

Communication

Intercropping Winter Oilseed Rape (*Brassica napus* L.) Has the Potential to Lessen the Impact of the Insect Pest Complex

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Abstract: Winter oilseed rape (*Brassica napus*) is a global major crop used for the production of vegetable oil. Typically sown in late summer and grown throughout winter and spring, it allows for interesting cultural practices, such as frost-sensitive intercropping with companion plants. This practice not only provides nitrogen resources much needed by the crop in the spring, but companion plants can also prevent weed growth in autumn, thereby reducing common herbicide use. Additionally, intercropping has the potential to protect the crop from insect pests. During winter 2019–2020, *B. napus* was grown alone (i.e., as a control) or intercropped with a mixture of faba bean (*Vicia faba*) and grass pea (*Lathyrus sativus*); because of the unusually clement weather conditions, the faba bean did not freeze, which allowed for the evaluation of the impact of these companion plants on the insect pest complex in spring. Insect damage by the beetles *Psylliodes chrysocephala*, *Ceutorhynchus napi*, and *Brassicogethes aeneus* was assessed in both treatments. The larval density of *P. chrysocephala* was significantly lower in the crop grown with service plants. Egg laying and damage by *C. napi* were significantly reduced when *B. napus* was intercropped, and the number of *B. aeneus* captured was significantly lower in the presence of service plants than in the control. Moreover, the yield from oilseed rape was significantly higher in the part of the field with service plants than in the pure crop control. The underlying mechanisms are only partially understood, but intercropping winter oilseed rape with frost-resistant service plants seems to be an ecologically sound practice with a very high level of potential to reduce insect pest pressure and increase crop yield. This may eventually reduce our reliance on chemical inputs in one of the most treated crops.

Keywords: cabbage stem flea beetle; rape stem weevil; pollen beetle; integrated pest management; functional biodiversity



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1. Introduction

Winter oilseed rape (WOR, *Brassica napus* L., Brassicales: Brassicaceae) is globally grown on ca. 35 billion hectares of land [1]. Arable land planted with this crop increased by ca. 5 billion hectares between 2007 and 2017 [1], along with the demand for vegetable oil and biofuel [2]. Despite numerous agronomic advantages (e.g., soil coverage over the winter and being a good crop in cereal rotation systems), WOR has high needs for nitrogen [3] and is sensitive to weed growth. To overcome these drawbacks, Theunissen [4] proposed growing *Brassica* sp. intercropped with companion plants. Sown together with WOR in late summer, companion plants provide better soil coverage and, as living mulch, control weeds [5]. Typically frost-sensitive intercropped plants freeze over winter and supplement WOR with nitrogen in spring [6]. In an experiment spanning over six WOR growing seasons, Verret et al. [5] showed that legume and non-legume companion plants reduced weed density by 52% and 38%, respectively. Certain companion plant mixes decreased weed abundance by up to 75% in WOR in the absence of herbicide application [7].

In addition to nitrogen supply and weed control, intercropping with companion plants has also been proposed for insect pest management [8], since WOR hosts a very diverse insect pest complex [9–12] that is typically controlled with synthetic insecticides [13,14]. In this context Breitenmoser et al. [15] demonstrated that companion plants (i.e., *Trifolium alexandrinum* L., Fabales: Fabaceae; *Lens culinaris* Medik., Fabales: Fabaceae; *Lathyrus sativus* L., Fabales: Fabaceae; *Vicia sativa* L., Fabales: Fabaceae; *Vicia faba* L., Fabales: Fabaceae; *Guizotia abyssinica* (L.f.) Cass., Asterales: Asteraceae; *Fagopyrum esculentum* Moench, Caryophyllales: Polygonaceae) significantly reduce damage from adult flea beetles (an insect complex mostly composed of *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae, cabbage stem flea beetle, CSFB) and *Phyllotreta* spp. (Coleoptera: Chrysomelidae), two of the major WOR insect pests).

In Europe, WOR is threatened by a very particular complex of insect pests. Starting in early autumn at plant stand, flea beetles cause economic damage to WOR as adults feed on the cotyledons or the seedlings [16]. Developing in the petioles and plant stems, the CSFB larvae cause major damage during winter. Attacked leaves exhibit darkened zones of necrosis, and eventually die and fall off the stem. Consequently, CSFB larvae migrate to the stem of the host plant prior to defoliation, where they resume feeding. Frost in the plant wounds can burst the mid-ribs or the stems and kill fed-on WOR plants [9,17]. Moreover, dry spring seasons and water stress may also kill CSFB-damaged plants [9,17]. In this biotic and abiotic context, an average of only one larva per plant can reduce WOR yield by ca. 40 kg ha⁻¹ [18] (average yield in Europe: 2000 kg ha⁻¹ [1]), and it is typical to have over five larvae on average per plant. In addition to CSFB, WOR is also exposed to a second wave of insect attacks in spring as clement weather resumes. The weevil complex is composed of rape stem weevil (*Ceutorhynchus napi* Gyll., Coleoptera: Curculionidae), the cabbage stem weevil (*C. pallidactylus* Marsh., Coleoptera: Curculionidae), and cabbage seed weevil (*C. obstrictus* Marsh., Coleoptera: Curculionidae) [11], where *C. napi* is the most impactful [10]. This weevil species lays eggs in the stem of WOR plants leaving only very small scars as evidence. Punctures by adults [19] and larval tunneling [20] in WOR may lead to significant yield loss. Finally, the pollen beetle *Brassicoglyphus aeneus* (F.) (Coleoptera: Nitidulidae) feeds on pollen within the developing flower buds or on the open flowers [11,21]. Complete destruction of the flower buds and severe damage to the ovaries [21] lead to major yield loss (up to EUR 25 million in Germany in 2006 [22]).

Since the ban of seed coatings containing neonicotinoids in Europe (EU Regulation No. 485/2013), the evolution of resistance to pyrethroids (see [14,23–27]), and a shift in agricultural practices, there has been an increasing need for ecologically sound strategies to sustainably manage insect pests in WOR. An unexpectedly mild winter (2019–2020, Supplementary Figure S1) offered us the unique opportunity to test the impact of companion plants on spring insect pests, as faba bean (*V. faba*) did not freeze. This study contributes to the global knowledge of insect pest population dynamics in WOR and the implementation of sustainable pest management practices. Because of the opportunistic aspect of the conducted measurements, only a low number of spatial replicates (and no temporal replicates) could be considered, yet we are confident that this study provides a strong basis for future research on sustainable pest management in WOR.

2. Materials and Methods

2.1. Field Site and Set-Up

A field trial was conducted at Agroscope (Nyon, VD, Switzerland) during the growing season of 2019–2020 on the research farm. Supplementary Figure S1 depicts temperature and precipitation over the course of the experiments. The rectangular field (36 × 91 m, 0.3 ha) was sown with *Brassica napus* subsp. *napus* on 26 August 2019 (var. Avatar, UFA Samen, FENACO, CH) (55 seeds m⁻², 50 cm row spacing). On the same date, one-half of the field was sown with a mixture of faba bean *Vicia faba* and grass pea *Lathyrus sativus* L. (Fabales; Fabaceae) (UFA Samen, FENACO, CH) as companion plants. Both species, as legumes, provide nitrogen after senescence and grass pea offer good soil coverage

against weeds during plant stand. The mixture was sown with a row spacing of 15 cm at 7.5 seeds m^{-2} and 9.9 seeds m^{-2} , respectively. The second half of the field remained with WOR only and served as a control. The control section of the field was sprayed once (August 2019) with herbicides (Devrinol Top (345 g L^{-1} Napropamide + 30 g L^{-1} Clomazone, Stähler Suisse SA, Zofingen, Switzerland), 3 l h^{-1}). The field was supplemented with nitrogen in February and March 2020, with 70 kg ha^{-1} and 50 kg ha^{-1} , respectively. No insecticides were applied on the intercropped or on the control sections of the field.

Both sections of the field, i.e., companion plants and control, were divided in four plots of 18 × 16 m. These plots served as spatial replicates for the duration of the field experiment. A buffer (width: 3 m) was established between the intercropped and control plots. Plants, rather than field, were considered as experimental units.

2.2. Impact of the Companion Plants on WOR

To evaluate the impact of the companion crop on WOR, *B. napus* density and stem diameter were recorded.

To measure the plant density, one metal frame (1 × 1 m) was thrown in a random direction from the center of the plot and aligned to the crop row. The density of WOR was measured by counting the number of standing plants within the frame. WOR density was evaluated once in each plot.

WOR stem diameter was measured on 3 × 4 consecutive plants randomly selected in each plot. The stem diameter was measured with calipers, 5 cm from the soil surface. Both measurements were performed in March 2020.

2.3. Impact of Intercropping on the Insect Pest Complex

2.3.1. *Psylliodes chrysocephala* Larval Density

To evaluate the number of CSFB larvae per plant, ten WOR plants were sampled in each plot at the end of winter (6 February 2020, BBCH 30). Plants were partially defoliated, keeping the stems, petioles, and main leaf veins intact, where larvae are located. Five plants were pooled into one modified Berlese trap (two traps per plot) [15]. Traps were made of copper funnels lined with mesh to prevent plant material from falling into a glass vial, which was placed at the end of the funnel neck (modified from Conrad et al. [28]). Glass vials were initially filled with 5 mL of 70% ethanol and topped up as ethanol evaporated. Berlese traps were kept for 30 days at 25 °C. Insect larvae escaping drying plant material were identified and counted under a dissecting microscope (Leica, Nidau, Switzerland). The number of larvae per plant was calculated and recorded.

2.3.2. *Ceutorhynchus napi* Oviposition Punctures and Damage

Oviposition sites of *C. napi* are characterized by small white dots on the stems (often surrounded by a small depression). In each plot, ten WOR plant stems were visually checked for oviposition punctures (10 March 2020, BBCH 35). Then, the number of attacked plants and the number of oviposition sites per plant was recorded.

C. napi larval development results in an increased tortuosity of the WOR stem. To evaluate this parameter, pictures of five WOR plants per plot were taken (1 April 2020, BBCH 60) from a distance of 0.8 m from the plant and a height of 1.2 m. In ImageJ [29,30], the total length of the stem was measured from its base to the tip of the flower buds (Lstem). In addition, the shortest distance between the base of the stem to the tip of the buds was recorded (orthoLstem). The tortuosity was calculated as:

$$\text{Tortuosity} = \frac{\text{Lstem}}{\text{orthoLstem}} \quad (1)$$

A perfectly straight stem is characterized by a tortuosity equal to 1, whereas values above 1 indicate a certain level of tortuosity.

2.3.3. Number of *Brassicogethes aeneus*

In each plot, 20 plants were randomly chosen (18 March 2020, BBCH 55). Each inflorescence was individually shaken in a small plastic bowl (20 cm diameter, 10 cm deep). *B. aeneus* were visually counted before being released. The number of insects per plant was recorded.

2.4. Yield

WOR was harvested on July 14, 2020 (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 WOR was then sampled and weighed (gross weight), and impurities (e.g., dust, non-WOR 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 in deciton per hectare (dt ha^{-1}). Technical limitations restricted the harvest to WOR only.

The Thousand Kernel Weight (TKW) was also evaluated for each plot. An aliquot of 500 kernels was weighed. The recorded weight was doubled to calculate the TKW.

2.5. Statistical Analyses

Student's paired t-tests were used to measure differences in density and tortuosity between treatments, using the presence or absence of companion crop as the main factor. As stem diameter, WOR yield, and TKW were not normally distributed, differences between treatments were assessed with a Mann-Whitney test. For all count data, a general linear model approach was adopted, with either a Poisson distribution (number of CSFB larvae, *C. napi* oviposition punctures per plant, and number of *B. aeneus*) or a binomial distribution (*C. napi* % plant attacked), using the same main factor. All tests were performed in R 4.1.0 [31].

3. Results

3.1. Impact of the Companion Plants on WOR

The density of *B. napus* was significantly lower in the WOR with companion plants ($21.5 \pm 0.57 \text{ plants m}^{-2}$) than in pure WOR ($28.25 \pm 0.75 \text{ plants m}^{-2}$) ($t = 7.18, p < 0.001$), with ca. 7 plants m^{-2} less. Whereas the density was lower, the WOR stem diameter was significantly larger when companion crops were present ($2.08 \pm 0.05 \text{ cm}$) as compared to pure WOR ($1.59 \pm 0.04 \text{ cm}$) ($U = 1270, p < 0.001$).

3.2. Impact of the Companion Plants on the Insect Pest Complex

3.2.1. *Psylliodes chrysocephala* Larval Density

More CSFB larvae per plant were collected from pure WOR than from WOR with companion crops (Figure 1, $\chi^2 = 54.122, p < 0.001$).

3.2.2. *Ceutorhynchus napi* Oviposition Punctures and Damage

There were significantly more WOR with *C. napi* oviposition punctures on control WOR than on intercropped plant stems (Figure 2a, $\chi^2 = 18.43, p < 0.001$). The ratio of attacked plants in intercropped WOR ($15 \pm 8.19\%$) remained lower than the Swiss economic threshold (Figure 2a, 45–65%, [19,32]). The total number of punctures per attacked *B. napus* was significantly lower on intercropped plants than on control WOR (Figure 2b, $\chi^2 = 25.25, p = 0.042$). Stem tortuosity was marginally different between WOR treatments (intercropped: 0.97 ± 0.01 , pure: 0.94 ± 0.01) ($t = 1.935, p = 0.059$).

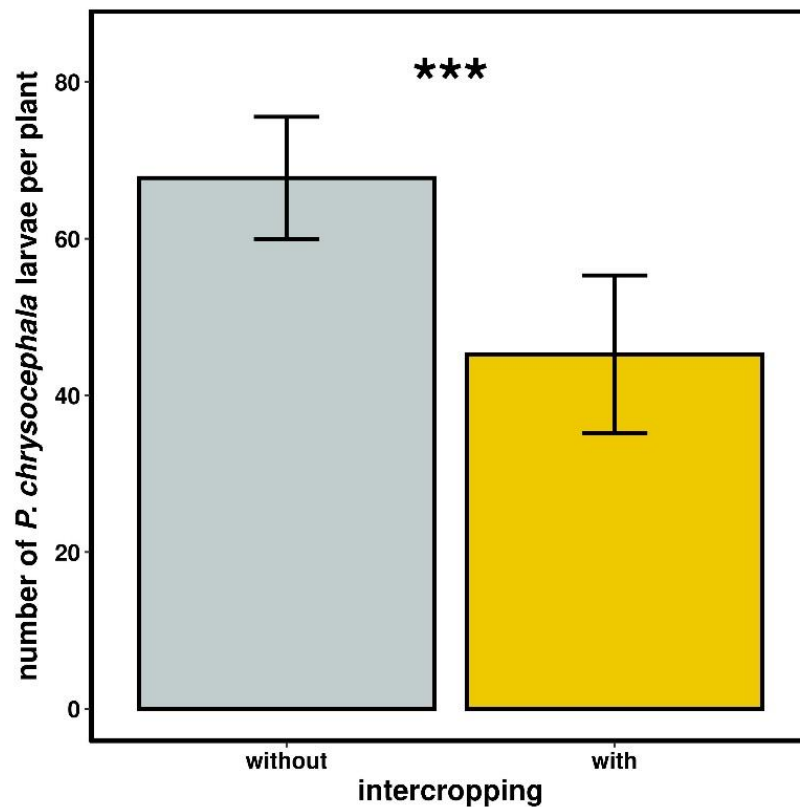


Figure 1. Intercropping *Brassica napus* reduces the number of *Psylliodes chrysocephala* larvae in plants when *Vicia faba* did not freeze over winter. Average number of *P. chrysocephala* per control (gray) and intercropped (yellow) *B. napus*. Bars represent SEM; *** indicates p -values < 0.001.

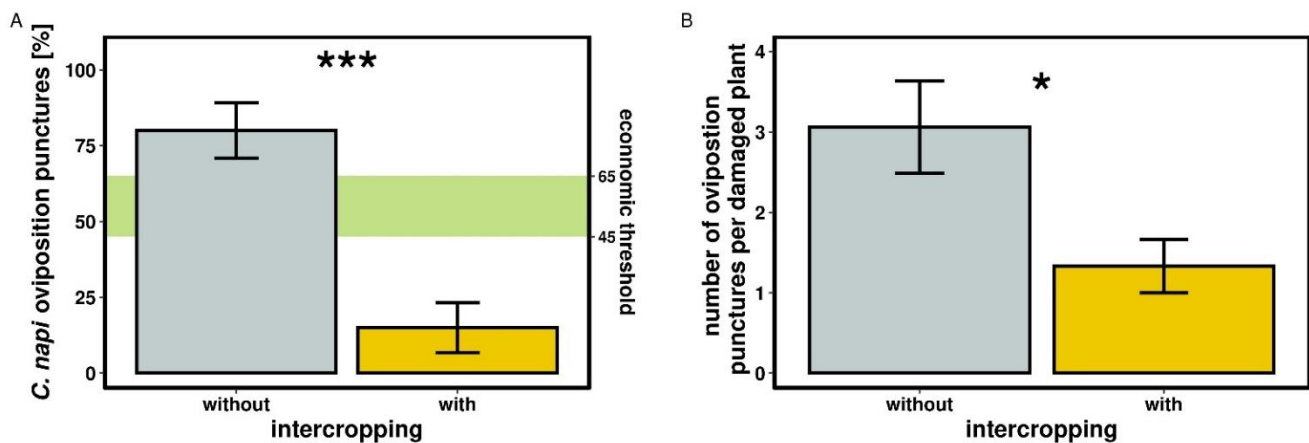


Figure 2. Intercropping *Brassica napus* improves management of *Ceutorhynchus napi* when *Vicia faba* did not freeze over winter. (A) Percentage of plants with oviposition punctures on control (gray) and intercropped (yellow) *B. napus*. (B) Average number of punctures on control (gray) and intercropped (yellow) damaged *B. napus* resulting from *C. napi* oviposition. Bars represent SEM; *** and * indicate p -values < 0.001 and < 0.05, respectively.

3.2.3. Number of *Brassicogethes aeneus*

The number of pollen beetles collected from flower buds was significantly higher in control WOR than in intercropped plots (Figure 3, $\chi^2 = 28.79$, $p < 0.01$).

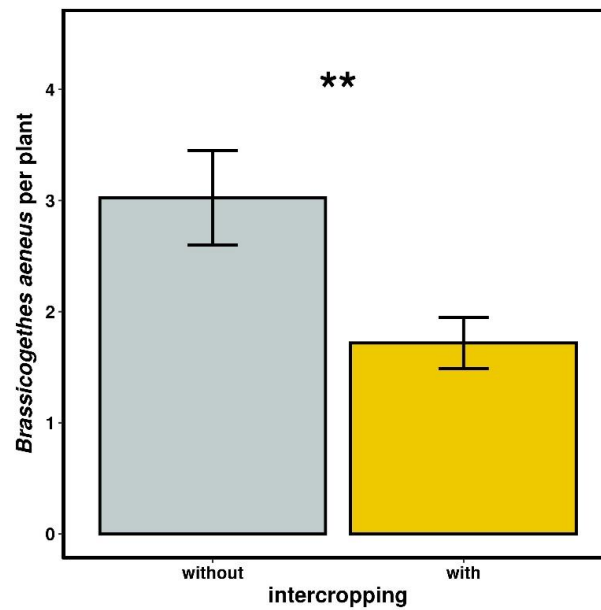


Figure 3. Intercropping *Brassica napus* reduces the number of *Brassicogethes aeneus* on flower buds when *Vicia faba* did not freeze over winter. Average number of *B. aeneus* per control (gray) and intercropped (yellow) *B. napus*. Bars represent SEM; ** indicates p -values < 0.01.

3.3. Yield

The total weight of harvested WOR seeds was significantly higher in the intercropped section of the field than in the control (Figure 4, $U = 16$, $p = 0.029$). Interestingly, the average yield of both the intercropped and control WOR were not significantly different from the Swiss national average for the same growing season (35.89 dt ha^{-2} [1]) ($U = 2$, $p = 1$ and $U = 4$, $p = 0.4$, respectively). Even though more seeds were produced by the intercropped WOR, the TKW was not different from the control WOR ($4.2 \pm 0.082 \text{ g}$ vs. $4.15 \pm 0.05 \text{ g}$, respectively, $U = 6.5$, $p = 0.739$).

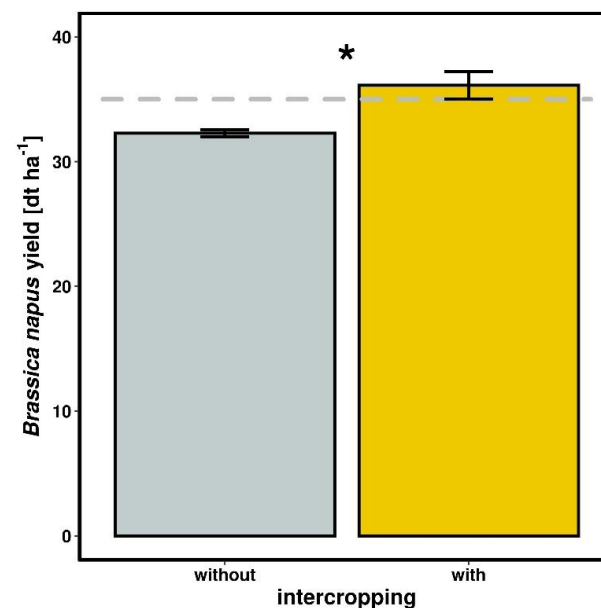


Figure 4. Intercropping supports *Brassica napus* yield when *Vicia faba* did not freeze over winter. Average yield of control (gray) and intercropped (yellow) *B. napus*. The dashed gray line represents the average Swiss *B. napus* yield for the same growing season. Bars represent SEM; * indicates p -values < 0.05.

4. Discussion

The present study highlights the potential of intercropping WOR to enhance crop protection from insect damage. Growing WOR with service plants was reported to decrease damage by flea beetle adults [15], despite having only a marginal effect on CSFB larval density. In the present study, WOR intercropped with a faba bean crop surviving the winter frost (and pea grass) hosted less larvae than controls. Intercropping also reduced the impact of *C. napi* and *B. aeneus*, two major insect pests in WOR. Intercropping WOR not only translated into a general negative effect on herbivorous insects, but it also resulted in a significantly higher crop yield in terms of seed weight ha^{-1} .

Intercropping has been tested against pests with various levels of success in diverse crops. Kloen and Altieri [33] showed that using mustard (*Brassica hirta* Moench, Brassicales: Brassicaceae) as an intercrop in broccoli (*Brassica oleracea* L., Brassicales: Brassicaceae) did not reduce the yield of the crop, though it improved insect predation on cabbage aphids, thereby reducing pest damage on broccoli. Intercropping WOR with faba bean also increases the survival of parasitoids by providing extrafloral nectar [34], an essential resource to several natural enemies [35–37]. In tobacco (*Nicotiana tabacum* L., Solanales: Solanaceae), WOR was used as a service plant to reduce the population of the aphid *Myzus persicae* Sulzer (Hemiptera, Aphididae), a vector of several diseases [38]. The authors noticed a significant increase in the density of natural enemies of the aphid and a significant reduction in the pest population in intercropped fields [38]. Whereas the present study did not look at the impact of intercropping WOR on beneficial insects, a reduction in pressure by CSFB, rape stem weevils, and pollen beetles was observed. This is in concordance with Breitenmoser et al. [15], who showed a significant impact of intercropping on CSFB adult damage, although this cultural practice only marginally affected their larvae. The mechanisms behind this pest reduction remain unclear, but it can be hypothesized that the presence of faba bean within WOR either (1) visually disrupts the foraging insects by increasing the landscape complexity, or (2) creates a chemical disruption by producing volatile cues repelling or confusing the pests. In addition to these potential direct impacts of faba bean on pests, the increased functional plant biodiversity might have resulted in a higher parasitism rate by parasitoids, therefore reducing insect pest pressure on WOR [39].

It is often assumed that intercropped fields may produce a lower yield because of interspecific competition, especially during crop plants' germination early on [40]. In the present study, in addition to lowering pest pressure, intercropping with faba bean and grass pea significantly decreased WOR density as the crop competed with the intercrop. Whereas there were on average ca. 7 plants m^{-2} less in intercropped WOR, the size of the intercropped plants (i.e., stem diameter) was ca. 0.5 cm bigger. Nonetheless, lower WOR density frequently increases WOR yield [30]. For instance, Momoh and Zhou [41] showed a two-fold increase in secondary branches on WOR planted at a density of 6.75×10^4 when compared to a plant density of 12.75×10^4 plant ha^{-1} . This also resulted in an increased number of seeds per pod [41]. Interestingly, intercropping with WOR increased the yield of tobacco by up to 34% [38]. It is notable that the yields reported here did not differ from the national seasonal average, despite no insecticide applications against insect pests, whereas insecticides are applied more than two times on average per growing season in Switzerland [42]. Last but not least, harvesting the faba bean in addition to WOR would increase the total economic return of the field, potentially rendering this approach even more beneficial [43]. The current study is not able to disentangle whether the yield increase results from the lower density of WOR or the reduction of pest pressure (visual or chemical disruption). It can be hypothesized that either of these aspects or a combination of both (bigger plants are more resilient to insect pest damage) increased yield in intercropped WOR as compared to the pure crop control.

5. Conclusions

Intercropping is increasing in popularity, especially to prevent weed growth and to provide soil coverage in autumn and winter and nitrogen in the spring, and intercropped

fields often show better soil health [44]. Furthermore, intercropping might also be useful to reduce pest pressure, as demonstrated herein. The underlying mechanisms remain unclear, but are likely to include behavioral disruption of the insect pest, provision of alternative food sources, and shelter for beneficial arthropods, such as parasitoids and predators. Thus, a better understanding of these mechanisms will allow for the use of combinations of service plant species favoring friends and hindering foes while boosting functional biodiversity in agricultural landscapes. The results reported herein show, for the first time, a potential advantage of a frost-resistant companion crop; one-and-a-half-fold fewer CSFB larvae per plant, a five-fold reduction in plant damage by *C. napi*, and two-fold fewer *B. aeneus* captured per plant. Despite lacking repetitions in time, these results could lead to the development of a new spring pest management strategy in WOR. Such an integrated approach can allow farmers to minimize pesticide reliance and lower the use of chemical inputs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12030723/s1>, Figure S1: provides details on the weather conditions during WOR growing season 2019–2020.

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