Thanks to farm staff at Rothamsted (UK) and Sorø, (DK) for facilitating sampling, and to Nigel Watts, Andrew Moss and Lucy Nevard for help sampling the UK fields. This work was funded in part by the UK Agriculture and Horticulture Development Board: Cereals & Oilseeds (Project 2140005; SMC & MTT), and the Danish Innovation Fund (9066-0051A; EB & LS). SMC is part-funded by AgZero+ (NE/W005050/1, jointly supported by UK NERC and BBSRC). Rothamsted research receives grant-aided support by the UK BBSRC.

CONFLICT OF INTEREST

SH, LStill, BB, RR, JL, and TN are currently or were previously employed by FaunaPhotonics ApS. during the study. EB was partially funded by FaunaPhotonics ApS. as per terms in the Danish Innovation Fund grant (9066-0051A).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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