Research Article

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Intercropping mitigates incidence of the oilseed rape insect pest complex

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Abstract

BACKGROUND: Oilseed rape (OSR, *Brassica napus*) is a major crop requiring numerous phytosanitary treatments. It is of paramount importance to find sustainable insect pest management to guarantee long term OSR availability. Therefore, we aimed to evaluate the effect of OSR intercropping with faba beans (FB, *Vicia faba*) on the OSR insect pest complex. In addition, we aimed to understand the underlying mechanisms of crop protection via intercropping by distinguishing between the effects of visual and physical disruptions caused by companion plants and those of olfactory disruptions in host location and selection, and to evaluate whether this effect is direct or indirect. In a field trial run over 2 years, OSR was grown either as a monocrop or intercropped with winter FB (WFB, frost-resistant), spring FB (SFB, frost-sensitive), or polyethylene artificial plants (ART) designed to mimic FB.

RESULTS: Compared to the monocropped OSR the OSR + FB intercropping systems significantly reduced the feeding damage caused by cabbage flea beetles (*Phyllotreta* spp.), the immigration of adult cabbage stem flea beetles (*Psylliodes chrysocephala*), the number of rape stem weevil (*Ceutorhynchus napi*) oviposition punctures on stems and the number of pollen beetle (*Brassicogethes aeneus)* per inflorescence. The intercropping with SFB had a stronger impact on *C. napi* and *B. aeneus* than intercropping with WFB. Compared to the monocropped OSR the ART treatment significantly reduced *Phyllotreta* spp. damage on leaves, *C. napi* oviposition punctures and the number of *B. aeneus*. The yield of OSR intercropped with SFB was higher than monocropped OSR, while the other treatments did not affect yield.

CONCLUSION: This study shows that intercropping reduces the incidence of the OSR insect pest complex. Mechanisms of action include the direct visual and physical disruption provided by the companion plants.

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

Keywords: mixed cropping; crop diversification; integrated pest management; companion planting; physical disruption

1 INTRODUCTION

It is now widely accepted that a transition towards more environmentally friendly agronomic practices is necessary.[1] In response, the European Union has banned a growing number of synthetic active ingredients, including several insecticides such as neonicotinoids.[2] Oilseed rape (OSR, *Brassica napus*) cultivation is particularly affected by these changes as it requires a high number of treatments against weed and insect pests.[3,4] Furthermore, the rapid evolution of insecticide resistance increases even more the need to develop sustainable alternative practices for pest management in OSR production.[5,6]

The complex of OSR insect pests in Europe, which strongly impacts OSR yield,[7–9] consists mainly of four Coleopteran insects, with very heterogenous life cycles and phenologies. The cabbage flea beetles belonging to the genus *Phyllotreta* spp. damage the very early development stage of the OSR plant by feeding on cotyledons.[10] The cabbage stem flea beetle adults (*Psylliodes chrysocephala*; Linnaeus, 1758) immigrate into OSR crops at the early stages of seedling development; they feed on the first leaves in autumn, before the larval stages develop in the petioles and stems over winter; both causing economic damage.[11] The rape stem weevil (*Ceutorhynchus napi*; Gyllenhaal, 1837) lays its eggs in the stems in spring, which can cause plant deformation and bursting.[12] The pollen beetle (*Brassicogethes aeneus*; Fabricius, 1775) immigrates into OSR fields in spring and perforates flower buds to feed on pollen; the larval stages also feed on flower buds.[13]

Intercropping practices in this article, describe an agricultural system where a service crop and a cash crop are grown together in the same field, interacting closely throughout a significant part

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of the cash crop life cycle.[14] The service crop, consisting of companion plants, provides benefits by improving the environmental and production performance of the cropping system, rather than being grown for yield. Intercropping OSR with legume companion plants such as common vetch (*Vicia sativa*) and faba beans (FB, *Vicia faba*), reduces weed competition and improves fertilization.[14–17] Intercropping OSR with clover (*Trifolium alexandrinum*),[18] a mixture of legumes and other frost-sensitive plants,[19,20] or cereals has been shown to reduce *Psylliodes chrysocephala* adult damage in autumn compared to monocropped OSR.[21] Furthermore, intercropping OSR with clover, FB,[18] cereals,[21] or a mixture of legumes was found to decrease *Psylliodes chrysocephala* larval infestation compared to OSR grown in monocropping systems.[22,23] There is a relative scarcity of studies evaluating intercropping on spring-active pests such as *C. napi* and *B. aeneus* and the literature is contradictory. Intercropping OSR with a mixture of frost-sensitive legumes resulted in fewer *C. napi* oviposition punctures per plant and fewer *B. aeneus* individuals per inflorescence compared to monocropped OSR.[22] However, Emery *et al.* estimated *B. aeneus* damage by the number of podless stalks in OSR intercropped with FB, clover or winter peas (*Pisum sativum*) and did not find differences compared to OSR in monocropping.[18]

Frost sensitive legumes were mostly studied for their capacity to cover the soil in autumn and to provide extra nitrogen nutrition in spring with their mineralization after death.[24] However, the companion plants destroyed by frost in winter were thought not to affect spring-active insect pests.[18,25] Studying the effect of the OSR cropping with FB companion plants, taking into account whether the FB is a winter (WFB, frost-resistant) or spring (SFB, frost-sensitive) variety, on the entire insect complex mentioned earlier would help evaluate their relative effectiveness in controlling various insects and maintaining yields – an area that has yet to be explored in the literature.

The reduction of insect pest attacks by the addition of companion plants may be the result of various mechanisms deriving from visual, physical or chemical or a combination of all properties.[26,27] Visual disruption encompasses the optical characteristics of the field and plants detected by the insects. The visual orientation of the pests can be disturbed by the reflectance and the shapes of the companion plants within the field.[28] Physical disruption includes the mechanisms hindering the movement of the pests.[29] The presence of mechanical barriers provided by the companion plants or a dilution effect of the host plant can disorient or increase the host–plant location time of the insect.[30] Chemical disruption involves confusing the insect's olfactory and gustatory mechanisms for host–plant location and acceptance, caused by compounds present in or released by the companion plant or by the host plant in response to the presence of companion plants.[31,32]

The disruption mechanisms listed earlier can be direct, with the companion plant acting directly on insect host–plant location. For example, the volatile organic compounds (VOCs) released by the companion plant could have a repellent effect on the pests or a masking effect of the host plant odor.[33] The disruption can also be indirect via the alteration of the host plant in several ways, providing a disruption via the cues of the host plant. For instance, the companion plants can alter the chemical profile of the host plant,[34,35] making its VOCs less recognizable by the pests or the plant less appealing for them.[36] Companion plants may also have an impact on ecophysiological traits of OSR crop plants[37]; phenological development, collar diameter and stem elongation were found to have an influence on *Psylliodes chrysocephala* and *C. napi* damage and plant infestations.[21,38,39] However, the disruption mechanisms involved in the companion planting FB with OSR (OSR + FB) are still not clear.

In our study we first aimed to evaluate the impact of WFB and SFB as companion plants on the prevalence of the OSR insect pest complex, with a specific focus on the viability of such a cropping system in terms of effect on OSR yield. Second, we aimed to investigate the mechanisms involved in the reduction of insect pests immigration or attacks, by distinguishing visual and physical disruption from chemical disruption. Thus, we conducted a field trial over 2 years recording the natural occurrences of insect pests within OSR either monocropped or with the addition of WFB, SFB companion pants or with artificial plants (ART). The ART simulated FB plants and were designed to provide visual and physical disruption without chemical cues. The addition of the ART with OSR allows us to disentangle the possible mechanisms at work.[40,41] Ecophysiological traits of OSR were recorded to evaluate the possible indirect impact of the companion plant on the insects.

2 MATERIAL AND METHODS

2.1 Field experiments – general set-up

This experiment took place in the 2022–2023 and 2023–2024 cropping seasons in open field conditions (Table 1). Two separate fields on the Agroscope Changins experimental farm were used in 2022–2023, the first (2022-P1, 2′509′390, 1′138′865) and the second (2022-P2, 2′507′033, 1′139′330) had three replicates for each treatment (Supporting Information, Table S1). In the 2023–2024 season all six replicates were in the same field (2023-P3, 2′507′120.3, 1′139′679.5). All fields had sandy clay loams soil with wheat as the previous crop.

Table 1. Summary table of the statistical analysis and model components used in this study

Response	Model	Link	Distribution	RANDOM factor
Number of *Phyllotreta* spp. feeding shotholes on cotyledons	GLMMs	Log	Negative binomial	Field/block
Number of *Psylliodes chrysocephala* per sticky trap	GLMMs	Log	Quasi Poisson	Week/field/block
Number of *Psylliodes* chrysocephala larvae per plant	GLMMs	Log	Negative binomial	Field/block
Number of *Ceutorhynchus napi* oviposition punctures	GLMMs	Log	Negative binomial	Field/block
Number of *Brassicogethes aeneus* per inflorescence	GLMMs	Log	Quasi Poisson	Field/block
Number of oilseed rape leaves	GLMMs	Log	Conway–Maxwell-Poisson	Field/block

Note: GLMM, generalized linear mixed model.

The general set up of those fields comprised plots (9 m^2) of winter OSR (var. Angelico, Limagrain, FR) alone or in additive intercropping with companion plants, WFB, frost-resistant (fields P1, P2 and P3), SFB, frost-sensitive (fields P2 and P3) or ART (fields P1, P2 and P3). All plots were buffered with 6 m of winter barley on every side, the treatments were arranged in latin square in 2022-P1 and in semi-randomized block design in 2022-P2 and 2023-P3 (Supporting Information, Fig. S1).

The winter OSR was sown on the 22 August 2022 and on the 18 August 2023 (40 seeds/m^2, 30 cm row spacing). The FB (SFB: var. Tiffanny, RAGT Semences, France; WFB: var. Augusta, NPZ, Germany) for the intercropping treatments were sown on the same date as OSR in the inter rows (15 seeds/m^2). ART were produced by hand to mimic the first phenological stage of FB development with four leaves (Fig. S2). Four oval-shaped pieces (4 cm length × 3 cm wide, dimensions averaged from the first four leaves of 20 FB plants) were cut from green polyethylene tarp (Jardin Royal bâche pro, Jumbo®, Switzerland). They were assembled on top of a green interlocking milestone post 33 cm height (Piquet jalon emboitable 33 cm, Rubalise.fr, France) with green Chatterton tape (tesa®, Switzerland). The ART were set in inter rows once the natural FB had two leaves with a density of 13 plants/m^2 (expected emergence density of the sown FB), on the 8 September 2022 and the 31 August 2023. They were raised to the FB average canopy level in the other treatments every week until the end of November (Fig. 1). The ART were left in the field through winter until the blooming period of OSR (the 12 April 2023 and the 2 April 2024).

All the fields received 50 m^3/ha of cattle manure in early August prior sowing, and were supplemented with nitrogen in February and March (90 kg/ha and 60 kg/ha, respectively) and with 30 units of sulfur in March (200 kg/ha Kieserite, Landor). A pre-emergence herbicide (3 L/ha Devrinol Plus, Staehler) was applied 5 days after sowing and two herbicides were spayed, one against cereal volunteers (1.5 L/ha Fusilade Max, Syngenta) after OSR emergence in early September and one against monocotyledous weeds (1.4 L/ha Arlit, Omya) in November. No insecticides or fungicides were applied. Observed densities of OSR and FB plants after emergence are shown in Fig. 1.

2.2 Sampling pest insect incidence and damage

*2.2.1 Cabbage flea beetle (*Phyllotreta *spp.) feeding damage on cotyledons*

Flea beetle feeding damage by both *Phyllotreta* cabbage flea beetles and *Psylliodes chrysocephala* is characterized by 'shotgun' feeding holes on OSR leaf material. Feeding damage was therefore estimated by counting the number of holes per plant at the development stage BBCH 12,[42] when two true leaves were unfolded (Fig. 1). In both years and across all fields, the crop reached this stage before the immigration of *Psylliodes chrysocephala*, as monitored by yellow water traps. Consequently, the damage was attributed to cabbage flea beetles belonging to the

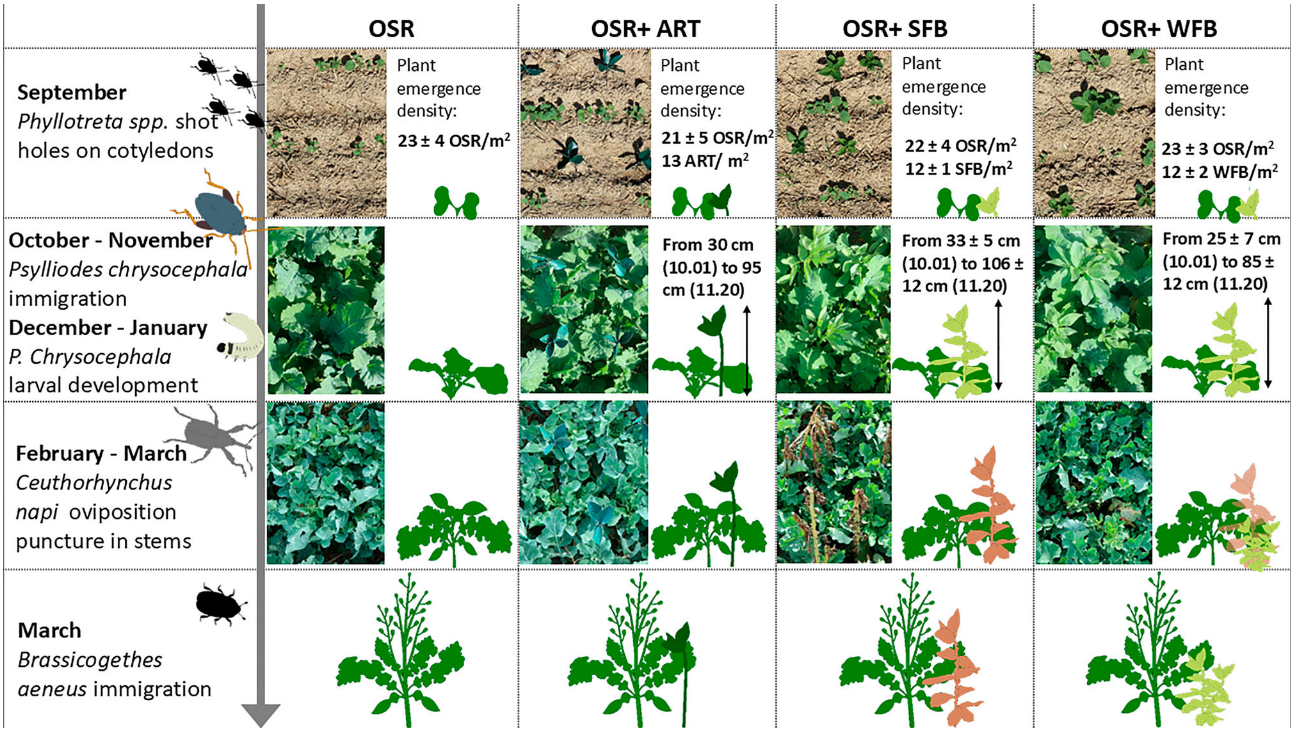

Figure 1. Graphical summary of the study to assess impacts of intercropping with companion plants on insect pest incidence and damage in oilseed rape (OSR), highlighting key periods for insect and plant development data collection. Four experimental treatments were compared: OSR (monocropped OSR) OSR + WFB (OSR intercropped with winter faba beans), OSR + SFB (OSR intercropped with spring faba beans), and OSR + ART (OSR grown with artificial plants). The first column indicates the monitoring periods for the studied insect pests: the cabbage flea beetle (*Phyllotreta* spp.), the cabbage stem flea beetle (*Psylliodes chrysocephala*), the rape stem weevil (*Ceuthorhynchus napi*) and the pollen beetle (*Brassicogethes aeneus*). The second row shows the mean ± standard error (SE) of plant emergence densities across replicates with a picture of the canopy for each treatment. The third row shows the mean ± SE of the height of the companion plants or artificial plants measured from early October to late November across replicates and a picture of the canopy for each treatment. The green plant illustrations represent growing plants, while the brown plant illustrations indicate plant remnants frozen during winter.

Phyllotreta genus caught in the traps.[10] In 2022, the number of feeding shotholes were counted on the 22 September on two series of five adjacent OSR plants ($n = 10$) per plot. In 2023, it was counted on the 11 September on four series of five adjacent OSR plants ($n = 20$) per plot.

2.2.2 *Cabbage stem flea beetle (*Psylliodes chrysocephala*) immigration and larval infestation*

To assess the attractiveness of treatments, we followed the immigration of *Psylliodes chrysocephala* with yellow sticky traps (Aeroxon®, Switzerland). Traps were placed in the center of each plot on the ground from the beginning of immigration to its end. Sticky traps were replaced every week and the number of *Psylliodes chrysocephala* recorded from the 22 September to the 20 October 2022 (BBCH 12 to BBCH 16) and from the 26 September to the 17 October 2023 (BBCH 15 to BBCH 19).

To evaluate the number of larvae per plant, a series of five adjacent OSR plants in the middle of the plot were sampled. Plants were cut at the base of the stem just below the soil surface at BBCH 30. In 2023, plants were collected on 15 February and placed together on a Berlese traps (described in Seimandi-Corda *et al.*)[43] for 3 weeks to collect and count *Psylliodes chrysocephala* larvae, while in 2024, plants were collected on 26 January and placed individually on Berlese traps for the same purpose.

2.2.3 *Rape stem weevil (*Ceutorhynchus napi*) oviposition punctures*

The oviposition damage of *C. napi*, characterized by punctures enclosed by a white mucus on the main stem of OSR plants, were counted on three series of five adjacent OSR plants ($n = 15$) per plot. In 2023 and in 2024 the counting of oviposition punctures was performed on the 1 March after the main flight peak of *C. napi* – determined by yellow water traps – at stem elongation stage (BBCH 32 in 2023 and BBCH 34 in 2024).

2.2.4 *Pollen beetle (*Brassicogethes aeneus*) abundance*

The number of *B. aeneus* per main inflorescence was counted by the beating method on three series of five adjacent OSR plants per plot. Each inflorescence was individually shaken in a small plastic bowl (20 cm diameter, 10 cm deep), and *B. aeneus* were counted before being released. This was done on the 21 March 2023 and on the 19 March 2024 before the first flowers bloomed (BBCH 57).

2.3 Characteristics and ecophysiological traits of oilseed rape plants

To provide a better understanding of the treatments and to explore possible indirect impacts, ecophysiological traits of OSR plants at the time of insect pest's occurrence through the cropping season were recorded (Fig. 1). In autumn, the ground covered by the crops (BBCH 12) was recorded. On the 15 September 2022 and the 11 September 2023 an overhead (zenithal) picture of 1 m^2 was taken for all plots for each field ($n = 12$ for each treatment). They were then analyzed with Canopeo (Canopeo Web©, V2.0) to provide an estimate of the ground covered by the OSR and the companion plants in the early crop development stage. For the OSR + ART treatment, the ART plant was recorded as vegetation and included in the ground covered estimation.

The number of leaves per plant was counted when OSR was estimated to have seven true leaves unfolded (BBCH 17) and before any true leaves started to fall. At the same time the collar diameter was measured with a digital caliper at the junction of the root and the stem. In 2022, the total number of true leaves were counted for two series of five adjacent OSR plants per plot and on four series of five adjacent OSR plants per plot in 2023 ($n = 180$ for each treatment).

Stem elongation was measured on the same date as *C. napi* oviposition punctures were counted, on the 1 March 2023 (BBCH 32) and 2024 (BBCH 34). It was assessed on the plants surveyed for *C. napi* oviposition punctures by measuring the new elongation of the stem after winter from the center of apical bud to the end of the newly elongated stem for three series of five adjacent OSR plants per plot, $n = 180$ plants for each treatment. The newly elongated stem is characterized by a fresh green color compared to the possible autumn elongation having purple shades.

The total stem height was measured at BBCH 57 before the first flowers bloomed when counting of *B. aeneus* was done on inflorescences. The stem length was measured from the ground to the highest flower bud on three series of five adjacent OSR plants per plot, $n = 180$ plants for each treatment.

The OSR was harvested on the 10 July 2023 and on the 4 July 2024 (BBCH 89–97), with an SP2100 experimental harvester (Baural, Champigny-en-Beauce, France). The harvester was equipped with an onboard data logger, HM800-ClassicGG version 2.1.1.12 (Harvest Master GrainGage, Juniper Systems, Logan, UT, USA), recording the mass density and total grain mass of the harvested plots. For each plot, an aliquot of 1 kg of OSR grain was then sampled and weighed (gross weight), and impurities (e.g., dust, non-OSR seeds, and stalk and leaf debris) were removed with a LA-LS-P (Westrup A/S, Slagelse, Denmark). Clean aliquots were weighed again (net weight), and their relative moisture was monitored (IntelliAg MVT, Dickey-John Europe, Colombes, France). Net grain yield (dried to the standard 6% relative moisture) was recorded. Technical limitations restricted the harvest to OSR only.[22]

2.4 Data analysis

All analyses were performed using R version 4.3.3.[44] The effects of our treatments on count responses were modeled with glmmTMB package,[45] model assumptions were checked with DHARMa package.[46] A Wald test on the generated models allowed the assessment of the effect of the treatments (car package),[47] followed by a pairwise comparisons of estimated marginal means with Tukey adjustment (emmeans package)[48] in case of significant Wald test ($P < 0.05$). The model parameters are presented in Table 1. The results were back transformed to the response scale to produce the figures and the percentage of the treatment effect compared to the control (OSR) was calculated using the estimated marginal means.

The effects of our treatments on continuous responses were analyzed via analysis of covariance (ANCOVA) with the block nested in the field as a random factor (rstatix package).[49] The collar diameters were square root transformed to satisfy analysis of variance (ANOVA) assumptions. The stem elongation and inflorescence height were standardized for each field [$x_{st} = (x - \text{mean}(x_{\text{field}}))/\text{SD}(x_{\text{field}})$] to allow a comparison of the effect of the treatments across fields. No transformations were needed for the data on ground-cover and the yield for which ANOVA assumptions were satisfied.

3 RESULTS

3.1 Cabbage flea beetle (*Phyllotreta* spp.) feeding damage on cotyledons

The treatments had a significant impact on *Phyllotreta* spp. feeding damage on OSR (Fig. 2(a); $\chi^2 = 39.47$, df = 3, $P < 0.001$). Both treatments OSR + SFB and OSR + WFB had significantly less

Phyllotreta spp. feeding shotholes on their cotyledons than monocropped OSR ($P < 0.001$ for both comparisons). The number of shotholes in the OSR + ART treatment was significantly lower compared to monocropped OSR ($P = 0.04$), but significantly higher than in OSR + WFB ($P = 0.014$). However, no significant difference in the number of feeding shotholes was observed between OSR + ART and OSR + SFB ($P > 0.05$). Compared to monocropped OSR, OSR + WFB and OSR + SFB had, on average, a reduction in the number of *Phyllotreta* spp. feeding shotholes of 35% and 38%, respectively. The treatment OSR + ART averaged a 19% reduction compared to monocropped OSR.

3.2 Cabbage stem flea beetle (*Psylliodes chrysocephala*) immigration and larval infestation

The treatments had a significant effect on the number of adult *Psylliodes chrysocephala* caught on yellow sticky traps in the plots (Fig. 2(c); $\chi^2 = 17.59$, df = 3, $P < 0.001$). OSR grown with FB (either OSR + SFB or OSR + WFB) had significantly fewer adult *Psylliodes chrysocephala* trapped than in the monocropped OSR ($P = 0.003$ and $P = 0.007$, respectively). Compared to monocropped OSR, OSR + WFB and OSR + SFB had, on average, a reduction of 37% and 32% in *Psylliodes chrysocephala* trapped adults, respectively. OSR with ART treatment presented an intermediate number of trapped *Psylliodes chrysocephala*, not significantly different from either monocropped OSR nor both companion planting scenarios of OSR with FB ($P > 0.05$ for all comparisons).

The larval infestation of *Psylliodes chrysocephala* was very low in our experiments, with an average across treatments of 1.63 ± 0.16 larvae per plant. The treatment had a significant effect on the larval infestation (Fig. 2(e); $\chi^2 = 12.46$, df = 3, $P < 0.01$). The treatment OSR + ART had significantly more larvae per plant than the OSR + SFB and OSR + WFB treatments ($P = 0.04$ and $P = 0.005$, respectively). Monocropped OSR had an intermediate larval infestation not significantly different from the other treatments.

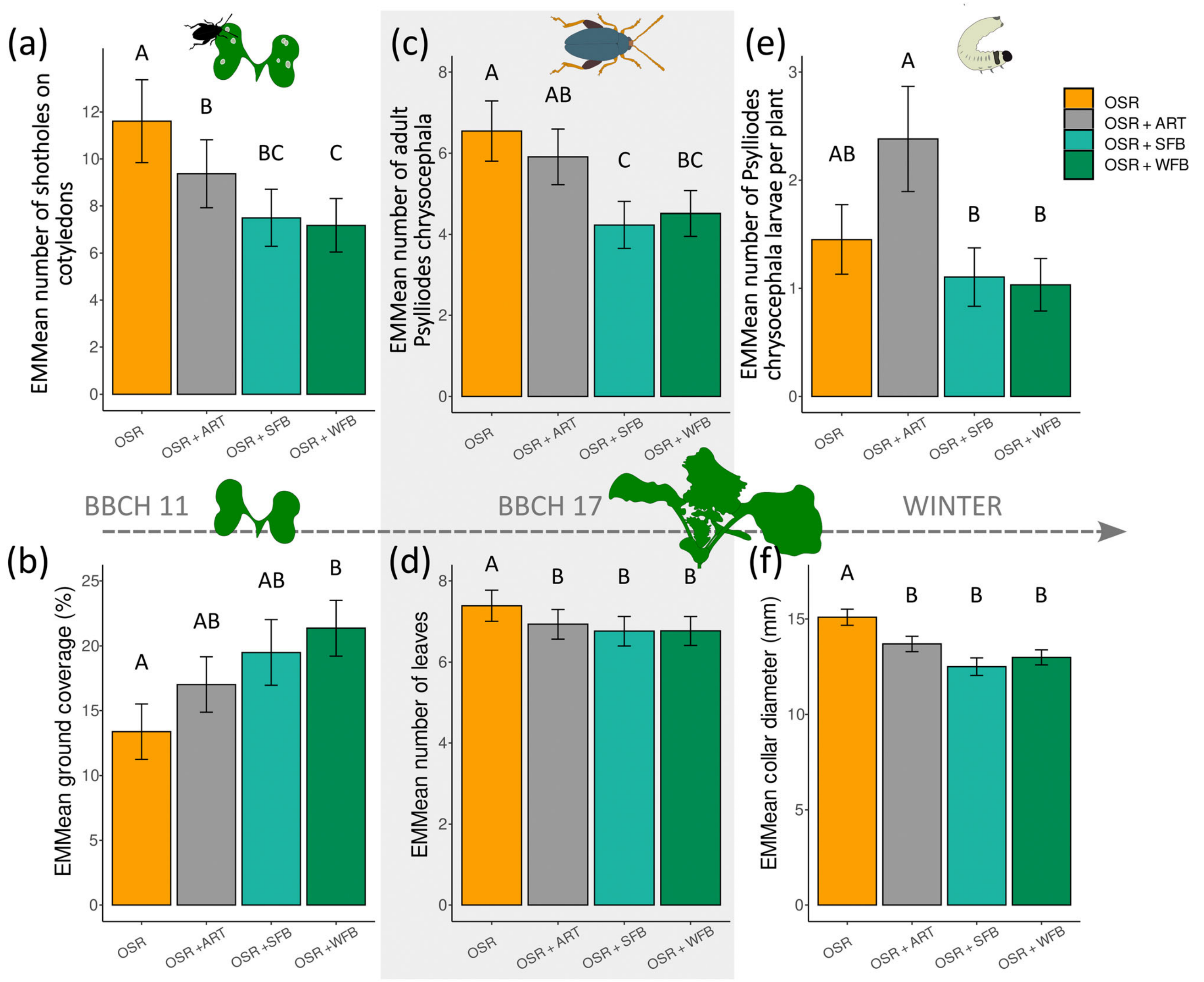

Figure 2. Estimated marginal mean (± standard error) of the incidence of autumn active pests, oilseed rape (OSR) crop development and ecophysiological traits across treatments. Four experimental treatments were compared: OSR (monocropped OSR), OSR + WFB (OSR intercropped with winter faba beans), OSR + SFB (OSR with spring faba beans), and OSR + ART (OSR grown with artificial plants). (a) Number of feeding shotholes on the cotyledons per OSR plant caused by cabbage flea beetle (*Phyllotetra* spp.). (b) Percentage of ground covered by vegetation (and artificial plants) at early development (BBCH 12). (c) Number of adult cabbage stem flea beetle (*Psylliodes chrysocephala*) trapped on yellow sticky traps placed on the ground per week over 4 weeks of monitoring. (d) Number of leaves per OSR plant in autumn before senescence. (e) Number of *Psylliodes chrysocephala* larvae per OSR plant in winter. (f) Collar diameter of OSR plants before winter.

3.3 Rape stem weevil (*Ceutorhynchus napi*) oviposition punctures

The number of *C. napi* oviposition punctures were significantly impacted by the treatments (Fig. 3(a); $\chi^2 = 96.57$, df = 3, $P < 0.001$). The monocropped OSR plants presented a greater number of oviposition punctures, significantly different from the other treatments ($P < 0.001$ for all comparisons). The treatments OSR + WFB and OSR + ART had intermediate numbers of stem punctures, both significantly different from OSR + SFB which had the lowest amount of oviposition punctures (Fig. 3(a); $P < 0.001$ for all comparisons). Compared to monocropped OSR, OSR + WFB and OSR + SFB had, on average, a reduction in *C. napi* oviposition punctures of 62% and 89%, respectively. The treatment OSR + ART had an average reduction in the number of *C. napi* oviposition punctures of 49% compared to the monocropped OSR.

3.4 Pollen beetle (*Brassicogethes aeneus*) abundance

The treatments had a significant effect on the number of adult *B. aeneus* per inflorescence (Fig. 3(c); $\chi^2 = 58.14$, df = 3, $P < 0.001$). Monocropped OSR had significantly more *B. aeneus* than the other treatments ($P = 0.002$ compared to OSR + WFB and $P < 0.001$ compared to OSR + SFB and OSR + ART). The OSR + SFB had significantly fewer *B. aeneus* per inflorescence than OSR + WFB ($P < 0.001$). Compared to monocropped OSR, OSR + WFB and OSR + SFB had, on average, a reduction in the number of *B. aeneus* per inflorescence of 30% and 58%, respectively. The treatment OSR + ART had an average reduction in the number of *B. aeneus* of 46% compared to monocropped OSR.

3.5 Characteristics and ecophysiological traits of oilseed rape plants

The treatments had a significant impact on the ground coverage at the early development stage (BBCH 12) of the OSR crops (Fig. 2

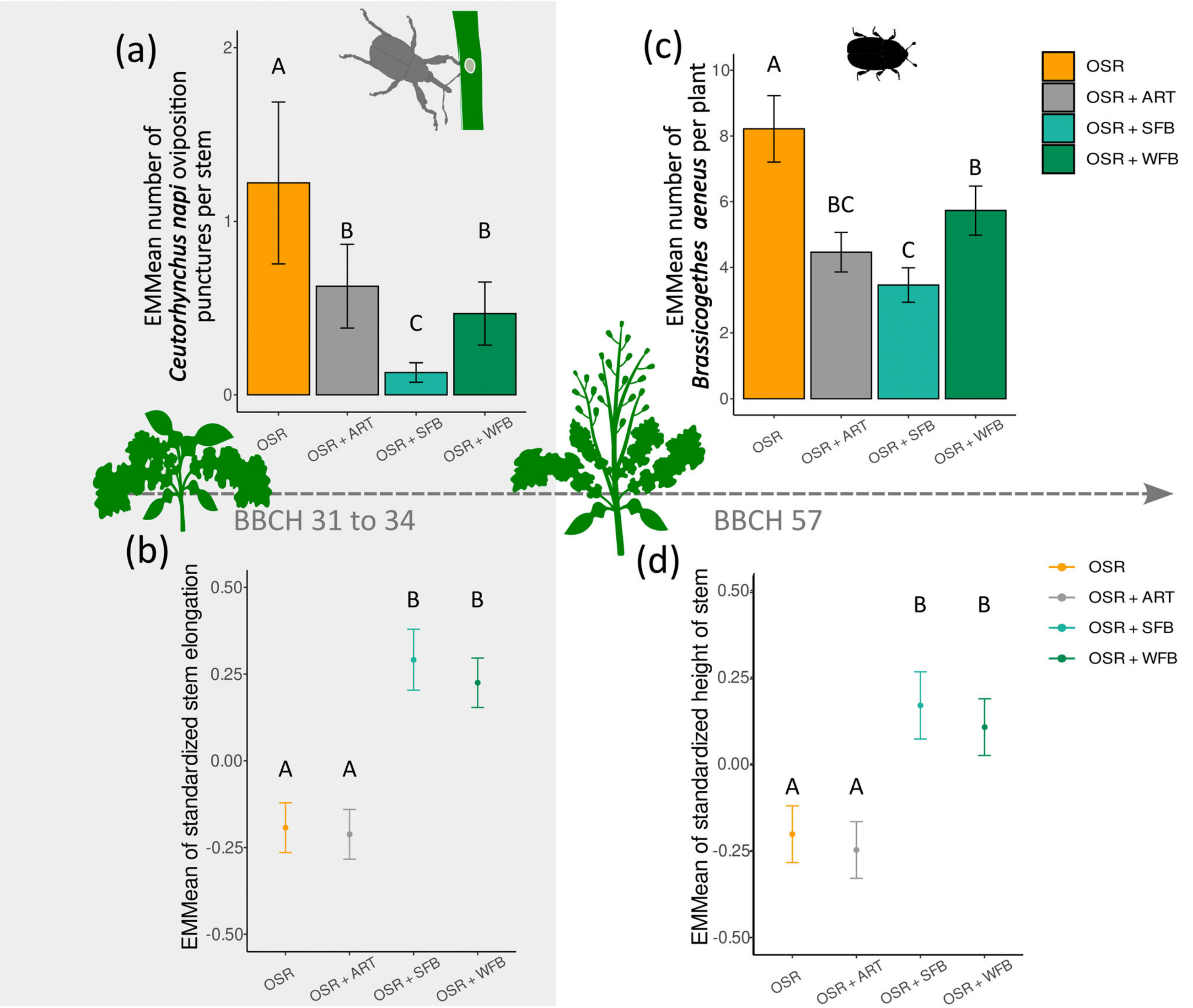

Figure 3. Estimated marginal mean (± standard error) of the incidence of spring-active insect pests of oilseed rape (OSR) and ecophysiological traits across treatments. Four experimental treatments were compared: OSR (monocropped OSR), OSR + WFB (OSR intercropped with winter faba beans), OSR + SFB (OSR with spring faba beans), and OSR + ART (OSR grown with artificial plants). (a) Number of rape stem weevil (*Ceutorhynchus napi*) oviposition punctures per plant. (b) Standardized OSR stem elongation at *C. napi* immigration. (c) Number of pollen beetle (*Brassicogethes aeneus*) per OSR inflorescence in spring. (d) Standardized height of the OSR inflorescence before bloom in spring.

(b); $F = 4.17$, df = 3, $P < 0.05$). OSR with WFB companion plants had significantly greater ground coverage than monocropped OSR ($P = 0.046$). The two other treatments had intermediate ground coverage, not significantly different from monocropped OSR nor OSR + WFB.

The treatments had a significant impact on the ecophysiological characteristics of OSR at the vegetative stage before winter (Fig. 2(d); $\chi^2 = 15.88$, df = 3, $P = 0.001$ for leaf number; and Fig. 2(f); $F = 7.36$, df = 3, $P < 0.001$ for collar diameter). Indeed, OSR grown with ART or intercropped with WFB and SFB had significantly fewer leaves than monocropped OSR ($P = 0.003$ and $P = 0.005$, respectively). Moreover, OSR plants in intercropping systems with both types of FB had a significantly smaller collar diameter than OSR plants when monocropped ($P = 0.001$ for WFB and $P < 0.001$ for SFB).

The treatments had a significant impact on the ecophysiological characteristics of the OSR plants at the stem elongation and budding stages after winter (Fig. 3(b); $F = 12.83$, df = 3, $P < 0.001$; and Fig. 3(d); $F = 7.62$, df = 3, $P < 0.001$, respectively). In the second year of the trial, OSR stem elongation at *C. napi* immigration and the inflorescence height before flowering were greater than in the first year of the trial (Table S2). However, with standardized data we observed a significant effect of the cropping system: OSR intercropped with SFB or WFB had a greater stem elongation than monocropped OSR and OSR + ART ($P < 0.001$ for all comparison). This difference was kept throughout the stem elongation stage (BBCH 31–34) and was also found regarding the total stem height before flowering (BBCH 57). Indeed, monocropped OSR had significantly smaller total stem height than the OSR intercropped with FB ($P = 0.01$ compared to OSR + WFB and $P = 0.006$ compared to OSR + SFB), and OSR + ART had significantly smaller total stem height than the OSR intercropped with FB ($P = 0.003$ compared to OSR + WFB and $P = 0.001$ compared to OSR + SFB).

3.6 Oilseed rape yield

Treatments had a marginally significant effect on the OSR yield (Fig. 4; $F = 2.61$, df = 3, $P = 0.07$) with a trend of OSR + SFB having greater yield than the OSR monocrop treatment ($P = 0.07$).

4 DISCUSSION

4.1 Effect of intercropping spring and winter faba beans on the insect pest complex in oilseed rape

Our study demonstrates that growing OSR together with FB reduces the incidence of insect pests in the crop. The additive intercropping system of OSR with SFB and WFB reduced *Phyllotreta* spp. feeding damage and reduced the numbers of adult *Psylliodes chrysocephala* in the plots in autumn. In spring, the number of oviposition punctures by *C. napi* as well as the number of adult *B. aeneus* on OSR inflorescences was reduced in intercropping systems with SFB and WFB compared to OSR when grown as monocrop. In our experiments, feeding damage on cotyledons was imputed to *Phyllotreta* spp. but our results are similar to previous studies on various companion plants and straw mulching, reducing feeding damage by *Psylliodes chrysocephala*.[18,19,21–23] Moreover, we showed a reduction in *Psylliodes chrysocephala* adult abundance in intercropped plots compared to plots of monocropped OSR, supporting the effect of companion plants on this species. However, the larval infestation of *Psylliodes chrysocephala* was very low in our study, falling well below the threshold of five larvae per plant typically associated with significant impact on OSR yield.[50] Additionally, there was no difference in larval infestation between the intercropping systems with FB and monocropped OSR.

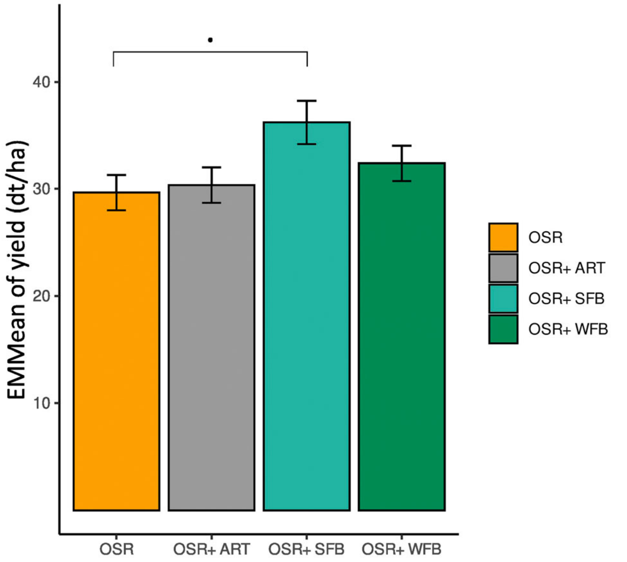

Figure 4. Estimated marginal means (± standard error) of oilseed rape (OSR) yield (dt/ha) across treatments. Four experimental treatments were compared: OSR (monocropped OSR), OSR + WFB (OSR intercropped with winter faba beans), OSR + SFB (OSR intercropped with spring faba beans), and OSR + ART (OSR grown with artificial plants). '•' Indicate $0.1 < P$-value < 0.05.

Intercropping OSR with SFB or WFB had a similar protective effect against the pressure of *Phyllotreta* spp. and *Psylliodes chrysocephala*. However, SFB had a higher protective effect against *C. napi* and *B. aeneus* compared to WFB. In spite of the SFB death after frost in winter, it provided a stronger effect on pest reduction than the frost resistant WFB with its regrowth after winter. This may be due to a combination of straw remaining standing in the field, providing visual and physical barriers, and the decomposition of its softer parts releasing VOCs, both reducing pests immigration.[51] This effect in spring requires a good development of the FB in autumn. This was achieved in this study with early sowing and mild temperatures before winter.

Despite the evidence of competition between the OSR and the companion plants, the intercropping system provided the same or marginally improved OSR yield compared to monocropped OSR. The OSR plants intercropped with FB showed a development delay in autumn with, on average, one leaf less than monocropped OSR. The intercropped OSR also expressed a shade avoidance syndrome in spring via faster and greater stem elongation than monocropped plants.[34] This competition was outbalanced by the protecting effect of the intercropping system which reduced insects pests damage, and the possible extra nitrogen brought by the legume companion plant.[52,53] The OSR yield marginal increase observed with the SFB intercropping is consistent with the results of Verret *et al.*[24]

4.2 Mechanisms involved in the reduction of insect pest incidence and damage

Growing OSR with ART reduced feeding damage caused by *Phyllotreta* spp., indicating a potential visual or physical disruption in the host plant location caused by the companion crop. However, intercropping with WFB provided even greater protection against this pest compared to ART, with a 19% reduction in damage for OSR + ART compared to a 38% reduction for OSR + WFB, relative to monocropped OSR. This may be due to the difference in ground coverage between the two treatments, with WFB providing greater ground coverage than ART. Seimandi-Corda *et al.* showed a correlation between the ground coverage of the companion plant and the feeding damage in *Psylliodes chrysocephala*, and Dover highlighted the role of height of the companion plant in providing visual and physical disruption.[21,41] The combination of both ground coverage and height of companion plants seem key factors to provide maximal visual and physical disruption to insect pest immigration and detrimental behavior.[54] The disruption provided by the companion plants reduced *Phyllotreta* spp. flea beetle feeding damage on OSR during its early development stages occurring mainly via direct visual and physical disruption, hinging on the volume of companion plants.

The incidence of adult *Psylliodes chrysocephala* in the plots of OSR was not reduced by the addition of the ART, but was significantly reduced by intercropping with FB. This may be due to the absence of olfactory cues from ART plants or due to the difference in volume between FB plants with side branches and the ART with only four leaves. Emery *et al*. and Seimandi-Corda *et al*. showed a disruption effect of straw and other companion plants such as clover and cereals on *Psylliodes chrysocephala*,[18,21] which may have very different olfactory cues than FB. This suggests that different VOCs blends may affect host–plant location, but does not exclude the possibility that the companion plant could influence *Psylliodes chrysocephala* host–plant location through visual and physical disruption, depending on its volume. ART plants had the same impact as FB on the OSR development in autumn, indicating that the disruption effect of the intercropping system on *Psylliodes chrysocephala* does not come from an indirect effect of the change in ecophysiological traits of intercropped OSR. The treatment OSR + ART had significantly more larvae per plant compared to plants intercropped with FB, possibly due to higher *Psylliodes chrysocephala* immigration into OSR + ART plots compared to intercropped plots. However, we observed only a non-significant trend of increased larval numbers in monocropped OSR compared to intercropping with FB, despite the high adult immigration in these plots. This could suggest that the sampling method of evaluating larval infestation using five plants per plot may not be sufficient when larval infestation levels are low, as variability can be quite high.

The oviposition incidence of *C. napi* on the stems of OSR plants was significantly reduced by 49%, on average, for OSR + ART compared to OSR monocropped. ART plants provided a similar level of disruption as WFB for *C. napi* oviposition. However, only the OSR plants intercropped with FB expressed a shade avoidance syndrome and had greater stem elongation than the monocropped OSR.[34] The companion plant thus provides a direct physical or visual disruption in host–plant location and/or acceptance for *C. napi*.

The number of adult *B. aeneus* per inflorescence was significantly reduced by 45%, on average, for OSR + ART compared to OSR grown as a monocrop. ART plants provided a similar level of disruption as both FB treatment for the immigration of *B. aeneus*. However, ART treatment did not trigger the greater OSR stem elongation observed for OSR intercropped with FB. Thus, we can dismiss the hypothesis of an indirect effect of the companion plant on *B. aeneus* via the change of OSR morphological traits. The intercropping system provided a direct physical or visual disruption on *B. aeneus* immigration.

SFBs intercropping had a significantly stronger impact on oviposition of *C. napi* and on the immigration of *B. aeneus* than the WFB intercropping. However, our experimental design does not allow us to conclude whether this effect is due to the release of VOCs from the decomposing companion plants, the companion plants indirect impact on OSR chemical traits, through nitrogen release, or from more effective visual or physical disruption.

Our study demonstrates that the intercropping system OSR + FB provides significant protection of OSR from insect pests via direct visual and physical disruption throughout the crops development. However, the ART had a weaker effect on the autumn pests and on *C. napi* than FB as a companion plant, suggesting potential other mechanisms at play such as olfactory disruption. The outcome of ART compared to FB companion plants may be explained by the lack of olfactory disruption (direct and indirect) or by the difference in volume compared to FB companion plants. Indeed, direct olfactory disruption from FB may still have an impact on insect pest incidence and may explain the differences in effect. This experiment did not allow us to evaluate indirect chemical disruption and part of the variation in effects between our treatments may come from such a mechanism.[37] Nevertheless, the volume of the companion plants in terms of ground covered and height seems to be a key factor of this disruption and the implementation of an ART having the same volume in the field as FB through the growing season was not experimentally possible.

5 CONCLUSION

The use of intercropping and/or companion planting systems to mitigate insect pest pressure is becoming a promising practice in integrated pest management. Our study was able to bring evidence of the protecting effect of OSR + FB intercropping on the OSR insect pest complex and its viability in terms of yield. Moreover, we demonstrated that well-developed SFB companion plants have an impact on spring pests even after plant death overwinter. The use of ART plants allowed us to demonstrate that the companion plant provided visual and/or physical disruption on insect pest incidence. However, this disruption may not fully explain the effect of FB companion plants and further research is needed, especially regarding the indirect effect of companion plants on OSR chemical traits. Understanding the mechanisms behind the protecting effect of intercropping systems will enable informed choices of companion plants which optimize crop protection and crop development to achieve successful sustainable cropping systems.

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DATA AVAILABILITY STATEMENT

All the data of this project and script used to analyze them are available on: https://github.com/LaurieMagnin/OSR_Pests_Intercropping.git.

AUTHOR CONTRIBUTIONS

Funding acquisition: AB, IH. Conceived and designed the experiments: LM, AB, IH, AJ. Analyzed the data/visualization/writing: LM. Supervision/revision: AB, AJ, IH.

SUPPORTING INFORMATION

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

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