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RESEARCH

Mating Disruption of a Flighted Spongy Moth, *Lymantria Dispar Japonica* (Motchulsky) in Japan

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Abstract

Mating disruption of a flighted spongy moth, *Lymantria dispar japonica* (Motchulsky)(Lepidoptera: Lymantridae), with a synthetic version of its sex pheromone, (+)-disparlure ([7*R*,8*S*] -*cis*-7,8-epoxy-2- methyloctadecane), was tested in the forests in Japan. Pheromone trap catches and the percentage mating of tethered females were measured in the pheromone-treated and untreated control forests. The attraction of male moths to pheromone traps placed at a height of 1.5 m was significantly disrupted when the pheromone dispensers were placed at 1.5 m height, but many moths were captured in control plots. Mating of tethered females placed at 1.5 m was inhibited entirely, while 44% of females were mated in an untreated control forest. We report the first trial of mating disruption against a flighted spongy moth, and these results suggest that mating disruption with the synthetic sex pheromone appears promising for reducing damage caused by *L. dispar japonica*.

Keywords Sex Pheromone · Mating Disruption · Flighted Spongy Moth · *Lymantria Dispar Japonica* · Japan

Introduction

The Flighted spongy moth (FSM) complex (formerly known as Asian gypsy moth) is a severe threat to countries where the moth is not native because, unlike the spongy moth (SM, formerly known as European gypsy moth), *Lymanria dispar*, female FSM can fly and can disperse considerable distances (USDA-APHIS-PPQ 2014). Global cargo transport provides the pathway by which FSM is readily moved to new areas of the world because females are attracted to lights at shipping terminals and oviposit egg masses on ship superstructures and departing cargo (Chen et al. 2016). Invasion and establishment of FSM have the potential to seriously affect agricultural and forest resources in newly invaded areas, as well as their natural landscapes. Because of these threats, vessels departing from the Russian Far East, northern China, Korea, and Japan to Argentine, Canada, Chile, New Zealand, and the USA have to be inspected and must obtain pre-departure certification of being free of FSM (Mastro et al. 2021; Ministry of Agriculture, Forestry and Fisheries, Japan, 2023).

In Japan, there are two subspecies of FSM: *Lymantria dispar japonica* (Motchulsky), distributed in western Hokkaido, Honshu, Shikoku, and Kyushu, and *L. dispar hokkaidoensis* (Goldschmidt), distributed in eastern Hokkaido (Kishida 2011). Similar to FSM populations on the Asian mainland, Japanese FSM has a broader host plant range, and most importantly, females are capable of flight, providing the possibility for rapid expansion of their geographic range once they are introduced into new regions. Both virgin and mated females of *L. dispar japonica* could fly or move 200 m on average and a maximum of 750 m during one night (Iwaizumi et al. 2010). Mated females stayed motionless at the copulation sites during the days and began to walk and/or fly actively in search of oviposition sites at dusk (Koshio 1996).

The sex pheromone of SM was identified as (+)-(7*R*,8*S*)-*cis*-7, 8-epoxy-2-methyloctadecane ([+]-disparlure, hereafter) (Bierl et al. 1970). (+)-Disparlure was detected from

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sex pheromone gland extracts of female Japanese FSM, *L. dispar japonica* (Gries et al. 2005), and synthetic (+)-disparlure attracted male *L. dispar japonica* (Gries et al. 2005; Minegishi et al. 2015).

Mating disruption (MD) of SM has been used to slow the spread of SM in the USA (Coleman and Liebhold 2023), but to our knowledge, MD of FSM has not been attempted. Therefore, the objective of the current work was to test MD with synthetic pheromone for the Japanese FSM, *L. dispar japonica*, one of the subspecies of FSM and a target of quarantine inspections. We determined whether MD inhibited attraction to pheromone-baited traps and the mating success of tethered females in the forests in Japan. In spongy moths, the dosages of 49.4 and 123.6 g AI/ha reduced male trap catch by > 90% relative to untreated control plots in the ground application (Onufrieva et al. 2019). We, therefore, tested the MD in FSM at the dosages of 1.5 g, 3 g, 10 g /1,000 m^2 (= 15, 30, 100 g /ha).

Materials and methods

Insects

We collected the egg masses of *L. dispar japonica* in Tsukuba, Ibaraki (N36.04, E140.11) in August 2013 and reared the larvae on an artificial diet (Shimazu 1991) under 16 L:8D and 25 ± 1 °C. When they pupated, we separated the sexes, and after emergence, adult moths were kept individually in plastic cups. Female moths were used as tethered females to evaluate the mating success in the fields.

Study Sites

The forests used for the present study and other trap site details are summarized in Table 1. Plots A, B, C, and D, more than 200 m apart from each other, were located in an urban forest containing gardens and an arboretum on the Matsudo campus of Chiba University, Matsudo-shi, Chiba prefecture, where trees of many different genera such as *Acer*, *Aesculus*, *Cerasus*, *Chamaecyparis*, *Cinnamomum*, *Ginkgo*, *Lithocarpus*, *Prunus*, *Quercus*, *Zelkova*, and so on are growing. Plots M and N, more than 1,500 m apart, were located in the mixed forest in "Nagaragawa Fureaino-Mori" park in Gifu-shi, Gifu prefecture, where major trees are *Clethra*, *Ilex* and *Quercus*.

Pheromone Dispensers for Mating Disruption

Ethylene-vinyl acetate co-polymer tubes containing Fe_2O_3 (inner diameter 1.1 ~ 1.3 mm; 100 cm length) were loaded with one gram of synthetic racemic disparlure, and both ends were sealed. This dispenser was designed to reduce the number of dispensers in the forests and was provided by Shin-Etsu Chemical Co., Ltd., Tokyo.

Attraction to Monitoring Traps Baited with Synthetic Sex Pheromone (Trap Shutdown Test)

As bait in monitoring traps, pure (+)-disparlure was used rather than the cheaper racemate because (-)-disparlure interferes with the attraction of male moths (Miller et al. 1977; Vité et al. 1977). Rubber septum lures loaded with 0.5 mg of synthetic (+)-disparlure were provided by Shin-Etsu Chemical Co., Ltd. and kept at 5 °C until use. For monitoring, we used the SE trap (Δ-shaped section, 29 × 32 × 8 cm, Sankei Chemical Co. Ltd., Tokyo, Japan) because this trap is much more effective than the standard USDA milk carton traps used in the USA (Minegishi et al. 2015).

In all treated plots, three monitoring traps baited with pheromone-impregnated septa were hung from tree branches at a height of 1.5 m in a triangle configuration at least 15 m apart from other monitoring traps and pheromone dispensers and rotated about once a week. Pheromone

Table 1 Details of forests used in the present study

Plot	Site	Location	Area (m^2)	Treated or control	Amount of pheromone treated (g/1,000m^2)[a]	Year tested
A	Matsudo campus, Chiba University	N35.77, E139.54	1,400	Control	0 g	2014
				Control	0 g	2015
				Treated	1.5 g	2016
B	Matsudo campus, Chiba University	N35.77, E139.89	1,300	Treated	9.2 g	2014
				Treated	9.2 g	2015
C	Matsudo campus, Chiba University	N35.78, E139.90	1,650	Control	0 g	2015
				Control	0 g	2016
D	Matsudo campus, Chiba University	N35.78, E139.90	1,100	Treated	10.0 g	2015
				Treated	10.0 g	2016
M	Nagaragawa Fureaino-Mori, Gifu	N35.47, E136.79	10,000	Control	0 g	2014
N	Nagaragawa Fureaino-Mori, Gifu	N35.46, N136.81	10,000	Treated	3.0 g	2014

[a] The amount of pheromone treated was calculated by dividing the total amount deployed in the plot by the area treated

dispensers were hung from tree branches at the height of 1.5~2 m above the ground at least 15 m apart. In all control plots, three monitoring traps were placed in a triangle configuration at least 15 m apart from other monitoring traps and rotated about once a week. In 2014, we set three traps in plots A and B on July 14, hung 12 dispensers in plot B (9.2 g AI/1,000 m^2) on July 15, and checked the traps every day until August 2. After we had observed that many FSM mature larvae had marched on the railing in plots M and N, we placed three traps in plots M and N and 30 dispensers in plot N (3 g AI/1,000 m^2) on July 1 and checked the traps once a week until August 27. The number was not monitored in plots M and N before MD treatment. In 2015, we placed three traps in plots A, B, C, and D on June 24 and set 12 and 11 dispensers, respectively, in plots B (9.2 g AI/1,000 m^2) and D (10.0 g AI/1,000 m^2) on July 7, and checked the traps every day until July 26. In 2016, three traps were placed in plots A, C, and D on June 22, two and 11 dispensers were hung in plots A (1.5 g AI/1,000 m^2) and D (10 g AI/1,000 m2), respectively, on July 5, and traps were surveyed every day until the end of July.

Mating of Tethered Females

We determined mating disruption efficacy by examining the mating rates of tethered females placed at heights of 1.5 m and at least 10 m apart from the pheromone dispensers in treated plots A and D, and untreated control plot C in 2016. During the MD-treated period in 2016, we set three to eight tethered females eight times between July 5 and July 19, which is the peak emergence of *L. dispar japonica* in Matsudo (Fig. 1).

Because females have significantly less pheromone in the pheromone gland on the day of emergence than on the second or later (Tang et al. 1992), we used females which had emerged > 24 h before. A cotton thread (10 to 15 cm long) was tied carefully to the base of the front forewing of each virgin female as a tether. The other end of the thread was connected to the tree bole with a stapler at a height of 1.5 m (Sharov et al. 1995). We placed the tethered females from 10:00 to 11:00 in the morning and left them for one day (Tcheslavskaia et al. 2005), after which they were retrieved and allowed to lay egg masses. Egg masses were kept at room temperature for 30 d, and egg embryonation was analyzed with a microscope. A female was considered fertilized if more than 5% of the eggs were embryonated (Thorpe et al. 2000).

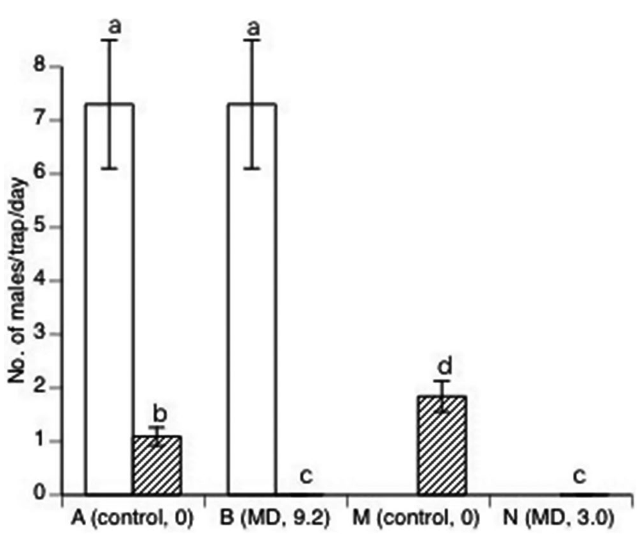

Fig. 1 Number of male moths captured (Mean/trap ± SE) in traps placed at the height of 1.5 m in the mating disruption (MD) (plots B and N) and control (plots A and M) forests in 2014. The numerals in the parentheses indicate the amount of pheromone treated (mg/1,000 m^2) in each plot. The white and hatched bars indicated the number of male moths before and after MD-treatment. The number was not monitored in plots M and N before MD treatment. Bars with the same letters are not significantly different ($P < 0.05$, Kruskal–Wallis test)

Statistics

Trap catch data were analyzed to detect significant differences between the control and the MD forest with the nonparametric Kruskal–Wallis test followed by the Steel-Dwass test. The mating rate of tethered females was compared by Fisher's exact probability test with the Bonferroni correction. Analyses were performed with R version 3.3.1 for Windows (R Development Core Team 2016).

Results

Trap Shutdown

In 2014, when MD pheromone dispensers were placed at a height of 1.5 m, monitoring traps placed at the same height in the MD forests captured no males. In contrast, in the control forests, significant numbers of males were captured in both Matsudo and Gifu (Fig. 1). In Matsudo, no males were captured in the MD plot (plot B) even though population densities were comparable between the two plots before the MD treatment. Delays in trap placement and MD treatment may have contributed to low catches in the control plot (plot A). Results were similar in 2015, with no males caught in treated plots versus large numbers of males caught in control plots after MD treatment (Figs. 2 and 3). Total trap catches in the control plots A and C were 77 and 95, respectively. In 2016, no males were captured in the MD forest with the same dose of pheromone treated as the previous year (10 g AI/1,000 m^2, Fig. 4). Significantly fewer males than in the control forest were captured in plot A treated with the lower dose of pheromone (1.5 g AI/1,000 m^2, Fig. 4). MD with

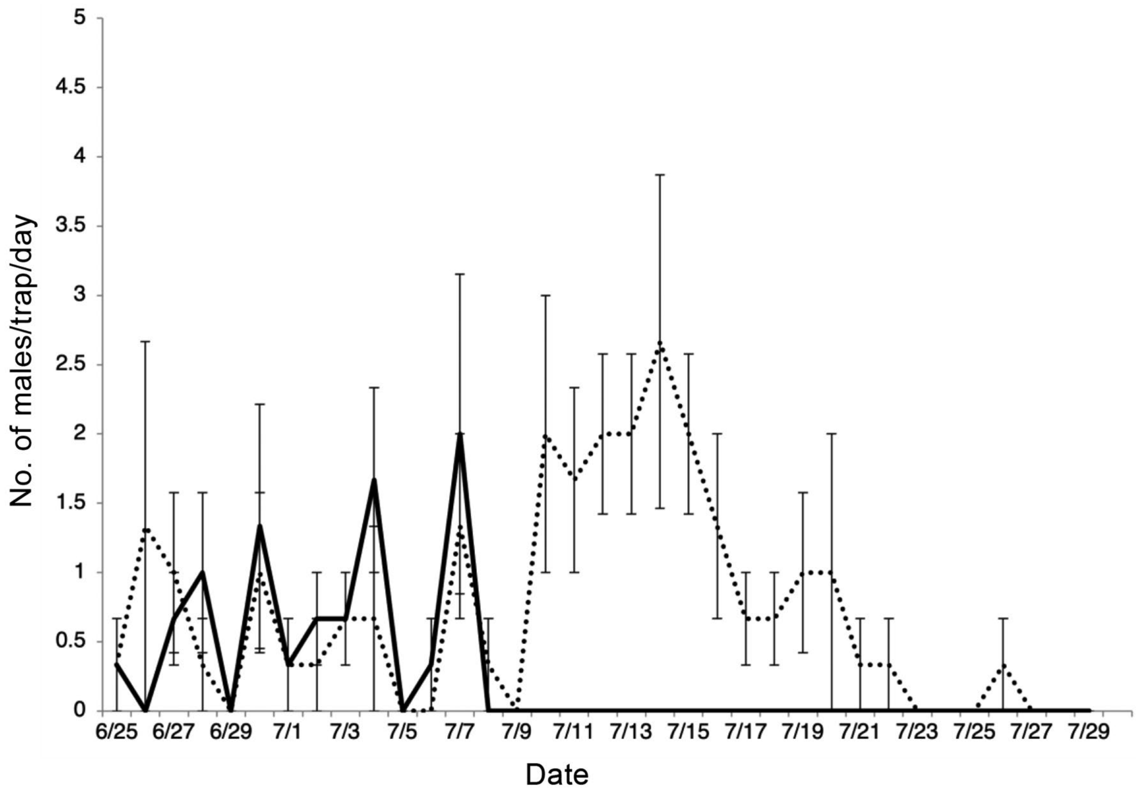

Fig. 2 Seasonal prevalence of male moth captured (Mean/trap ± SE) in control plot A (broken line) and treated plot B (solid line) in 2015

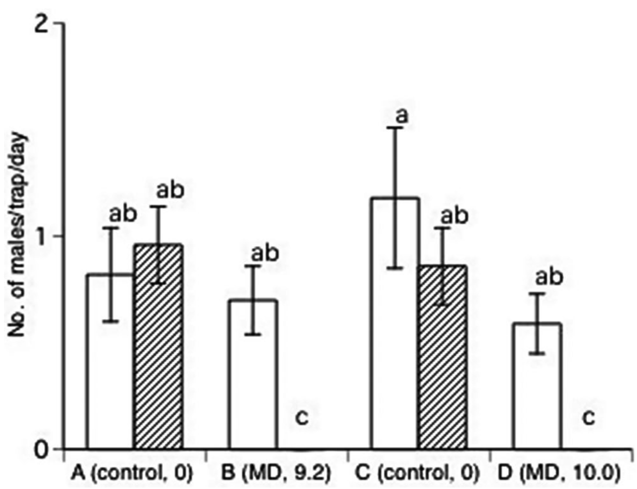

Fig. 3 Number of male moths captured (Mean/trap ± SE) in traps placed at the height of 1.5 m before (white bar) and after (hatched bar) MD-treatment in the MD (plots B and D) and control (plots A and C) forests in 2015. The numerals in the parentheses indicate the amount of pheromone treated (mg/1,000 m^2) in each plot. Bars with the same letters are not significantly different ($P < 0.05$, Kruskal–Wallis test)

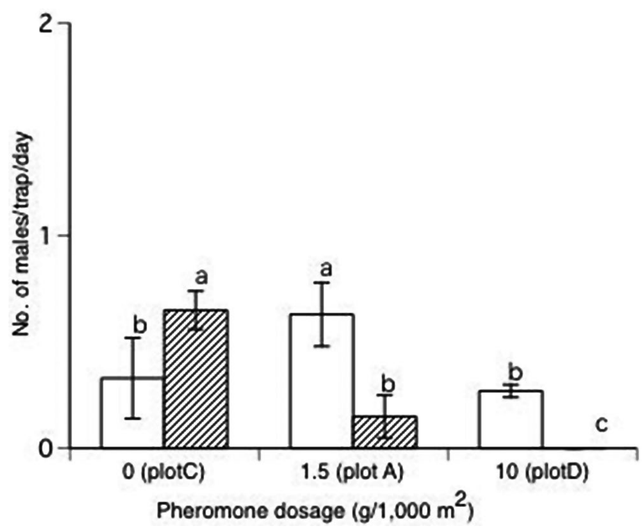

Fig. 4 Number of male moths captured (Mean/trap ± SE) in traps placed at a height of 1.5 m before (white bar) and after (hatched bar) MD-treatment in the MD (plots A and D) and control (plot C) forests in 2016. Bars with the same letters are not significantly different ($P < 0.05$, Kruskal–Wallis test)

the lower dose of pheromone decreased the total trap catch by 86% when compared to the untreated plot C. In contrast, 0.7 ± 0.1 ($n = 3$) males per day were caught in the control forests (plot C, Fig. 4).

Reduction of Mating in Tethered Females

We also examined mating disruption efficacy in virgin females tethered at heights of 1.5 m in the MD and control plots in 2016. No tethered females were mated in the MD plot (plot D, 10 g AI/1,000 m^2, $n=30$), and 9.4% of females were mated in the MD plot A with the lower dose (1.5 g AI/1,000 m^2, $n=32$), whereas in the control plot C, 44% of the females were mated ($n=34$, $P<0.05$).

Discussion

We conducted a study to verify the effectiveness of mating disruption using sex pheromones against FSM in Japan. The results showed that the mating disruption treatment inhibited the attraction of male moths during the peak flight period in 2014 and throughout the flight period in 2015 and 2016 to pheromone-baited monitoring traps and eliminated mating by tethered females in the final year of testing. The trap catches at the peak flight period in 2016 were 8, 15, and 8 males per 10 days in three traps, while Iwaizumi et al. (2009) reported that trap catches at the peak flight period in 2005 were 20 to 50 males/10 days/trap. Therefore, the density of the FSM in the present study seemed to be lower than that reported by Iwaizumi et al. (2009). In combination, these results suggest that mating disruption using synthetic pheromones effectively controls low-density populations of FSM. In plot A in 2015, adult emergence occurred from late June to the end of July, peaking in mid-July, which is consistent with the results by Iwaizumi et al. (2009), reporting that adult emergence occurred from the beginning of June to the early August in 2005 and 2006 in a port in Kanto district, where our study site (plots A to D) belongs.

Outbreaks of FSM in hardwood forests in Japan occur at intervals of about ten years, and outbreaks usually end within 2 to 3 years due to pandemics of a nucleopolyhedrosis virus (NPV) (Higashiura and Kamijo 1978). Therefore, no control measures are thought to be necessary in forests. However, in exporting ports and nearby forests where a low density of FSM has to be kept to suppress the oviposition of egg masses on ship superstructures and departing cargo, mating disruption is an effective measure to meet their requirements.

The amount of pheromone deployed in the present mating disruption trials varied from 1.5~10 g/1,000m^2 (=15~100 g/ha). To optimize the economics of MD for FSM control, mating disruption of FSM with lesser amounts of pheromone should be tested.

In particular, even though MD may not be deployed in Japan because of the endemic presence of the NPV, MD may be effective as a means of controlling FSM infestation in areas that FSM might invade. In North America, since the first FSM egg masses were discovered near the port of Vancouver in British Columbia, Canada, in 1991, there have been at least 20 confirmed FSM invasions in locations across the United States from 1991 to 2014, and eradication has been costly (USDA-APHIS, 2016). In recent years, 14 adult FSM males (10 moths in Washington state, two moths in Oregon, one moth in Georgia, and one moth in South Carolina) were attracted to pheromone traps in 2015 (USDA-APHIS, 2016), and monitoring levels have increased in such areas. If FSM male adults continue to be caught in these areas, control through mating disruption may be an effective means of controlling and possibly eradicating small localized populations before they can spread.

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Author Contributions KN contributed to the conception and design of the study. HM and AO contributed to data collection and analysis. KN wrote the manuscript, which was reviewed and approved by HM and AO.

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Declarations

Competing Interests The authors declare no competing interests.

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