Field evaluation of the combined deterrent and attractive effects of dimethyl disulfide on Delia radicum and its natural enemies

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A B S T R A C T

Delia radicum (L. 1758) is a major pest of cabbage crops in northern Europe. Due to more constraining laws relating to insecticide use, new strategies to control this pest are urgently needed. Manipulating insect behavior through infochemicals is a promising approach. The recent identification of dimethyl disulfide (DMDS) as a compound that both attracts the main predators of D. radicum and inhibits oviposition by the fly gives a challenging opportunity to develop such strategy. The aim of the present study was to confirm such potential of DMDS, in the field. Through the 8 weeks of the first egg laying peak of the fly we assessed, the potential of artificially increasing the levels of this molecule in the close vicinity of broccoli plants to 1/attract predators, 2/stimulate predatory activity and 3/limit damage done by the fly. Despite a lower number of D. radicum eggs as food resource, DMDS effectively increased predator catches in treated plots (119 Aleochara bilineata (Gyllenhal, 1810) caught in treated plot, while only 21 in control plots). However, damages done by the fly were of the same magnitude order in treated plots than in control ones. Number of D. radicum larvae and pupae recovered in plant roots were similar, despite the important decrease in eggs laid. This result, together with the observation that the numbers of eggs pre-dated in artificial patches were lowered in the presence of the molecule, seems to indicate that increasing DMDS amounts disturbed the foraging activity of the fly predators. Consequences of these findings for the future of DMDS use in crop protection against D. radicum are discussed.

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1. Introduction

Interactions among organisms are under the strong influence of chemical compounds. In insect–plant interactions, volatile chemicals play a key role in foraging activities. These volatiles, originating either from conspecifics, preys or their habitat, are often used by foragers to make useful decisions for finding resources or avoiding danger (e.g., Bell and Cardé, 1984; Vet and Dicke, 1992; Dicke and van Loom, 2000. The importance of these infochemicals in insect–insect and plant–insect interactions led to the emergence of new pest management practices (e.g. Lewis and Martin, 1990; Nordlund et al., 1981; Foster and Harris, 1997. In particular, artificially added infochemicals were used with success as pest attractants in mass trapping strategies involving Lepidopteran sex pheromones and Coleopteran aggregation pheromones (e.g. Howse et al., 1996. Using the deterrent activity of some synthetic volatiles like alarm pheromones (E-ß-farnesene) also gave successes in crop protection against aphids (Griffiths et al., 1989; Pickett et al., 1997). Another strategy often considered is the use of volatiles which are attractive for natural enemies of the pest (parasitoids and/or predators) to enhance their colonization of crops and their natural limiting effect on pest populations (e.g. Dicke et al., 1990; Degenhardt et al., 2003; Turlings and Ton, 2006. Although this strategy was used early to manipulate parasitoids (Lewis and Nordlund, 1984), it was only recently used with success to attract predators by James and co-authors in hop yards (2005). In addition, to increase the potential of such approach it has been suggested to combine different strategies using infochemicals (Pickett et al., 1997). For example, the stimulo deterrent diversionary strategy (SDDS) proposes the use of different odors to simultaneously push a pest from harvestable crops while, at the same time, pull them in a sacrificed plot (Miller and Cowles, 1990; Cook et al., 2007).

Nevertheless, except for the case of mass trapping strategies, using infochemicals generally lacked efficiency when compared to conventional crop protection with insecticides. However, there is growing evidence of adverse effects of the intensive use of insecticides (Cooper, 1991); Edwards and Tchounwou, 2005, leading to more and more constraining laws. New solutions based on our increasing knowledge of pest biology and ecology need to be found.

Delia radicum, the cabbage root fly, is an economically important pest of brassicaceae crops in northern countries of the Hol-arctic region. The fly lays its eggs on the soil in the vicinity of
brassicaceae stems. Larval development takes place inside the plant roots and can lead to important yield losses. There are typically two to three peaks of eggs laying by the fly during the whole season of crop plantation (from the middle of March to the end of September). The first peak, corresponding to the emergence of hibernating pupae, is of major concern. Damages occur on a very valuable crop (early vegetables) and can cause up to 100% losses due to the small size of plants at the time of attack. The use of the main insecticide (the organophosphorous Chlorfenvinphos, i.e. 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) against this pest has been banned in all the European Union since December 2007. Recent studies have showed the potential use of dimethyl disulfide (DMDS) in a biocontrol strategy against D. radicum (Ferry et al., 2007, in preparation). This compound is emitted in large amounts by roots heavily infested by the cabbage root fly. In the field, we found that DMDS is attractive for the main natural enemies of the fly (Ferry et al., 2007), i.e. carabidae belonging to the genus Bembidion (Latreille, 1802) acting as generalist predators of eggs and larvae, and Aleochara bilineata (Gyllenhal, 1810) and A. bibustulata (L., 1761), two staphilinids that are both generalist predators and parasitoids of the fly pupae. DMDS was also found to be an oviposition repellent for the fly (Ferry et al., in preparation). These two coupled effects (repel the pest and attract the predators) may offer a great advantage in comparison to other strategies using infochemicals targeting only one of the two. The present study investigates the potential for using DMDS as an infochemical against D. radicum. A field trial was conducted to investigate under cropping conditions the potential of DMDS for both limiting oviposition by the fly and improving predator occurrence and activity. The effect of DMDS use on crop quality was also assessed.

2. Materials and methods

2.1. Plot description

The field experiment took place in the experimental station of La Rimbaudais, Saint Méloir des Ondes, France, in the heart of an important zone of cabbage production (001°54 W/48°39 N). The field consisted of a broccoli (Brassica oleracea, L. 1753; var. Monopoly) plot cultivated in a traditional way. One month old seedlings were planted in the field the 12th of April at a density of 4.3 plants per m² on a 40 x 80 m field. One week after plantation, the plot was treated at 2L/Ha with the herbicide Butisan® (active compound: matazachlore) to limit further machine intervention in the experimental plot. No insecticide was used, neither as seed coating (frequently used by non-organic farmers) nor as sprays in the field. The crop was maintained until the 1st of June, at a date corresponding to the end of the first peak of D. radicum egg laying.

We used a randomized block design consisting of four blocks with two treatments in each block. Blocks were separated from each other by 5 m of bare soil. Elementary plots in the blocks were also separated by a non cropped band of 3 m. Elementary plots consisted of a 14 x 15 m cultivated surface (16 rows with around 18 plants per row). Treatments were applied in the 10 middle rows and the 12 middle plants of each row of these plots, leading to an extra buffer zone of untreated plants around the treated ones (three rows and 2.5 m at the end of the rows) (Fig. 1). The firsts rows and the firsts plants in rows at the boundary of the treated zone were counted as ‘margin’ and where not taken into account for the measurements.

Treatments consisted of adding an open Eppendorf tube filled with 0.5 mL of DMDS diluted in paraffin oil (1:250), at a distance
of 2 cm from each plant of the treated areas. Pure paraffin oil was placed in the same way in the vicinity of control plants. Eppendorf tubes were changed three times per week, on Monday, Wednesday and Friday to ensure a constant emission of DMDS.

2.2. Measurements

2.2.1. Presence of predators

The presence and abundance of fly predators in the elementary plots was monitored using pitfall traps (10 cm diameter) filled with 50% alcohol. A diagonal of four pitfalls was placed in each elementary plot (Fig. 1). They were placed on rows and spaced from each other by two empty rows and 3–4 plants. Pitfall n°1 was placed at a corner of the elementary plot, on the untreated buffer zone, spaced by one row and 2–3 plants from the uncropped band. Pitfall n°2 was placed in a corner of the treated zone, spaced by one row and 1–2 plants from the untreated buffer zone. Pitfalls n°3 and n°4 were respectively on the 5th and 8th row of the treated zone, spaced by 5–6 and 2–3 plants from the untreated buffer zone. In each bloc the diagonal of pitfalls was oriented in a different direction, thereby avoiding any unwanted directional effect such as the ones resulting from dominant winds. Alcohol vials where insects are trapped were changed once a week on Friday. Removed vials were brought back to the lab for analyses. We essentially focussed on A. bilineata and A. bipustulata, that were counted and sexed (using the presence of parameres in the male genital apparatus), and on carabidae belonging to the genus Bembidion. For these three taxa, the treatment effect was assessed using the mean number of individuals caught by each of the three pitfalls placed in the treated areas (n°2, 3 and 4) and looking at the spatial distribution of the catches along the whole transect of each elementary plot (including pitfall n°1 placed in the untreated buffer zone).

2.2.2. Oviposition by D. radicum

Egg laying by D. radicum was monitored using felt bands traps (Bligaard et al., 1999). Each Friday, traps were gently removed and the eggs found inside were counted and removed. Traps were then replaced on another plant for a week. The usual threshold of a mean number of seven eggs per trap (above which farmers are advised to apply a pesticide) was used to assess the ending of the first peak of egg laying by D. radicum. Eight felt traps were used in each elementary plot, on one plant of each central row of the treated area. The traps were placed both around the stem of the plant and the Eppendorf containing the paraffin solution. Analyses were carried out on the number of eggs found in each trap.

2.2.3. Egg predation

Egg predation on the plots was assessed using artificial patches of eggs. These patches consisted of a 1 × 3 cm piece of black paper folded in the middle and pinned down to the soil surface. Five D. radicum eggs collected in our rearing facility were deposited in the horizontal V made by the folded paper. Sixteen patches were placed on each elementary plot, in the immediate vicinity of two plants of each of the eight central rows of the treated area (avoiding the ‘margin’ plants and rows of the treated area). Patches were maintained in the plots for 48 h before egg predation was checked. We measured the percentage of patches where predation occurred (at least one egg predated per patch) and the mean number of predated eggs when predation occurred. This experiment was repeated three consecutive weeks (the 19th and 27th of April and the 4th of May).

2.2.4. Crop damage by D. radicum

Sixteen plants of each elementary plot (two plants in each of the eight central rows of the treated areas) were randomly assigned to be noted on their appearance and were checked for D. radicum larvae and pupae. Plant condition was scored according to the above ground appearance only between 1 and 4: 1 = good, 2 = medium, 3 = bad, 4 = dead or dying. Roots and surrounding soil (in a 10 cm radius around the plant) were sampled and brought back to the lab to count the number of larvae and pupae present. We measured the mean number of D. radicum larvae and pupae found per root and the percentage of medium to good quality plant (i.e. plants scored 1 or 2).

The operation was repeated twice, the two last weeks of the experiment (the 25th of May and the 1st of July). A total of 32 plants by elementary plots (128 plants by treatment) were scored for plant quality and D. radicum infestation. The data obtained on the two dates of sampling were pooled for analysis.

2.3. Statistical analysis

All statistical analyses were carried out with R software (R Development Core Team, 2007).

GLM analyses (function ‘glm’) used the following factors: treatment (two modalities: DMDS and control), block (four modalities), date (eight modalities corresponding to the 8 weeks of measurement for egg count and predator catches, and three modalities for the data on predation), and all interactions between these factors. The family distribution of the analysed data, used to define the link function in GLM analyses, was chosen to give the best residuals. These family distributions were: Poisson for the predator catches, and the number of D. radicum larvae and pupae per root, negative binomial for the number of D. radicum eggs per plant (function ‘glm.nb’ instead of ‘glm’), Gaussian for the mean number of predated eggs per patch and binomial for the percentage of predated patches and the percentage of good quality plants. For all GLM analysis, the normality of residuals was checked using a normal quantile–quantile plot. This plot used the residuals for data following a normal distribution, and the randomized quantile residuals for data following a Poisson or a binomial distribution (function ‘residuals’, following Dunn and Smyth, 1996). The significance of factor effects was assessed by analysis of deviance (function ‘anova.glm’) using a χ²-test.

Predator catches were compared between treatments for each date or according to the position of the pitfalls by an analysis of contrast (function ‘esticon’ of package ‘doBy’, following Højsgaard (2004). Sex-ratio bias on A. bilineata and A. bipustulata were tested using a χ²-test.

3. Results

3.1. Presence of predators

3.1.1. A. bilineata

A total of 164 A. bilineata were caught in pitfall traps. Sex ratio was slightly but not significantly biased toward males (93 males, 71 females, χ² = 2.689, df = 1, P = 0.1010). With almost six times more individuals caught on DMDS treated plots (119 versus 21 in control plots), a GLM analysis of the insects caught in the pitfalls inside the treated areas (pitfalls n°2, 3 and 4) reveals a clear effect of the presence of DMDS (Table 1). Other significant effects on the mean number of A. bilineata caught per trap are block, date, and their interaction, indicating a strong spatiotemporal variation at our experimental scale. Non-significant effects of interactions involving the treatment as a factor (e.g. treatment × block, treatment × date and treatment × block × date) were found. This emphasizes the role of DMDS as the main explanatory factor for explaining A. bilineata catches repartition. Mean numbers of individuals caught per date revealed a late colonization of the plot, starting in DMDS treated plots the third week after crop plantation (Fig. 2A). A maximum number of insects were trapped on the 6th
week after crop plantation, at the end of the *D. radicum* egg laying peak.

Numbers of *A. bilineata* caught per trap along the pitfall transects made in elementary plots revealed that DMDS was attractive at this scale. While there was no difference between the center and the margin in control plots, DMDS treated plots showed a structured spatial distribution of the catches with a higher number of insects trapped in the center of the treated area (Fig. 2B).

### 3.1.2. *A. bipustulata*

A total of 209 *A. bipustulata* were caught during the experiment. Sex ratio for this species appeared balanced (106 males, 103 females, $\chi^2 = 0.0191, df = 1, P = 0.89$). Inside of the treated areas (pitfalls n°2, 3 and 4), 50% more individuals were caught in DMDS treated plots (107 versus 69 in control plots), leading to a significant global effect of the treatment in the GLM analysis (Table 1). For *A. bipustulata* all tested factors but the date × block interaction had a significant effect. This indicates a spatiotemporal variation of the catches, and a variability of the DMDS action among blocks and among dates. This variability is notably highlighted by the large unexpected increase of catches in DMDS plots the 6th week of the experiment (Fig. 3A). Except for this date, the number of *A. bipustulata* seemed to gently decrease over all the experiment period, with a maximum of catches the first week after crop plantation.

DMDS attractiveness at the plot scale was revealed by observing the catches of *A. bipustulata* along the pitfall transects (Fig. 3B). In the control plots, the mean number of individuals caught in the untreated buffer zone (e.g. pitfall n°1) did not differ from the mean number of individuals caught in the central area. On the contrary, pitfalls placed in the central area of DMDS treated plots caught significantly more *A. bipustulata* than those in the untreated buffer zone (which did not catch more individuals than similarly placed traps of control plots).

### 3.1.3. *Bembidion spp.*

Overall, 1289 carabid beetles belonging to the genus *Bembidion* were caught. Inside of the treated zones (pitfalls n°2, 3 and 4), catches were slightly higher in DMDS plots (539 versus 462 in control plots) but differences between treatments varied widely from date to date/during the experiment. All tested effects significantly influenced the number of individuals caught inside the treated zones (Table 1). Accordingly, despite of a treatment effect detected by the GLM analysis, interaction effects among date and treatment seemed even more important, leading to changes between the preferred treatment among dates (see for example the two last weeks of the experiment; (Fig. 4A).

The unclear effect of DMDS in explaining the number of *Bembidion* spp. caught was even more striking when looking at the number of individuals caught along the transect pitfalls (Fig. 4B). Indeed, there was no evident spatial pattern distinguishing control plots from DMDS-treated ones. There was no significant difference between the buffer zone and the treated zone, or between the DMDS and the control plots. Only pitfall n°4 in DMDS treated plots caught a significantly higher number of *Bembidion* spp. than all other pitfalls, but this was mainly due to two data points, the 25th of May, in block A and D (mean number of insect caught in these two points = 26.5 while mean number of individuals per pitfall = 6.48 overall).

### 3.2. Effect of DMDS on *D. radicum*

#### 3.2.1. Oviposition

Over all the experimental period the mean number of eggs laid per plant in DMDS plots was lowered by 60% compared to control plot (respectively, 4.23 and 10.84 eggs per plant) (Fig. 5) Main results of GLM analysis are given in Table 1.
Mean number of A. bilineata caught per pitfall trap ± standard deviation. (A) Mean number of individuals caught in pitfalls 2, 3 and 4 at each date. Stars indicate a significant difference at: * = 0.05, ** = 0.01, *** = 0.001. (B) Mean number of individuals caught in each pitfall according to their position in the transect (1 = untreated buffer zone; 2 = next to the margin of the treated zone; 3–4 = in the center of the treated zone). Different letters indicate a significant difference at * = 0.05.

Mean number of Bembidion spp. caught per pitfall trap ± standard deviation. (A) Mean number of individuals caught in pitfalls 2, 3 and 4 at each date. Stars indicate a significant difference at: * = 0.05, ** = 0.01, *** = 0.001. (B) Mean number of individuals caught in each pitfall according to their position in the transect (1 = untreated buffer zone; 2 = next to the margin of the treated zone; 3–4 = in the center of the treated zone). Different letters indicate a significant difference at * = 0.05.
3.2.2. Larvae and pupae

*D. radicum* larvae and pupae found in plant roots at the end of the experiment was not negatively affected by DMDS (Fig. 5). Total number of larvae or pupae found in DMDS plots was even higher than in control plots, although this difference was not significant (161 versus 136 respectively). Furthermore GLM analysis revealed a strong spatial effect (block) that was absent for the number of eggs recovered (Table 1).

3.3. Egg predation

Among the 374 egg patches deposited over the three series of predation experiments, only one third were visited and predated, with no difference between treatments (56 patches in DMDS versus 54 in control plots; Fig. 6A). According to GLM analysis, the only factors affecting patch discovery by predators were spatio-temporal (e.g.: block, date and block x date interaction; Table 1).

The mean number of eggs predated per patches that were discovered (i.e. when at least one egg was predated) revealed an effect of the presence of DMDS in the plots. Interestingly, with 2.07 ± 0.18 eggs predated per patch in DMDS plots versus 2.61 ± 0.19 in control plots, presence of DMDS negatively affected the number of eggs predated (Fig. 6B). No interaction involving the treatment as a factor significantly affected the result (Table 1), indicating a quite stable spatiotemporal effect of DMDS on this result. Other factors affecting the number of predated eggs per patches were the date and the block x date interaction.

3.4. Plant quality

Overall, plant quality in our experimental plot was poor. Good and medium quality plants only represented 50% and 40% of the sampled plants in DMDS and control plots, respectively (Fig. 7). Despite this difference, the treatment effect was not significant (Table 1). Only block factors significantly affected the result.

4. Discussion

The field bioassays presented here confirm the importance of volatiles for structuring insect communities, as well as their potential for pest control practices (Foster and Harris, 1997). The same monomolecular compound, i.e. DMDS, was shown, in the field, to potentially deter a pest while attracting its predators. Artificially increasing DMDS emissions near broccoli plants significantly decreased egg laying by *D. radicum* and increased pitfall catches of key predators of this pest (i.e. *A. bilineata* and *A. bipustulata*) in the vicinity of treated plants. However, in spite of these two very interesting coupled effects, adding DMDS did not efficiently limit damages done by *D. radicum* larvae on the crop since both the number of recovered larvae/pupae and the quality of plants did not differ significantly between treated and control plots at the end of the experiment. As discussed below, insufficient plant quality may have been a strong limiting factor for the development of the fly on both treated and control plants in our experiments. However, predation measurements, indicating a decrease of eggs...
predated per patches in the DMDS treated plots, highlight a potential adverse effect of the odor use that could explain the contradictory results obtained.

The present study demonstrates the capacity to increase pitfall catches of *D. radicum* predators in plots containing plants artificially associated with higher amounts of DMDS. Local attractiveness of DMDS towards *A. bilineata*, and *A. bipustulata* was already demonstrated (Ferry et al., 2007). Repartition of catches along the pitfall transects in plots, reveals that this molecule can also have a longer range action. Indeed, the mean number of insects trapped in pitfalls gently increase when going further inside the treated zones while they remain constant in control plots. However, with the doses used in our experiments, the attraction range of the molecule did not seem to exceed a few meters, like presumed by Ferry et al. (2007): pitfalls in the untreated margin of DMDS treated plots, only one row away (i.e. 80 cm) from the first treated broccoli row, did not catch more insects than pitfalls placed in control plots. Surprisingly, DMDS, that was shown to attract *Bembidion* carabid beetles in Ferry et al. (2007), had no clear effect on this taxon in the current study. The lack of response observed here could be related to the amount of DMDS present in our plots. Indeed, in previous study, *Bembidion* sp were shown to no longer respond to DMDS when the molecule concentration was too high. Although the net quantity of 2 μL of DMDS used in the present experiment was shown to be attractive for the beetles when placed in odorous traps, it is likely that the different means of odor release in the present experiment led to higher amounts released in the environment, and weakened the attraction of the carabid beetles. Other factors such as the timing of the experiment or differences in the set-up and the trapping method used could also explain the contrasting results obtained.

Before concluding on the effect of DMDS as an effective attractant of *D. radicum* predators, the limits of pitfall traps for measuring insect abundance should not be forgotten. Pitfall trap counts depend on local abundance of the target arthropod, but also on its locomotor activity (Greeneslade, 1964). The higher number of *A. bilineata* found in DMDS treated plots can indistinctively be due to a higher density of this species or to a higher locomotor activity in these plots, or to both factors. Increased locomotor activity due to DMDS has already been shown in parasitoids (Lecomte and Thibout, 1984).

The plant quality data and the number of *D. radicum* found in roots at the end of the experiment clearly indicate that DMDS did not sufficiently prevent pest damage caused by the larvae. Despite a decrease in the number of eggs laid by the pest and an apparent increase in numbers of biocontrol agents in DMDS treated plots, numbers of larvae and pupae found in roots were not significantly lower than in untreated plots, and plant quality was not significantly improved. A first explanation of this result could be that the poor quality of our plants (both in the treated and in the control plots) was the factor limiting the number of larvae that could feed upon the plants. With almost half of the plants looking bad or already dead after one and a half month of culture, our plots were globally of poor quality. According to the experimental station technicians, the crop would have been harvested one month later than a ‘normal’ commercial crop. Repeated stamping of the soil surrounding the plants by experimenters and the absence of hoeing (that is often done in traditional cropping, allowing to aerate the soil for a better root development) due to experimental constraints, could be the main explaining cause. The poor quality of the plants, assessed by looking at their above-ground part, is probably linked with a small root biomass, and consequently, a limited food resource for the fly. We can not exclude that this limitation of resources restricted the number of larvae able to feed upon plants. Broccoli plants at the same age were usually shown to carry up to five times more larvae and pupae in previous years (unpublished results). This could explain why, even with a higher number of eggs laid, the numbers of larvae and pupae in roots of control plots were not higher than those in DMDS treated plots.

However, even on a low number of larvae, predatory activity of the biocontrol agents should have led to some difference between the two treatments. The second explanation, well supported by the data on egg predation, is a perturbing effect of DMDS on predators. Indeed, while the number of patches visited by predators was the same in the two treatments, the mean number of predated eggs per patches was significantly decreased in DMDS treated plots. The same decrease in predatory activity in DMDS treated plots could be responsible for a lower predation of the eggs naturally laid by *D. radicum*, leading to no difference between control and DMDS plots when considering the number of larvae and pupae present in roots. Lowered predatory activity in DMDS treated plots could have two main origins. First, the large amount of the molecule present in treated plots might have hidden other volatile cues to the predators, thereby diminishing their efficiency in finding eggs. Another possibility would be that DMDS presence acted as a diversionary cue. DMDS abundance in treated plots could indicate the presence of abundant resources that were actually not present. Predators might have been confused by this signal, spending time searching for high quality patches that did not exist. The lower number of predated eggs in treated plots could be due to a lower time spent on egg patches, perceived as having a poor quality compared to what could be expected globally in the environment, in agreement with optimal foraging theory (Charnov, 1976). Increased search activity in DMDS plots could also partially explain the higher number of catches in pitfalls placed in these areas. Such diversionary problem, when using an odor to increase biocontrol agent activity against a pest, has already been shown in different systems involving parasitoids (examples in Powell, 1986). The present study could be the first evidence of this undesirable phenomenon occurring also with predators. Of course, in field experiments such as the ones we conducted, results are influenced by many uncontrolled biotic and abiotic factors, making it hard to conclude on which factors were the most influential in explaining the data obtained. Lab experiments, working on isolated individuals and in controlled conditions would be necessary to confirm such an effect.

Even if, in the conditions of this field experiment, DMDS was no efficient in protecting the crop against *D. radicum*, its effect on egg laying diminution is an encouraging step toward its potential use for controlling this pest. Furthermore this study still confirms the possibility of odor use in manipulating insect community. Despite the fact that our study highlights the main risk of such an approach, the results obtained are encouraging and show a potential deserving complementary studies. Such studies should permit to better face current and future problems of crop protection given the ever limited use of insecticides.

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