Long-Term Field Trial to Control the Invasive Argentine Ant (Hymenoptera: Formicidae) With Synthetic Trail Pheromone

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ABSTRACT Previous short-term experiments showed that trail following behavior of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), can be disrupted by a high concentration of synthetic trail pheromone component (Z)-9-hexadecenal. In this study, a long-term field trial was conducted in 100-m^2 plots of house gardens in an urban area of Japan to see whether the control effect on Argentine ants can be obtained by permeating synthetic trail pheromone from dispensers. The dispensers were placed in the experimental plots during the ant's active season (April–November) for 2 yr with monthly renewal. To estimate Argentine ant population density, foraging activity of Argentine ants in the study plots was monitored by monthly bait surveys. Throughout the study period, Argentine ant foraging activity was suppressed in the presence of the dispensers, presumably via trail forming inhibition. In contrast, the level of foraging activity was not different between treatment and no-treatment plots when the dispensers were temporarily removed, suggesting that treatment with pheromone dispensers did not suppress Argentine ant density in the treatment plots. Population decline may be expected with larger-scale treatment that covers a significant portion of the ant colony or with improvement in the potency of the disruptant.

KEY WORDS Argentine ant, *Linepithema humile*, synthetic trail pheromone, (Z)-9-hexadecenal, control

Invasive alien ants are well adapted to disturbed habitats and have become significant urban pests around the world (Holway et al. 2002). They are not only nuisance pests that frequently intrude into structures but also agricultural pests that cause outbreaks of homopterans and they cause damage to ecosystems by displacing native species from diverse taxa. Control of invasive ants with toxic baits, insecticides, or both is very difficult, primarily because of their characteristic of forming large-scale colonies (supercolonies) (Silverman and Brightwell 2008). For effective control, treatment with toxic baits, insecticides, or both should be areawide corresponding to supercolony size, but in most cases it is virtually impossible to apply toxic agents over wide-ranging habitats from the viewpoint of human and animal health, environmental conservation, and economics. Local treatment within a supercolony may give a temporary effect in the treated area, but there will be immediate reinfestation by immigration of ants from the surrounding untreated area.

Recent studies on the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), proposed the use of synthetic trail pheromone as a novel and species-specific control agent for pest ants (Tatsuki et al. 2005; Tanaka et al. 2008, 2009; Suckling et al. 2008). The Argentine ant is a representative of invasive ants (Sunamura et al. 2010), which has spread from South America to many parts of the world (Suarez et al. 2001, Wetterer et al. 2009). The major component of its trail pheromone is (Z)-9-hexadecenal (Z9-16:Ald) (Cavill et al. 1979, 1980). This compound is virtually nontoxic to most organisms when used as mating disruptant: it has long been used for mating disruption of Asiatic rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) (Tatsuki 1990, 2009). So far, inducement to artificial trails (e.g., Van Vorhis Key and Baker 1982) and mixing with liquid baits and consequent enhancement of recruitment to the baits (Greenberg and Klotz 2000) have been suggested as the uses of Z9-16:Ald in controlling Argentine ant. Recent studies have started to target trail following behavior of the Argentine ant and have been attempting to use synthetic Z9-16:Ald as a disruptant of this behavior (Tatsuki et al. 2005).

We confirmed in 2003 the trail pheromone activity of synthetic Z9-16:Ald on Argentine ant workers that had colonized in an urban district of western Japan, and we observed that their trail following behavior was greatly disrupted when a high concentration of

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the synthetic compound was applied (Tatsuki et al. 2005, Tanaka et al. 2008). Furthermore, short-term (4-d) treatment with polyethylene tube dispensers containing synthetic Z9–16:Ald over a 100-m^2 field largely suppressed worker recruitment to baits (Tanaka et al. 2009). Similar results were reported independently with Argentine ants in a natural ecosystem in Hawaii by using synthetic Z9–16:Ald formulated as short-life pheromone carriers (Suckling et al. 2008, 2010). Based on these data, we expected that a long-term treatment with synthetic trail pheromone would make Argentine ants run short of food and, therefore, decrease their density.

Here, we conducted for the first time a long-term field treatment with long-life synthetic trail pheromone dispensers to test whether 1) the foraging suppression effect lasted for a long period, and 2) the effect lead to local population decline by using relatively small treatment plots in a large outbreak area. Dispensers were applied to 100-m^2 plots of urban house gardens during the active season of Argentine ants (spring to autumn) for 2 yr. Argentine ant foraging activity in the study plots was monitored monthly 1) to determine foraging suppression by the dispensers and 2) to estimate change in population densities.

Because our present trial was conducted mostly to evaluate the effectiveness of a novel control method, labor and economical cost were largely neglected. Development of dispenser type more suitable for ant control and of treatment method will give more economical as well as more effective control of the ants.

Materials and Methods

Study Sites. The experiment was conducted in an urban district of Iwakuni City, Yamaguchi Prefecture, western Japan, which we had visited regularly and had carried out various investigations on Argentine ant biology and synthetic trail pheromone since 2003 (Tatsuki et al. 2005; Nishisue et al. 2006; Terayama et al. 2006; Tanaka et al. 2008, 2009). In this study, we had limited availability of experimental fields but could use three private kitchen gardens with the consent of the owners. The gardens were located in the central part of the infested area, where Argentine ants were most abundant and had almost displaced native ants (Nishisue et al. 2006, Terayama et al. 2006) and were separated from each other by >150 m. A 10- by 10-m experimental plot was set for each garden. The plot size is very small compared with the foraging range of the Argentine ant, which may exceed 61 m (Markin 1968, Ripa et al. 1999, Vega and Rust 2003). The three experimental plots were named P-1, C-1, and C-2. In 2005, P-1 was used as a treatment plot and C-1 was used as a no-treatment plot. In 2006, P-1 was again used as a treatment plot. In this year, we initially used C-1 as a no-treatment plot but were compelled to change the no-treatment plot to C-2 in May, because pesticide was applied to C-1 in May and Argentine ants were temporally eliminated from the plot. Otherwise, there was no other case of pesticide treatment in the experimental plots.

Pheromone Dispensers. "Rope" type pheromone dispensers were used (Shin-Etsu Chemical, Tokyo, Japan; see details in Tanaka et al. 2009). Each dispenser was a closed 20-cm-long polyethylene tube and contained 75–80 mg of synthetic Z9–16:Ald. The lifespan of each dispenser was >1 mo (the manufacturer guaranteed 2 mo based on data for usual moth disruption, but our preliminary experiments on Argentine ants showed that in some cases the effectiveness decreased by 2 mo). Dispensers were applied to the treatment plot from 20 April to 4 November in 2005 and from 29 April to 2 November in 2006, with monthly renewal. The treatment period covered the active season of the Argentine ant in Japan (early spring to late autumn; Terayama 2008). The dispensers were tied to plastic poles at ≈40 cm above ground level. Although placement of the dispensers to a lower position may have given more pronounced disruptive activity, it might have caused severe damage to the dispensers with mud and dust within a short period. The dispensers were deployed every 1 m (121 dispensers in 100 m^2; pheromone release rate, 70–100 $\mu g/h/m^2$). This dispenser density, extremely high compared with usual moth mating disruption, was adopted based on our preliminary field experiments by using the same dimension.

Ant Counts at Baits. During the monthly renewal of dispensers, the foraging activity of Argentine ants in the experimental plots was measured by recruitment to baits. In the center of each plot, nine bait stations were set with 2-m spacing. Each bait station consisted of a water-repellent, 15-cm-diameter paper dish coated by ≈1 ml of sugar water (≈50%). The number of Argentine ants on each dish was counted 40 min after sugar water application. One to five (2.4 ± 1.0) replicate bait counts were conducted in each of the following three phases: 1) old dispenser phase: in the presence of dispensers applied in the previous month, except the first survey in each year; 2) pheromone-free phase: after removal of the old dispensers, i.e., dispenser-free conditions; and 3) new dispenser phase: in the presence of newly applied dispensers, except the final survey in each year. Foraging activity, i.e., number of ants recruited to sugar water, during phase 2 was regarded as an indicator of population density. During phase 2, ants should be able to exhibit their natural foraging ability, under pheromone-free condition. Meanwhile, in phases 1 and 3, suppression of foraging activity by synthetic pheromone is expected, and so the ant count data obtained in these phases cannot be used as the indicator of population density. Instead, relative foraging activity during phase 1 or 3 to that during phase 2 was used as an indicator of the foraging suppression effect of the pheromone dispensers. The date of surveys were as follows: 2005-1, 20–21 April; 2005-2, 20–21 May; 2005-3, 17–18 June; 2005-4, 22–24 July; 2005-5, 24 August–1 September; 2005-6, 2–4 October; 2005-7, 4–5 November; 2006-1, 29–30 April; 2006-2, 3–4 June; 2006-3, 1–3 July; 2006-4, 31 July–2 August; 2006-5, 28–30 August;

2006-6) 29 September–1 October; and 2006-7, 1–2 November. The first count trials for phases 2 and 3 were conducted after at least 30 min passed from the removal or application of the dispensers, and replicate trials in each phase were conducted with at least 40-min intervals. The period of pheromone-free condition, phase 2, was usually <24 h but was sometimes as long as 48 h, depending on weather conditions. Count trials were conducted simultaneously in the treatment and no-treatment plots and were not performed on rainy days. Paper dishes and sugar water were replaced with new baits in each count trial.

To examine whether foraging activity was affected by dispenser treatment and differed between plots, likelihood ratio tests in generalized linear mixed models (GLMM) were performed (Bates and Sarkar 2006) by using software R (R Development Core Team 2007). For each of the monthly investigations, a model was constructed with Poisson error distribution, Laplace approximation, and a logarithmic link. The response variable was the number of Argentine ants on a bait dish. Presence/absence of dispensers, dispenser age (old, no treatment, or new), and plot was entered as fixed factors, and bait station and count trial were entered as random factors.

To express the relative change in foraging activity between phases in the treatment plot, we first calculated the average number of ants on a dish in one count trial for each phase and monthly monitoring. The numbers for phases 1 and 3 were divided by that for phase 2, respectively. To evaluate the difference of foraging activity among phases that stemmed from factors other than synthetic pheromone (e.g., temperature and time zone), the same calculative procedure also was conducted for the no-treatment plot. The value for the treatment plot was then divided by that for the no-treatment plot, to compensate for the effects of such nonpheromone factors on foraging activity. In this way, the ratio of foraging activity under pheromone disruption compared with pheromone-free condition was calculated. By subtracting this value from 100%, foraging suppression rate by synthetic pheromone was obtained.

Results and Discussion

In all of the monthly monitoring surveys during the 2 yr, Argentine ant recruitment to baits in the treatment plot was suppressed when pheromone dispensers were present (GLMM, $P \leqq 0.001$). This suggests that trail following disruption by synthetic trail pheromone lasts for a long period. Ant recruitment to baits during phases 1 and 3, i.e., pheromone treatment phase (only in the treatment plot), was 0.32 ± 0.26 in the treatment plot (averaged across monthly surveys) and 1.09 ± 0.77 in the no-treatment plot, compared with that during phase 2, i.e., pheromone-free phase. When the value for the treatment plot was controlled for the fluctuation of foraging activity due to nonpheromone factors, the foraging suppression rate by synthetic pheromone was calculated as $70 \pm 27\%$ (Fig. 1). Effects of the dispensers on ant foraging activity differed significantly depending on whether the dispensers were old (applied in previous month) or new (newly applied) in six monthly monitoring surveys (GLMM, $P \leqq 0.001$). It is possible that old dispensers show less disruptive effect due to aging. If this was the case, then foraging suppression rate would have been generally higher in phase 3 (new dispensers) than in phase 1 (old dispensers). However, the suppression rate was lower in phase 1 on three occasions and lower in phase 3 on the other three occasions. Thus, the detected significant difference was caused by some unknown factor(s), and the effect of dispensers may have been stable for 1 mo (from application to renewal).

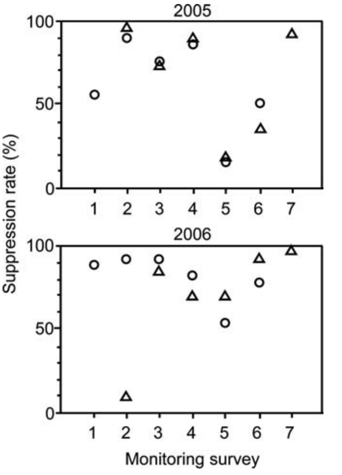

Fig. 1. Suppression rate of Argentine ant foraging activity by synthetic trail pheromone dispensers. Dispensers were applied to 100-m^2 experimental plots of Japanese house gardens during the active season of Argentine ant in 2005 and 2006, with monthly renewal. Seven monthly bait surveys (x-axes) were conducted in each year (top, 2005; bottom, 2006). Suppression rate (y-axes) was obtained by calculating relative foraging activity (recruitment to baits) under pheromone-treatment condition to temporal pheromone-free condition, compensating it for fluctuation of foraging activity due to nonpheromone factors, and subtracting this value from 100%. Triangles, results for phase 1 (old dispensers applied in the previous month are present); and circles, results for phase 3 (newly applied dispensers are present).

Despite the persistence of foraging suppression because of the pheromone dispensers, the long-term treatment seemed to have had little effect on Argentine ant density (Fig. 2). Throughout the study period, plot was not related to the variation in ant recruitment to baits (i.e., ant number did not differ significantly between treatment and no-treatment plots, when foraging suppression effect by dispensers was statistically deducted) except in three monthly surveys (2005, seventh survey: GLMM, $P < 0.005$; 2006, third survey: $P < 0.001$; and 2006, seventh survey: $P < 0.05$). Foraging activity in phase 2 was lower in the treatment plot than in the no-treatment plots in two of the three cases (Fig. 2), but this was unlikely to reflect robust control effects, because the two cases did not occur in consecutive months. In fact, during the 2 yr of the

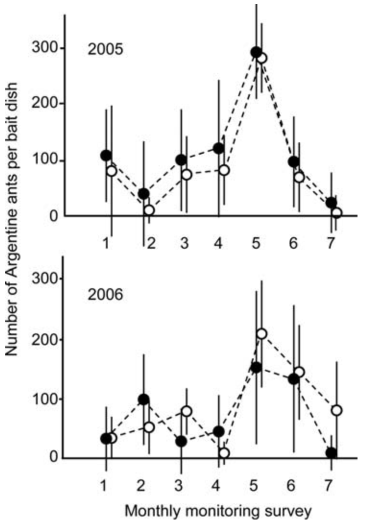

Fig. 2. Argentine ant foraging activity in the study plots during a temporary removal of synthetic trail pheromone dispensers in 2005 and 2006. Mean ± SD ant number per bait dish is shown for phase 2, i.e., pheromone-free phase. Results for the treatment plot are shown with black circles, and results for the no-treatment plots are shown with white circles. The numbers of surveys on the x-axes corresponds to those in Fig. 1.

study, foraging activity in phase 2 was generally higher in the treatment plot: the monthly foraging activity in phase 2 in the treatment plot was 1.54 ± 1.10 of that in the no-treatment plot (Fig. 2).

Several hypotheses can be advanced to explain the failure to suppress Argentine ant density with long-term synthetic trail pheromone treatment. First, the experimental plot may have been too small compared with the area infested by Argentine ants. In general, good control effects with synthetic pheromone can be obtained when the treated area is wide enough (e.g., see Ogawa 1990 for mating disruption of moth pests). In addition, the control of invasive ants is difficult with local treatment (Forschler 1997, Silverman and Brightwell 2008). Argentine ants form vast supercolonies consisting of numerous nests that tolerate each other (Tsutsui et al. 2000, Giraud et al. 2002, Sunamura et al. 2009), and individuals move among nests ranging up to 650 m^2 (Heller et al. 2008). An influx of individuals and nutrition from outside might have maintained the population density in the treatment plot. Individuals can survive and work for the colony by entering whichever nest they come across in the treatment plot, even if synthetic trail pheromone prohibits them from distinguishing the trail to their original nests. Second, trail following disruption by the dispensers may have been insufficient. Complete trail disruption would cause severe damage to resource acquisition by ants, as well as suppressing ant movement between inside and outside of the treated area. In the current study, ant recruitment to baits was greatly reduced by the pheromone dispensers, but a certain number of ants reached the baits (phases 1 and 3; Fig. 1). Indeed, workers were occasionally observed to form vague trails and follow them awkwardly (they frequently came to stand or walked in zigzags). The concentration of synthetic trail pheromone in the field atmosphere may have been unstable (e.g., it could be affected by wind conditions), particularly in a small area treatment, with the present dispenser type and treatment method (Suckling et al. 2010 obtained satisfactory results with different formulation of Z9–16:Ald, although for a limited period). In addition, minor components of the trail pheromone, if they exist (Van Vorhis Key and Baker 1982), might have allowed Argentine ants to distinguish their natural trails from synthetic Z9–16:Ald. Even though the dispensers lowered foraging efficiency, Argentine ants might have had plenty of time to collect food resources in the absence of important competitors (i.e., native ants they had displaced; Terayama et al. 2006). In both 2005 and 2006, foraging suppression by the dispensers was notably weak in the fifth monthly surveys (August), when ant activity was highest (Figs. 1 and 2). In Japan, Argentine ant density peaks in late summer, when the brood produced in late spring by new queens pupate, and the density diminishes in winter (Terayama 2008). The pattern of foraging activity observed in the current study corresponded well to this life history cycle (Fig. 2). Weaker trail following disruption against Argentine ant trails with larger traffic density was also observed in our previous study (see fig. 1 of Tanaka et al. 2009). These data were consistent with the general rule that the control effect by synthetic pheromone is weak when the density of the target pest is high (Cardé 1990), although the specific mechanism for synthetic trail pheromone needs to be addressed.

We suggest two factors, small treatment area and incomplete trail disruption, as responsible for the control failure, but there are some other possible explanations. For example, the treatment might have actually reduced Argentine ant density, but because ant recruitment to baits from nests located outside of the treatment plot was not disrupted during pheromone-free phase, it might have masked the population decline. However, this is unlikely because we confirmed the decrease in ant recruitment to baits associated with population decline in a more recent study that used the same size plots as the current study (unpublished data). In addition, starving ants that had survived in the treatment plot might have foraged more vigorously during the absence of the dispensers than ants in the no-treatment plots. Evidence that could exclude this possibility was not obtained. It was desirable to use another method that does not concern foraging behavior to measure population density, but an easy and nondestructive method has not been developed to date, and we tentatively used bait count, a prevailing measure of foraging activity and density (e.g., recommended by Alder and Silverman 2004). Furthermore, the specific treatment plot used in this study (P-1) might have had some conditions that made it unsuitable for the pheromone treatment. However, the application of dispensers to a different experimental plot in our later study did not achieve population control (unpublished data). Our data at least show that the present pheromone treatment condition does not necessarily exhibit a control effect for every environment.

In conclusion, this study demonstrated not only the long-term utility of synthetic trail pheromone as a disruptant of ant trail following behavior but also showed that population control is difficult with the present treatment conditions. The results of this first long-term field treatment with synthetic trail pheromone provide valuable findings for future studies. First, application of synthetic trail pheromone may need to be areawide so that the treated area covers at least a considerable portion of the effective colony size within the target supercolony (i.e., the range of nests within which individual ants actually move). Second, development of a formulation that enables such areawide application, as well as improvement in the disruptive effect, is necessary. Our results also suggested that when ant density is extremely high, the application of synthetic trail pheromone alone may not achieve population control. Under such circumstances, synthetic trail pheromone may need to be combined with other control agents (e.g., suppression of ant density by toxic baits before pheromone application or simultaneous treatment with synthetic pheromone and toxic baits which include potent attractant to Argentine ants and are readily detected by them even under trail disruption). Finally, the introduction of competitors (native ants which are not affected by Z9–16:Ald) to the pheromone-treated area may be an effective way to suppress resource acquisition by Argentine ants.

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