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Field evaluation of CLB as an attractant for beneficial parasitoid wasps attacking mealybug pests

Yuma Sugawara and Takatoshi Ueno

Abstract
Cyclolavandulyl butyrate (CLB, thereafter) is a chemical compound discovered by chance during the synthesis process of the sex pheromone of a serious pest mealybug, *Planococcus kraunhiae*. Notably, it is known that CLB strongly attracts natural enemy parasitoid wasps such as *Anagyrus* spp. (Hymenoptera: Encyrtidae) attacking the pest mealybug. However, it was not clear what species of *Anagyrus* parasitoids were exactly attracted with CLB trapping, partly due to taxonomic problems. In the present study, we first confirmed whether CLB attracted *Anagyrus* spp. and then examined the species composition of the parasitoids in relation to localities, seasons and habitat types. Our surveys showed the attraction of *A. subalbipes* and *A. sawadai* to CLB traps; both species occurred widely and commonly in orchards though the former was much more abundant in traps. They were few in early spring but increased the populations as the season went on. *A. sawadai* showed a habitat preference to the cedar tree shelterbelt but *A. subalbipes* was mostly found in perssimon and apple orchards. We discuss the usefulness of CLB trapping systems in monitoring beneficial parasitoids in IPM of pest mealybugs.

Keywords: Seasonal prevalence, natural enemy attractant, cyclolavandulyl butyrate, *Anagyrus subalbipes, Anagyrus sawadai*

Introduction

Insect parasitoids use chemical cues such as synomones and kairomones for locating their potential host [1, 2]. Herbivore-induced plant volatiles (HIPVs) are such a synomone that is produced by the host plant in response to herbivore attack. Also, host-associated volatiles (HAVs) emitted by the hosts, such as host sex pheromones and aggregation pheromones, are among the major kairomones [3, 4, 5, 6]. Because HAVs directly indicates the presence of host, many parasitoids commonly use them for host location. In other word, such HAVs can strongly attract the associated parasitoids [7, 8, 9]. Because of this, HAVs have been applied as a tool for monitoring beneficial parasitoids and/or enhancing their activity in integrated pest management programs [10, 11, 12].

Planococcus kraunhiae, a mealy bug species commonly found in orchards, is a serious pest of fruit trees such as Japanese persimmon [13]. Because chemical control often does not work sufficiently, integrated pest management has recently been developed to control this mealybug pest [13]. Conservation biological control with the native parasitoid complex is one of the key elements for the successful management. Recently, a sex pheromone of *P. kraunhiae* has been identified and applied to its trap monitoring system and to pheromone-based mating disruption control of this pest. The mealybug sex pheromone also attracts its parasitic wasps: *Anagyrus fujikona* (Hymenoptera: Encyrtidae) [14]. Furthermore, a cyclization product of the sex pheromone, (-)-cyclolavandulyl butyrate (CLB, thereafter), was discovered to attract other mealybug parasitoid wasps including the members of the genus *Anagyrus* [15]. It is well known that *Anagyrus* parasitoids include a number of natural enemies of pest mealybugs, some of which are at least significantly beneficial in mealybug control. Thus, *Anagyrus* parasitoids appear to use host sex pheromones or similar chemicals as HAVs to locate their mealybug hosts.

However, *Anagyrus* is a taxonomically confusing group and the exact identification of the species is often difficult. Although it is known that CLB notably attracts *Anagyrus* parasitoids, it is not known so far what species in the genus are included in the trapped parasitoids. We have previously demonstrated that there are two species of *Anagyrus* parasitoids in trapped specimens (Sugawara *et al*., in revision). One was *A. subalbipes* Ishii and the other is *A.*

sawadai Ishii, which had been considered to be synonymous with the former [16, 17]. However, Japoshvili *et al*. (2016) [18] and our previous study (Sugarawa *et al*., in revision) suggested that the two parasitoids are distinctly different species.

Thus, CLB can lure these two *Anagyrus* parasitoids but its attractiveness to each species is unknown. In addition, there are no information or data on the seasonal prevalence and habitat preference of the two parasitoids. For the application of CLB in integrated pest management of the mealybug, it is required to examine its attractiveness to each *Anagyrus* species in detail. In the present study, therefore, we conducted field surveys in order to investigate the efficiency of CLB to attract the two *Anagyrus* species in relation to seasonality and habitat type. Our study will help evaluate potential of two parasitic wasps as biological control agents.

Materials and Methods

Attractant and trap designs

We prepared and purified a racemic mixture of CLB following the method of Tabata *et al*. (2011) [15]. The compound (0.160mg) was dissolved into 0.1ml of hexane and was then impregnated into rubber septa (2 mm outside diameter × 20 mm length; Shin-Etsu Chemical Co., Tokyo, Japan.), which were used as an attractant. To monitor the parasitic wasps, one white delta trap (SE trap; 29 × 33 × 9 cm; Sankei Chemical Co., Kagoshima, Japan) with a white sticky board (10 × 20 cm; Sankei Chemical Co.) baited with a CLB attractant was hung in the field ca. 1.2 m above the ground. The sticky boards were collected and replaced weekly to investigate the number of parasitoids trapped. Only in Fukuoka Prefecture, a funnel trap (Agrisense BCS LTD, Glamorgan, UK) baited with the CLB attractant was used. The monitoring of captured parasitoid wasps was thus conducted at intervals of 7 days. The CLB attractant was replaced monthly.

Habitat types and seasonality

Surveys were made in 2015, 2017, and 2018. We monitored the parasitoids from May to October using the trapping system mentioned above. To investigate the effect of habitat types on the two parasitoid species, eight localities were selected in Japans: Aomori Prefecture (Botandaira, Kuroi-shi, Apple Research Center, peer orchard), Nagano Prefecture 1 (Ushimaki, Shimoina-gun, Takamori-machi, persimmon orchard), Nagano Prefecture 2 (Ogawara-chô, Suzaka-shi, apple orchard), Yamanashi Prefecture (Ezohara, Yamanashi-shi, Yamanashi Fruit Tree Experiment Station, grape orchard), Gifu Prefecture (Nonnou-chô, Kaidu-shi, persimmon orchard), Tottori Prefecture (Yurashuku, Touhaku-gun, Hokuei-chô, Tottori Prefecture Horticultural Research, Center, cherry orchard), Yamaguchi Prefecture (Ouchinagano, Yamaguchi-shi, Yasmaguchi Prefectural Agriculture & Forestry General Technology Center, cedar green belt).

Table 1: Sampling localities and habitat types

Localities	Habitat types	Years	Periods
Aomori (40.6˚N, 140.6˚E)	Pear	2017	May - October
Nagano 1 (35.5˚N, 137.8˚E)	Persimmon	2017	May - October
Nagano 2 (36.6˚N, 138.3˚E)	Apple	2017	May - October
Yamanashi (35.7˚N, 138.6˚E)	Grape	2017	May - October
Gifu (35.2˚N, 136.6˚E)	Persimmon	2015	May - August
Tottori (35.4˚N, 133.7˚E)	Cherry	2017	May - October
Yamaguchi (34.1˚N, 131.5˚E)	Cedar	2018	June - November
Fukuoka (33.5˚N, 130.5˚E)	Cedar, persimmon	2018, 2019	May - November

The locations and corresponding geographic coordinates are presented in Table 1. In all sampling sites except for in Yamaguchi, the field surveys were conducted in sprayed fruits orchards. In Yamaguchi, we hung the traps on cedar trees in the shelterbelt around persimmon orchards. In our preliminary observations, the infestations of mealybugs were not severe in all sampling sites. After collection of sticky boards, we carefully checked the parasitoid wasps trapped on the boards under a binocular microscope and identified each attracted wasp to the species level. After that, we counted the numbers of parasitoids that had been trapped. To identify whether the wasp was *A. sawadai* or *A. subalbipes*, we used the character of scape in the antenna, which was a reliable index for identification (Sugawara *et al*., in revision).

Habitat type

In Fukuoka prefecture (Yoshiki, Chikushino-shi, Fukuoka Agriculture and Forestry Research Center), we also conducted field sampling in different habitat conditions to examine how habitat types affected the trapping system in the same sampling site. We settled one funnel trap in a persimmon tree in a persimmon orchard, and another trap was placed in a cedar tree in the shelterbelt around the orchard. Traps were hung in the field at intervals of 100 m. This field monitoring was replicated two years, *i.e*., 2018 and 2019; the traps were set on the same trees in the two years survey.

Statistical analyses

We statistically analyzed the numbers of wasps captured per one month with a logarithmic Poisson generalized linear mixed models (GLMMs). The effect of habitat types was analyzed using parasitoid species and habitat types as explanatory variables and month (= season) as a random variable. When the seasonal prevalence was analyzed, we used parasitoid species and month as explanatory variables and prefecture as a random variable. Familywise errors associated with multiple comparisons were controlled with Holm's method or Holm-Bonferroni sequential correction method [19]. The calculation was performed by the function "glmer" of the program package lme4 in the software R version 3.6.0 [20].

Results

Habitat types and seasonality

In the present study, 70 trap samples were obtained, and the samples included 4114 encyrtid parasitoid wasps in all. Then, we first confirmed that both *A. subalbipes* and *A. sawadai* were captured in our trapping systems, and other members of the genus were not detected. The monthly mean numbers of *Anagyrus* parasitoids that had been trapped varied greatly depending on habitat types (Fig. 1). This was particularly true for *A. subalbipes*, and the mean numbers of capture were much greater than *A. sawadai*, regardless of the habitat types (Fig. 1; df = 1, LR-χ = 1272.6, *P*<0.0001)). Habitat types have a significant effect on the numbers of *Anagyrus* parasitoids attracted (df = 5, LR-χ = 1687.1, *P*<0.0001). In *A. subalbipes*, the numbers of capture were larger in the order of persimmon > apple > cherry = grape = cedar > pear (Fig. 1; *P*<0.05). In *A. sawadai*, the numbers of capture were larger in the order of cedar $\geq$ persimmon $\geq$ apple > cherry > grape > pear (Fig. 1; *P*<0.05).

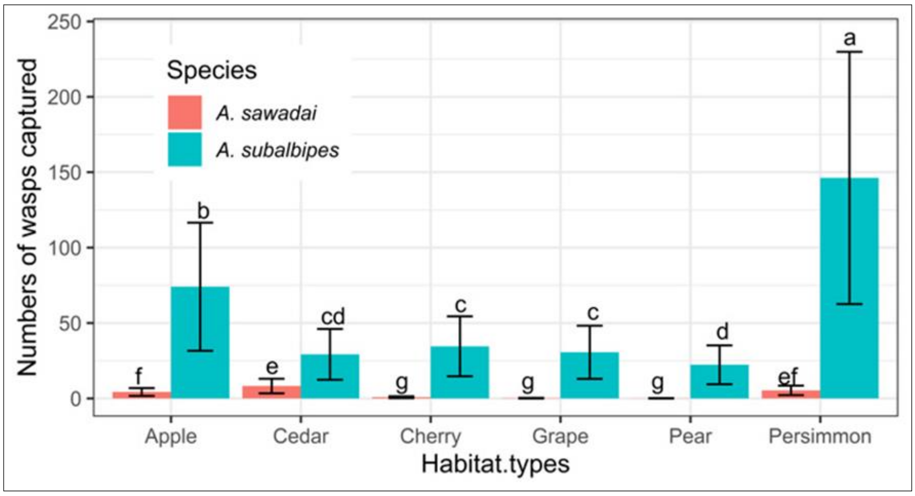

Fig 1: The mean numbers of trapped wasps in relation to habitat types. Lines above bars indicate standard errors predicted by Poisson GLMM. Different letters on lines show significant differences among the groups (*P*<0.05).

Also, the mean numbers of captured parasitoids varied significantly depending on the months (df = 4, LR-χ = 1127.2, *P*<0.0001). In *A. subalbipes*, the numbers of capture were higher in the order of August > September > July = June > May (Fig. 2; *p*<0.05). In *A. sawadai*, the order was same (Fig. 2; *P*<0 .05). Except for May, the numbers of *A. subalbipes* were much higher than *A. sawadai*.

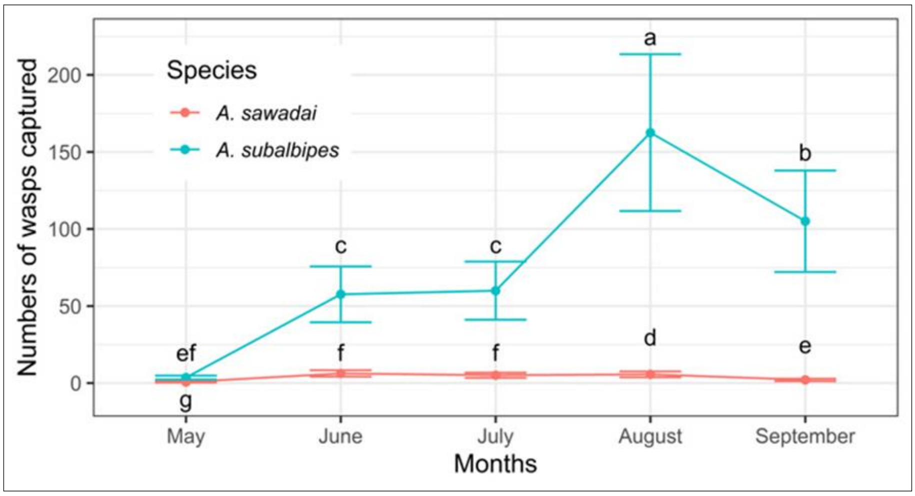

Fig 2: Relationship between the months and the numbers of captured wasps. Means and the standard errors predicted by Poisson GLMM are shown. Different letters on bars indicate significant differences among the groups (*P*<0.05).

Habitat type in the same sampling site

Even within the same sampling site, the habitat type was a highly significant factor affecting the monthly numbers of parasitoids trapped (Fig. 3; df = 1, LR-χ = 62.2, *P*<0.0001). The mean numbers of *A. subalbipes* were much larger in persimmon orchards than in windbreak cedar belts. On the other hand, the numbers of *A. sawadai* were rather abundant in the cedar belts than in persimmon orchards. In both habitats, the numbers of *A. subalbipes* were much greater than those of *A. sawadai* (Fig. 3; df = 1, LR-χ = 255, *P*<0.0001).

Discussion

In the present study, we first confirmed that two *Anagyrus* parasitoid wasps, *A. subalbipes* and *A. sawadai*, were captured in the trap system used here, and the total numbers of capture were fairly great, demonstrating that the trap system with CLB was effective in attracting these mealybug natural enemies. Second, we revealed that *A. subalbipes* was much more abundant species than *A. sawadai*. In the previous studies, CLB has been concluded to attract mainly *A. sawadai* [13, 14, 21]. However, as shown in the present study, CLB attraction is much stronger for *A. subalbipes*. When the above mentioned previous studies had been carried out, there were taxonomic confusions between the two parasitoids, due to morphological similarities. Later, we made a series of molecular-based and morphological-based, detailed studies of the two parasitoid species and found that *A. sawadai* included a cryptic species, *A. subalbipes* (Sugawara *et al*., in revision). Thus, CLB traps can attract two different *Anagyrus* species with *A. subalbipes* more abundantly captured, regardless of sampling habitat types and seasons.

Although *A. subalbipes* was much more abundant, both *Anagyrus* parasitoids were common and cohabit in orchard environments of Japan (Fig.1). This suggests that the two parasitoids can play an important role in suppressing pest mealybug populations in the orchard. If this is the case, CLB trapping systems can be useful in monitoring these parasitoid species to assess in what degree the conservation biological control or IPM program can work in the orchard.

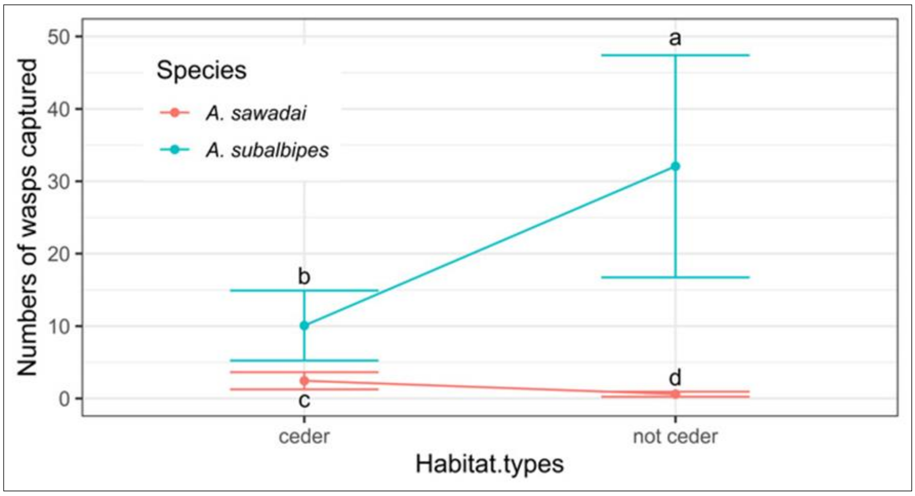

Fig 3: Habitat effect on the numbers of wasps captured per month. Means and the standard errors predicted by Poisson GLMM are shown. Different letters on bars indicate significant differences among the groups (*P*<0.05).

In our study, both parasitoids were rather rare in May but they gradually increased in number as the season went (Fig. 2). We do not know what factors are involved in the seasonal prevalence of the parasitoids. The parasitoids may migrate from the outside of orchards in early spring and then build their populations inside the orchards to increase the number later in the season. Alternatively, they are simply low in number in the orchard because the host mealybugs are also low in density in early spring and then increase their numbers as host abundance increases with the season. Host-parasitoid population dynamics will be a subject addressed in future studies.

We found that the environment around traps also affected the proportion of the attracted numbers of parasitoids, even when each trapping site was close to each other. Notably, the numbers of trapped *A. sawadai,* which was found much less abundant than *A. subalbipes,* were much higher when the traps were set on cedar trees inside the shelterbelt than when in the orchard. Host records suggests that *A. sawadai* prefers, as the host, *Atrococcus flavidus* (Kanda), which infests cedar trees [22]. We therefore suppose that *A. sawadai* is abundant in the environment where cedar trees (and *A. flavidus*) are abundant, and, hence, *A. sawadai* is more likely to be attracted to the CLB traps set on the cedar tree in the shelterbelt than in the orchard. If this is the case, this parasitoid may have a minor role in suppressing pest mealybugs in the orchard. Thus, we consider that *A. subalbipes* can be the principal biological control agent in Japanese persimmon orchards.

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