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E-β-farnesene synergizes the influence of an insecticide to improve control of cabbage aphids in China

Liang-Liang Cui[a], Jie Dong[a], Frédéric Francis[b], Ying-Jie Liu[a], Stéphanie Heuskin[c], Georges Lognay[c], Ju-Lian Chen[d], Claude Bragard[e], John F. Tooker[f], Yong Liu[a,*]

[a] College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, PR China
[b] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liége, Passage des Déportés 2, 5030 Gembloux, Belgium
[c] Laboratory of Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium
[d] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 2 West Yuanmingyuan Road, Beijing 100193, PR China
[e] Earth and Life Institute, Applied Microbiologye-Phytopathology, Catholic University of Louvain, 1348 Louvain-la-Neuve, Belgium
[f] Department of Entomology, The Pennsylvania State University, 501 ASI Bldg, University Park, PA 16802, USA

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ABSTRACT

Extensive use of pesticides to control insect pests can have negative effects on the environment, natural enemies and food safety. The aphid alarm pheromone, E-β-farnesene (Eβf), appears to hold strong potential for controlling a wide variety of aphid pests. To understand the control potential of Eβf, we used field experiments in a factorial design to test its influence and that of the insecticide imidacloprid on populations of aphids *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) on Chinese cabbage, *Brassica rapa pekinensis* (Brassicales: Brassicaceae). Our results showed imidacloprid treatment alone can significantly decrease aphid populations, and that combining insecticide with Eβf further reduced numbers of apterous aphids at distances of 5 m from pheromone emitters in two years of our experiments. Our results demonstrate that imidacloprid can be effective in reducing the abundance of aphids in Chinese cabbage fields, but the degree of control can be even stronger in the presence of Eβf.

1. Introduction

Conventional pesticides can be an effective tool to control pest populations, but over-reliance on synthetic compounds carries environmental and human-health risks that render their repeated use unsustainable (Hart et al., 2003; Pimentel, 2005). Therefore, it is prudent to explore alternatives to conventional pesticides to improve the sustainability of pest management. By altering herbivore behavior or manipulating natural enemy populations, insect-derived semiochemicals have been recognized as potential alternative tools for the control of insect pest populations (Rodriguez and Niemeyer, 2005). Among semiochemicals relevant for improved aphid control, aphid alarm pheromone is among the most promising (Schwartzberg et al., 2008).

Alarm pheromone is emitted from the cornicles of aphids when they are attacked by natural enemies and signals the risk of attack to others (Edwards et al., 1973). When other aphids detect this cue, they remove their stylets from the host plant and fall, jump, or walk away to escape potential danger (Edwards et al., 1973; Montgomery and Nault, 1977; Roitberg and Myers, 1978; Wohlers, 1981; Braendle and Weisser, 2001). Aphids that sense alarm pheromone do not appear to release additional pheromone (Hatano et al., 2008; Verheggen et al., 2008b). Alarm pheromones are emitted by nearly all aphid species, and the sesquiterpene E-β-farnesene (Eβf), the most common component, has been found in more than 40 aphid species (Xiangyu et al., 2002). Eβf is the only volatile compound identified in the alarm pheromone of 13 aphid species (Francis et al., 2005). For other aphid species, the production of Eβf is often accompanied by other minor components (Nishino et al., 1977; Pickett and Griffiths, 1980). Although Eβf is used by many aphid species, some species are more responsive to alarm pheromone than others (Losey and Denno, 1998), with variation in response even among different clonal lines of the same species (Muller, 1983; Braendle and Weisser, 2001). Aphid alarm pheromone release influences the responses of aphids in the presence of predators and parasitoids, but also has longer-term consequences for aphid colony composition and dispersal (Su et al., 2006, Schwartzberg et al., 2008).

Studies of the ecological importance of Eβf under natural conditions are necessary to demonstrate its applicability to pest

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* Corresponding author. Tel.: +86 0538 8248208.
E-mail address: liuyong@sdau.edu.cn (Y. Liu).

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control; however such studies can be challenging because synthetic Eβf is unstable when exposed to air (Dawson et al., 1990). To overcome this challenge to developing Eβf as a pest control tool, it is prudent to explore natural sources of Eβf. Various plant families also emit Eβf, including Poaceae (Turlings et al., 1998), Solanaceae (Agelopoulos et al., 1999), Malvaceae (Röse and Tumlinson, 2004), Lamiaceae (Bruce et al., 2005) and Asteraceae (Heuskin et al., 2009), and in some plant species, such as wild potato, *Solatium berthaultii* Hawkes, Eβf may act as a natural aphid deterrent (Gibson and Pickett, 1983). A transgenic line of *Arabidopsis thaliana* (L.) Heynh (Brassicales: Cruciferae) has even been produced that carries the genes for synthesizing Eβf (Beale et al., 2006). Plant essential oils with high Eβf content are a promising source of material, particularly because formulations of essential oils are likely to prevent aerial oxidation of Eβf (Bruce et al., 2005). Although some essential oils with Eβf content have been identified from a few plant species (Dawson et al., 1990; Bruce et al., 2005; Heuskin et al., 2009, 2010), few experiments have been conducted with these oils to test their influence on aphid populations under field conditions. One report demonstrated that the number of pea aphids, *Acyrthosiphon pisum* (Harr.), on spring sown field beans, *Vicia fabae* L., could be reduced by use of the essential oil of *Hemizygia petiolata* Ashby with Eβf content (Bruce et al., 2005).

In this paper, Eβf, with a high purity degree, which was isolated from *Matricaria chamomilla* L. (Asterales: Asteraceae) essential oil by means of a fast and simple process (Heuskin et al., 2009, 2010), was applied to control two aphid species, *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), which are major pests of cruciferous crops, such as Chinese cabbage *Brassica rapa pekinensis* (Brassicales: Brassicaceae), the primary vegetable crop in northern China. *M. persicae* and *L. erysimi* are usually found concurrently on Chinese cabbages in fields of Shandong province. Populations of these aphid species damage crops by removing photoassimilates from phloem and vectoring numerous plant viruses (Harris and Maramorosch, 1977; Foster et al., 2000; Verheggen et al., 2009; Summers et al., 2010). Therefore, the primary goal of this study was to test the potential of *M. chamomilla*-derived Eβf to improve control of two aphid species, *L. erysimi* and *M. persicae* in Chinese cabbage fields.

2. Materials and methods

2.1. Field experimental design

Field experiments were conducted on experimental farmland at Shandong Agricultural University, Shandong Province of China in 2009 and 2010. Four treatments were (a) control; (b) Eβf [83.8% ± 0.3, extracted by flash chromatography from essential oil of *M. chamomilla* L. (Heuskin et al., 2010) provided by Dr. S. Heuskin and Prof. F. Francis] only; (c) a low dose of imidacloprid (10 g ai ha^{-1}, 2.5%, EC, Guangxi Tianyuan Biochemistry Co., Ltd; recommended dose: 20–22.5 g ai ha^{-1}) treated plots; and (d) low dose of imidacloprid with Eβf. A completely randomized design was used with three replications. The plots measured 10 m × 25 m, and were separated along all edges by paths (10 m). Chinese cabbage ('Beijing new 3′, Beijing Jingyanyinong Sci–Tech Development Center) was planted in rows with 60-cm spacing. The plants were sown on August 15th, 2009 and August 17th, 2010. All plots were fertilized with 110–50–150 (N–P–K) kg ha^{-1} and no herbicides were used during the growing season. A total of five applications of imidacloprid were made per season. The first application occurred seven days before the first sampling date in treatment plots (c) and (d), and the other four applications occurred after every sampling date. Eβf was released using 1-cm diameter rubber septa, allowing the chemical to be slowly released. One hundred microlitres of Eβf solution in paraffin oil was added to the rubber septa and this amount was added again every seven days. In laboratory conditions at 20 °C, relative humidity of 65% and air flow 0.5 L min^{-1}, 76 μg of Eβf was released from the formulation per seven days (Dr. S. Heuskin, unpublished data).

To sample alate aphid populations, yellow pan traps (26 cm diameter, 10 cm depth) were placed on platforms 10 cm above the ground in all plots. The traps were filled with a dilute detergent solution (Diao Brand, Nice Group Co., Ltd.). In plots (b) and (d), an Eβf releaser was mounted centrally close to the liquid surface of the trap. A transparent plastic cover was placed above the septa to protect them from rain. The treatments began when the Chinese cabbage reached the rosette stage (15 September 2009 and 22 September 2010). The plots were irrigated when plants were at the rosette and heading stages.

2.2. Cabbage aphid sampling

To quantify the influence of treatments on aphid populations, we determined the number of cabbage aphids present on cabbage leaves in the different plots every seven days for five sampling dates. Within each plot, we chose four sampling sites that were located 5, 10, 15 and 20 m from the yellow traps. From each sampling site, we counted the number of aphids (alatae and apterae) on leaves of 10 plants.

On each sampling date, the contents of yellow traps were collected. All aphids in the traps and on 12 Chinese cabbage leaves from a randomly chosen cabbage plant in each plot were brought back to laboratory and identified under a dissecting microscope. The numbers and species of insects, including natural enemies, were recorded.

2.3. Data processing and statistical analysis

Two-way analysis of variance (ANOVA, general linear model procedure) was used for aphid counts (summed across sampling dates) with treatment and distance as fixed effects. Mean separation tests (Duncan test) were used for all fixed effects. All data were transformed to meet assumptions of normality using $\log_{10}(x+1)$ (SPSS, version 16.0 for Windows). The relationship between the deterrent rate (Y) and distance from the Eβf releaser (x) was determined using cubic regression models: $Y = b_0 + b_1x + b_2x^2 + b_3x^3$, with:

$$Y = \frac{\text{Nck} - \text{Ntr}}{\text{Nck}} \times 100\%$$

Nck is the number of aphids on 10 plants from control and imidacloprid plots, and Ntr is from Eβf and 'Eβf + imidacloprid' plots. Data from control and Eβf plots, and imidacloprid and 'Eβf + imidacloprid' plots were combined as two groups to analyze the deterrent rate. Cubic models were generated using SPSS. With the exception of aphid densities on plants and in traps, the aphid data represented the mean of five samples.

3. Results

3.1. Population dynamics of the two main aphid species

The dynamics of aphid populations over the two years of our experiments appeared similar between the two dominant aphid species (Fig. 1; *M. persicae* and *L. erysimi*), so the data were combined for analysis. *L. erysimi* was predominant from the middle of September to the end of October, but the proportion of *M. persicae* increased as the temperatures decreased (Fig. 1). A few