Original article KOREAN JOURNAL OF APPLIED ENTOMOLOGY

한국응용곤충학회지
Korean J. Appl. Entomol. 59(4): 333-339 (2020)
DOI: https://doi.org/10.5656/KSAE.2020.10.0.057

pISSN 1225-0171, eISSN 2287-545X

Disruption of Chemical Communication of *Synanthedon tenuis* (Lepidoptera: Sesiidae) by Sex Pheromone Dispensers in Sweet Persimmon Orchards

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단감원에서 성페로몬 방출기에 의한 애기유리나방의 화학통신 교란 효과

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ABSTRACT: Pheromone-based techniques are becoming a viable strategy of insect pest management as facilitated by the exponential increase in numbers of pheromone identifications from many insect pests. This is the report on the efficacy of pheromone-mediated chemical communication disruption (PCD) technique against the Korean population of smaller clearwing moths, *Synanthedon tenuis* (Butler) (Lepidoptera: Sesiidae) using its female sex pheromone component, (*Z*, *Z*)-3, 13-octadecadien-1-ol. The PCD trials were carried out four times during 2016 and 2017 in persimmon orchards located at Suncheon and Jinju Cities in Korea, and the PCD efficacy was expressed as the mean differences in the seasonal catches of *S. tenuis* males in the PCD and control plots. The seasonal male moth catches in monitoring traps installed in the PCD plots were significantly lower as compared with those installed in the control plots. Consequently, the PCD efficacy in the experimental orchards ranged from 95.2-100% with an average efficacy of 98.8 ± 1.2%, revealing a future possibility of pheromone-based management of *S. tenuis*.

Key words: Mating disruption, Chemical communication disruption, Persimmon pests, Pheromone trap, (*Z*, *Z*)-3, 13-octadecadienol

초 록: 페로몬에 기반한 해충방제 기술은 페로몬이 동정되는 곤충의 수가 지수적으로 증가함에 따라 더욱 성공가능성이 높은 전략이 되고 있다. 이 연구는 애기유리나방, [*Synanthedon tenuis* (Butler) (Lepidoptera: Sesiidae)]의 성페로몬((*Z*, *Z*)-3, 13-octadecadien-1-ol.)에 의한 화학통신교란(pheromone-mediated chemical communication disruption, PCD)의 효과에 대한 것이다. 본 PCD법은 우리나라 전남 순천과 경남 진주의 2곳의 단감 과수원에서 2016년과 2017년에 총 4회 수행되었으며, PCD의 효과는 성페로몬 처리구와 무처리구에서의 평가용 트랩 포획수로 나타내었다. 성페로몬 처리구의 평가용 트랩에 유인된 수컷 성충수는 무처리구에 유인된 수보다 유의하게 적었으며, 유인수 감소효과는 95.2~100% (평균 98.8±1.2%)이었다. 이러한 결과로 볼 때 성페로몬에 기반한 애기유리나방의 방제가 가능할 것으로 판단된다

검색어: 교미교란, 화학통신 교란, 감 해충, 페로몬 트랩, (*Z, Z*)-3, 13-octadecadienol

Pheromone-based techniques such as mating disruption and attract-and-kill have become increasingly popular methods for managing insect pests (Yamanaka, 2007; Witzgall et al., 2010; El-Sayed, 2019). It is ever increasing in popularity, largely because of the growing dissatisfaction with the conventional chemical insecticides. A key benefit of pheromone-based programs is that they are highly selective, with species-specificity, without non-target effects on biological control agents, and without resistance and resurgence of pest species (Welter et al., 2005). Area-wide implementation of pheromone-based management strategies, including mating disruption, have resulted in significant

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Received September 7 2020; **Revised** October 12 2020
Accepted October 14 2020

reductions in pesticide use and improved pest control efficacy (McGhee et al., 2011). This is further demonstrated by near exponential increase in the use of mating disruption since the 1990s', and the area of agricultural crops managed by using mating disruption reached 770,000 ha in 2010 worldwide (Ioriatti et al., 2011; Witzgall et al., 2010). A decade (from 2002 to 2012) mating disruption increase was 75% as reported by Miller and Gut (2015). One of the most successful cases is in controlling codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), where mating disruption has been reportedly used over 90% of apples and pears grown in Washington (Hansen, 2015).

The smaller clearwing moth, *Synanthedon tenuis* is one of the 27 species under 12 genus of clearwing moths found in Korea (Arita et al., 2004; Lee et al., 2004). In southern part of Korea, its population peaks twice a year, once in late May and another in mid or late September when monitored with the sex pheromone (Cho et al., 2016). Their larval damage on the persimmon trees by feeding in the cambium layer beneath the outer bark (Yang et al., 2012; Lee and Park, 2003). In Korea, its presence with somewhat damaging level has already been described from Gimhae and the other southern parts of the country (Cho et al., 2016; Yang et al., 2012). As their larvae are largely destructive, labor and monetary efforts are required for its control. Furthermore, it is normally required to clean off the feces and wooden dust prior to the application of insecticides (Yang et al., 2012; Lee and Park, 2003).

The female sex pheromone of *S. tenuis* was identified as (*Z*, *Z*)-3, 13-octadecadien-1-ol (*Z*3, *Z*13-18:OH) (Yang et al., 2012). Trapping systems baited with this pheromone component have been successfully used for monitoring the population density of *S. tenuis* in Korea (Cho et al., 2016). In the present study, we have evaluated the reduction in male *S. tenuis* catches in the monitoring (pheromone) traps after the dense application of female sex pheromone dispensers in a treatment plots as comparing with the control plots in persimmon orchards located in southern part of Korea. Efficacy of pheromonal chemical communication disruption (PCD), measured by the trap catches may direct the future investigations prospects to more environmentally-friendly and economically-sound pheromone-based *S. tenuis* management strategies.

Materials and Methods

Preparation of Pheromone Dispensers and Lures

The sex pheromone of *S. tenuis* (*Z*3, *Z*13-18:OH, 98% pure) was synthesized by saponification of the corresponding acetate (Shin-Etsu, Japan) based on the method described in Theodorou et al. (2007). As a pheromone dispenser for PCD, a red rubber septum (11 mm O.D., Wheaton Scientific, Millville, NJ, USA) impregnated with 5 mg of the *Z*3, *Z*13-18:OH dissolved in 200 µL of hexane was kept in a fume hood until all the hexane had completely evaporated. The pheromone dispenser was then kept in a refrigerator at 5°C until use for the field experiments. The prepared dispensers were placed in a meshed muslin cloth sachet for PCD tests (Fig. 1). The muslin sachets were used to protect the dispensers from harsh weather conditions, to ensure continuous release of the pheromone from the dispensers, and to facilitate handling of the dispensers when installing and uninstalling in the field.

Preliminary Monitoring of *S. tenuis*

Before conducting PCD trials, the population densities of *S. tenuis* were monitored at the experimental orchards of Suncheon (34°55'42.7"N 127°27'20.6"E, Chonnam Province, Republic of Korea) and Jinju (35°08'28.55"N 128°8'12.02"E, Gyeongnam Province, Republic of Korea) (Fig. 2). Three bucket traps per orchard with pheromone lures as prepared above were used to confirm the presence of *S. tenuis* population in the experimental

Fig. 1. A pheromone dispenser (A) and a bucket trap with a pheromone lure for monitoring *Synanthedon tenuis* males (B) used in the communication disruption experiment of *S. tenuis* at three orchards; Suncheon, Jinju A and Jinju B located in southern part of Korea.

persimmon orchards. The monitoring experiments were carried out at Suncheon orchard from April 23 to June 24 in 2015 and Jinju orchard from April 31 – June 25 in 2015. The number of adults attracted to traps was recorded every week. Pheromone lures in the traps were changed only once during the monitoring period, after a month of trap installation.

Efficacy of Pheromone Communication Disruption

A total of four PCD trials were carried out at two sweet persimmon orchards for two consecutive years (2016 and 2017), each in two occurring seasons (spring and fall) of *S. tenuis* (Fig. 2). In Suncheon orchard, two season trials [spring (April 22 ~ June 23) and fall (July 21 ~ September 28)] were conducted in 2016. The elevations of the experimental orchards for the two seasons were 100 ~ 110 and 100 ~ 125 m above sea level, respectively, for spring and fall season. The areas of PCD testing plots were 2,862 and 9,861 m^2, respectively for spring and fall season while those of control plots were 2,796 and 6,309 m^2, respectively. The Suncheon orchard was convex in topography. In total, 120 and 400 dispensers were hung each on a persimmon tree for spring and fall season, respectively. Three monitoring traps were installed in each of the PCD and control plots at least 20 m apart from each other.

In Jinju orchard also, two season trials in two years (July 27 ~ October 19, 2016 and July 21 ~ October 28, 2017) were carried out. Elevation of the orchard for both the experimental seasons was 70 ~ 90 m above the sea level. In the fall season 2016, the areas of PCD and control plots were 9,822 and 3,554 m^2, respectively, while in the fall of 2017, the areas were 9,828 and 4,383 m^2 respectively. The topography of Jinju orchard was also convex. In total, 400 and 415 dispensers were hung on every persimmon trees of the PCD plots of fall 2016 and fall 2017, respectively. At the initial stage of experiment in the fall 2017, we found that the trap catches in a neighboring non-experimental orchard was very low. Thus, the amount of

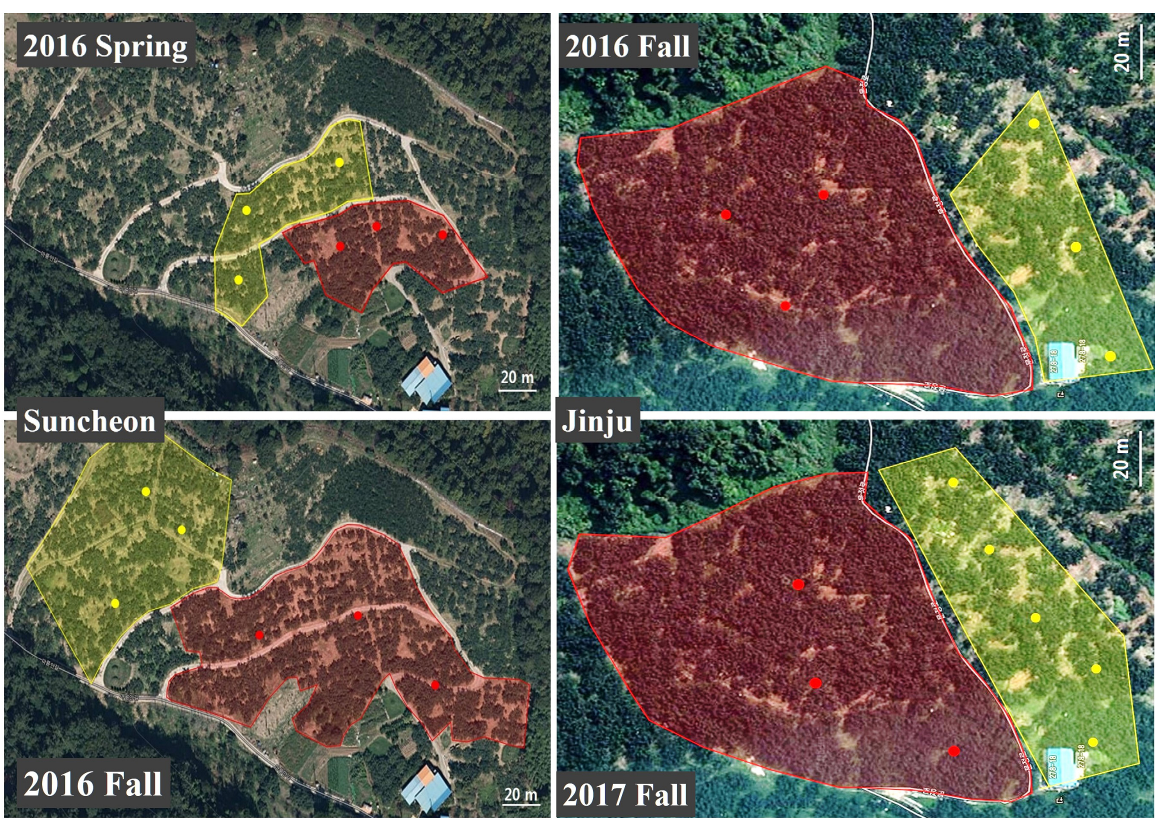

Fig. 2. Aerial photographs of experimental persimmon orchards from three different locations of southern parts of Korea: Suncheon and Jinju. Experiments were conducted at Suncheon during 2016 spring and fall seasons, at Jinju during 2016 and 2017 fall seasons. The dark red and the light yellow highlights represent for communication disruption (CD) and control plots, respectively. The red and yellow circles denote for the traps with pheromone lures installed for monitoring *Synanthedon tenuis* males.

pheromone in each dispenser was doubled to 10 mg per dispenser, and monitoring traps in control plots were increased to five mg.

No dispenser was used in control plots. As in preliminary monitoring, the trap catches were recorded every week, lures in the monitoring traps were changed every month, and the dispensers were used once for whole experimental season.

For the monitoring of *S. tenuis* males, pheromone traps (green bucket trap, Green Agro Tech Co., Ltd., Andong, Republic of Korea) equipped with rubber septa baited with pheromone lures containing 1 mg of *Z*3, *Z*13-18:OH each were deployed in both PCD and control plots.

Both the dispensers and pheromone traps were hung 1.5 m above ground either on branches of each persimmon tree or on the steel wires within each orchard following the methodology described by Yang et al. (2012). In the PCD plots, the number of dispensers were equal to the number of trees (spaced at the distance of ~ 5 m) of the plot because one dispenser per tree was installed.

However, three to five monitoring traps were installed both in PCD and control plots. For a suitable pheromone communication disruption technology, the dispensers should be labor efficient, cheaper and durable. Keeping these in mind, the cost of dispensers were used only once for the whole experimental period of each season. However, the pheromone lures used for monitoring were changed once a month.

Statistical Analyses

Difference in mean male *S. tenuis* catches in the PCD and control plots was analyzed with Student's *t*-test using SAS (SAS Institute, 2011). The four trials in Suncheon and in Jinju for two consecutive years were considered as replications. The number of trap catches in the four PCD plots and the four control plots were pooled separately, and the average male captures in monitoring traps in PCD and control plots were used for the calculation of PCD efficacy. The efficacy (%) of PCD was calculated by using Abbott's formula (Abbott, 1925).

$$PCD\ efficacy(\%) = \frac{[No.of\ catches\ in\ control - No.\ of\ catches\ in\ treatment]}{No.\ of\ catches\ in\ control} \times 100$$

Results

Preliminary Monitoring of *S. tenuis*

In the preliminary monitoring, 19.4 and 8.0 males of *S. tenuis* were trapped in the pheromone traps installed in Suncheon and Jinju orchard, respectively (Fig. 3). This result confirmed the presence of *S. tenuis* population in both of the experimental orchards. Based on this result, the PCD efficacy trials were carried out.

Comparison of Trap Catches of *S. tenuis* in Experimental Orchards

The season-long weekly pheromone-trap catches of *S. tenuis* males in PCD and control plots are presented in Fig. 4. In Suncheon orchard, the average number (of three monitoring traps) of weekly catches of *S. tenuis* males in control plot overtopped the number of males caught in PCD plot both in the 2016 spring and fall of 2016. Similar result was observed in the fall of 2016 and 2017 in Jinju orchards.

Efficacy of Pheromone Communication Disruption

The PCD efficacy expressed as differences in the mean number of seasonal catches of *S. tenuis* males in PCD and control plots is shown in Fig. 5. The overall PCD efficacy was

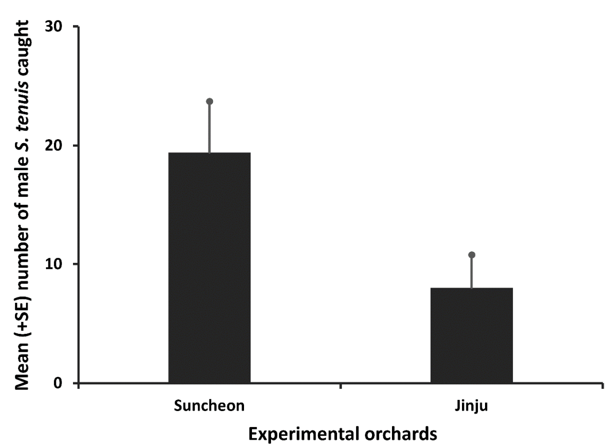

Fig. 3. Weekly catches of *Synanthedon tenuis* males in green bucket traps baited with its female sex pheromone (5 mg) lures in a preliminary monitoring.

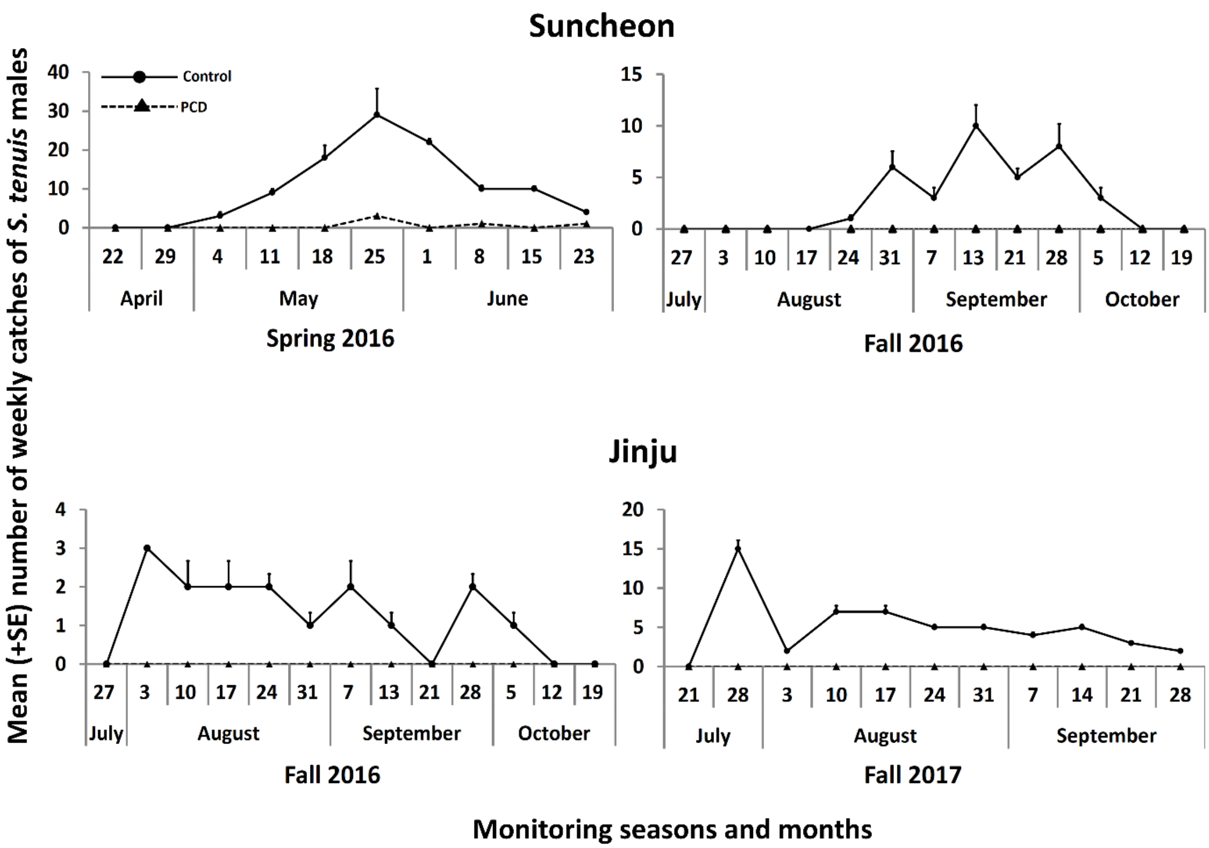

Fig. 4. Weekly catches of *Synanthedon tenuis* males in control and PCD persimmon orchards of Suncheon and Jinju in 2016 and 2017 as monitored with its synthetic sex pheromone traps.

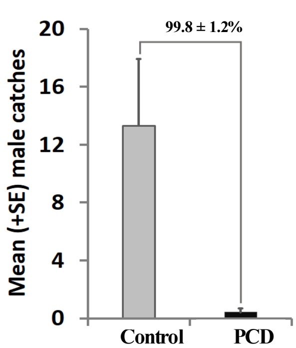

Fig. 5. Mean (+SE) catches of male *Synanthedon tenuis* in PCD and control plots. The significant difference in the seasonal mean catches between the two plots is denoted by asterisks. (*** = $P < 0.0001$ by *t*-test). The mean ± SE value above bars in the figure represents the mean efficacy (%) of PCD of *S. tenuis* at Suncheon and Jinju sweet persimmon orchards.

measured as 98.8% with the significantly lower male catches in PCD plots compared with the control plots ($t = 4.67$, $df = 10$, $P < 0.001$).

Discussion

Since the first pheromone identification in 1960s, pheromones for over 1000 species of insect species have been identified (Witzgall et al., 2010). Mating disruption using over-dosed pheromone lures become the most successful strategies in pest management practices. The pheromone communication and mating in the kin species of *Synanthedon tenuis*; *S. (Aegeria) myopaeformis* (Kyparissoudas and Tsourgianni, 1993), *S. hector* (Matsumoto et al., 2007), *S. exitiosa* (Johnson et al., 1991), and *S. pictipes* (McLaughlin et al., 1976); and other sesiids like *Vitacea polistiformis* (Weihman and Liburd, 2006), *Sanninoidea exitiosa* (McLaughlin et al., 1976), and *Ichneumonoptera chrysophanes* (Vickers, 2002), were reported successfully disrupted with the pheromone-based behavior modifying tactics. However, the present paper is the first report on the efficacy of PCD against *S. tenuis,* one of the most serious pests in Korea. Females of *S. tenuis* moths produce *Z*3, *Z*13-18:OH as a sex pheromone to attract males (Yang et al., 2012). By evaluating the number of male *S. tenuis* catches in paired PCD and control plots in two orchards located at different geographic locations through two-year periods with two different seasons, we found a very promising PCD efficacy rate.

In Jinju orchard, the increased (by × 2) dose of dispensers in the fall of 2017 was equally effective as in the fall of 2016 because there were no captures in the PCD plots. The diffusion of the pheromone into the surroundings may affect the effective attraction radius (EAR) (Byers, 2007), the distance from the point of pheromone source.

On the other hand, several studies have shown that the effectiveness of pheromone-based strategies like mating disruption is strongly correlated with an insect population density. For instance, at a low density of *C. pomonella*, the mating disruption was found satisfactory (Barnes et al., 1992) but failed at a high population density (Witzgall et al., 1999). Cardé and Minks (1995) also reviewed the success of mating disruption in many moth species, including *Pectinophora gosipiella*, *Grapholita molesta*, *Epiphyas postvittana* etc. They further suggested that the inefficacy of mating disruption at high population is due to the higher density of moth pests, which was also demonstrated by Borchert and Walgenbach (2000) in the tufted apple bud moth, *Platynota idaeusalis* (Tortricidae).

The results presented in this study are based on male catches of *S. tenuis* in the pheromone traps in paired PCD and control plots in persimmon orchards. It may suggest that the mating disruption strategy could be a future practical tool for the control of *S. tenuis* in sweet persimmon orchards. Further experiments on evaluating female mating success and quantifying damage levels of the persimmon fruits in PCD and control plots are required to justify the level of management with pheromone dispensers. This non-pesticide method of *S. tenuis* management may provide persimmon growers with an effective control strategy and benefit people living near orchards by providing a more ecofriendly environment.

Acknowledgements

This study was partially supported by RuralDevelopment Administration, Republic of Korea (PJ013356).

Statements for Authorship Position & contribution

Chiluwal, K.: Nepal Agricultural Research Council, Researcher; Designed the research, conducted experiments, and wrote the manuscript

Kim, J.: National Institute of Forest Science, Researcher; Designed the research and synthesized the pheromones

Park, C.G.: Gyeongsang National University, Professor; Designed the research, critically reviewed and finalized the manuscript

Roh, G.H.: United States Department of Agriculture, post-doctoral researcher; Designed the research, conducted experiments, and wrote the manuscript

All authors read and approved the manuscript.

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