Combining *E*-β-farnesene and methyl salicylate release with wheat-pea intercropping enhances biological control of aphids in North China

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Abstract

Combining intercropping with the release of semiochemicals may strengthen biological control of aphids as a push-pull strategy that simultaneously repels aphids and attracts their natural enemies. This hypothesis was tested in the Henan Province of China in 2016 where aphids, their natural enemies and mummies were trapped and observed on crops in three treatments: wheat-pea strip intercropping solely (control), intercropping combined with the release of *E*-β-farnesene (EBF) and intercropping combined with the release of methyl salicylate (MeSA). Each treatment was repeated four times. The abundance of aphids throughout the growing season (9 weeks between March and May) was significantly decreased and the abundance of natural enemies and mummies were significantly increased in treatments with releases of semiochemicals compared to intercropping solely. The effect was stronger with MeSA than with EBF on the control of *Rhopalosiphum padi* and pea aphids as well as on the attraction of lacewings and hoverflies. Indeed, lacewings and hoverflies were on average twice more numerous in MeSA than in the other treatments. These results show that combining wheat-pea intercropping with the release of EBF or MeSA can significantly reduce aphid density and attract their natural enemies and that this effect is strengthen with MeSA when compared to EBF.

Key words: Biological control · Integrated pest management · push-pull strategy · semiochemical

1 Introduction

Aphids (Hemiptera: Aphididae) are the most dominant and destructive insect pests in wheat (*Triticum aestivum* L.) production regions in China (Cai et al. 2004), the two main species being *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) (Ma et al. 2006; Wang et al. 2009; Zhao et al. 2009). Aphids cause severe damages to wheat by feeding on leaves and developing ears, as well as by transmitting the barley yellow dwarf virus (Fereres et al. 1989). Lopes et al. (2016) reported that, in most of cases, the total aphid number are reduced in wheat-based intercropping systems, compared to pure-stand crops. Hence, intercropping is a promising practice to control aphids without chemical pesticides, which are harmful to health and the environment (Grung et al. 2015; Kim et al. 2017). Intercropping is defined as the cultivation of at least two plant species simultaneously in the same field, without necessarily being sown and/or harvested at the same time (Lithourgidis et al. 2011). It has been practiced in China for more than a thousand years and the benefits of mixing crops are being rediscovered in the light of the sustainability challenges agriculture faces (Knörzer et al. 2009). Among crops to be associated with wheat, pea (*Pisum sativum* Linn.)—as a legume—presents the interest of fixing atmospheric nitrogen and transferring it to the associated cereal plants, complementing or supplementing fertilizers (Hauggaard-Nielsen et al. 2008; Bedoussac and Justes 2010). Previous studies showed that the maintenance of pea cover between rows of wheat crop reduces populations of the wheat aphid *S. avenae* compared to pure-stand wheat (Zhou et al. 2009a, 2009b).

In addition to intercropping, the deployment of semiochemicals (i.e. informative molecules used in insect-insect or plant-insect interactions) has been widely considered within Integrated Pest Management (IPM) programs (Rodríguez and Niemeyer 2005; Heuskin et al. 2012a, b; Mensah et al. 2014; Sarles et al. 2015; Nakashima et al. 2016). Laboratory and field studies have demonstrated that releasing semiochemicals has the potential to simultaneously repel pests and attract natural enemies (i.e. 'push-pull' plant protection strategy) (Ninkovic et al. 2003; Zhou et al. 2016). Among other semiochemicals, methyl salicylate (MeSA) is a herbivore-induced plant volatile that is repellent to *R. padi* and other cereal aphids (Glinwood and Pettersson 2000; Ninkovic et al. 2003). It is moreover attractive to aphid predators such as ladybeetles (Coleoptera: Coccinellidae; e.g. *Coccinella septempunctata* Linnaeus) (Zhu and Park 2005; Saona et al. 2011), lacewings (Neuroptera: Chrysopidae; e.g. *Chrysopa nigricornis* Burmeister) (James 2003a), hoverflies (Diptera: Syrphidae) (Mallinger et al. 2011) and aphid parasitoid wasps (Hymenoptera: Braconidae, Aphelinidae) (Orre et al. 2013; Martini et al. 2014). Additionally, *E*-β-farnesene (EBF)—the major component of the alarm pheromone in several aphid species (Francis et al. 2005)—can act as a repellent for plant herbivores and attracts predatory ladybeetles (e.g. *Harmonia axyridis*) (Francis et al. 2004; Verheggen et al. 2007), hoverflies (Verheggen et al. 2008), lacewings (Boo et al. 1998) and parasitoids (Foster et al. 2005). To assess the effectiveness of different types of semiochemicals in repelling pests and attracting their natural enemies, field experiments under production conditions are needed (Daems et al. 2016).

In their review, Lopes et al. (2016) highlighted that intercropping alone may not enhance pest natural enemies. Conversely, the use of semiochemicals in pure-stands may not be consistently successful and may even negatively influence natural enemies in low pest density situations (Wang et al. 2011). Hence, combining semiochemicals with intercropping may bridge these problems. A previous experiment conducted in Belgium showed promising results toward the reduction of aphids and the increase of their natural enemies when wheat-pea intercropping was combined with the release of semiochemicals, compared to intercropping solely (Xu et al. 2018). The present study aims at evaluating this tactic in the context of China, by (i) determining if combining wheat-pea strip intercropping with the release of EBF or MeSA can better repel aphids and simultaneously attract their natural enemies than intercropping alone and (ii) evaluating the comparative efficacy of two types of semiochemicals (i.e. EBF and MeSA).

2 Materials and methods

Field layout

This study was conducted in a field of the Xinxiang experimental station of the Institute of Plant Protection, Chinese Academy of Agricultural Science, Henan Province of China (34°55′N, 114°15′E) in 2016. Three treatments, repeated four times, were tested: (1) wheat-pea strip intercropping (Control), (2) wheat-pea strip intercropping with EBF release formulated in oil (EBF), and (3) wheat-pea strip intercropping with MeSA release (MeSA). Repeated plots measured 80 m^2 (10 m × 8 m) and were placed in a completely randomized design within the field (Fig. 1). Each plot was composed of three strips of winter wheat (variety 'Jimai 22', 225 kg seeds/ha) and two strips of spring pea (variety 'Zhongwan 4', 150 kg seeds/ha), each strip being 2 m wide. The area between the experimental plots was rows of wheat (same variety). The two varieties of wheat and pea are currently used commercially in

Huang-Huai-Hai plain, China. Wheat and pea were separately sown on 20 October 2015 and 15 February 2016, respectively, and were harvested in June 2016. All plots were irrigated during the growing season as it is commonly practiced in this region of China. The field was surrounded by strips of wheat (same variety) in order to limit the interactions with the surrounding fields. No pesticides (except fungicides: tebuconazole EC) were used in the experimental area.

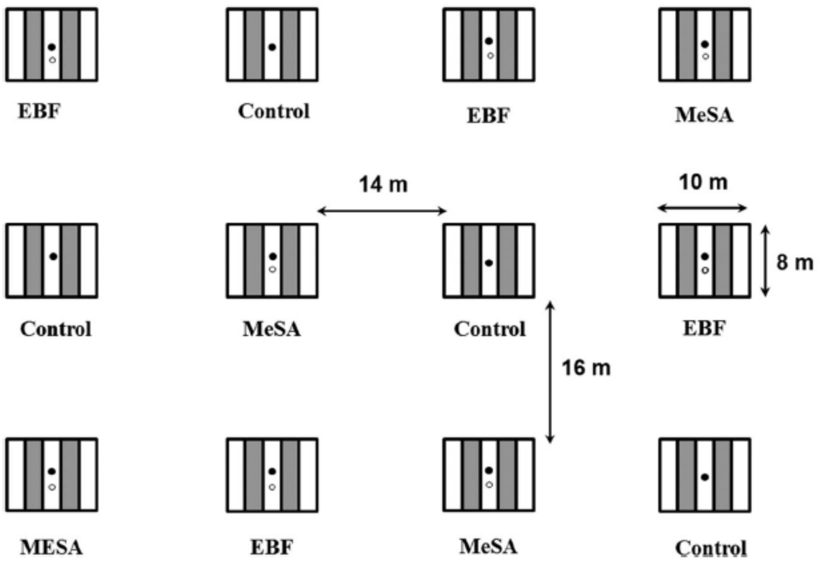

Figure 1. Experimental design: treatments are wheat-pea intercropping (Control), wheat-pea intercropping with EBF release using oil (EBF) and wheat-pea intercropping with MeSA release (MeSA).

E-β-farnesene and methyl salicylate dispensers

EBF was provided by the Laboratory of Functional and Evolutionary Entomology of Gembloux Agro-Bio Tech (University of Liège, Belgium) and was formulated in paraffin oil at a concentration of 10 mg/mL while MeSA (purity 99%) was purchased from Sinopharm Chemical Reagent Co., Ltd in China. For the experiment, 100 μL of EBF oil (10 mg/mL) for the EBF treatment, and 400 μL (468 mg) of pure MeSA for the MeSA treatment, was placed in a 1 cm-diameter rubber septum that was fixed to a trap stake in the middle of each plot. All release devices were placed under a plastic roof (35 × 35 cm) to protect them from the rain and they were changed every seven days.

The chosen volumes of EBF (i.e. 100 μL every week) and MeSA (i.e. 400 μL per week) used in the experiment were based on previous studies. Heuskin et al. (unpublished data) measured a release rate of 0.6 ± 0.1 μg/h of EBF from 100 μL EBF oil in rubber spectum in laboratory conditions (20ºC, 65% relative humidity, airflow 0.5 L/min) during 21 days (i.e. 100.8 μg over seven days). No peak of emission was observed during the 21 days of experiment. Regarding MeSA, James (2003a) used 2 mL per month (i.e. 400 μL per week) of 99% pure MeSA to obtain significant results. The first application of semiochemicals was on 21 March 2016.

Monitoring of aphids and their natural enemies

Aphids (all instars), their predators (i.e. larvae of ladybeetles, hoverflies and lacewings) and mummified aphids (mummies) were counted on pea plants and wheat tillers every seven days from 21 March 2016 to 28 May 2016 (9 weeks). Ten pea plants and 10 wheat tillers were randomly selected for counting insects and mummies at four different locations in each plot (totally 40 pea plants and 40 wheat tillers in each plot). Adults of ladybeetles, hoverflies, lacewings and alate aphids were collected using yellow pan traps (Flora®, 27 cm diameter and 10 cm depth). Traps were attached to fiberglass stakes, positioned at 10 cm higher than wheat, and filled with water and few drops of detergent to reduce water surface tension. A single trap was placed in the middle of each plot. Traps were emptied and refilled weekly during the same period. Trap contents were decanted through a 0.5 mm mesh sieve and collected insects were transferred to plastic vials containing 75 % ethanol. Aphid predators and alate aphids trapped were identified in the laboratory to species level, using specific identification keys: Taylor (1981) for aphids, Ren et al. (2009) for ladybeetles, He and Li (1992); Li (1988); van Veen (2010) for hoverflies, and Yang (1974) for lacewings. The number of individuals of each species was recorded.

Statistical analyses

First, generalized linear mixed models (GLMM, function 'glmer', package 'lme4', Bates et al. 2014) assuming a Poisson error distribution (log-link function) were fitted to assess the effect of treatments (i.e. Control, EBF and MeSA) on the abundance of aphids (i.e. *S. avenae*, *R. padi*, pea aphids observed), their natural enemies (trapped adults of ladybeetles, lacewings, hoverflies) and mummies. Treatments were included as a fixed factor and the plots as a random factor as measurements were repeated each time in the same plots. The effect of treatments on insect and mummy abundance was tested using a likelihood-ratio test ($P < 0.05$) and mean abundances were compared between treatments by using the post-hoc test of Tukey ($P < 0.05$, function 'glht', package 'multcomp', Hothorn et al. 2008). Second, linear regressions were used to analyze the relationship between aphid and natural enemy abundances. Total abundance of each taxon over the sampling period for each treatment separately was summed, considering each repetition in each treatment, then $\log_{10}$ (n+1) -transformed prior to analysis. All analyses were performed using R 2.6.2 (R Core Team 2017).

3 Results

One aphid specie was observed on pea plants (*Acyrthosiphon pisum* Harris) and four on wheat tillers (*S. avenae, R. padi, Metopolopium dirhodum* Walker, *Schizaphis graminum* Rondani). Four species of ladybeetles and hoverflies as well as two species of lacewings were trapped (Table 1).

The treatments significantly affected the abundance of pea aphids (df = 2; $\chi^2 = 17.3$; $P < 0.001$, Fig. 2(a)), *S. avenae* (df = 2; $\chi^2 = 14.9$; $P < 0.001$, Fig. 2(b)) and *R. padi* (df = 2; $\chi^2 = 30.4$; $P < 0.001$, Fig. 2(c)) observed on plants. Post-hoc tests of Tukey show that pea aphids, *S. avenae* and *R. padi* were significantly more abundant in the Control plots than in EBF and MeSA treatments (Fig. 2(a), (b), (c)). *R. padi* were also less abundant in plots where MeSA was released than in all other treatments (Fig. 2(c)). Additionally, the density of mummies was significantly affected by the treatments (df =2; $\chi^2 = 20.9$; $P < 0.001$), being significantly less abundant on wheat tillers of the control plots than in EBF and MeSA treatments (Fig. 3). In traps, treatments significantly affected the abundance of ladybeetles (df = 2; $\chi^2 = 20.9$; $P < 0.001$, Fig. 4(a)), lacewings (df = 2; $\chi^2 = 30.7$; $P < 0.001$, Fig. 4(b)) and hoverflies (df = 2; $\chi^2 = 20.2$; $P < 0.001$, Fig. 4(c)). Post-hoc tests of Tukey show that ladybeetles and lacewings were significantly less abundant in the Control plots than in EBF and MeSA treatments (Fig. 4(a), 4(b)). Moreover, lacewings were significantly more abundant in plots where MeSA was released, compared to those with EBF (Fig. 4(b)). As for hoverflies, no differences were observed between EBF and Control treatments, but they were significantly more abundant in MeSA plots than in all other treatments (Fig. 4(c)). More generally, aphid natural enemies were about two times more trapped in the MeSA treatment than in the Control (Fig. 4). Predatory larvae observed on both wheat and pea, as well as mummified aphids on pea, were very few abundant which did not allow performing any further statistical analysis.

Finally aphids observed on plants and tillers were significantly negatively correlated with the densities of natural enemies trapped (adult ladybeetles, lacewings and hoverflies) and mummies observed (Table 2).

Table 1 Diversity of aphids and their trapped natural enemies

Order: Family	Species
Aphids (Hemiptera: Aphididae)	*Sitobion avenae* (Fabricius)
	Rhopalosiphum padi (Linnaeus)
	Metopolophum dirhodum (Walker)
	Schizaphis graminum (Rondani)
	Acyrthosiphon pisum (Harris)
Ladybeetles (Coleoptera: Coccinellidae)	*Harmonia axyridis* (Pallas)
	Coccinella septempunctata (Linnaeus)
	Propylaea japonica (Thunberg)
	Adonia variegate (Goeze)
Hoverflies (Diptera: Syrphidae)	*Episyrphus balteata* (De Geer)
	Metasyrphus corollae (Fabricius)
	Sphaerophoria scripta (Linnaeus)
	Scaeva pyrastri (Linnaeus)
Lacewings (Neuroptera: Chrysopidae)	*Chrysopa sinica* (Tjeder)
	Chrysopa septempunctata (Wesmael)

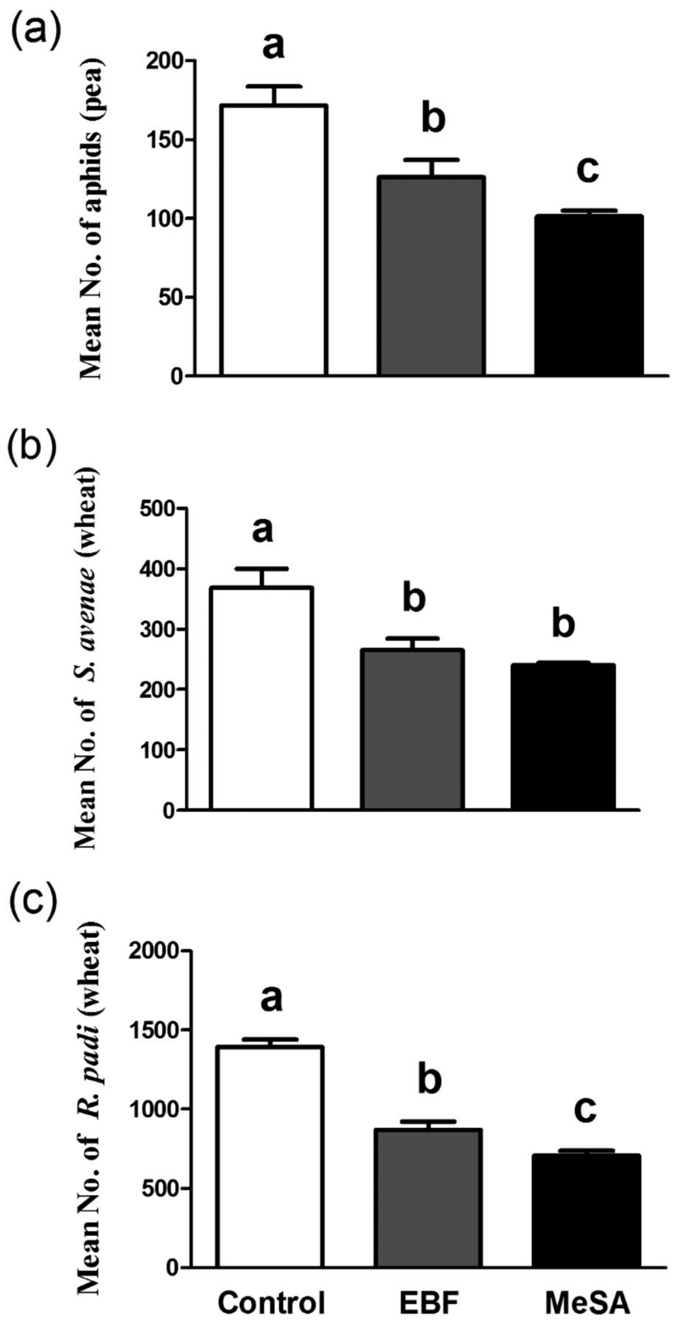

Figure 2. Mean numbers (and standard error) of aphids observed on plants in the different treatments throughout 2016 growing season. (a) aphids on pea plants, (b) *S. avenae* on wheat tillers, (c) R. padi on wheat tillers.

Note: Letters indicate significant differences based on post-hoc tests of Tukey performed on GLMMs (P < .05).

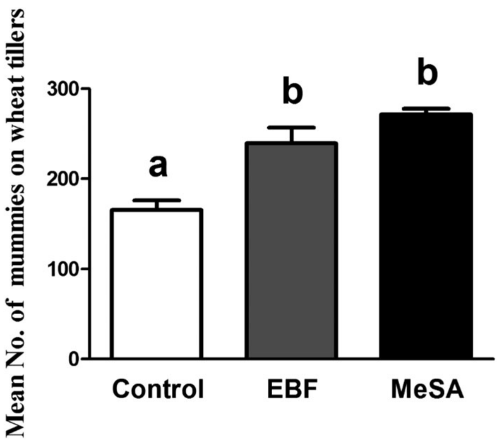

Figure 3. Mean numbers (and standard error) of mummies on wheat tillers in the different treatments. Note: Letters indicate significant differences based on post-hoc tests of Tukey performed on the GLMM (P < .05).

Table. 2 Linear regressions between the abundances of aphids, predators (adults and larvae) and mummies without distinguishing treatments, * p < 0.05, ** p < 0.01, *** P < 0.001

	Estimate	R^2	$F_{1\text{-}10}$	*p*-value
Ladybeetles				
A. pisum	-0.98	0.53	11.4	0.007 **
R. padi	-0.92	0.7	23.1	< 0.001 ***
S. avenae	-1.07	0.48	9.3	0.012 *
Lacewings				
A. pisum	-1.59	0.84	50.9	< 0.001 ***
R. padi	-1.27	0.8	39.6	< 0.001 ***
S. avenae	-1.6	0.64	17.9	0.002 **
Hoverflies				
A. pisum	-1.45	0.63	17.2	0.002 **
R. padi	-1.14	0.58	13.7	0.004 **
S. avenae	-1.47	0.49	9.63	0.011*
Mummies				
R. padi	-0.65	0.68	21.8	< 0.001 ***
S. avenae	-0.84	0.58	14	0.004**

4 Discussion

Releasing EBF or MeSA allowed significantly reducing aphid density and attracting their natural enemies in the present wheat-pea intercropping system in the Henan Province of China. The beneficial effect of aphid reduction may be due to two factors. First, EBF and MeSA may have repelled aphids, and/or induced the development of wings, an effect that would accelerate aphid dispersal (Ninkovic et al. 2003; Kunert et al. 2005; Hatano et al. 2010; Thieme and Dixon 2015). Second, the increased number of aphid predators may have preyed on aphids, reducing their populations. As for natural enemies, ladybeetles and lacewings, which were the most abundant aphid natural enemies trapped, were positively attracted by both semiochemicals, confirming previous studies (Cui et al. 2012; Francis et al. 2004; James 2003a, 2006; Zhu and Park 2005). Regarding the effect of EBF on lacewings, few experiments have been conducted in field conditions to our knowledge. Our present observations in fields are nevertheless not consistent with previous laboratory experiments using Y-tube olfactometer on the Asian lacewing *Chrysopa cognata* (Boo et al. 1998) and *Chrysopa pallens* (Li et al. 2017). However, previous electroantennogram results showed that the antennae of *Chrysoperla carnea* are highly sensitive to EBF (Zhu et al. 1999), which can support the increased abundance of lacewings observed in EBF treatment compared to Control. Concerning hoverflies, they were not affected by EBF compared to Control, which contradicts previous findings reporting that EBF is an important olfactory cue for aphid localization by hoverflies (Verheggen et al. 2008). However, hoverflies were positively affected by MeSA, which is consistent with James (2003b). Finally, even though parasitoids were not identified from traps, the number of mummified aphids on wheat was higher in treatments with MeSA or EBF compared to Control, suggesting that releasing such semiochemicals in fields can increase parasitoid abundance and/or enhance the host-finding ability of aphid parasitoids and leading to an improved pest control.

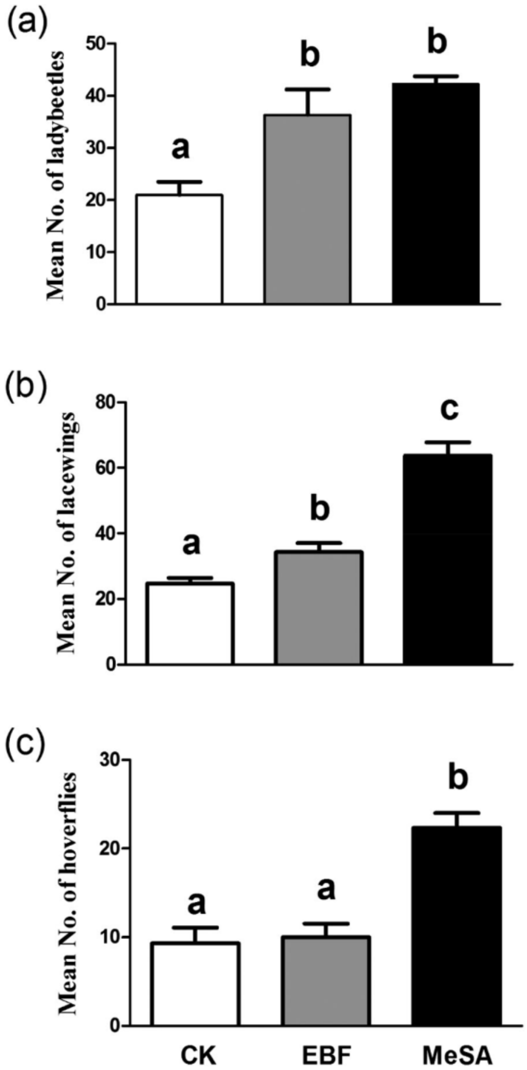

Figure 4. Mean numbers (and standard error) of natural enemies in the traps in the different treatments throughout 2016 growing season. (a) ladybeetles, (b) lacewings, (c) hoverflies.

Note: Letters indicate significant differences based on post-hoc tests of Tukey performed on GLMMs ($P < .05$).

The present experiment also reveals that MeSA attracted twice as many hoverflies and lacewings (and to a lesser extent ladybeetles) than EBF. This may explain the better control on *R. padi* in MeSA compared to EBF plots. To our knowledge, few studies previously compared the release of these two semiochemicals in wheat-pea intercropping systems toward biological control of aphids. Xu et al. (2018) showed in Belgium that ladybeetles were significantly more abundant in treatment with EBF in oil than with MeSA, while no significant differences were reported for lacewings and mummies, and hoverflies were increased in only one over the two years. This previous study also reported that pea aphids were about ten times more abundant than wheat aphids, while the contrary was observed here. These different results recall that insect dynamic may vary from a location to another, highlighting the need to test tactics of biological control in various contexts. Nevertheless, in both studies, the release of the two semiochemicals led to a reduced abundance of aphids on both pea plants and wheat tillers, confirming their interest for IPM strategies.

Despite that this study was conducted over only one growing season, the results show that releasing semiochemicals in intercropping systems can reduce aphids and increasing their natural enemies in this region of China. These results were stronger when MeSA was released, compared to EBF. Wheat-pea intercropping was previously shown to enhance associational resistance to aphids (Ndzana et al. 2014) and the addition of semiochemical releases can improve crop protection further by enhancing natural enemies while simultaneously repelling aphids.

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