

Kairomone-assisted trap cropping for protecting spring oilseed rape (*Brassica napus*) from pollen beetles (Coleoptera: Nitidulidae)

Gunda Thöming,^{a*}  Knut A Solhaug^b and Hans R Norli^a



Abstract

BACKGROUND: Pollen beetles are key pests in oilseed rape (OSR) production. These beetles use visual and olfactory cues to locate their host plants at specific phenological stages, hence trap cropping may represent an alternative pest control strategy. In this study, a trap crop strategy for spring OSR was developed. To elaborate such a trap cropping system, a pest control measure that eradicates the attracted beetles in the trap crop before they migrate further into the main crop was included in the final trap cropping strategy.

RESULTS: Testing yellow-flowering turnip rape and one yellow- and two cream-coloured flowering OSR cultivars as potential crops in different trap cropping strategies, we found that pollen beetles clearly preferred turnip rape over the cream-coloured and yellow OSR cultivars, and preferred the yellow OSR cultivar over the two cream-coloured cultivars. This behaviour was related to the plant growth stage and associated volatile and colour signals of the tested cultivars. Using turnip rape as a trap crop and testing kairomone- or insecticide-assisted trap cropping as the pest control strategy was as effective as compared with whole fields treated with a standard pesticide.

CONCLUSION: Combining a turnip rape cultivar as trap crop with kairomone traps placed in the trap crop as a killing agent may enable renunciation of pesticides in spring OSR production.

© 2020 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry. Supporting information may be found in the online version of this article.

Keywords: *Brassicogethes* spp.; *Brassica rapa*; turnip rape; oilseed rape; trap crop; semiochemicals

1 INTRODUCTION

Oilseed rape (OSR) *Brassica napus* L. (Brassicaceae) is a key crop in arable systems in temperate regions, and its production has risen significantly over past decades.^{1,2} In Europe, mostly winter OSR is cultivated, whereas in Norway, 87% of the OSR production is spring OSR, and only 13% is winter OSR. In the northern margin of OSR cultivation areas, OSR is of increasing interest, due to its value in crop rotation enabling more diverse cropping systems.^{3,4} The major pests in OSR are pollen beetles, *Brassicogethes* spp. Stephens (Coleoptera: Nitidulidae, previously *Meligethes* spp.).⁵ These beetles locate their host plants using visual and olfactory cues, which comprise the yellow colour of the buds and flowers, and volatiles mainly noted as floral and crucifer-specific compounds.^{5,8} Females lay their eggs in the flower buds, and both adults and larvae feed on the pollen. Oviposition and feeding leads to bud abscission. Yield reduction of > 50% has been reported.^{5,9} Currently, insecticides are used to control pollen beetles, these pests have been observed to develop resistance to insecticides, and thus demand for alternative pest control strategies is increasing.^{10–13}

Trap cropping is a traditional tool in plant protection that has received increasing attention in recent years,^{14–17} also in OSR production.^{8,18–22} The use of trap crops in pest management can reduce the need for insecticides and in rare cases may even enable a renunciation of pesticides, although few trap cropping

strategies have been successful so far.¹⁶ Numerous studies have explored the mechanisms underlying the success of a trap cropping strategy in protecting OSR against the pollen beetle *Brassicogethes aeneus* Fabricius.^{7,8,18,22–31} Early flowering turnip rape (*Brassica rapa* L.) appears to be a promising trap crop in OSR production due to its early flowering character and highly attractive visual and olfactory cues.^{8,22,32,33} Several assessments using live OSR plants or artificial substrates have compared the attractiveness of different colours to the pollen beetle, although results have varied.^{26,30,34} However, it has been suggested that a trap crop system with late cream-coloured-flowering *B. napus* plants as the main crop and early yellow-flowering *B. napus* as a trap crop may reduce pollen beetle populations in the main crop.²⁶

Both *B. rapa* and *B. napus* are host plants for pollen beetles and are not dead-end trap crops ('plants that are highly attractive to insects but on which they or their offspring cannot survive'¹⁶).

* Correspondence to: G Thöming, Norwegian Institute of Bioeconomy Research, PO Box 115, NO-1431 Ås, Norway. E-mail: gunda.thoeming@nibio.no

a Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Ås, Norway

b Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway

To improve such trap crop systems, an effective killing agent that can eradicate the attracted beetles in the trap crop before they can migrate further into the main crop can be included. For that purpose, synthetic toxicant insecticides can be used to reduce the pest pressure in the trap crop before the pest insects move into the main crop. Additionally, natural enemies of the pest insects can be attracted to or used in combination with the trap crop.^{16,17} Another option is semiochemical-assisted trap cropping, which entails 'trap crops whose attractiveness is enhanced by application of semiochemicals or regular crops that can act as trap crops after application of semiochemicals'.^{16,35–37} Semiochemicals are chemical signals for the purpose of communication between individuals of the same species (intraspecific; pheromones) or between different species (interspecific; allelochemicals).¹⁷ Mauchline *et al.*⁷ reviewed possible semiochemical-based alternatives to insecticides for pollen beetle management, considering the semiochemical-mediated impact on behaviour of pollen beetles throughout their entire life cycle.

Based on the above-mentioned knowledge in this area, our aim was to develop a trap crop strategy for spring OSR production that can be successful in applied pest management. To improve the trap cropping system, we combined a trap crop with a killing agent to reduce the pest population in the trap crop, thereby creating a novel trap-and-kill-strategy which is resilient and effective against pollen beetles.

2 MATERIAL AND METHODS

2.1 Insects

Naturally occurring pollen beetle populations were assessed in our experiments. We identified pollen beetle species that occurred in the experimental locations and near the OSR production areas by collecting pollen beetles from *B. napus* crops in the experimental field station in Ås in Akershus County and at four different locations in OSR production areas in Akershus County (Kråkstad) and Østfold County (Askim, Rakkestad, Sarpsborg) in east Norway, in 2015. At each location, samples of pollen beetles were collected from at least 50 randomly selected plants by beating each plant on a tray to dislodge the insects.³⁸ Beetles were transferred to the laboratory and identified taxonomically using a binocular microscope,³⁹ which revealed the co-occurrence of five different species of the subfamily Meligethinae (Coleoptera: Nitidulidae) in all five sampling areas. In this species complex, *B. aeneus* predominated (60–91%) followed by *B. viridescens* Fabricius (8–23%) and *B. coeruleovirens* Förster (5–16%); only small numbers of *B. subaeneus* Sturm (0–4%) and *Astylogethes subrugosus* Gyllenhal (0–2%) were found. This species composition is considered when pollen beetles are mentioned in this study.

2.2 Plants

Yellow-flowered OSR (*B. napus* cv. Majong), cream-coloured OSR (*B. napus* cv. Lyside and cv. Silver Shadow), and yellow turnip rape (*B. rapa* cv. Valo) were used in the experiments. These cultivars were selected because they grow well under northern conditions. When growing OSR crops in Norway, yield and oil content are essential aspects, although early establishment of the plants can be regarded as the most important criterion for successful cultivation. A cultivar screening trial focused on suitability for cultivation of OSR in Norway was conducted in east Norway.⁴⁰ This showed lower yield for the two cream-coloured-flowering cultivars Lyside and Silver Shadow (26.5 and 27.1 dt ha⁻¹, respectively) compared with well-established cultivars such as Majong (32.4 dt ha⁻¹). That

screening also found late establishment of cv. Silver Shadow and particularly cv. Lyside compared with all yellow-flowering cultivars tested.⁴⁰

For the odour and spectral reflectance analyses, all four cultivars were sown in pots (20 cm diameter, three plants/pot) in commercial substrate (Gojord, Degernes Vekstjord, Degernes Torvstrøfabrikk, Degernes, Norway) and cultivated in a polycarbonate greenhouse (20 ± 2 °C, 68% relative humidity, 16:8 light/dark photoperiod, cultivation period March–May). Cultivars were arranged in discrete blocks in the greenhouse during growth. Whenever the photosynthetic photon flux in the greenhouse fell below ~150 μmol m⁻² s⁻¹ (as on cloudy days), artificial light was automatically added by high-pressure metal-halide lamps (HPI-T Plus 400 W/645). Plants were used for analysis when they reached one of the following stages on the BBCH scale: 29 (formation of side shoots), 52 (green bud) or 65 (full flower).⁴¹

2.3 Odour analysis

Volatiles were collected from whole cut plants in the following phenological stages: BBCH 29 (*n* = 4 Valo, *n* = 4 Majong, *n* = 4 Lyside and *n* = 4 Silver Shadow), BBCH 52 (*n* = 6 Valo, *n* = 4 Majong, *n* = 4 Lyside and *n* = 4 Silver Shadow), and BBCH 65 (*n* = 8 Valo, *n* = 4 Majong, *n* = 4 Lyside and *n* = 4 Silver Shadow). Headspace collection and chemical analyses were performed according to previously reported protocols.⁴² The plant material was placed in a 2000-mL glass jar closed with a ground glass fitting. Charcoal-filtered air was pushed through the jar (220 mL min⁻¹) and then through a Porapak filter (35 mg adsorbent, 80/100 mesh; Alltech, Deerfield, IL, USA). Filters were rinsed sequentially with 6 mL hexane, 6 mL methanol, and an additional 6 mL hexane, and dried at room temperature before use. Headspace collection was done for 3 h at 18 ± 2 °C. Volatile compounds were desorbed from the filter by rinsing with 0.3 mL hexane. To each sample, 500 ng heptyl acetate and 500 ng undecyl acetate were added as internal standards. Samples were crimp-capped and stored at -80 °C pending further analysis. Chemical analyses were carried out on an Agilent 6890 N gas chromatograph connected to an Agilent 5973 mass spectrometer, using an autosampler. The chromatograph was operated in splitless mode at 250 °C with an injection volume of 1 μL. A fused silica Agilent J & W Scientific DB-Wax separation column (Agilent Technologies, Santa Clara, CA, USA; 30 m long, internal diameter of 0.25 mm, 0.25 μm film thickness) was used, and a 2.5-m methyl-deactivated precolumn (Varian Inc., Lake Forest, CA, USA) with the same internal diameter was connected to the analytical column via a press-fit connector (BGB Analytik AG, Boeckten, Switzerland). After injection of a sample, the temperature was held at 40 °C for 2 min and subsequently raised 6.9 °C min⁻¹ to 160 °C and then 21.5 °C min⁻¹ to 250 °C. Thereafter, the temperature was held constant at 250 °C for 3.6 min. The total running time was 27.18 min.⁴²

Identification and quantification of volatile compounds were achieved by combined gas chromatography and mass spectrometry (GC-MS).⁴³ Volatile compounds were identified using Deconvolution Reporting Software (DRS, ver. A.03.0.84; Agilent Technologies), which combines automatic MS deconvolution and identification software (AMDIS version 2.71, NIST) with an MS library (NIST05 database) and GC-MS software (ChemStation ver. D.03.00) (Agilent Technologies). The AMDIS database contained 1279 volatile compounds, 277 of which connected to Kovats retention indices.⁴⁴ To obtain comparable retention times for all samples, retention time was locked and referenced

according to the internal standard heptyl acetate at 10.75 min using the ChemStation retention time-locking program. Peaks present in the chromatogram but not identified by the DRS were interpreted manually using the NIST05 database. To ensure reliable identification, a match factor of ≥ 70 was employed.⁴⁵ Identification of compounds was verified by comparing mass spectra and Kovats indices with those obtained for synthetic standards on the same column. Relative amounts of identified compounds were calculated by dividing the peak area (from the total ion chromatogram) by the area of the internal standard heptyl acetate. The compounds were acquired as standards from Aldrich (Oslo, Norway), Fluka (Munich, Germany) and Chiron AS (Trondheim, Norway).

2.4 Reflectance analysis

Petals (BBCH 65) were removed carefully from plants of each of the four cultivars immediately before the analyses. Reflectance spectra of a single petal were measured with the petal fixed in position with forceps at the port of an integrating sphere (ISP-50-RE FL, Ocean Optics, Dunedin, FL, USA). The integrating sphere was connected with a 400- μm fibre to an Ocean Optics SD 2000 diode array spectrometer. Reflectance spectra (300–1000 nm) were measured by illuminating the sample with visible and UV light from a DH 2000 deuterium/halogen light source (Ocean Optics) with a 600- μm fibre connected to the collimating lens on the sphere. Reference reflectance spectra were recorded with a reflectance standard (WS-2, Ocean Optics).

2.5 Experiment 1

A preference experiment was designed to provide pollen beetles with a relatively natural situation under field conditions that offered a choice between different cultivars of *B. rapa* and *B. napus*. The cultivars were sown at the same time and thus exposed beetles to the different phenological stages of the plants and their corresponding volatile and visual cues. The preference of pollen beetles for yellow (cv. Majong) and cream-coloured (cv. Lyside and cv. Silver Shadow) OSR or yellow turnip rape (cv. Valo) was tested at the experimental field station in Ås, Akershus, east Norway, in 2015. The four cultivars were grown in replicated plots (three of each type) in a randomized block design: the size of each plot was 1.2 \times 7 m, with a distance of 0.5 m between plots and 2 m between blocks. All plots were sown on 17 April 2015 at a density of 200 seeds m^{-2} for OSR and 400 seeds m^{-2} for turnip rape, which are seed rates according to farmers practice.

The colonisation preference of adult pollen beetles and the BBCH stage were assessed weekly in each plot. The number of beetles per plant was determined by the beating method for 15 randomly selected plants per plot.³⁸

2.6 Experiment 2

Based on the results from the odour and reflectance analyses and Experiment 1, two trap crop systems for pollen beetle management were designed to be tested in Experiment 2: yellow OSR cv. Majong with the early flowering yellow turnip rape cv. Valo as trap crop, and cream-coloured OSR cv. Silver Shadow with yellow OSR cv. Majong as trap crop. To select the best trap crop system for Norwegian conditions, we conducted a field experiment in 2017, which was replicated three times at different locations in the counties Akershus and Østfold in east Norway. At each location, an experimental field was established with one treatment plot per field of the following four treatments: (i) yellow-flowering OSR cv. Majong without trap crop (Y-OSR); (ii) yellow-flowering

OSR cv. Majong with yellow-flowering turnip rape cv. Valo as trap crop (Y-OSR-TC); (iii) cream-coloured-flowering OSR cv. Silver Shadow without a trap crop (C-OSR); and (iv) cream-coloured-flowering OSR cv. Silver Shadow with yellow-flowering OSR cv. Majong as trap crop (C-OSR-TC). The C-OSR-TC treatment was based on what is called the Flower Power System[®], a cultivation method for OSR using a white flowering OSR variety as main crop and a yellow flowering variety as trap crop (developed by Knold & Top ApS, Odder, Denmark). Each plot was 40 \times 40 m, with at least 200 m between plots, and the plots were surrounded by cereals (wheat or barley). In the plots with trap crops, the main crop was 38 \times 38 m and was surrounded by a 2-m wide border of trap crop (i.e. the total area of each plot was 40 \times 40 m; Fig. S1). According to farming practice, the OSR and turnip rape were sown at densities of 200 and 400 seeds m^{-2} , respectively, on 21 April 2017. Colonisation by adult pollen beetles and BBCH stage were assessed weekly in each plot. The number of beetles per plant was recorded for nine sampling points in the main crop (i.e. the inner 38 \times 38 m) and eight sampling points in the trap crop (i.e. the surrounding 2-m wide border) (Fig. S1). Three plants were selected randomly at each sampling point, and beetles were captured, counted and recorded as described above.

2.7 Experiment 3

Based on the results of Experiment 2, yellow-flowering OSR (cv. Majong) as a main crop together with early flowering yellow turnip rape (cv. Valo) as trap crop appeared to be the best trap crop system for further study in Experiment 3. As pest control measures for the final trap crop strategy, we tested a synthetic insecticide and a semiochemical-based approach using kairomone traps. As a control, we used a plot sown with OSR (cv. Majong) but without a trap crop and treated only with synthetic insecticides according to the farmers' common practice. As insecticide treatment, Steward[®] (active ingredient indoxacarb) was used. For the semiochemical-based approach, we employed CSALOMON[®] VARb3z + kairomone traps (Csalomon, Plant Protection Institute, MTA ATK, Budapest, Hungary) with sticky liners at the inlets. These traps contained a three-component blend of the synthetic floral volatile compounds (*E*-anethol, (*E*)-cinnamyl alcohol and (*E*)-cinnamaldehyde combined with fluorescent yellow visual stimuli in a funnel trap designed for monitoring purposes.⁴⁶ Preliminary studies have shown that these volatile food signals are more effective in attracting pollen beetles than OSR-specific volatile compounds.⁴⁶ Pollinating insects were excluded from trap catches by use of a bee screen.

Experiment 3 was carried out in 2018 and comprised one treatment plot per field of the following three treatments: (i) yellow-flowering OSR cv. Majong without a trap crop and treated with synthetic insecticide (as a control, C); (ii) yellow-flowering OSR cv. Majong with early flowering yellow turnip rape cv. Valo as trap crop and with insecticide treatment of the trap crop only (TC-I); and (iii) yellow-flowering OSR cv. Majong with early flowering yellow turnip rape cv. Valo as trap crop and kairomone traps placed in the trap crop (TC-K). This field experiment was replicated three times at different locations in Akershus and Østfold counties. Each experimental field had an area of ~ 4 ha and had forest on one or two sides, and grassland on two or three sides. Each plot was 40 \times 40 m and was surrounded by cereals (wheat, barley, or oats), and there was at least 250 m between the plots without kairomone treatment and > 500 m between the plot with the kairomone traps and the other two plots (Fig. S2). As in Experiment 2, when a trap crop was included, it consisted of a 2-m wide

Table 1. Volatile compounds in headspace collections from oilseed rape (OSR) and turnip rape plants (yellow *Brassica rapa* cv. Valo, yellow *B. napus* cv. Majong, and cream-coloured *B. napus* cv. Lyside and cv. Silver Shadow) in different phenological stages

Compound name ^a /CAS #	Yellow turnip rape cv. Valo				Yellow OSR cv. Majong				Cream-coloured OSR cv. Lyside				Silver Shadow			
	RT ^b /RI ^c	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE
α-Pinene	4.15	0.13 ± 0.05	0.95 ± 0.14	0.15 ± 0.06	0.29 ± 0.1	0.21 ± 0.05	0.19 ± 0.03	0.17 ± 0.03	0.09 ± 0.05	0.14 ± 0.05	0.08 ± 0.05	0.64 ± 0.23	0.22 ± 0.05			
80-56-8	1018															
Dimethyl disulphide	4.9		0.08 ± 0.07													
624-92-0	1067															
Sabinen	5.78			0.08 ± 0.05			0.21 ± 0.05	0.18 ± 0.05	0.06 ± 0.04	0.02 ± 0.02	0.16 ± 0.03		0.23 ± 0.05			
3387-41-5	1116															
<i>p</i> -Xylene	5.99	0.03 ± 0.03		0.01 ± 0.01			0.16 ± 0.05			0.08 ± 0.05			0.16 ± 0.04			
106-42-3	1128															
β-myrcene	6.62			0.17 ± 0.06			0.85 ± 0.25	0.02 ± 0.02		0.23 ± 0.03			0.33 ± 0.13			
123-35-3	1161															
(+/-)-Limonene	7.25			0.05 ± 0.05	0.12 ± 0.05		0.27 ± 0.06	0.17 ± 0.05	0.09 ± 0.03	0.1 ± 0.04	0.17 ± 0.04		0.27 ± 0.07			
138-86-3	1194															
Z-3-hexenyl acetate	9.61	2.11 ± 0.69	0.62 ± 0.43	1.71 ± 0.61	0.32 ± 0.09	0.16 ± 0.11	0.93 ± 0.79	0.2 ± 0.04	0.2 ± 0.09	0.05 ± 0.05	0.05 ± 0.05	0.23 ± 0.23	0.59 ± 0.26			
3681-71-8	1314															
Dimethyl trisulphide	10.7		5.58 ± 5.58	0.08 ± 0.08												
3658-80-8	1374															
Z-3-hexenol	10.97	0.12 ± 0.06		0.41 ± 0.19			0.17 ± 0.17						0.17 ± 0.09			
928-96-1	1384															
Benzaldehyde	13.37		0.03 ± 0.03	1.31 ± 0.42	0.21 ± 0.21		0.69 ± 0.32		0.01 ± 0.01	0.5 ± 0.1			0.55 ± 0.22			
100-52-7	1516															
Linalool	13.96		1.35 ± 1.35	0.31 ± 0.08			1.27 ± 0.39			0.16 ± 0.04			0.17 ± 0.09			
78-70-6	1547															
Methyl benzoate	15.12			0.6 ± 0.3			0.65 ± 0.27			0.5 ± 0.11			1.72 ± 0.73			
93-58-3	1617															
Phenylacetaldehyde	15.42			6.8 ± 2.58			3.26 ± 2.51						0.27 ± 0.27			
122-78-1	1635															
(Z,E)-α-farnesene ^g	16.92			0.37 ± 0.06			0.33 ± 0.17						0.14 ± 0.06			
26 560-14-5	1721															
(E,E)-α-farnesene	17.28	0.15 ± 0.1	2.05 ± 0.41	0.02 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	1.95 ± 0.73	0.18 ± 0.07	0.17 ± 0.09	0.83 ± 0.29			1.16 ± 0.4			
502-61-4	1742															
Methyl salicylate	17.63			0.19 ± 0.09			0.06 ± 0.05			0.35 ± 0.08			0.96 ± 0.48			
119-36-8	1770															
Phenylethyl alcohol	19.78			0.17 ± 0.09			0.31 ± 0.13						0.49 ± 0.18			
60-12-8	1912															
Phenethyl isothiocyanate ^g	22.39			0.04 ± 0.04									0.01 ± 0.01			
2257-09-2	2216															

Compound name ^a /CAS #	Yellow turnip rape cv. Valo			Yellow OSR cv. Majong			Cream-coloured OSR cv. Lyside			Cream-coloured OSR cv. Silver Shadow		
	RT ^b /RI ^c	Leaf ^d % ± SE	Bud ^e % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE	Leaf ^d % ± SE	Bud ^e % ± SE	Flower ^f % ± SE
Indole 120-72-9	23.56 2459	0.38 ± 0.14		3.86 ± 2.34			0.01 ± 0.01	1.55 ± 0.49		0.71 ± 0.3		

^a Volatile compounds were collected from oilseed rape plants in different phenological stages.
^b Mean release rates (%) per 100 g of fresh plant material are relative to the internal standard heptyl acetate (500 ng).
^c RT, retention time.
^d RI, Kovats retention index, DB-Wax.
^e Leaf (BBCH 29).
^f Bud (BBCH 52).
^g Flower (BBCH 65).
^h Compounds that were not verified by comparison with synthetic standards.

border that completely surrounded the main crop. The OSR and turnip rape were sown on 26 April 2018 at seed densities described in Section 2.6. The insecticide Steward[®] was applied at a level of 8.5 g/day on 28 May and 6 June according to the Norwegian damage threshold for pollen beetles in OSR, i.e. when observations in the crop indicated a mean per plant of > 0.5–1 beetle/plant at early bud stage (BBCH 50–52), > 1–2 beetles/plant at middle bud stage (BBCH 53–55), and > 2–3 beetles/plant at late bud stage (BBCH 57–59).^{13,41} On 26 May, eight kairomone traps were installed in the trap crop (two traps at each side of the plot) at the top level of the canopy and adjusted with the height of the growing plants (Fig. S2). The CSALOMON[®] VARb3z + traps were designed for monitoring purposes and not for mass trapping, thus we inspected the traps on a daily basis, counted the beetles that were caught, and, if necessary, emptied the traps and replaced the sticky inlet liners. Odour dispensers were changed every second week. The number of trapped pollen beetles and the BBCH stage were assessed weekly in each plot, as described for Experiment 2.

2.8 Statistics

The number of beetles counted per plant in all three field experiments (Experiments 1–3) were analysed using a generalised linear mixed model (GLMM) with a negative binomial distribution (PROC glimmix, SAS 9.4), considering dates of evaluation as a repeated parameter, using area under the curve (AUC) estimation,⁴⁷ with the following as fixed factors: treatment (cultivar in Experiment 1; treatment in Experiments 2 and 3), block (block in Experiment 1; location in Experiments 2 and 3), and interaction between treatment and block. After establishing the significance of the factor treatment, Tukey's test was used for multiple comparisons between the levels of the factor treatment. To identify particular time intervals during which treatment effects differed, individual GLMMs were used for each date of evaluation. A significance level of $\alpha = 0.05$ was selected in all analyses.

3 RESULTS

3.1 Volatile profiles

Analysis of volatiles collected from the four tested cultivars during the three tested growth stages, respectively, revealed qualitative

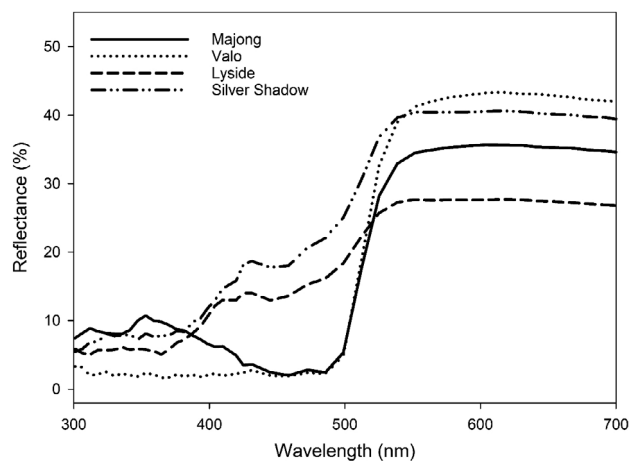


Figure 1. Spectral reflectance of petals of yellow turnip rape cv. Valo, yellow oilseed rape (OSR) cv. Majong, and cream-coloured OSR cv. Lyside and cv. Silver Shadow.

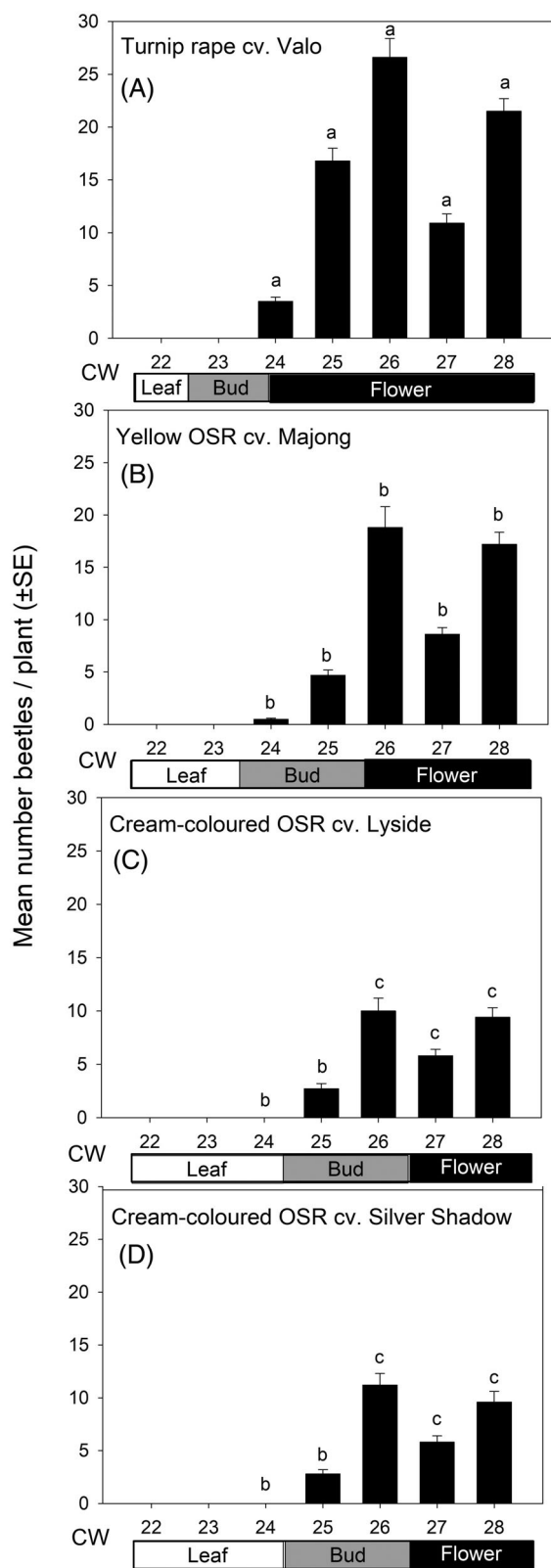


Figure 2. Preference for oilseed rape (OSR) and turnip rape cultivars in plant colonisation by pollen beetles in the field. Results are shown as weekly mean number of beetles per plant on (A) yellow turnip rape cv. Valo, (B) yellow OSR cv. Majong, (C) cream-coloured OSR cv. Lyside, and (D) cream-coloured OSR cv. Silver Shadow. Phenological plant stages are indicated as leaf development–stem elongation (white bar), bud development (grey bar), and flower development (black bar). Differences (Figure legend continues on next column.)

and quantitative differences between the odour profiles (Table 1). In total, 19 compounds were identified, the majority of which were detected during the flowering stage for all cultivars. Considering the major differences between plants in the flowering and bud growth stages with regard to the chemical composition of their odours, the flowering plants showed higher relative amounts and/or presence of the compounds *p*-xylene, β -myrcene, *Z*-3-hexenol (except OSR cv. Lyside), benzaldehyde, methyl benzoate, phenylacetaldehyde (except OSR cv. Lyside), (*Z,E*)- α -farnesene (except OSR cv. Lyside), (*E,E*)- α -farnesene, methyl salicylate, phenylethyl alcohol (except OSR cv. Lyside), and indole than in the bud stage (Table 1). Dimethyl disulphide, dimethyl trisulphide and phenethyl isothiocyanate were found mainly in the flowering stage of the turnip rape cultivar. The analyses also showed dimethyl trisulphide in the bud stage of turnip rape and phenethyl isothiocyanate in the flowering stage of OSR cv. Silver Shadow (Table 1).

3.2 Spectral reflectance

The spectral reflectance of the four tested cultivars differed markedly in both the UV range (300–400 nm) and the visible light range (400–700 nm) (Fig. 1). The turnip rape cultivar showed very low reflectance at 300–500 nm (< 5%), and maximum reflectance at 610 nm (44%). The yellow and two cream-coloured OSR cultivars showed slightly higher reflectance in the UV range (6–11%). In the blue light range (~ 380–500 nm), both yellow cultivars (turnip rape and OSR) had a stable and very low (< 5%) reflectance. By contrast, the two cream-coloured OSR cultivars (Lyside and Silver Shadow) showed much higher reflectance, which increased from 10% at 400 nm (both cultivars) to 20% (cv. Lyside) and 27% (cv. Silver Shadow) at 500 nm. In the range 550–700 nm, turnip rape had the highest reflectance (41–44%), followed by the cream-coloured OSR cv. Silver Shadow (~ 40%), the yellow OSR cv. Majong (~ 35%), and the cream-coloured OSR cv. Lyside (~ 27%) (Fig. 1).

3.3 Experiment 1: colonisation preferences between cultivars

Considering crop development, the turnip rape plants were consistently at a more advanced growth stage than plants of the three tested OSR cultivars. Furthermore, the plants of the yellow-flowering OSR cultivar were at a more advanced stage than the plants of the two cream-coloured-flowering OSR cultivars, whereas both of the cream-coloured cultivars showed similar developmental stages throughout the experimental period (Fig. 2). The first pollen beetles were found in calendar week (CW) 24 in the turnip rape cv. Valo (plants in late bud stage, BBCH 55) and yellow-flowering OSR cv. Majong (early bud stage, BBCH 50). Pollen beetles were not found in the two cream-coloured OSR cultivars Lyside and Silver Shadow until CW 25, when plants had reached the early bud stage, BBCH 50. Throughout the entire experimental period, pollen beetles were recorded at higher densities in the turnip rape plots (Fig. 2A) than in any of the OSR plots (Fig. 2B–D) ($F_{3,168} = 107.94$; $P < 0.0001$). The yellow-flowering OSR cultivar was more extensively infested than the two cream-coloured cultivars ($F_{3,168} = 107.94$; $P < 0.0001$). Numbers of

(Figure legend continued from previous column.)

between the four cultivars during specific weeks are indicated by lower case letters; consecutive columns with the same letter within the same calendar week represent values that are not significantly different (Tukey's test).

Table 2. Occurrence of pollen beetles in Experiment 2 presented as mean number of pollen beetles/plant (\pm SE) over an 8-week experimental period for the four different treatments: (1) yellow oilseed rape (OSR) cv. Majong with no trap crop (TC) (Y-OSR); (2) yellow OSR cv. Majong with yellow turnip rape (TR) cv. Valo as TC (Y-OSR-TC); (3) cream-coloured OSR cv. Silver Shadow with no TC (C-OSR); (4) cream-coloured OSR cv. Silver Shadow with yellow OSR cv. Majong as TC (C-OSR-TC). The corresponding phenological stages of the plants are indicated in white (leaf development--stem elongation), grey (bud development), and black (flower development)

Field area	OSR/TR	Cultivar	Colour	Crop/TC	Number of pollen beetles/plant (mean \pm SE)										Total				
					23	24	25	26	27	28	29								
1	Centre	Majong	Yellow	Crop	1.07 \pm 0.28	9.48 \pm 1.46	a	7.70 \pm 0.84	a	8.48 \pm 0.73	a	4.19 \pm 0.72	ab	3.41 \pm 0.55	a	2.33 \pm 0.45	ab	4.58 \pm 0.02	A
	Border	Majong	Yellow	Crop	0.75 \pm 0.22	5.25 \pm 0.74	b	5.38 \pm 0.68	c	7.38 \pm 0.71	a	1.25 \pm 0.30	b	1.29 \pm 0.33	a	1.92 \pm 0.48	a	2.90 \pm 0.02	C
2	Centre	Majong	Yellow	Crop	0.59 \pm 0.2	5.07 \pm 0.78	c	7.04 \pm 0.86	a	8.78 \pm 0.97	a	3.04 \pm 0.63	b	2.41 \pm 0.36	ab	2.56 \pm 0.43	ab	3.69 \pm 0.02	B
	Border	Valo	Yellow	TC	3.12 \pm 0.59	13.0 \pm 1.20	a	11.92 \pm 0.96	a	5.38 \pm 0.71	b	2.08 \pm 0.49	ab	2.58 \pm 0.70	a	2.33 \pm 0.48	a	5.05 \pm 0.03	A
3	Centre	S. Shad.	Cream	Crop	0.59 \pm 0.22	6.26 \pm 0.77	bc	7.33 \pm 0.66	a	6.37 \pm 0.71	a	1.96 \pm 0.51	b	1.44 \pm 0.45	b	1.15 \pm 0.31	b	3.14 \pm 0.02	C
	Border	S. Shad.	Cream	Crop	0.33 \pm 0.14	4.88 \pm 1.07	b	5.04 \pm 0.57	c	2.71 \pm 0.38	c	0.58 \pm 0.23	b	1.67 \pm 0.37	a	1.75 \pm 0.39	a	2.12 \pm 0.02	D
4	Centre	S. Shad.	Cream	Crop	0.71 \pm 0.21	8.67 \pm 0.87	ab	10.22 \pm 1.16	a	7.37 \pm 0.86	a	5.48 \pm 0.73	a	3.44 \pm 0.48	a	3.11 \pm 0.48	a	4.88 \pm 0.02	A
	Border	Majong	Yellow	TC	1.21 \pm 0.31	7.29 \pm 1.43	b	7.88 \pm 0.82	b	6.92 \pm 0.83	ab	3.58 \pm 0.96	a	2.38 \pm 0.68	a	2.12 \pm 0.63	a	3.92 \pm 0.03	B
	TR	Valo	Yellow		Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Flower	Flower	Flower	Flower		
	OSR	Majong	Yellow		Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Flower	Flower	Flower	Flower		
	OSR	S. Shad.	Cream		Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Flower	Flower	Flower	Flower		

Means per crop (in each treatment) followed by the same letter within columns are not significantly different (Tukey). Differences between treatments of the fields during particular time intervals (calendar weeks) are indicated for the centre of the fields by lower case letters and for the border of the fields by upper case letters in bold italics. Differences between treatments over the entire experimental period are indicated for the centre of the fields by lower case letters and for the border of the fields by upper case letters in bold italics.

Table 3. Occurrence of pollen beetles in Experiment 3 presented as mean number of pollen beetles/plant (\pm SE) over an 7-week experimental period for the three treatments: (1) yellow oilseed rape (OSR) cv. Majong with no trap crop (TC) and with insecticide treatment (as a control, C); (2) yellow OSR cv. Majong with yellow turnip rape (TR) cv. Valo as TC and insecticide treatment of the TC only (TC-); (3) yellow OSR cv. Majong with yellow TR cv. Valo as TC and kairomone traps in the TC (TC-K). The corresponding phenological stages of the plants are indicated in white (leaf development--stem elongation), grey (bud development), and black (flower development)

Field area	OSR/TR	Cultivar	Colour	Treatment	Number of pollen beetles/plant (mean \pm SE)										Total				
					21	22	23	24	25	26	27								
1	Centre	Majong	Yellow	Crop	0	0.37 \pm +0.11	a	3.04 \pm 0.66	b	3.67 \pm 0.49	a	3.93 \pm 0.56	a	0.25 \pm 0.1	a	0	1.61 \pm 0.01	A	
	Border	Majong	Yellow	Crop	0	0.67 \pm 0.18	b	4.5 \pm 0.82	b	9.17 \pm 1.31	a	6.54 \pm 0.79	a	0.25 \pm 0.12	b	0	3.02 \pm 0.03	C	
2	Centre	Majong	Yellow	Crop	0	0.33 \pm 0.13	a	6.56 \pm 1.31	a	3.11 \pm 0.48	ab	3.04 \pm 0.48	a	0.37 \pm 0.12	a	0	1.92 \pm 0.02	A	
	Border	Valo	Yellow	TC	0	3.79 \pm 0.58	a	24.71 \pm 3.13	a	3.92 \pm 0.77	b	6.29 \pm 0.73	a	1.29 \pm 0.25	a	0	5.71 \pm 0.06	B	
3	Centre	Majong	Yellow	Crop	0	0.37 \pm 0.13	a	3.30 \pm 0.67	b	2.04 \pm 0.31	b	4.22 \pm 0.58	a	0.33 \pm 0.11	a	0	1.47 \pm 0.01	A	
	Border	Valo	Yellow	TC	0	3.58 \pm 0.57	a	28.29 \pm 2.44	a	11.21 \pm 0.98	a	4.08 \pm 0.44	b	1.33 \pm 0.26	a	0	6.93 \pm 0.06	A	
	TR	Valo	Yellow		Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Flower	Flower	Flower	Flower		
	OSR	Majong	Yellow		Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf	Flower	Flower	Flower	Flower		

Means per crop (in each treatment) followed by the same letter within columns are not significantly different (Tukey). Differences between treatments of the fields during particular time intervals (calendar weeks) are indicated for the centre of the fields by lower case letters and for the border of the fields by upper case letters in bold italics. Differences between treatments over the entire experimental period are indicated for the centre of the fields by lower case letters and for the border of the fields by upper case letters in bold italics.

beetles generally increased in all treatments over time, with a peak in CW 26, and then subsequently decreased. At the peak of pollen beetle infestation in CW 26, the turnip rape plants were in full bloom (BBCH 65), whereas the yellow OSR cultivar had just started to bloom (BBCH 62), and the two cream-coloured OSR cultivars were in the late bud stage (BBCH 59) (Fig. 2).

3.4 Experiment 2: selection of best trap crop system

In both of the trap crop experiments (Experiments 2 and 3), plants varied in growth stage over the entire observation period, both between and within treatments, as well as between replicates. Overall, the turnip rape plants were consistently at a more advanced growth stage than the plants of the OSR cultivars (Tables 2 and 3), and the plants of the yellow-flowering OSR cultivar were at a more advanced stage than the plants of the cream-coloured-flowering OSR cultivar (Table 2).

Table 2 outlines the colonisation of the border and central areas of the experimental plots by pollen beetles over time for the four treatments: (i) yellow OSR cv. Majong without trap crop (Y-OSR); (ii) yellow OSR cv. Majong with yellow turnip rape cv. Valo as trap crop (Y-OSR-TC); (iii) cream-coloured OSR cv. Silver Shadow without a trap crop (C-OSR); and (iv) cream-coloured OSR cv. Silver Shadow with yellow OSR cv. Majong as trap crop (C-OSR-TC). Pollen beetles were first found in CW 23 in all four treatments (centre and border), although on average, development of the OSR cultivar plants was still at the leaf stage. At that time, significantly higher numbers of beetles were present in the border area with turnip rape as trap crop (Y-OSR-TC: 3.12 ± 0.59) compared with the other three border treatments ($F_{3,84} = 12.04$; $P = 0.0003$ Y-OSR-TC versus C-OSR-TC; $P < 0.0001$ Y-OSR-TC versus Y-OSR; $P < 0.0001$ Y-OSR-TC versus C-OSR), whereas no significant differences in number of beetles per plant were noted for the central areas (i.e. main crops) of the plots in the four treatments (Table 2). In CW 24, significantly larger numbers of beetles were again found in the turnip rape trap crop (Y-OSR-TC: 13.0 ± 1.20) compared with the other three border treatments ($F_{3,84} = 10.97$; $P = 0.0006$ Y-OSR-TC versus C-OSR-TC; $P < 0.0001$ Y-OSR-TC versus Y-OSR; $P < 0.0001$ Y-OSR-TC versus C-OSR). At that particular time, significantly lower numbers of beetles were recorded in the central area of Y-OSR-TC plots compared with the centre of the corresponding Y-OSR plots without a turnip rape trap crop ($F_{3,96} = 4.00$; $P = 0.0031$; Table 2). Even in CW 25, the border areas showed significantly larger numbers of beetles in the treatment with turnip rape trap crop (Y-OSR-TC: 11.92 ± 0.96) compared with the other three border treatments ($F_{3,84} = 18.67$; $P = 0.0002$ Y-OSR-TC versus C-OSR-TC; $P < 0.0001$ Y-OSR-TC versus Y-OSR; $P < 0.0001$ Y-OSR-TC versus C-OSR). No significant differences in number of beetles per plant were noted for the central areas of the four treatments (Table 2). In CW 26–29, numbers of beetles were not significantly different in either the borders or the centres of the plots with yellow-flowering OSR treatments with or without a trap crop. By contrast, during CW 27–29, larger numbers of beetles were found in the central areas of the plots C-OSR-TC compared with C-OSR (CW 27, $F_{3,96} = 5.45$, $P = 0.0002$; CW 28, $F_{3,96} = 4.30$, $P = 0.0027$; CW 29, $F_{3,96} = 3.69$, $P = 0.0017$). In addition, during CW 25–27, larger numbers of beetles were found in the border areas of the plots C-OSR-TC compared with C-OSR (CW 25, $F_{3,84} = 18.67$, $P = 0.0078$; CW 26, $F_{3,84} = 9.73$, $P < 0.0001$; CW 27, $F_{3,84} = 5.22$, $P = 0.0003$) (Table 2).

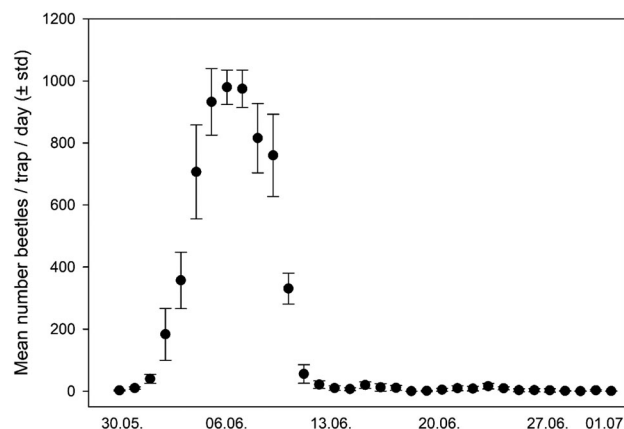


Figure 3. Kairomone trap catches over time in the field experiments conducted in 2018, shown as mean number of beetles per trap and day (\pm SD).

3.5 Experiment 3: best trap crop strategy with a control measure in the trap crop

Table 3 shows the occurrence of pollen beetles over time in the border and central areas of plots with the three treatments: (i) yellow OSR cv. Majong without a trap crop and treated with synthetic insecticide (as a control, C); (ii) yellow OSR cv. Majong with yellow turnip rape cv. Valo as trap crop and with insecticide treatment of the trap crop only (TC-I); and (iii) yellow OSR cv. Majong with yellow turnip rape cv. Valo as trap crop and kairomone traps placed in the trap crop (TC-K). Comparing the pollen beetle colonisation of the border areas over the entire experimental period, numbers of beetles were significantly higher in the border area of TC-K compared with C and TC-I ($F_{2,63} = 21.56$; $P < 0.0001$ C versus TC-K; $P = 0.0468$ TC-I versus TC-K) and in TC-I compared with C ($P < 0.0001$). When considering the central areas of the plots, we found no significant differences between the treatments over the entire experimental period (Table 3). The first pollen beetles were observed in CW 22 in all treatments (centre and border of plots), but numbers were significantly higher in the border areas of trap crop treatments TC-I and TC-K than in treatment C without a trap crop ($F_{2,63} = 12.97$; $P < 0.0001$). Regarding the average plant development at that time, the OSR cultivar was still in the leaf stage, whereas the turnip rape was already in the bud stage (Table 3). In CW 22–26, numbers of beetles in the border areas varied between the treatments, although there was a trend towards the highest numbers occurring in the border area of the trap crop treatment with kairomones (TC-K, except in CW 25) and the lowest numbers in control treatment (C, except in CW 24 and CW 25) (Table 3). By contrast, similar numbers of beetles were found in the central areas of the three treatment plots during this period (CW 22–26). Significant differences in the central areas were recorded only in CW 23, with the highest number of beetles in TC-I compared with the two other treatments ($F_{2,72} = 5.00$; $P = 0.0059$ TC-I versus C; $P = 0.0105$ TC-I versus TC-K). Pollen beetles were caught in the kairomone traps mainly during CW 22–24, with a peak in CW 23 (871–1045 beetles/trap/day on 6–7 June; Fig. 3).

4 DISCUSSION

In this study, we investigated the possibility of a kairomone-assisted trap cropping strategy to manage pollen beetles at the northern margin of OSR production in Norway.

Volatile cues, particularly those emitted from bud and flowering stages, have previously been shown to be important for the attraction and host selection of *B. aeneus*.^{7,8,22} We found the majority of volatile compounds emitted during the flowering stage followed by the bud stage, irrespective of the cultivar tested, as shown in previous studies.^{7,8,22} All the compounds detected in this investigation have been found previously in OSR plants,^{7,48–50} and many of them are known to be involved in odour-mediated behaviour of pollen beetles,^{6–8,51} as well as other organisms.^{52,53} In our study, the *B. rapa* plants most often preferred by pollen beetles had an odour profile that differed from that of the *B. napus* plants.^{8,22,25} The Brassicaceae-specific compounds dimethyl disulphide, dimethyl trisulphide and phenethyl isothiocyanate were recorded primarily for turnip rape (bud and flowering stages). These odour differences may partly explain the preferences in host selection behaviour of pollen beetles that we observed. Cook *et al.* suggested that the odour of turnip rape is more preferred than that of OSR, due mainly to phenylacetaldehyde and (*E,E*)- α -farnesene.⁸ These two compounds were also found in larger amounts in turnip rape than OSR in our study.

In addition, a behavioural preference for a particular shade of yellow may be involved in the host selection process of pollen beetles. Several studies have noted that the host-finding behaviour of pollen beetles comprises a preference for yellow over other colours, including cream and white.^{26,30,34,54} The yellow colours of the *B. napus* cv. Majong and *B. rapa* cv. Valo we used in our investigation may appear to be very similar to the human eye, but spectral reflectance analysis showed that the two yellow cultivars differed not only in the colour spectrum (~ 400–700 nm), but also in the UV region (~ 300–400 nm). For *B. aeneus*, spectral sensitivity has been measured with peaks at ~ 520–540 nm and 370 nm.³⁰ Döring *et al.* found evidence for UV, blue and green receptors, and concluded that colour preferences of pollen beetles could be determined by a green *versus* blue colour opponent mechanism, with an input to a green receptor at 540 nm and an input to a blue receptor at 440 nm.³⁰ Cook *et al.* verified that the UV region is important for pollen beetles regarding their host selection behaviour.²⁶ Based on these findings, colour preference of pollen beetles can be determined by considering a green *versus* blue colour opponent mechanism and spectral sensitivity in the UV region, which would give preferences as found in this study: yellow turnip rape \geq yellow OSR > cream-coloured OSR cv. Silver shadow > cream-coloured OSR cv. Lyside.

In the arranged field situation in our preference test in Experiment 1, more beetles were found on the turnip rape plants over the three tested OSR cultivars throughout the entire experimental period, suggesting a preference for turnip rape as stated previously.^{8,22,31–33} This suggests that the cues emitted by turnip rape during early flowering and onwards until late flower development send signals to the beetles that those plants offer more suitable buds/flowers for egg laying and/or food compared with OSR plants that are consistently in earlier phenological stages. Additionally, in Experiment 1, more beetles were found on the yellow OSR plants over the cream-coloured OSR plants, possibly because the latter were always in an earlier phenological stage than the yellow OSR plants. Perhaps the signals produced by the yellow OSR inform the beetles that such plants offer better oviposition and food sources compared with the cream-coloured OSR plants that are consistently in earlier phenological stages. It is known from previous studies that the phenological stage of a plant is important for host selection by pollen beetles, particularly for the females.^{22,55} Cook *et al.* showed in a semi-field assay that

B. aeneus clearly preferred *B. rapa* over *B. napus* if the plants were in the bud stage, no preference if both of these plant species were in the flowering stage, and always a preference for the flowering stage over the bud stage irrespective of tested cultivar.⁸

Results from the odour and reflectance analyses and Experiment 1 indicate that pollen beetles do show preference for turnip rape over the three tested OSR cultivars, and for the yellow OSR cultivar over the two cream-coloured cultivars, and these preferences are related to plant growth stage and the associated volatile and colour signals. Considering these findings, we designed two trap crop systems for pollen beetle management to be tested in trap crop field experiments: Y-OSR-TC using yellow OSR with an early flowering yellow turnip rape as trap crop, and C-OSR-TC using cream-coloured OSR (cv. Silver Shadow) with yellow OSR as trap crop. During the period when the trap crop was in the bud or early flowering stage while the main crop was still in the leaf or early bud stage, both tested trap crop systems showed significantly larger numbers of beetles in the border area (= trap crop) of each field plot compared with the border areas of the corresponding control fields without a trap crop (Y-OSR-TC, CW 23–25; W-OSR-TC, CW 25–27). These results concur with previous findings from other parts of Europe demonstrating the potential of early flowering turnip rape (*B. rapa*) as trap crop in OSR production^{8,22,32,33} and yellow OSR as a trap crop for cream-coloured OSR.³⁴ However, our results also demonstrated that pollen beetles migrated further from the trap crop into the main crop when upon reaching a high population in the trap crop. Therefore, it was only during a very short period (CW 24) that the number of beetles was significantly lower in the central areas of the plots with a turnip rape trap crop (Y-OSR-TC) compared with the central areas of control plots without a trap crop. Furthermore, for the trap crop system using cream-coloured OSR as the main crop and yellow OSR plants as trap crop (C-OSR-TC), we found significantly larger numbers of beetles in the central areas of the fields compared with the centres of the corresponding control fields without a trap crop (CW 27–29). These 'spill-over' effects noted in our study have been observed previously,^{7,16,17} and hence were expected, because both of the cultivars we used as trap crops are known to be host plants for pollen beetles and not dead-end crops. In light of the very rapid spill-over effect from the trap crop into the main crop using the C-OSR-TC system, and the poor suitability (i.e. low yields and very late plant development) of the cream-coloured cultivars under Norwegian conditions,⁴⁰ we used the turnip rape trap crop system Y-OSR-TC in the subsequent studies. This conclusion is in accordance with previous studies in which no difference between numbers of pollen beetles on white and yellow OSR plants were found.²⁶

The need for an effective control measure in the trap crop is highlighted by the mentioned rapid spill-over effect we found in Experiment 2. We compared kairomone-assisted trap cropping with insecticide-assisted trap cropping using conventional application of insecticides on the whole OSR field plot as a control. Remarkably, throughout the entire experimental period, beetle density was not significantly higher in the central areas of the plots with kairomone-assisted trap cropping compared with central areas in the other two treatments. We found significantly larger numbers of beetles in the trap crop regions of the two tested trap crop treatments compared with the OSR control. However, both the kairomone and the insecticide strategy effectively reduced the number of beetles in the trap crop to such an extent that it prevented a spill-over effect into the main crop. This suggests that both kairomone mass trapping-assisted and insecticide-assisted trap

cropping can be effective alternative strategies to control pollen beetles and thereby reduce the use of synthetic insecticides in OSR production. These findings are based on only one year of field experiments. Inasmuch as the weather conditions during the OSR growing season show considerable annual variation in Norway, experiments conducted over several years are needed to achieve a fair evaluation of this pest control approach. However, the results clearly support earlier studies on trap cropping in OSR in other parts of Europe.^{8,22,31–33}

Our studies emphasized that a key to successful pest control in spring OSR production is correct timing of the control measure. This applies to control of pollen beetles in general (traditional use of spraying pesticides), but more particularly to measures used in the trap crop to avoid a spill-over effect into the main crop. Pesticide treatment of a trap crop, as we did in our study, required very proper timing to be an effective pest control tool. By comparison, kairomone-assisted trap cropping used for this purpose was somewhat more robust with respect to timing: the traps have to be installed early enough to catch the first beetles that arrive in the trap crop, and thereafter the traps will be active for at least 3–4 weeks. We recorded very high trap catches in the beginning of June (CW 23), noted at > 1000 pollen beetles/trap/day. It should be noted that the traps used in our study were developed for monitoring and not for mass trapping, and thus we had to check and empty the traps daily to ensure their efficacy, which would not be practical for conventional OSR production. A trap designed for mass trapping is therefore required, if kairomone-assisted trap cropping is adopted on a commercial scale.

5 CONCLUSIONS

Our results concerning trap crop strategies for protecting spring OSR from pollen beetles in a northern climate suggest that kairomone-assisted trap cropping can reduce the need for insecticides in OSR production or in some cases may even make it possible to refrain from pesticides completely. As also noted in an earlier study,⁸ we observed that the success of turnip rape as trap crop in OSR production is related to early plant development of *B. rapa*, which includes inflorescence emergence occurring much earlier than in OSR and the association with plant volatile emission. We also found indications that the visual cues of *B. rapa* can offer important support in the mechanism underlying the pronounced attraction of pollen beetles to turnip rape compared with the tested OSR cultivars, even though the flowers of both the trap crop and the main crop plants in our final trap crop strategy were both yellow.

ACKNOWLEDGEMENTS

We are grateful to the farmers who hosted our field experiments. We also thank Toril Sagen Eklo for identifying the pollen beetle species, Andrew Dobson for help with the experiments, Torfinn Torp for statistical advice, and Andrea Ficke for valuable comments on the manuscript. This study was funded by the Research Council of Norway through the project BRAKORN (project no. 244210).

SUPPORTING INFORMATION

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

REFERENCES

- Carré P and Pouzet A, Rapeseed market, worldwide and in Europe. *OCL* **21**:D102 (2014).
- Zajac T, Klimek-Kopyra A, Oleksy A, Lorenc-Kozik A and Ratajczak K, Analysis of yield and plant traits of oilseed rape (*Brassica napus* L.) cultivated in temperate region in light of possibilities of sowing in arid areas. *Acta Agrobot* **69**:1696 (2016).
- Peltonen-Sainio P, Jauhiainen L and Venäläinen A, Comparing regional risks in producing turnip rape and oilseed rape – today in light of long-term datasets. *Acta Agr Scand* **59**:118–128 (2009).
- Peltonen-Sainio P and Niemi JK, Protein crop production at the northern margin of farming: to boost, or not to boost. *Agric Food Sci* **21**:370–383 (2012).
- Williams IH, The major insect pests of oilseed rape in Europe and their management: an overview, in *Biocontrol-Based Integrated Management of Oilseed Rape Pests*, ed. by Williams IH. Springer, Dordrecht, pp. 1–44 (2010).
- Williams IH and Cook SM, Crop location by oilseed rape pests and host location by their parasitoids, in *Biocontrol-Based Integrated Management of Oilseed Rape Pests*, ed. by Williams IH. Springer, Dordrecht, pp. 215–244 (2010).
- Mauchline AL, Hervé MR and Cook SM, Semiochemical-based alternatives to synthetic toxicant insecticides for pollen beetle management. *Arthropod Plant Interact* **12**:835–847 (2018).
- Cook SM, Rasmussen HB and Birkett MA, Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). *Arthropod Plant Interact* **1**:57–67 (2007).
- Čuljak TG, Pernar R, Juran I, Ančić M and Bažok R, Impact of oilseed rape crop management systems on the spatial distribution of *Brassicogethes aeneus* (Fabricius 1775): implications for integrated pest management. *Crop Prot* **89**:129–138 (2016).
- Thieme T, Heimbach U and Müller A, Chemical control of insect pests and insecticide resistance in oilseed rape, in *Biocontrol-Based Integrated Management of Oilseed Rape Pests*, ed. by Williams IH. Springer, Dordrecht, pp. 331–336 (2010).
- Slater R, Ellis S, Genay J-P, Heimbach U, Huart G, Sarazin M *et al.*, Pyrethroid resistance monitoring in European populations of pollen beetle (*Meligethes* spp.): a coordinated approach through the insecticide resistance action committee (IRAC). *Pest Manag Sci* **67**:633–638 (2011).
- Zimmer CT and Nauen R, Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. *Pest Manag Sci* **67**:599–608 (2011).
- Johansen NS, Bosque Fajardo M, Folkedal Schjøll A, Thöming G and Strand E, Glansbillene er resistente – hva nå? *Norsk Landbruk* **01**:50–51 (2019).
- Hokkanen HMT, Trap cropping in pest management. *Annu Rev Entomol* **36**:119–138 (1991).
- Javadi I and Joshi J, Trap cropping in insect pest management. *J Sustain Agric* **5**:117–136 (1995).
- Shelton AM and Badenes-Perez FR, Concepts and applications of trap cropping in pest management. *Annu Rev Entomol* **51**:285–308 (2006).
- Cook SM, Khan ZR and Pickett JA, The use of push and pull strategies in integrated pest management. *Annu Rev Entomol* **52**:375–400 (2007).
- Hokkanen HMT, Biological and agrotechnical control of the rape blossom beetle *Meligethes aeneus* (Coleoptera: Nitidulidae). *Acta Entomol Fenn* **53**:25–30 (1989).
- Buntin GD, Cabbage seedpod weevil (*Ceutorhynchus assimilis* Paykull) management by trap cropping and its effect on parasitism by *Trichomalus perfectus* (Walker) in oilseed rape. *Crop Prot* **17**:299–305 (1998).
- Veromann E, Metspalu L, Williams IH, Hiisaar K, Mand M, Kaasik R *et al.*, Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping. *Arthropod Plant Interact* **6**:385–394 (2012).
- Vinatier F, Gosme M and Valantin-Morison M, A tool for testing integrated pest management strategies on a tritrophic system involving pollen beetle, its parasitoid and oilseed rape at the landscape scale. *Landsc Ecol* **27**:1421–1433 (2012).
- Cook SM, Smart LE and Martin JL, Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomol Exp Appl* **119**:221–229 (2006).

- 23 Ekbohm B and Borg A, Pollen beetle (*Meligethes aeneus*) oviposition and feeding preference on different host plant species. *Entomol Exp Appl* **78**:291–299 (1996).
- 24 Ruther J and Thiemann K, Response of pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. *Entomol Exp Appl* **84**:183–188 (1997).
- 25 Cook SM, Bartlett E, Murray DA and Williams IH, The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomol Exp Appl* **104**:43–50 (2002).
- 26 Cook SM, Skellern MP, Döring TF and Pickett JA, Red oilseed rape? The potential for manipulation of petal colour in control strategies for the pollen beetle (*Meligethes aeneus*). *Arthropod Plant Interact* **7**:249–258 (2013).
- 27 Mauchline AL, Osborne JL, Martin AP, Poppy GM and Powell W, The effects of non-host plant essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. *Entomol Exp Appl* **114**:181–188 (2005).
- 28 Mauchline AL, Cook SM, Powell W and Osborne JL, Effects of non-host plant odour on *Meligethes aeneus* during immigration to oilseed rape. *Entomol Exp Appl* **146**:313–320 (2012).
- 29 Jönsson M, Rosdal K and Anderson P, Response to olfactory and visual cues by over-wintered and summer generations of pollen beetle, *Meligethes aeneus*. *Physiol Entomol* **32**:188–193 (2007).
- 30 Döring TF, Skellern M, Watts N and Cook SM, Colour choice behaviour in the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae). *Physiol Entomol* **37**:360–368 (2012).
- 31 Skellern MP and Cook SM, The potential of crop management practices to reduce pollen beetle damage in oilseed rape. *Arthropod Plant Interact* **12**:867–879 (2018).
- 32 Hokkanen HMT, Granlund H, Husberg G-B and Markkula M, Trap crops used successfully to control *Meligethes aeneus* (Coleoptera: Nitidulidae), the rape blossom beetle. *Ann Entomol Fenn* **52**:115–120 (1986).
- 33 Büchi R, Investigations on the use of turnip rape as trap plant to control oilseed rape pests. *IOBC/wprs Bull* **13**:32–39 (1990).
- 34 Giamoustaris A and Mithen R, The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomol Exp Appl* **80**:206–208 (1996).
- 35 Aluja M, Jimenez A, Camino M, Piñero J and Aldana L, Habitat manipulation to reduce papaya fruit fly (Diptera: Tephritidae) damage: orchard design, use of trap crops and border trapping. *J Econ Entomol* **90**:1567–1576 (1997).
- 36 Pair SD, Shreffler J and Motes J, Utilizing trap crops for insect suppression in watermelon, in Proceedings of the 17th Annual Horticultural Industry Conference, ed. by McCraw BD, Stillwater, Oklahoma State University, pp. 287–290 (1997).
- 37 Vernon RS, Kabaluk JT and Behringer AM, Aggregation of *Agriotus obscurus* (Coleoptera: Elateridae) at cereal bait stations in the field. *Can Entomol* **135**:379–389 (2002).
- 38 Williams IH, Büchi R and Ulber B, Sampling, trapping and rearing oilseed rape pests and their parasitoids, in *Biocontrol of Oilseed Rape Pests*, ed. by Alford DV. Blackwell Science Publications, Oxford, pp. 145–160 (2003).
- 39 Audisio P, Cline AR, De Biase A, Antonini G, Mancini E, Trizzino M *et al.*, Preliminary re-examination of genus-level taxonomy of pollen beetle subfamily *Meligethinae* (Coleoptera: Nitidulidae). *Acta Ent Mus Nat Pra* **49**:341–504 (2009).
- 40 Abrahamsen U, Sortsfoersøk i vårraps, in *Jord- og Plantekultur 2016 – Forsøk i korn, olje- og proteinvekster, engfrøavl og potet 2015*, ed. By Strand E, NIBIO BOK, Vol. 2:1, Nibio, Ås, pp.162–165 (2016).
- 41 Meier U, *Growth Stages of Mono- and Dicotyledonous Plants*, Federal Biological Research Centre for Agriculture and Forestry. Germany, Berlin (2001).
- 42 Thöming G, Norli H, Saucke H and Knudsen GK, Pea plant volatiles guide host location behaviour in the pea moth. *Arthropod Plant Interact* **8**:109–122 (2014).
- 43 Dalen M, Knudsen GK, Norli HR and Thöming G, Sources of volatiles mediating host location behaviour of *Glypta haesitator*, a larval parasitoid of *Cydia nigricana*. *Biol Control* **90**:128–140 (2015).
- 44 Kovats E, Gas-chromatograpische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. *Helv Chim Acta* **4**:1915–1932 (1958).
- 45 Stein SE, An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. *J Am Soc Mass Spectrom* **10**:770–781 (1999).
- 46 Toth M, Szarukán I, Marczali Z and Csonka ÉB, Non-sticky trap for *Meligethes* (Coleoptera, Nitidulidae) combining visual and chemical stimuli, in Proceedings of the 31st conference of the International Society of Chemical Ecology; 29th June–3rd July, Stockholm, Sweden, pp. 363 (2015).
- 47 Zerbe GO, Randomization analysis of the completely randomized design extended to growth and response curves. *J Am Stat Assoc* **74**:215–221 (1979).
- 48 Butcher RD, MacFarlane-Smith W, Robertson GW and Griffiths DW, The identification of potential aeroallergen/irritant(s) from oilseed rape (*Brassica napus* spp. *oleifera*): volatile organic compounds emitted during flowering progression. *Clin Exp Allergy* **24**:1105–1114 (1994).
- 49 McEwan M and MacFarlane Smith WH, Identification of volatile organic compounds emitted in the field by oilseed rape (*Brassica napus* spp. *oleifera*) over the growing season. *Clin Exp Allergy* **28**:332–338 (1998).
- 50 Knudsen JT, Eriksson R and Gershenzon J, Diversity and distribution of floral scent. *Bot Rev* **72**:1–120 (2006).
- 51 Smart LE and Blight MM, Response of the pollen beetle, *Meligethes aeneus* to traps baited with volatiles from oilseed rape, *Brassica napus*. *J Chem Ecol* **26**:1051–1064 (2000).
- 52 Blight MM, Le Métayer M, Delègue MHP, Pickett J, Marion-Poll F and Wadhams LJ, Identification of floral volatiles involved in recognition of oilseed rape flowers, *Brassica napus* by honeybees, *Apis mellifera*. *J Chem Ecol* **23**:1715–1727 (1997).
- 53 Shannon RWR, Félix A-E, Poppy GM, Newland PL, van Dam NM and Hanley ME, Something in the air? The impact of volatiles on mollusk attack of oilseed rape seedlings. *Ann Bot* **117**:1073–1082 (2016).
- 54 Blight MM and Smart LE, Influence of visual cues and isothiocyanate lures on capture of pollen beetle, *Meligethes aeneus* in field traps. *J Chem Ecol* **25**:1501–1516 (1999).
- 55 Frearson DJT, Ferguson AW, Campbell JM and Williams IH, The spatial dynamics of pollen beetles in relation to inflorescence growth stage of oilseed rape: implication for trap crop strategies. *Entomol Exp Appl* **116**:21–29 (2005).