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Citation for final published version:

Nakashima, Yoshitaka, Ida, Takashi Y., Powell, Wilf, Pickett, John , Birkett, Michael A., Taki, Hisatomo and Takabayashi, Junji 2016. Field evaluation of synthetic aphid sex pheromone in enhancing suppression of aphid abundance by their natural enemies. *BioControl* 61 (5) , pp. 485-496. 10.1007/s10526-016-9734-3

Publishers page: <http://dx.doi.org/10.1007/s10526-016-9734-3>

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Field evaluation of synthetic aphid sex pheromone in enhancing suppression of aphid abundance by their natural enemies

Yoshitaka Nakashima · Takashi Y. Ida · Wilf Powell ·

John A. Pickett · Michael A. Birkett · Hisatomo Taki ·
Junji Takabayashi

Abstract The effects of lures containing aphid sex pheromone components (4aS,7S,7aR)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol on abundance of pea aphid, *Acyrtosiphon pisum* Harris, aphid parasitoids, predators and hyperparasitoids in alfalfa fields were investigated over three years. Although aphid abundance was variable among years, pheromone lure treatment significantly decreased aphid abundance. Among natural enemies of the aphids, only parasitism by the aphid parasitoids *Aphidius ervi* Haliday and *Praon barbatum* Mackauer was affected by pheromone lure treatment, with parasitism rates being significantly increased. In contrast, no pheromone lure effects on abundance were detected for predacious species and hyperparasitoids. These results indicate that slow-release formulations of synthetic aphid sex pheromone can attract primary aphid parasitoids and enhance their ability to suppress aphid abundance in

the field, and that negative effects on biological control by hyperparasitoids and intraguild predation are not promoted by pheromone lure treatment.

Keywords Aphid parasitoid Host location Parasitism Biological control Intraguild predation Hyperparasitism

Introduction

Accumulated knowledge of semiochemicals to aid parasitoids/predators in locating their hosts/prey has created prospects for these chemicals as tools to enhance activity of natural enemies by means of manipulation of parasitoid or predator behaviors (Powell 1986; Lewis and Martin 1990). Several studies have shown that parasitoids (Hardie et al. 1991; Powell et al. 1993; Mizutani et al. 1997; James and Grasswitz 2005; Simpson et al. 2011) and predators (McEwen et al. 1994) were attracted in the field by artificial lures releasing semiochemicals derived from their hosts/prey, suggesting treatment of the crop with appropriate semiochemicals might increase the chance of host/prey encounter rates by parasitoids/predators. Several studies have shown that semiochemical applications increased parasitism rates in the field (Glinwood et al. 1998; Uefune et al. 2012). For example, application of a hexane extract of moth scales or synthetic sex pheromone improved percentage

Y. Nakashima (&) · T. Y. Ida · J. Takabayashi
Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga 520-2113, Japan e-mail: nksm@ecology.kyoto-u.ac.jp

W. Powell · J. A. Pickett · M. A. Birkett
Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

H. Taki
Department of Forest Entomology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

parasitism of *Heliothis zea* eggs by *Trichogramma* species (Lewis et al. 1972). These results suggest that semiochemical application is a promising approach for enhancing biological control, but effectiveness of behavioral manipulation of natural enemies in terms of suppression of pest population has not been fully demonstrated (Mallinger et al. 2013). Thus, population trends of both pests and natural enemies in plots with/without semiochemical application should be evaluated throughout several seasons, because abundance of hosts/prey and parasitoids/predators are variable between years and within seasons.

Enhancing aphid parasitoid activities via behavioral manipulation using synthetic aphid sex pheromone has received significant attention (Powell and Pickett 2003; Birkett and Pickett 2003). The sex pheromones of a number of aphid species comprise (4aS,7S,7aR)-nepetalactone and (1R,4S,4aS,7S,7aR)-nepetalactol (Dawson et al. 1990; Pickett et al. 2013). Attraction of female aphid parasitoids, *Praon* spp., to synthetic aphid sex pheromone components, particularly (4aS,7S,7aR)-nepetalactone was demonstrated by experiment using water traps in cereal fields (Hardie et al. 1991; Powell et al. 1993; Hardie et al. 1994). Behavioral and electrophysiological studies in the laboratory also demonstrated that a range of economically important aphid parasitoids, including *Aphidius*, *Dieaeretiella*, *Ephedrus* and *Praon* species, responded to aphid sex pheromone components (Powell and Pickett 2003; Glinwood et al. 1999b). Glinwood et al. (1998) showed that aphid sex pheromone components increased rates of parasitism of aphids by *Praon volucre* on potted plants. However, the potential of the sex pheromone to stimulate suppression of aphid populations in the field has not been fully determined.

Suppressive effects of parasitoids on aphid populations may be affected by intraguild predators and hyperparasitoids (Rosenheim 1998; Brodeur and Rosenheim 2000). Intraguild predation (IGP), trophic interactions between organisms sharing the same resource, potentially changes the extent to which top-down forces by predator and parasitoid guilds affect herbivore populations. Aphids are associated with a large assemblage of insect natural enemies (Wheeler 1977; Nakashima and Akashi 2005). Furthermore, aphid parasitoids are usually the intraguild prey because parasitized hosts are potentially consumed by predators (Wheeler et al. 1968; Hoelmer et al. 1994; Wells et al. 2001). Primary aphid

parasitoids are also attacked by a broad range of hyperparasitoid species (Sullivan 1988). Hyperparasitism rates are often very high (Sullivan and van den Bosch 1971; Horn 1989), suggesting that hyperparasitoids may decrease the degree of pest suppression exerted by primary aphid parasitoids (Vickerman and Wratten 1979; Dean et al. 1981). Thus, for a better understanding of enhancing biological control by semiochemical application, it is crucial to determine how intraguild predators as well as hyperparasitoids of the target aphid respond to the semiochemicals.

The pea aphid, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae), is an important pest of alfalfa, *Medicago sativa* L., and other leguminous crops in many parts of the world (Cuperus et al. 1982). Aphids are attacked by a large assemblage of insect natural enemies such as aphidiine parasitoids, and coccinellid, syrphid, nabid, anthocorid, chrysopid and carabid predators (Takada 1968; Wheeler 1977; Ekbom 1994; Nakashima and Akashi 2005). Among these natural enemies, the seven-spot ladybird beetle *Coccinella septempunctata* L. (Ekbom 1994) and the parasitoids *Aphidius ervi* Haliday and *Praon barbatum* Mackauer are reported as particularly important natural enemies of *A. pisum* on alfalfa (Senoo et al. 2002; Nakashima and Akashi 2005). These two species of aphid parasitoid are attacked by hyperparasitoids, mainly *Dendrocerus carpenteri* (Curtis) and *Asaphes suspensus* (Nees), and total hyperparasitism rates can become very high (approximately 70–80 %) (Senoo et al. 2002). These two species attack immature aphid parasitoids within mummified aphids (Sullivan 1988).

The aim of this study was to measure the effects of aphid sex pheromone components on aphid abundance, parasitism rates of aphids, aphid predator abundance, and hyperparasitism rates in the field, and so evaluate the enhancing effects of the pheromone components on biological control of *A. pisum*. Field experiments were carried out in two alfalfa fields over three years.

Materials and methods

Aphid sex pheromone lure

Two aphid sex pheromone components, (4aS,7S,7aR)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol, were

formulated into separate flexible plastic polymer ropes (Birkett and Pickett 2003). *Nepeta cataria* L. essential oil [containing approximately 90 % (4aS,7S,7aR)-nepetalactone by GC] and (1R,4aS,7S,7aR)-nepetalactol were obtained from Botanix Ltd (Paddock Wood, Kent, UK), as described previously (Hooper et al. 2002) and formulated into separate plastic polymer ropes (5 % w/w loading; Agrisense-BCS Ltd, Pontypridd, Wales, UK). The sex pheromone of *A. pisum* is composed of a 1:1 ratio of these two components (Dawson et al. 1990). To adjust release rates of the two compounds to a 1:1 ratio, 4 and 8 cm lengths of plastic polymer rope were used as a unit of the pheromone lure for nepetalactone and nepetalac-tol, respectively. The short segments, giving a release rate for each compound of approximately 200 lg per day (Graves et al. Graves 2003) were used in traps in field experiments. Hereafter the unit is referred to as ASPL (aphid sex pheromone lure). Samples of (4aS,7S,7aR)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol used to prepare baits were analyzed by coupled gas chromatography–mass spectrometry (GC–MS) using a capillary GC column (50 m 9 0.32 mm ID 9 0.32 lm film thickness; J&W Scientific) directly coupled to a magnetic sector mass spectrometer (Micromass Autospec Ultima). Ionisation was by electron impact (70 eV, 250 LC).

Study field

Field studies were conducted throughout the 2004, 2005 and 2006 growing seasons in alfalfa fields at Obihiro University of Agriculture and Veterinary Medicine (42L92⁰ N, 143L22⁰ E) in Hokkaido, Japan. Two 4 ha fields separated by ca. 100 m were used (designated A and B). The crop was harvested three times in the season: mid-June, late July and early September. Aphids and their natural enemies are most abundant during the period before the first harvest (early May to mid-June) (Senoo et al. 2002; Naka-shima and Akashi 2005), and so the experiments were conducted during this period.

Sixteen plots (10 9 10 m) were established in each field, arranged as two columns by eight rows, with 40 m separating adjacent plots (both in rows and columns). Sex pheromone lures attached to poles were placed at the center of each treated plot and set at a height of 70 cm. Plots with or without ASPL altered in both rows and columns.

Abundance of aphids, their natural enemies and hyperparasitoids

Samples were taken approximately every four days from early May to the middle of June, with a total of seven sampling occasions each year. On each occasion, ten sweep samples were taken from each plot, using a 40 cm diameter sweep net. Each sweep sample covered a 2 9 5 m area, starting from the side of the plot and moving toward the center. The side of plots from which sweeping was started was randomly chosen on each sampling date. Samples were placed in plastic boxes (28 9 13 9 7 cm), kept in coolers with ice, and transported to the laboratory, where aphids and their natural enemies were sorted and counted.

Aphids collected from each plot were reared on broad bean seedlings, *Vicia fabae* L. (Fabaceae), at the 4 to 6-leaf stage, growing in vermiculite in plant pots (height 7.0 cm, diameter 7.5 cm). Three to four seedlings were grown in each pot, contained in a cylindrical transparent acrylic tube (diameter 8.5 cm, height 25.0 cm). The top of the cage was covered by mesh, thus enclosing the infested seedlings securely. Thirty aphids from each plot were put on plants, but all aphids were reared when the number of collected aphids was less than 30. Plants with aphids were maintained for 12–14 days in a growth room at 20 ± 1 LC and a photoperiod of 16:8 L:D. Parasitism rates of *A. pisum* were estimated from the number of mummified aphids formed during this period.

To estimate hyperparasitism, mummies were collected from 2 9 5 m areas in each plot for 2 min. Collected mummies from each plot were labeled and kept individually in gelatin capsules (No. 00, Eli Lilly, Co) at 20 ± 1 LC and a photoperiod of 16:8 L:D for 40 days. Emerged primary parasitoids and hyperparasitoids were identified. The hyperparasitism rates for each hyperparasitoid species were calculated by dividing the number of emerged hyperparasitoids by the total number of emerged primary parasitoids and hyperparasitoids.

Abundance of carabid beetles

Carabid beetles were sampled using pitfall traps in 2004 and 2005. Each trap consisted of a plastic cup, 6.5 cm in diameter and 9.0 cm deep, half filled with 50 % methanol. A single pitfall trap was placed at the

center of each plot. Pitfall samples were taken from each plot approximately every seven days beginning 15 May, 22 May, 29 May in 2004, and 19 May, 26 May, and 2 June in 2005. All carabid beetles collected were identified to either genus or species. Taxa with less than five individuals collected across the survey were excluded from the analysis.

Data analysis

All analyses were done with generalized linear mixed models [GLMM: Stroup 2013: F test and test of partial regression coefficient (b) = 0, with degree of freedom calculated according to Kenward and Roger (1997)] as implemented with the GLIMMIX procedure of SAS version 9.4 (SAS Institute 2013). Analyses of numbers of individuals (aphids, foliar foraging predators and carabid beetles) and parasitism rates (aphid parasitoids and hyperparasitoids) involved negative binomial distribution (log link function) and binomial distribution (logit link function), respectively, and included $\ln(\text{doy})$ [logarithm of day of year] as a covariate. For analyses of predators and parasitism of aphid parasitoids, $\ln(\text{aphids})$ was also included as a covariate.

We compared numbers of insects and parasitism rates by year [2005 vs. 2006 for carabid beetles and hyperparasitoids (see below), and 2004, 2005, vs. 2006 for others], field (A vs. B), and ASPL (with vs. without ASPL) with orthogonal, planned contrasts ($\alpha = 0.05$). Mixed models were necessary for these analyses because samples of insects were repeatedly obtained on each plot. Therefore, we used GLMM with a model of compound symmetry to account for repeated measurements of individual plots (Fitzmaurice et al. 2004). In all cases, non-significant interactions between the fixed effects and covariates were eliminated from the final model by backward elimination ($\alpha = 0.05$).

In analyses of foliar foraging predators, numbers of different taxonomic groups in each plot were summed before the analysis because of their low density. Additionally, one dominant species, *Coccinella septempunctata* L. was separately analyzed with data in 2004 and 2006. For the analyses of carabid beetles, both total numbers of different taxonomic groups and numbers of each species/genus in each plot were analyzed with the above-mentioned models.

We investigated the hyperparasitism rates with the same statistical model, but hyperparasitoids were

scarce during 2005 and only a small number of hyperparasitoids emerged from mummies of *P. barbatum*. Hence, we analyzed total hyperparasitism rates, which were calculated as division of the total number of emerged *D. carpenteri* and *A. suspensus* by total *A. ervi* and *P. barbatum* combined in each plot. Hyperparasitism rates of *A. ervi* by *D. carpenteri*, which was the dominant hyperparasitoid-aphid parasitoid combination, were also analysed.

To facilitate interpretation, we present results for a particular factor adjusted for the effects of other components of the statistical models. For categorical factors, we present least-squares means and their SE (Milliken and Johnson 1984). To illustrate the effects of a continuous independent variable, we adjusted observations of the dependent variable to account for the effects of other independent variables.

Results

Abundance of aphids and predators

The number of aphids significantly varied with ASPL treatment (Fig. 1a; Table 1), fields and years (Fig. 1b; Table 1). Overall, aphid abundance in plots without ASPL was 32 % more than those with ASPL (Fig. 1a). Furthermore, aphids tended to increase with the observation dates, but differently among fields and years (Table 1). In field A, aphid numbers remained relatively high and constant during 2006 (partial regression coefficient, $b \pm \text{SE} = 0.738 \pm 0.920$, $t_{271.3} = 0.80$, $P = 0.42$), whereas aphids increased over time to a similar extent in 2004 and 2005 (2004: $b = 20.073 \pm 1.143$, $t_{483.6} = 17.56$, $P \setminus 0.001$, 2005: $b = 20.043 \pm 2.559$, $t_{77.92} = 7.83$, $P \setminus 0.0001$). In field B, aphids increased over time more rapidly in 2005 ($b = 18.281 \pm 1.278$, $t_{283.6} = 14.32$, $P \setminus 0.0001$) than in 2004 ($b = 15.606 \pm 1.004$, $t_{529.2} = 15.54$, $P \setminus 0.0001$) and 2006 ($b = 13.165 \pm 1.213$, $t_{323.2} = 10.85$, $P \setminus 0.0001$). Thus, ASPL treatment tended to decrease the aphids irrespective of the differences among fields and years.

Foliar foraging predators, coccinellids (*C. septempunctata*, *Hippodamia tredecimpunctata* Capra, *Propylea quatuordecimpunctata* (L.) and *P. japonica* (Thunberg)) and heteropteran predators (*Orius* spp. and *Nabis stenoferus* Hisao) occurred in both fields.

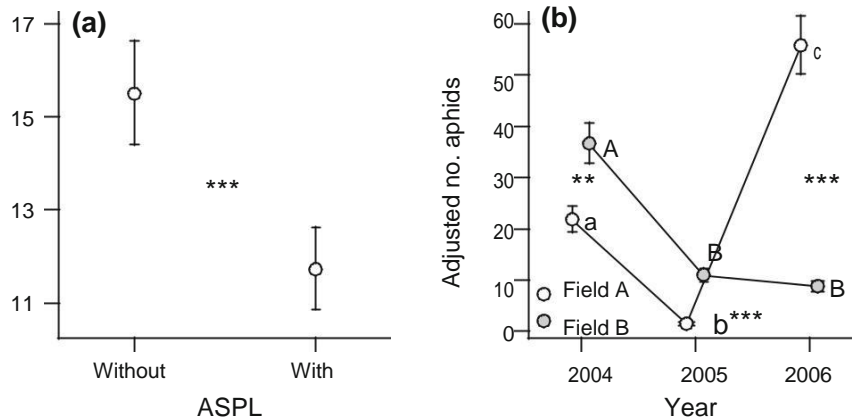


Fig. 1 Mean (\pm SE) number of aphids per plot in response to ASPL treatment (a) and years and fields (b). Asterisks indicate significant difference between ASPL treatments (a) and between fields within each year (b) (** $P \leq 0.001$, ** $P \leq 0.01$). Different letters in (b) indicate significant

difference among years within each field. Lower-case and Upper-case letters represent the field A and B, respectively. Mean values in a and b were adjusted for the variations in effects of other factors in each model

Table 1 Results of generalized linear mixed models of the effects of year, field and aphid sex pheromone lure (ASPL) on the number of aphids, foliar foraging predators and carabid beetles per plot

	Aphids	Foliar foraging predators	Carabid beetles
Year	$F_{2,218.5} = 60.84^{***}$ $F_{1,172.8} = 2.81$	$F_{2,602.1} = 14.09^{***}$ $F_{1,111.8} = 0.22$	$F_{1,128.2} = 13.51^{***}$
Field	$F_{1,101.4} = 8.47^{**}$	$F_{1,112.2} = 0.19$	$F_{1,56.86} = 16.44^{***}$ $F_{1,157.09} = 1.22$
ASPL	$F_{2,218.5} = 34.28^{***}$ $F_{1,296.76} = 1.25$	$F_{2,135.9} = 16.95^{***}$ $F_{1,2109.9} = 0.68$	$F_{1,91.77} = 5.38^*$ $F_{1,156.07} = 2.09$
Year 9 field	$F_{1,101.4} = 0.32$	$F_{1,110.9} = 0.72$	$F_{1,56.12} = 0.05$
Year 9 ASPL	$F_{1,296.76} = 0.79$	$F_{1,2109.7} = 1.15$	$F_{1,56.04} = 1.11$
Field 9 ASPL	$F_{1,177.4} = 603.18^{***}$	$F_{1,648.4} = 1.77$	$F_{1,132.5} = 18.39^{***}$
Year 9 field 9 ASPL	$F_{2,222.7} = 60.86^{***}$	$F_{2,599.5} = 14.40^{***}$	$F_{1,128.4} = 13.15^{***}$
Ln (doy)	$F_{1,177.4} = 3.00$		
Ln (doy) 9 year	$F_{2,222.7} = 32.51^{***}$	$F_{1,581.2} = 0.29$	$F_{1,171.2} = 1.88$
Ln (doy) 9 field			
Ln (doy) 9 year 9 field			
Ln (aphids)			

All of analyses explicitly account for effects of observation days (day of year: doy) and number of aphids by the inclusion of $\ln(\text{doy})$ and $\ln(\text{aphids})$ as a covariate, respectively

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

No chrysopid was collected. In total, 255 predators were collected throughout the survey and the dominant predator, *C. septempunctata*, formed 57.8 % of the total. In contrast to aphids, ASPL did not affect the number of foliar foraging predators (Fig. 2a; Table 1). Predator abundance varied among years and fields with the lowest density during 2005 in field A (Fig. 2b) and these patterns were similar to those of

aphids (Fig. 1a; Table 1). Furthermore, the number of the predators varied over time differently among years (Table 1): they increased in 2004 ($b = 8.692 \pm 2.578$, $t_{632.5} = 3.37$, $P \leq 0.001$) and 2006 ($b = 12.172 \pm 2.207$, $t_{519.0} = 5.52$, $P \leq 0.0001$), whereas they decreased in 2005 ($b = -12.453 \pm 4.398$, $t_{533.5} = -2.83$, $P \leq 0.01$). Aphid abundance did not affect the number of foliar foraging predators

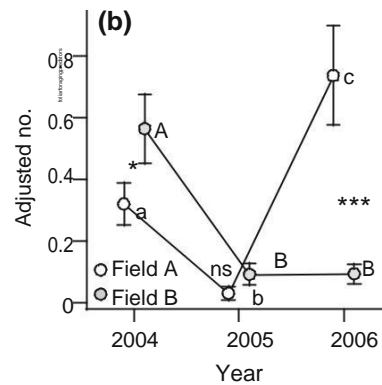
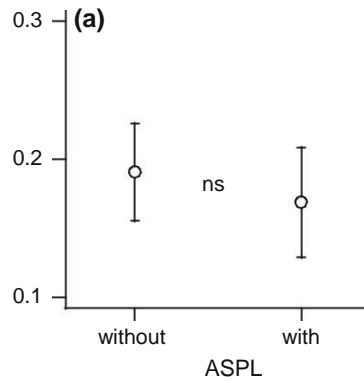


Fig. 2 Mean (\pm SE) number of foliar foraging predators per plot in response to ASPL treatment (a) and years and fields (b). In b, asterisks and different letters indicate significant difference between fields within each year (** $P < 0.001$, * $P < 0.05$) and significant difference among years within each field,

respectively. Lower-case and upper-case letters represent the field A and B, respectively. ns no significant difference between ASPL treatment (a) and between fields within each year (b). Mean values in a and b were adjusted for variations in effects of other factors in each model

(Table 1). These patterns correspond to the trends in numbers of the dominant predator, *C. septempunctata* (Table 2).

Sixteen species of carabid beetles were collected by pitfall traps, but eight species of them were removed from the analysis because of small numbers (less than five; Table 2). In total, 2543 carabid beetles were used for the analysis (Table 1). ASPL treatment did not influence carabid numbers (Table 1). Carabid beetles were more abundant in 2005 (256 % change) and in field A (249 % change) than in 2006 and field B (Table 1), respectively, and their seasonal patterns varied between years (Table 1). During 2005 carabid numbers decreased with observation date ($b = -7.409 \pm 1.406$, $t_{105.5} = -5.27$, $P < 0.0001$), whereas they tended to remain stable over time during 2006 ($b = -0.849 \pm 1.231$, $t_{109.8} = -0.69$, $P = 0.49$). Aphid abundance did not affect the number of carabid beetles (Table 1). The analysis of each taxonomic group, in general, showed similar results to that of total carabid beetles (Table 2).

Parasitism rates of aphid parasitoids and hyperparasitoids

The parasitism rate of aphids by *Aphidius ervi* was 66 % greater in plots with ASPL than in those without ASPL (Fig. 3a; Table 3), and this was more pronounced during late season (Table 3) because parasitism rates decreased over time more rapidly in plots without ASPL ($b = -9.275 \pm 1.844$, $t_{558.2} = -5.30$,

$P < 0.0001$) than in plots with ASPL ($b = -5.164 \pm 1.760$, $t_{554.5} = -2.93$, $P < 0.01$). The variations in parasitism rate by *A. ervi* varied among years and fields (Fig. 3b; Table 3), and these trends were similar to those for aphid abundance (Fig. 1b). Temporal variations also differed greatly among years (2004: $b = -1.999 \pm 1.696$, $t_{565.8} = -1.18$, $P = 0.24$, 2005: $b = -7.107 \pm 4.222$, $t_{548.8} = -1.68$, $P = 0.09$, 2006: $b = -12.554 \pm 1.244$, $t_{544.2} = -10.09$, $P < 0.0001$) and fields (A: $b = -5.067 \pm 1.939$, $t_{553.6} = -2.61$, $P < 0.01$, B: $b = -9.373 \pm 1.864$, $t_{559.5} = -5.03$, $P < 0.0001$). The number of aphids did not affect the parasitism rate by *A. ervi* (Table 3).

The parasitism rate by *P. barbatum* was 51 % greater in plots with ASPL than in those without ASPL (Fig. 3c; Table 3) and differed among fields and years with the greatest difference between fields during 2006 (Fig. 3d; Table 3). The parasitism rate increased over time differently among years (2004: $b = 6.546 \pm 1.743$, $t_{565.8} = 3.76$, $P < 0.001$, 2005: $b = 6.408 \pm 2.474$, $t_{558.4} = 2.59$, $P < 0.01$, 2006: $b = 0.461 \pm 1.434$, $t_{540.9} = 0.32$, $P = 0.75$). Aphid abundance did not affect the parasitism rate by *P. barbatum* (Table 3).

In total, 332 hyperparasitoids, *D. carpenteri* and *A. suspensus*, emerged from *A. ervi* (323) and *P. barbatum* (9) throughout this study. As approximately 78 % (259/332) were *D. carpenteri* emerging from *A. ervi*, the trends in hyperparasitism rates were similar for total hyperparasitoids and for *D. carpenteri* alone (Table 3; Fig. 4). The hyperparasitism rate did not differ between plots with and without ASPL (Fig. 4a,

Table 2 Results of generalized linear mixed models of the effects of year, field and aphid sex pheromone lure (ASPL) on *Coccinella septempunctata* (CS) and carabid beetles per plot^a

	Cs	Pp	Ht	Ai	Ph	Ac	Ase	Bm	Ba
Year	F 1,73.49 = 0.83	F 1,40.50 = 10.48**	F 1,54.97 = 1.59	F 1,68.10 = 16.87***	F 1,73.83 = 1.81	F 1,64.38 = 0.31	F 1,57.78 = 22.82***	F 1,65.36 = 36.40***	F 1,65.67 = 4.00*
Field	F 1,76.27 = 5.46*	F 1,34.32 = 11.78**	F 1,47.45 = 23.32***	F 1,55.45 = 28.18***	F 1,61.13 = 3.61	F 1,53.4 = 0.01	F 1,50.16 = 0.58	F 1,59.45 = 0.87	F 1,58.40 = 16.69***
ASPL	F 1,72.58 = 0.22	F 1,34.56 = 3.23	F 1,47.88 = 2.88	F 1,55.50 = 0.78	F 1,61.53 = 0.99	F 1,53.73 = 0.07	F 1,50.27 = 0.06	F 1,59.73 = 0.12	F 1,59.01 = 0.14
Year 9 field	F 1,86.07 = 14.02***	F 1,54.45 = 38.94***	F 1,71.82 = 12.35***	F 1,89.19 = 0.49	F 1,93.47 = 0.22	F 1,84.90 = 8.18**	F 1,70.89 = 0.61	F 1,75.97 = 0.94	F 1,80.84 = 1.51
Year 9 ASPL	F 1,72.15 = 0.42	F 1,34.05 = 2.27	F 1,47.17 = 0.78	F 1,54.45 = 0.90	F 1,60.44 = 0.00	F 1,52.98 = 1.17	F 1,49.78 = 0.99	F 1,59.27 = 0.01	F 1,58.02 = 0.31
Field 9 ASPL	F 1,71.93 = 0.29	F 1,34.07 = 0.81	F 1,47.19 = 1.48	F 1,54.49 = 0.59	F 1,60.50 = 0.03	F 1,53.00 = 0.64	F 1,49.83 = 0.34	F 1,59.32 = 0.42	F 1,58.09 = 4.22*
Year 9 field 9 ASPL	F 1,72.03 = 0.57	F 1,34.03 = 0.65	F 1,47.14 = 0.02	F 1,54.37 = 0.01	F 1,60.35 = 0.21	F 1,52.90 = 0.96	F 1,49.77 = 0.38	F 1,59.25 = 0.90	F 1,58.00 = 0.61
Ln (doy)	F 1,435.6 = 13.79***	F 1,103.3 = 5.92*	F 1,66.85 = 3.43	F 1,136.6 = 27.56***	F 1,149.9 = 37.17***	F 1,139.1 = 6.52*	F 1,98.01 = 3.72	F 1,113.0 = 17.51***	F 1,125.5 = 9.21**
Ln (aphids)	F 1,429.4 = 0.72	F 1,173.6 = 1.23	F 1,177.3 = 1.58	F 1,167.2 = 0.15	F 1,172.9 = 0.06	F 1,110.5 = 2.57	F 1,134.1 = 0.08	F 1,96.83 = 0.01	F 1,120.2 = 4.80 ^{#2}

All of analyses explicitly account for effects of observation days (day of year: doy) by the inclusion of ln(doy) as a covariate. *Coccinella septempunctata* L. and carabid beetles were analyzed with data in 2004 and 2006 and data in 2005 and 2006, respectively. *Anisodactylus signatus* (Panzer), *Pterostichus samurai* (Lutshnik), *Hemicarabus tuberculatus* Dejean et Boisduval, *Campalita chinense* Kirby, *Loricera pilicornis* Fabricius, *Chlaenius pallipes* Gabler, *Pterostichus adstrictus* Eschscholtz and *Agonum chalconum* Bates were removed from the analysis because of small number of collected individuals (5)

^a Cs *Coccinella septempunctata* L., Pp *Pterostichus* (*Poecilus*) *planicollis*, Ht *Hemicarabus tuberculatus*, Ai *Agonum impressum*, Ph *Pterostichus haptoderoides*, Ac *Amara chalcites*, Ase *Asaphidion semilucidum*, Bm *Bembidion morawitzi*, Ba *B. articulatum*

^b Partial regression coefficient; $b \pm SE = -0.190 \pm 0.087$, $t_{120.2} = -2.19$, $P \setminus 0.05$

*** $P \setminus 0.001$; ** $P \setminus 0.01$; * $P \setminus 0.05$

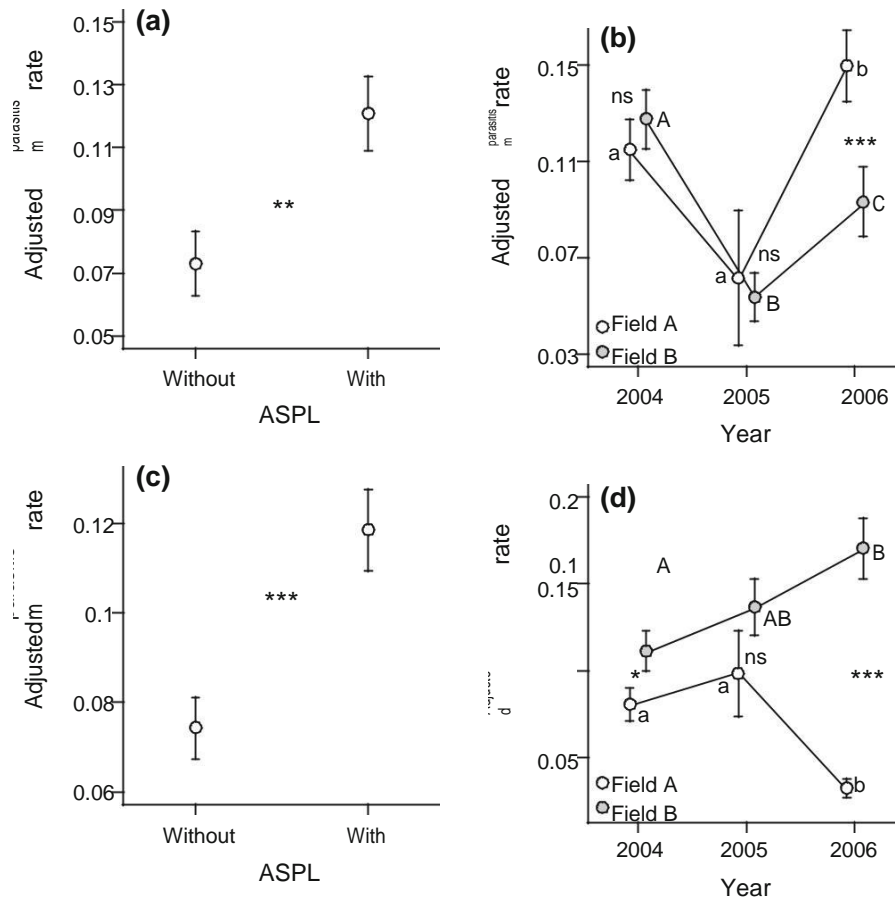


Fig. 3 Mean (\pm SE) proportion of aphids parasitized by *Aphidius ervi* (a, b) and *Praon barbatum* (c, d) per plot in response to ASPL treatment (a, c) and years and fields (b, d). Asterisks indicate significant difference between ASPL treatments (a, c) (*** $P \leq 0.001$, ** $P \leq 0.01$). In (b, d), asterisks and different letters indicate significant difference between

fields within each year (*** $P \leq 0.001$, * $P \leq 0.05$) and significant difference among years within each field, respectively. Lower-case and upper-case letters represent the field A and B, respectively. ns no significant difference between fields within each year (b, d). Mean values in a, b, c and d were adjusted for variations in effects of other factors in each model

c; Table 3), whereas it differed between years and fields with the lowest rate during 2006 in field B (Fig. 4b, d; Table 3), and increased over time more rapidly during 2006 than 2004 (2004: $b = 6.985 \pm 2.336$, $t_{173.5} = 2.99$, $P \leq 0.01$, 2006: $b = 29.160 \pm 2.550$, $t_{171.3} = 11.44$, $P \leq 0.0001$).

Discussion

It was clear that aphid sex pheromone lures (ASPL) increased parasitism of *A. pisum* by *A. ervi* and *P. barbatum*. It has previously been demonstrated in the laboratory (Glinwood et al. 1999a, b) and in semi-field experiments (Glinwood et al. 1998) that aphid

parasitoids, including *A. ervi*, are attracted to aphid sex pheromone components. Additionally, much greater declines in aphid abundance occurred in plots with ASPL than in those without ASPL. To our knowledge, this is the first demonstration of enhancing biological control in terms of suppression of pest population via behavioral manipulation using ASPL in the field. Numbers of aphids and parasitism rates by primary parasitoids varied among years, observation dates within each year and fields. However, ASPL effects on parasitism rates and aphid abundance were not affected by these temporal and spatial differences, suggesting the behavioral manipulation of aphid parasitoids by ASPL has robust enhancing pest suppression effects.

Table 3 Results of generalized linear mixed models of the effects of year, field and aphid sex pheromone lure (ASPL) on parasitism rates by *Aphidius ervi* and *Praon barbatum* and hyperparasitism rates per plot

	Parasitism rate		Hyperparasitism rate	
	<i>Aphidius ervi</i>	<i>Praon barbatum</i>	Total	<i>Dendrocerus carpenteri</i>
Year	F _{2,549.5} = 12.52***	F _{2,562.2} = 4.87**	F _{1,172.5} = 41.19*** F _{1,139.6} = 2.50	F _{1,135.2} = 8.68** F _{1,143.9} = 2.21
Field	F _{1,543.5} = 4.62* F _{1,531.0} = 6.51*	F _{1,143.2} = 52.53*** F _{1,141.9} = 19.54***	F _{1,139.6} = 0.54 F _{1,134.5} = 0.54	F _{1,143.9} = 0.53 F _{1,134.4} = 2.85
ASPL	F _{2,164.6} = 3.25* F _{2,133.0} = 0.65	F _{2,164.0} = 16.63*** F _{2,129.5} = 2.44	F _{1,139.6} = 5.80* F _{1,134.5} = 0.61	F _{1,143.9} = 0.11 F _{1,134.4} = 0.58
Year 9 field	F _{1,169.1} = 2.33 F _{2,132.6} = 0.30	F _{1,140.2} = 0.13 F _{2,129.1} = 0.70	F _{1,135.2} = 0.55 F _{1,135.2} = 2.80	F _{1,136.2} = 0.58 F _{1,136.2} = 2.05
Year 9 ASPL	F _{1,560.4} = 19.77*** F _{2,548.7} = 12.56***	F _{1,564.7} = 13.24*** F _{2,561.0} = 5.02**	F _{1,172.3} = 109.25*** F _{1,172.3} = 41.12***	F _{1,135.1} = 47.97*** F _{1,135.1} = 8.50**
Field 9 ASPL	F _{1,542.4} = 4.73* F _{1,530.1} = 6.92**	– –	– –	– –
Year 9 field 9 ASPL	F _{1,520.7} = 3.41	F _{1,533.5} = 0.20	–	–
Ln (aphids)				

*** P \ 0.001; ** P \ 0.01; * P \ 0.05

Many aphid species, including *A. pisum*, pass through a sexual phase in the autumn and the sexual female attracts the winged male by releasing a sex pheromone consisting of the two main chemical components used in our study. *Aphidius* and *Praon* species enter diapause and overwinter as larvae in mummified aphids (Polgár and Hardie 2000). Thus, responses to these components for the location of aphid colonies late in the season would be important for parasitoid adaptiveness. On the other hand, aphid predators, both foliar foraging predators and epigeal carabid beetles, do not need to locate aphids in order to overwinter successfully. Thus, responses to aphid sex pheromones may not have evolved in aphid predators. Aphid hyperparasitoids also overwinter in mummies, but for mummy hyperparasitoids like *D. carpenteri* and *A. suspensus*, which attack immature aphid parasitoids after mummy formation, aphid sex pheromone components are not likely to be direct host location cues. So far, limited evidence that aphid sex pheromone components attract aphid predators has been reported (Birkett and Pickett 2003). One exception is for lacewings, but only male lacewings were attracted, suggesting these compounds or analogous structures may play a pheromonal role in intra-species sexual communication and that their similarity to aphid sex pheromones may be simply incidental (Koczor et al. 2010). Little is known about foraging

process of hyperparasitoids (Sullivan and Vo'lk 1999; but see Poelman et al. 2012). Olfactory cues for host location have not been determined for *D. carpenteri* and *A. suspensus* (Buitenhuis et al., 2005).

Recently, direct effects of ASP on asexual female aphids themselves are suggested (Fernandez-Grandon et al. 2013). They found that virginoparae of *Myzus persicae* (Sulzer) are repelled in y-tube olfactometer assays only when using a very high concentration of nepetalactone (10 mg ml⁻¹). The dose used in their five-minute olfactometer assays was 10 lg, whereas the dose released from our slow-release formulation in a similar 5-minute period was calculated to be 0.7 lg (200 lg per day; Birkett and Pickett 2003). In the Fernandez-Grandon (2013) paper, it was shown that a broadly similar dose to ours (1 lg) was not behaviorally active in olfactometer assays, and so we did not expect to get a direct repellent effect versus aphids in our field experiments. This expectation was also supported by the fact that, in the Fernandez-Grandon (2013) paper, population growth of *M. persicae* was not affected by the compound in their laboratory experiments. Also, our experiments show that ASP increased parasitism rates of two aphid parasitoids, supporting the hypothesis that the indirect effect of ASP via enhancing parasitoid activity is important in suppression of aphid abundance in the field.

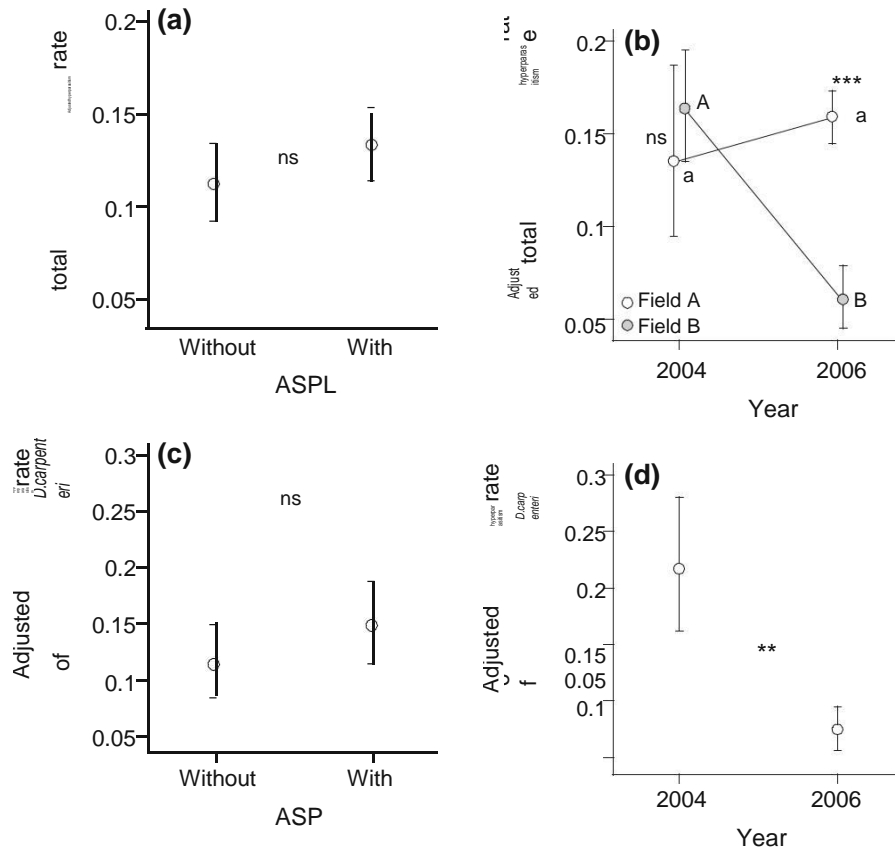


Fig. 4 Hyperparasitism rates for total hyperparasitoids (a, b) and *Dendrocerus carpenteri* (c, d). Mean (\pm SE) proportion of hyperparasitism per plot in response to ASPL treatment (a, c) and years and fields (b, d) is represented. Asterisks indicate significant difference between fields within each year (b) and years (d) (** $P < 0.001$, ** $P < 0.01$). Different letters indicate

significant difference between years within each field (b). ns no significant difference between ASPL treatment (a, c) and between fields within each year (b). Lower-case and upper-case letters represent the field A and B, respectively. Mean values in a, b, c and d were adjusted for variations in effects of other factors in each model

Intraguild predation and hyperparasitism may decrease the effectiveness of pest suppression by insect parasitoids (Rosenheim 1998). Aphid parasitoids are attacked by various predators (Brodeur and Rosenheim 2000) and hyperparasitoids (Senoo et al. 2002), and this may decrease the extent to which top-down forces by aphid parasitoids affect herbivore populations. Our field experiments showed that the ASPL increases only parasitism rates of primary aphid parasitoids and can enhance suppression of aphid densities in the field, but negative effects on biological control by hyperparasitoids and IGP would not be promoted by ASPL treatment.

ASPL comprises a potentially useful tool to enhance natural control of pest aphids which often fails because of annual variability of parasitoid and

aphid abundance, and a lack of synchrony between aphid and parasitoid populations. In this study, although ASPL was provided in fields in initial and growing crop season, additional use in autumn in managed grass/wild flower field margins with the aim of establishing overwintering reservoirs of parasitoids (Powell 1986; Powell and Pickett 2003) should be confirmed. Importance in the timing of ASPL deployment as well as effective area of ASPL for aphid suppression should be studied to help elucidate a system development to use ASPL in commercial fields. Further studies on determining combined effects of the ASPL with floral resource supplementation (Simpson et al. 2011) and other semiochemicals for the manipulation of pests and parasitoids (Pickett et al. 2013) are needed.

Acknowledgments We would like to thank the laboratory students of Obihiro University of Agriculture and Veterinary Medicine for field survey assistance. We have also appreciated the support from the staff at the University farm. This study was partially founded by Grants-in-Aid for Regional R&D Proposal-Based Program from Northern Advancement Center for Science

& Technology of Hokkaido, Japan. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom.

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- Yoshitaka Nakashima is interested in behavior and ecology of pest insects and their natural enemies in agricultural landscapes.
- Takashi Y. Ida studies the ecology and evolution of plant reproductive strategy, with focuses on the mutualism between plants and insects.
- Wilf Powell is an applied entomologist with interests in the foraging behavior of insect parasitoids and predators and in arable farmland ecology.
- John A. Pickett is a biological chemist having pioneered many pheromone identification studies and now interested particularly in plant–insect interactions.
- Michael A. Birkett is a biological/organic chemist working on the isolation, identification and delivery of semiochemicals involved in plant/pest and plant/plant interactions.
- Hisatomo Taki is interested in the biodiversity and ecosystem services in agricultural and forest landscapes.
- Junji Takabayashi is a chemical ecologist studying interaction networks in ecosystem.